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Sujay Rakshit
Yi-Hong Wang *Editors*

The Sorghum Genome

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The Sorghum Genome

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In memory of Dr. Neelamraju Ganaga Prasad Rao (1927–2016), father of Indian sorghum research. He had a pioneering role to bring quantum jump in the productivity of sorghum in India. The legacy he has left behind will inspire the generations to come.

Foreword

Agricultural practices need to be climate resilient so that the production system remains sustainable over a long period of time in spite of changes in climatic conditions. Millets in general, with their inherent drought tolerance and ability to yield substantially under the most neglected crop management, are climate resilient. Besides, they are a good source of micronutrients and have low glycemic indices. Thus, they fit best in the current food and agricultural scenario.

Sorghum or the 'big millet' represents nearly 60% of area being covered by millets across the world, mainly being cultivated by the resource-poor farmers of semi-arid tropics of Asia and Africa. In addition to meeting the calorific requirement of the human population particularly in rainfed regions, sorghum commands an important place as a source of feed and fodder for large animal populations in this region. In recent past, sorghum assumed renewed importance as an alternate source of biofuel. With its C4 photosynthetic pathway, sorghum has become a model plant system to understand the genetic architecture of grass family through comparative 'omics' studies.

With publication of its genome sequence sorghum has turned out to be the most sought-after system to understand the genomic architecture of C4 plants. Since 2009, a good number of studies on re-sequencing of sorghum genotypes have brought out a fair understanding of the genome organization and sequence diversity, which are being deployed in association mapping. The available information on genomic variation has its potential use in genomic selection of sorghum and allied crops. However, all these

achievements have been possible through initial studies on cytogenetics, germplasm characterization, construction of genetic and physical maps, and identification of QTLs for traits of interest.

I appreciate the efforts of the editors to organize 15 chapters in this book in such a way that all these developments have been brought out in the right perspective by eminent groups of scientists across the world. I am sure that this book will be of utility to the students, scientists working on sorghum and other field crops, science managers and policy makers.

New Delhi, India
September, 2016

T. Mohapatra

Preface

The biggest challenge facing agricultural scientists is to feed an ever-increasing human population, which is expected to reach 9.1 billion by 2050. The demand for cereals, for both food and animal feed uses, is projected to reach around 3 billion tons by 2050 from 2.1 billion tons today. This gap is to be filled in spite of decreasing availability of arable land, deteriorating soil fertility, and increased incidences of climatic extremes. Under that context, agriculturists need to make the agricultural practices more climate resilient. Sorghum is the fifth most important cereal crop after rice, wheat, maize, and barley, and is extensively grown in the semi-arid tropics of the world thanks to its inherent ability to tolerate harsh environments. Thus, this is a model crop among grass species to study stress response and ensuring food security for millions of poor masses living in the most impoverished drought-prone regions of the world. Sorghum not only provides food and feed but also serves as an important source of fodder for large cattle with its dry stover. Green plants are also a source of forage for cattle. In recent years, sweet sorghum has turned out to be a source of ethanol production and second-generation lignocellulose-based biofuels. Thus, sorghum has the potential to provide food, feed, fodder, and fuel.

Unlike other cereals such as rice, wheat, and maize, sorghum received lesser attention with regard to genetic and genomics studies in the past. The lesser economic importance of sorghum is the principal reason behind this. However, over the last two and a half decades much progress has been made in this area. After publication of the rice genome sequence, sorghum turned out to be a natural complement to rice in understanding the complexity of the genomes of this most important group of crop plants, that is, the grass family. With its proximity not only to cereal crops but also to commercial crops including sugarcane, sorghum has turned out to be a model crop to initiate genomics research through syntenic studies. With publication of the sorghum genome sequence in 2009, the scenario was revolutionized and this neglected crop started receiving prominence in genomics studies. Stress tolerance of the crop proved to be an added advantage for its popularity.

Over the period of a few decades many reports on sorghum genomics as well as transgenic research have come into the public domain, which deals with almost all traits related to the crop. These studies have exhibited promise to improve the crop further in terms of stress tolerance and yielding ability.

This has also opened up opportunity to improve other related crops as well, using the genomics information generated in sorghum.

The current volume, Compendium of Plant Genomes: The Sorghum Genome, comprises 15 chapters. Chapter 1 deals with the global status of the crop and its economic importance. It has been observed that sorghum yield levels have increased in almost all the sorghum-growing regions except Africa, and this has been achieved both due to genetic gains in the released cultivars and better crop management. Consumption of sorghum as food is declining because of changes in food habits and consumer preference. However, use for animal feed and other industrial purposes is increasing. The world sorghum trade is mainly linked to demand for livestock products. Chapter 2 is devoted to the botany, floral biology, and classification of sorghum and their implications for the breeding methods to be used. It highlights how understanding of botany and taxonomy could be effectively used for improving sorghum yield and nutritional quality.

Genomic studies of a crop are partially dependent on availability of cytogenetic information on it. Due to inherent small sizes of sorghum chromosomes such studies are scanty. Chapter 3 details the progress in molecular cytogenetics that has paved the way for genome sequencing of the crop and for understanding its genetic architecture. Furthermore, sorghum germplasm is best characterized among crop plants, which have been grouped into core and mini-core collections and a genotyping-based reference set. These have been characterized systematically to identify sources of resistance against various stresses and quality traits. All these developments are narrated in Chap. 4.

Completion of sorghum genome sequencing after that of Arabidopsis and rice is a big step leading to widespread genomics applications. Chapter 5 elaborates international private and public efforts leading to sorghum genome sequencing. The chapter also discusses a postgenomic scenario in the context of next-generation sequencing and beyond. Progress in sorghum genomics leading to elaborate syntenic studies with allied and model genomes as well as the computation needs and implications have been described in Chap. 6. Progress in crop genomics has forged a new path of gene mapping in the form of association mapping, paving the way for genomic selection. As compared to fine cereals and maize, progress in this regard in sorghum is meager. The current status is dealt with in Chap. 7.

Although sorghum is relatively stress tolerant, like other crop plants its productivity is affected by various stresses, including biotic and abiotic stresses. Chapter 8 explores the application of genomic approaches such as large-scale genotyping and high-throughput sequencing towards genetic linkage mapping, association studies, and marker-assisted selection for biotic stresses. Chapter 9 describes similar progress for abiotic stresses, for which less success have been recorded. Chapter 10 provides the current status of the application of genomics tools in improving sorghum grain quality, be it starch quality or composition of seed proteins and nonstarch polysaccharides. Underground root architecture plays a vital role in moisture and nutrient acquisition by the plants from the soil, which most commonly remains

unexplored. Chapter 11 focuses on sorghum root architecture, its screening tools, and the status of QTL analysis.

Overexpression and gene knockout studies play a vital role in gene discovery and their characterization; both are dependent on efficient transformation protocols. Chapter 12 examines studies that improve transformation efficiency in sorghum and enhance biotic and abiotic stress tolerance and nutritional quality using transgenic approaches. Chapter 13 reviews positional or map-based cloning of economically important genes/alleles and their characterization leading to their effective deployment in improvement of sorghum. The chapter further describes cloning strategies used to identify the underlying mutations of economic significance. TILLING, a reverse genomics tool, and its variant eco-TILLING, are novel tools for the discovery of genes and/or their mutant forms. Chapter 14 provides an account of this new dimension in TILLING/Eco-TILLING and its implication in sorghum genomics. Plant–microbiome interaction is a very dynamic phenomenon, being influenced by environmental stimuli. Such studies are limited in sorghum, which are elaborated in Chap. 15. Genomics and transcriptomics studies, which can be designed to understand the microbial communities associated with sorghum, are also described in this chapter.

The chapters of this book have been authored by a team of scientists who are expert in their respective fields of research in sorghum involving both conventional and genomics tools. Sincere efforts have been made to avoid overlapping in contents, however, some overlapping in isolated spots is unavoidable.

Some books in this area have already been published in the recent past by an international group of scientists. We have made efforts to include updated information in the chapters, and we believe that this book will be of much use to the sorghum research community. Any omission in the book is our responsibility and will be addressed in future editions.

We express our sincere thanks to the 42 contributors for their chapters. We sincerely appreciate their continuous cooperation starting from first submission of drafts to revision of their chapters matching with the reviews. We also thank our family members for bearing with us throughout the process of editing and finalization of this book.

Finally, we put on record our most sincere thanks to the series editor, Prof. C. R. Kole for giving us this opportunity to edit this book and Springer-Verlag and its entire staff, particularly Dr. Jutta Lindenborn and Ms. Abirami Purushothaman, for their kind understanding and help in publication and promotion of this book. We hope that this book will be useful to students, scientists both in academia and industry, and policy makers.

Hyderabad, India Sujay Rakshit
Lafayette, USA Yi-Hong Wang

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1 Economic Importance of Sorghum

K. Hariprasanna and Sujay Rakshit

Abstract

Sorghum acts as a dietary staple for millions of people living in about 30 countries in the subtropical and semi-arid regions of Africa and Asia. It is a source of food and fodder, mostly in the traditional, smallholder farming sector. It also finds a place in the high-input commercial farming sector as a feed crop, and is fast emerging as a biofuel crop. More than 80 % of the global sorghum area is characterized by low yield levels contributing to slightly above half of total grain output whereas the rest comes from the developed world with high yield levels. Though sorghum cultivation is reported from more than 100 countries, only eight countries have over 1 million ha area under sorghum, which together contribute more than 60 % of world sorghum production. In Africa, although only a few countries contribute a major share of area, sorghum is widely distributed and is a major staple food grain in large parts of the continent. In spite of its economic importance, sorghum cropped area around the world has declined over the last four decades at a rate of over 0.15 million ha per year. However, in some countries including Brazil, Ethiopia, Sudan, Australia, Mexico, Nigeria, and Burkina Faso it is expanding, mainly because of new land brought under sorghum cultivation or diversion of a portion of area planted to other crops such as maize and wheat. Global sorghum production peaked during the mid-1980s, and thereafter it declined by about 13–15 %, but not steadily. In almost all the sorghum growing regions except Africa yield levels have been enhanced over the years as a result of improved cultivars, higher input use, better resources, and crop management. Most of the sorghum is consumed in the countries where it is produced and world trade is mainly linked to demand for livestock products, which is governed by the feed requirements and prices in developed countries. Consumption of sorghum for food purposes is declining because of a change in food habits and consumer preference

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brought about by economic status, whereas use for animal feed and other industrial purposes is increasing. Under a changing climate regime sorghum would assume renewed importance as a food and industrial crop, and therefore concerted focus is necessary on such marginalized crops to ensure food and nutritional security in a sustainable manner in the years to come.

1 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal in terms of production and area harvested in the world. It is one of the most important dryland food crops grown in marginal lands in more than 100 countries, and is a dietary staple of more than 500 million poor and the most food-insecure people living in about 30 countries in the subtropical and semi-arid regions of Africa and Asia (Kumar et al. 2011). The *Sorghum* genus has many species and subspecies. There are several types of sorghum, including grain sorghums, forage sorghums (for pasture and hay), sweet sorghums (for syrups and biofuel), and Broomcorn. The crop is agronomically suited to hot and dry agroecologies where other food grains fail to yield substantially or are even difficult to grow. In these agroecologies sorghum is a dual-purpose crop, as both grain and stalks or stover are highly valued for human and animal consumption, respectively. In developed countries such as the United States, Japan, and Australia, and in some developing countries including China and Mexico, grains are important as animal and bird feed. In large parts of the developing world, stover represents up to 50 % of the total value of the crop, particularly in drought years (ICRISAT & FAO 1996).

The sorghum-based world economy has two distinct segments: a traditional, smallholder farming sector (largely in Asia and Africa as subsistence farming), and a modern high-input large-scale farming sector (principally in the developed countries and in Latin America; ICRISAT & FAO 1996). More than 80 % of the global sorghum area of 42.12 m ha (FAO 2015) lies in developing countries on the African and Asian continents (Fig. 1), where sorghum is grown primarily for food by low-income farmers. The remaining area of 16–20 % is predominantly in the developed world, especially cultivated by large-scale commercial farms, which produce sorghum mainly for animal feed. The yield levels are high in the latter sector because of the use of modern agricultural practices. Africa and Asia together account for approximately 56 % of global sorghum production, whereas the Americas contribute nearly 38 % of global output from just about 16 % of the global harvested area (Fig. 2). Production in Africa is characterized by low productivity and extensive, low-input cultivation, whereas production is generally more intensive in Asia, where improved cultivars and fertilizers are used more widely (ICRISAT & FAO 1996). Though developed countries in general are feed producers, some developing countries such as Mexico and Argentina are also major producers of sorghum for the feed market.

2 Origin and Distribution

The origin and early domestication of sorghum is hypothesized to have taken place in northeastern Africa or at the Egyptian–Sudanese border around 5000–8000 years ago (Mann et al. 1983). The largest diversity of cultivated and wild sorghum is observed in this part of Africa. From the site of early domestication, sorghum later spread to other parts of Africa and eventually to Asia including India, the Middle East, and China (Doggett 1970). Among the five different cultivated races, *durra* types presently extend from Ethiopia along the river Nile to the Near East, and farther to Thailand and across India. The *durra* types were probably introduced to Arabia as early as the Sabian Empire (1000–800 BC),

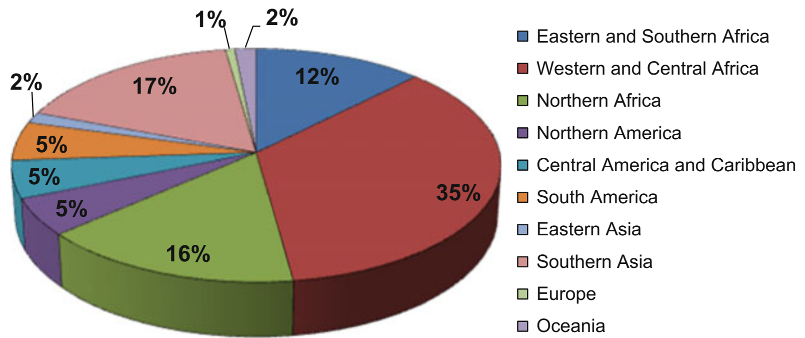


Fig. 1 World sorghum area (3-year average for 2011–2013)

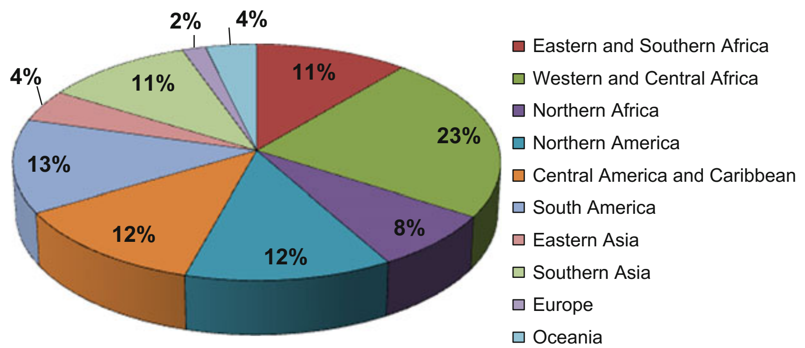


Fig. 2 World sorghum production (3-year average for 2011–2013)

and later spread to the Near East along trade routes (House 1985). Sorghum probably reached India by both land and sea routes. The secondary center of the origin of sorghum is the Indian subcontinent, with evidence for early cereal cultivation discovered at an archaeological site in western parts of Rojdi (Saurashtra) dating back about 4500 years (Vavilov 1992; Damania 2002).

The first written record of sorghum is from the first century AD, found in writings by the Roman, Pliny (Martin 1970; Smith and Frederiksen 2000). Possibly sorghum was introduced to the Near East at about the same time it appeared in Italy. Pliny recorded (in approximately 60–70 AD) that the crop was introduced to Italy from India. Distribution suggests that sorghum was probably introduced to China from India about the third century AD. The presence of *durra* types in Korea and adjacent Chinese provinces suggests that it may have been introduced there via the ancient Silk Routes from Asia Minor (House 1985). Sorghum is relatively new to the American continent. It is supposed that grain sorghum was first introduced to North America through the West Indies by African slaves during the seventeenth century. Even after widespread distribution in the Americas, production slowly dropped off until it almost disappeared (Maunder 1999). The reintroduction of grain sorghum into America occurred in California in 1874, and shortly after that it became widespread in the southern Great Plains and other arid regions of the United States as it was recognized as a drought-tolerant crop that would outperform maize (Smith and Frederiksen 2000). It was extensively used in the early 1900s for syrup (Doggett 1965). Its cultivation in Central and South America has become significant only since 1950 (House 1985).

3 Cropping Area

Sorghum is cultivated in 105 countries (Rakshit et al. 2014). Among these, 37 countries have more than 0.1 m ha sorghum harvested area and eight countries (Sudan, India, Nigeria, Niger, United States, Ethiopia, Burkina Faso, and Mexico in decreasing order) have over 1 m ha area under sorghum, which together contribute 71 % of world sorghum harvested area. In western and central Africa, sorghum is grown between the Sahara desert in the north and the equatorial forests in the south. In southern and eastern Africa it is grown predominantly in drier regions (FAO & ICRISAT 1996). Sudan has the largest area under sorghum in northern Africa and the area has increased more than four times during 2011–2013 compared to 1961–1963 (Table 1). In Asia, geographically only India and China are important sorghum-growing countries. In the Americas, the United States has the largest area followed by Mexico. In South America, Argentina and Brazil have some appreciable acreage under sorghum. Australia grows sorghum on more than 0.6 m ha. A few countries in Africa contribute the major share of area, sorghum is widely distributed, and is a major staple food grain in large parts of the continent.

In spite of the importance of the crop, over the last four decades the sorghum cropped area around the world has reduced at a rate of over 154,000 ha per year (Rakshit et al. 2014). In those countries with the maximum area, such as China, the United States, and India, the cropped area has dropped drastically. In the case of China the sorghum-growing area has declined more than 11-fold compared to 1961–1963 levels, whereas in India the area decline is almost to the tune of 60–65 % of the area sown to sorghum during the early 1960s. In the United States, the area has come down by more than 55 % in 2011–2013 compared to 1961–1963 levels. Another western Asian country with appreciable area under sorghum is Yemen, which also recorded area reduction of over 50 % of the 1961 levels. On the other hand, in some countries area has tremendously increased over the years. Brazil recorded maximum proportional increase in area compared to the 1970 level followed by Ethiopia, Sudan, Australia, Mexico, Nigeria, and Burkina Faso. In Brazil, sorghum area has increased significantly since 1995, whereas in Mexico the area has increased since the 1970s by more than 60 % compared to 1971–1973 but recorded a slow increase from 1981 onwards. The loss in area in Asia is attributed to the change in food habits, low profitability of the crop, and lack of government support, whereas that in the United States is due to government policy allowing marginal lands to be placed under the Conservation Reserve Program and competition from genetically modified maize hybrids. On the other hand, an increase in area in countries such as Australia is because of new land being brought under cultivation, higher cropping intensity with better water management, and in the recent past due to allocation of land under wheat and barley to sorghum. The steady increase in sorghum area, particularly since the 1990s in African countries is attributed to new land being brought under cultivation and some from former maize acreage. In South American countries some portion of the area planted with maize, wheat, and other crops was gradually brought under sorghum leading to an increase in sorghum area (Rakshit et al. 2014).

4 Production

Developed countries produce more than one-third of the world's sorghum and the remainder comes from the rest of the world where more than 70 % of the sorghum area is geographically located. With over 16 % of global output the United States is the world's largest producer. In the United States, sorghum cultivation is concentrated in the central and southern Great Plains where rainfall is low and variable. Nearly 90 % of the grain sorghum in the United States is produced in five states: Kansas, Oklahoma, Texas, Nebraska, and Missouri. Sorghum production in Central America and the Caribbean is dominated by Mexico, which accounts for 94 % of the region's total production. In South America production is concentrated in Argentina (56 % of the region's total) and in the dry areas

Table 1 Sorghum area and production by region/country[a]

	Area (lakh ha)						Production (lakh tonnes)					
	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013
Africa	**134.76**	**141.00**	**137.29**	**209.65**	**238.45**	**255.25**	**109.46**	**104.81**	**124.66**	**164.23**	**211.75**	**239.77**
Northern Africa	**17.86**	**22.62**	**39.37**	**55.08**	**61.21**	**63.22**	**21.24**	**24.61**	**30.59**	**40.97**	**50.56**	**44.61**
Egypt	1.95	2.05	1.66	1.44	1.57	1.47	6.90	8.46	6.23	7.40	9.08	7.82
Morocco	1.32	0.67	0.35	0.31	0.18	0.07	0.80	0.77	0.22	0.18	0.11	0.06
Sudan (former)	14.44	19.74	37.20	53.28	59.42	61.65	13.49	15.28	24.08	33.36	41.36	36.71
Western Africa	**74.62**	**74.05**	**56.39**	**110.30**	**126.61**	**121.83**	**56.81**	**44.48**	**55.22**	**89.20**	**112.87**	**110.44**
Benin	1.13	0.90	0.98	1.43	1.83	1.10	0.60	0.59	0.58	1.10	1.75	1.19
Burkina Faso	9.52	10.38	10.73	12.98	15.46	17.57	4.60	4.89	6.26	12.70	14.52	17.90
Cote d'Ivoire	0.14	0.31	0.34	0.48	0.58	0.68	0.10	0.15	0.18	0.27	0.34	0.48
Gambia	0.07	0.07	0.07	0.11	0.24	0.31	0.07	0.07	0.07	0.11	0.26	0.23
Ghana	1.56	2.18	2.11	2.93	3.37	2.34	1.05	1.64	1.21	2.76	3.11	2.75
Guinea	0.20	0.20	0.20	0.18	0.35	0.36	0.24	0.25	0.24	0.27	0.34	0.48
Guinea-Bissau	0.04	0.06	0.35	0.14	0.16	0.24	0.03	0.03	0.23	0.13	0.13	0.23
Mali	5.15	3.73	5.34	8.91	8.16	11.61	3.49	2.84	4.52	7.16	6.29	10.74
Mauritania	2.10	1.71	1.11	1.28	1.36	1.27	0.82	0.35	0.31	0.67	0.53	0.50
Niger	4.67	5.31	10.75	23.14	23.72	30.30	3.16	2.00	3.45	3.80	6.83	11.57
Nigeria	48.88	47.92	22.16	55.39	67.40	52.97	41.72	30.72	35.99	57.76	75.44	68.32
Senegal	1.09	1.22	1.13	1.19	1.94	1.41	0.81	0.87	1.23	0.98	1.52	1.05
Sierra Leone	0.07	0.05	0.09	0.37	0.16	0.30	0.12	0.06	0.13	0.23	0.18	0.34
Togo			1.03	1.77	1.85	2.18			0.81	1.27	1.63	2.50
Central Africa	**10.54**	**9.06**	**7.69**	**10.43**	**12.83**	**20.18**	**7.34**	**5.67**	**5.08**	**7.20**	**11.20**	**20.82**
Angola					0.53	1.65					0.17	0.40
Cameroon	3.01	3.43	4.04	5.10	4.11	7.78	2.55	2.43	2.66	3.90	5.40	11.33
Central African Rep.	0.62	0.47	0.54	0.17	0.47	0.40	0.33	0.38	0.43	0.20	0.42	0.47

(continued)

Table 1 (continued)

	Area (lakh ha)						Production (lakh tonnes)					
	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013
Chad	6.53	4.86	2.73	5.07	7.62	8.60	4.20	2.59	1.66	3.05	5.14	8.55
DR of the Congo	0.39	0.30	0.38	0.09	0.10	0.09	0.26	0.27	0.34	0.05	0.06	0.07
Eastern Africa	**26.26**	**30.31**	**30.09**	**30.52**	**36.14**	**48.44**	**20.48**	**24.76**	**29.64**	**23.11**	**34.20**	**62.00**
Burundi	0.20	0.21	0.53	0.58	0.57	0.57	0.20	0.20	0.53	0.66	0.71	0.50
Eritrea				14.03	1.83	2.56	0.00	0.00	0.00	0.45	0.57	0.78
Ethiopia	10.23	9.87	9.06	4.48	12.76	18.27	8.11	9.32	13.25	6.28	16.26	39.65
Kenya	1.61	2.04	0.97	1.17	1.43	2.29	1.77	2.27	0.61	1.09	1.20	1.55
Malawi	0.71	1.20	0.28	0.34	0.56	0.86	0.45	0.96	0.16	0.15	0.40	0.75
Mozambique	2.17	2.50	3.33	4.08	2.95	6.28	1.71	2.09	1.97	1.23	1.68	2.79
Rwanda	1.04	1.30	1.78	1.37	1.79	1.09	1.32	1.42	1.98	1.49	1.77	1.49
Somalia	3.83	3.80	4.64	3.10	3.58	2.43	1.32	1.33	1.92	1.06	1.33	1.71
Uganda	2.94	3.04	1.92	2.50	2.86	3.62	2.78	3.85	3.32	3.74	4.24	3.57
UR of Tanzania	1.79	3.38	5.00	6.42	5.99	7.87	1.78	1.73	4.93	6.39	5.09	8.26
Zambia	0.68	0.74	0.20	0.40	0.27	0.20	0.41	0.47	0.13	0.23	0.18	0.16
Zimbabwe	1.02	2.20	2.35	1.12	1.53	2.43	0.61	1.12	0.81	0.62	0.75	0.76
Southern Africa	**5.48**	**4.97**	**3.76**	**3.31**	**1.67**	**1.58**	**3.59**	**5.28**	**4.13**	**3.75**	**2.92**	**1.91**
Botswana	0.87	0.97	0.65	0.72	0.23	0.65	0.28	0.51	0.12	0.29	0.21	0.35
Lesotho	0.65	0.70	0.52	0.30	0.35	0.16	0.55	0.42	0.35	0.27	0.23	0.06
Namibia	0.12	0.22	0.31	0.29	0.21	0.17	0.02	0.05	0.07	0.07	0.07	0.07
South Africa	3.64	3.05	2.27	1.99	0.86	0.59	2.63	4.28	3.59	3.12	2.41	1.47
Asia	**267.39**	**230.92**	**205.34**	**152.14**	**114.49**	**82.61**	**167.08**	**178.47**	**200.07**	**169.65**	**113.17**	**99.49**
Eastern Asia	**67.17**	**51.03**	**27.19**	**13.79**	**8.01**	**5.97**	**65.12**	**87.12**	**73.65**	**51.64**	**30.04**	**25.40**
China	66.70	50.67	27.00	13.43	7.83	5.32	64.67	86.67	73.30	50.41	29.62	20.22
DP Rep. of Korea	0.31	0.23	0.12	0.09	0.13	0.21	0.34	0.27	0.18	0.11	0.21	0.37

(continued)

Table 1 (continued)

	Area (lakh ha)						Production (lakh tonnes)					
	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013
Southern Asia	**188.37**	**168.69**	**168.62**	**130.88**	**98.29**	**68.07**	**92.40**	**82.61**	**118.02**	**109.99**	**74.35**	**62.14**
India	183.46	163.35	164.69	127.04	94.65	66.04	89.92	79.29	115.78	107.73	72.13	60.88
Pakistan	4.89	5.32	3.91	3.84	3.63	2.03	2.46	3.31	2.23	2.25	2.21	1.28
Southeastern Asia	**0.08**	**0.76**	**2.57**	**1.71**	**3.00**	**2.54**	**0.20**	**1.42**	**2.90**	**2.36**	**2.88**	**2.72**
Thailand	0.08	0.70	2.51	1.71	0.69	0.29	0.20	1.29	2.79	2.36	1.24	0.55
Myanmar					2.31	2.25					1.64	2.16
Western Asia	**11.76**	**10.44**	**6.96**	**5.68**	**5.13**	**6.01**	**9.37**	**7.33**	**5.50**	**5.53**	**5.78**	**9.16**
Iraq	0.06	0.07	0.06	0.02	0.04	0.22	0.04	0.07	0.06	0.01	0.03	0.70
Israel	0.16	0.09	0.04	0	0.04	0.06	0.39	0.30	0.17	0	0.20	0.32
Saudi Arabia	1.10	1.60	1.10	1.32	1.62	0.70	1.62	0.68	0.66	1.47	2.43	2.16
Yemen	10.35	8.66	5.75	4.24	3.35	4.88	7.25	6.26	4.60	3.93	2.95	4.37
Americas	**62.65**	**99.33**	**101.12**	**72.73**	**69.88**	**63.14**	**155.27**	**298.74**	**336.19**	**259.11**	**224.92**	**215.51**
Northern America (United States)	**48.40**	**60.77**	**51.01**	**41.60**	**31.70**	**20.79**	**133.43**	**219.51**	**186.14**	**168.84**	**108.89**	**72.00**
Central America	**3.68**	**13.39**	**17.86**	**15.48**	**21.32**	**19.56**	**5.40**	**30.98**	**56.27**	**45.46**	**64.61**	**69.06**
El Salvador	0.96	1.25	1.15	1.35	0.87	0.95	0.88	1.53	1.28	1.94	1.44	1.40
Guatemala	0.31	0.41	0.40	0.68	0.43	0.27	0.19	0.41	0.82	0.80	0.52	0.44
Honduras	0.42	0.43	0.56	0.73	0.58	0.33	0.50	0.46	0.54	0.76	0.66	0.41
Mexico	1.44	10.76	14.99	12.11	18.86	17.46	3.30	27.99	52.24	40.81	60.78	65.69
Nicaragua	0.50	0.45	0.47	0.51	0.52	0.44	0.47	0.45	0.92	0.88	1.08	0.79
Caribbean	**2.24**	**2.04**	**1.67**	**1.28**	**1.23**	**1.27**	**2.13**	**2.05**	**1.42**	**1.10**	**0.94**	**1.15**
Haiti	2.00	1.98	1.57	1.20	1.19	1.23	1.84	1.87	1.14	0.92	0.87	1.09
South America	**8.33**	**23.13**	**30.58**	**14.37**	**15.63**	**21.52**	**14.30**	**46.20**	**92.35**	**43.72**	**50.48**	**73.30**
Argentina	8.25	20.74	23.77	7.21	5.62	9.39	14.17	41.40	78.83	26.26	28.14	41.16

(continued)

Table 1 (continued)

	Area (lakh ha)						Production (lakh tonnes)					
	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013
Bolivia			0.04	0.22	0.53	1.42	0.00	0.00	0.13	0.70	1.36	3.75
Brazil	0.00	0.50	1.17	1.59	5.55	7.39	0.00	0.85	2.24	2.74	11.69	20.07
Colombia	0.04	1.04	2.65	2.34	0.71	0.13	0.08	2.43	5.65	7.08	2.36	0.52
Ecuador				0.02	0.06	0.09			0.01	0.03	0.10	0.14
Paraguay	0.03	0.05	0.08	0.14	0.29	0.25	0.03	0.06	0.10	0.19	0.37	1.26
Uruguay		0.70	0.62	0.40	0.23	0.56		1.18	1.43	1.21	0.88	2.35
Venezuela		0.05	2.15	2.39	2.64	2.22		0.06	3.63	5.33	5.59	3.87
Europe	**1.52**	**2.11**	**2.86**	**2.18**	**1.71**	**2.77**	**1.73**	**5.62**	**7.07**	**8.50**	**6.67**	**9.87**
France	0.13	0.68	0.59	0.85	0.67	0.46	0.36	2.58	2.76	4.89	3.63	2.67
Italy	0.05	0.03	0.21	0.32	0.33	0.42	0.18	0.09	1.05	1.85	1.96	2.50
Romania	0.06	0.02	0.16	0.06	0.04	0.18	0.08	0.03	0.20	0.05	0.04	0.42
Russian Federation (USSR)	0.77	0.57	1.25	1.10	0.32	0.77	0.62	0.68	1.30	1.21	0.32	0.92
Spain	0.01	0.43	0.27	0.11	0.08	0.08	0.01	1.72	1.12	0.58	0.28	0.43
Ukraine				0.11	0.15	0.66				0.09	0.17	2.23
Oceania	**1.36**	**6.30**	**6.73**	**4.59**	**7.51**	**6.30**	**2.33**	**11.83**	**11.62**	**9.17**	**18.11**	**21.39**
Australia	1.36	6.29	6.71	4.58	7.49	6.29	2.32	11.81	11.60	9.15	18.07	21.34
World	**467.68**	**479.66**	**453.33**	**441.29**	**432.03**	**410.07**	**435.86**	**599.47**	**679.61**	**610.65**	**574.61**	**586.04**

[a]Each figure is a 3-year average for the respective period, for example, 1961–1963

of Brazil (27 % of the region's total). Production in Europe is limited to small areas in France, Italy, and Ukraine. In Oceania, Australia is the only significant producer. Production in Asia is far more concentrated in just two countries, China and India, which together contribute more than 85 % of the regional total (Table 1). In India, the main sorghum-producing states are Maharashtra, Karnataka, Telangana, Madhya Pradesh, and Gujarat. In the recent past, sorghum has been gaining increased popularity in coastal Andhra Pradesh under a rice-fallow situation (Chapke et al. 2011). Sorghum production in China is concentrated in the drier regions of the north and especially the northeast. However, it is distributed from Taiwan in the east, Xinjiang in the west, to Aihui county in Heilongjiang in the northeast, and to Sisha Island in the south (Gao et al. 2010). In northern Africa, Sudan is the largest sorghum producer, and production levels have nearly tripled compared to production during 1961–1963. Nigeria is the major sorghum producer in western Africa and production has increased there by more than 60 % in the period 2011–2013 over 1961–1963. However, a lot of variation has been observed over the years both in area harvested and production. In central Africa, Cameroon is the largest producer whereas in eastern Africa, Ethiopia is the biggest sorghum producer with all other countries far behind with respect to quantity of grain produced.

Global sorghum production peaked during 1985 with 77.57 m tons of grain, nearly 90 % more than the production levels recorded in the early 1960s. During the period of 1981–1983 to 2011–2013 global sorghum production fell by 13–15 % with a mean of 0.75 % per annum and the area declined during the corresponding period by 9.9 %. The decline in sorghum production is in contrast to annual increases in the production of other major grains such as rice, wheat, and maize. During the 50-year period from 1961, production grew mostly in Africa especially in Sudan, Burkina Faso, Ghana, Mali, Niger and Nigeria (North and Western Africa), Cameroon, Chad, and Ethiopia (Central and Eastern Africa), but declined in most other parts of the world, particularly in North America and Asia. Mexico in Central America and Brazil in South America exhibited significant growth in sorghum production during the period. Argentina had a significant increase in production till 1983, commensurate with the increase in area under sorghum followed by a decline in area but a gradual increase in production, indicating an increase in productivity. The sorghum production in Argentina fell from 8 m tons in 1983 to 2 m tons in 1990, as a result of a drastic fall in imports by the former USSR (ICRISAT & FAO 1996).

In Asia, production has fallen over the past three decades largely because of sharp declines in area, particularly in China and India. The drop in per capita consumption of sorghum as food occurred mainly because an increase in income levels, urbanization, changing food habits and preferences has led to a decline in cultivated area. In India, production grew by almost 5 % per annum during the 1970s, remained unchanged during the next two decades, and then started declining as sorghum has been replaced by more profitable crops such as soybean, cotton, and maize. The loss in area was partly compensated by higher productivity obtained through use of improved varieties and better management. In Africa, in contrast, production increase was due to area expansion into drier lands as a result of population growth although yield levels did not increase. Among developing countries, in Argentina and Mexico, production fell by nearly 40 % during the late 1980s, essentially because of policy interventions that led to a reduction in sorghum area.

5 Productivity

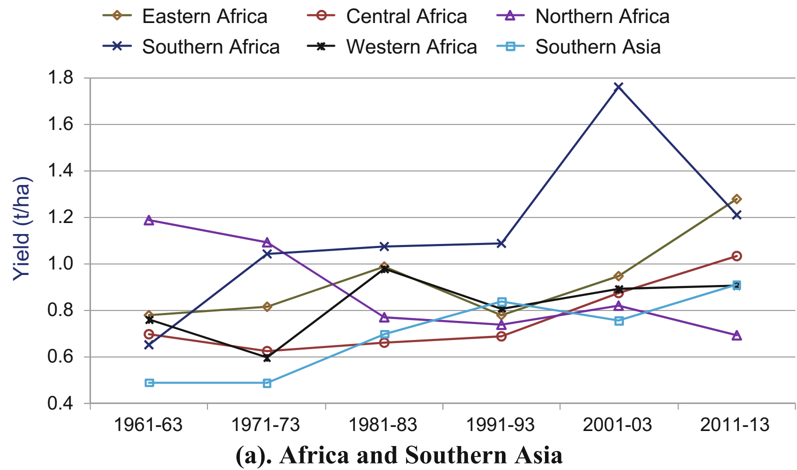
Enhanced yield levels have been observed in almost all the sorghum-growing countries as a result of improved cultivars, higher input use, and better resource and crop management. The exception is Africa, where yields declined by 14 % during the 1980s before increasing in the early 1990s. In India, productivity varies widely between regions depending on rainfall, soil type, and season. India has two adaptive types, rainy

season sorghum and post-rainy season sorghum (Rakshit et al. 2012; Patil et al. 2013). Yields in rainy season sorghum range between 2.5 and 3.5 t/ha in areas with deep soils and assured moisture, but post-rainy season yields are less than 1 t/ha as the crop is raised predominantly under receding soil moisture in low-depth soils. There are sharp differences in productivity between regions/countries essentially due to the degree of commercialization and the corresponding adoption of new technologies. For example, yield levels (2011–13 average) were more than 1.2 t/ha in Eastern and Southern Africa, less than 0.7 t/ha in Northern Africa, and less than 1 t/ha in Southern Asia (Fig. 3a), whereas they were more than 3.4 t/ha in the Americas, more than 3.7 t/ha in Europe, and more than 4 t/ha in Eastern Asia (Fig. 3b). Dramatic growth in Mexico has been recorded where average yields rose to over 3.7 t/ha by 2013 from about 2.5 t/ha in the early 1960s. In a number of developed countries, the use of hybrid seed, fertilizer, and irrigation have ensured that yield levels have increased even from a high base level (ICRISAT & FAO 11996). In contrast, in many of the developing countries most of the sorghum is produced on small and fragmented plots, poor soils, and where there is generally limited use of purchased inputs due to the economic status of the growers. In Sudan the yield levels have dropped over the years (Rakshit et al. 2014), and this is principally due to the expansion of cultivated area to more marginal lands leading to a decline in overall productivity of Northern Africa (Fig. 3a). However, Sudan and some other developing countries including Zimbabwe also produce sorghum on large farms for commercial purposes using high inputs and irrigation. Under such conditions, yield levels up to 3 t/ha have been recorded by commercial farmers compared to the national average of 500 to 670 kg/ha.

Hybrids are most widely adopted in areas where sorghum is commercially produced and in countries with a well-developed private seed industry and complementary legislation. In most of the developing countries except China, India, Thailand, Sudan, and Zimbabwe, the use of hybrids is negligible. Most hybrids are developed for feed sorghum. However, hybrids are also being developed for food grain purposes in some of the developing countries, especially in India where hybrids occupy more than 85 % of sorghum area during the rainy season (Reddy et al. 2006). In many African countries, population growth forced expansion of the sorghum area into drier and more hostile lands, resulting in a reduction in productivity. However, in some other countries government policies have also led to the reduction in sorghum productivity as a result of relocation of productive sorghum fields to maize or other crops and pushing sorghum to more marginal lands.

Among the top sorghum producers, which produce more than 1 m tons (2011–2013) of grains annually, the highest yield levels are recorded in Argentina followed by China, Mexico, the United States, and Australia (Table 2). In Argentina, the yield levels rose by 155 % during 1961–1963 and 2011–2013, and in China the yield rose threefold during the same period. The relative rise in productivity levels is lower in the United States (25 %) and Mexico (60 %), but in Australia productivity almost doubled in the 50 years between 1961–1963 and 2011–2013. Among the other top producers, the jump in yield levels ranged from 9 % (Brazil) to 174 % (Ethiopia), whereas in Sudan the yield levels have gone down by 38 %, a clear-cut indication of the spread of sorghum to less-productive soils and poor management.

Analysis of yield gain over the years in the top 10 sorghum-producing countries from 1970 to 2009 by Rakshit and others (2014) indicated that relative to yield level of 1970, sorghum productivity increased annually at 0.96 % per year across the top 10 countries. China (100.9 kg/ha/year) and Nigeria (48.6 kg/ha/year) experienced phenomenal yield gain before reaching a plateau. Overall yield gain was not associated with increased yield stability in a majority of countries except Ethiopia. In fact, in China and India (post-rainy season sorghum), the yield variability increased over time. Genetic gain for grain yield over the years in the Indian sorghum improvement program was prominent in the rainy season (over 18 kg/ha/yr for hybrids and 90 kg/ha/yr for varieties till the 1980s),



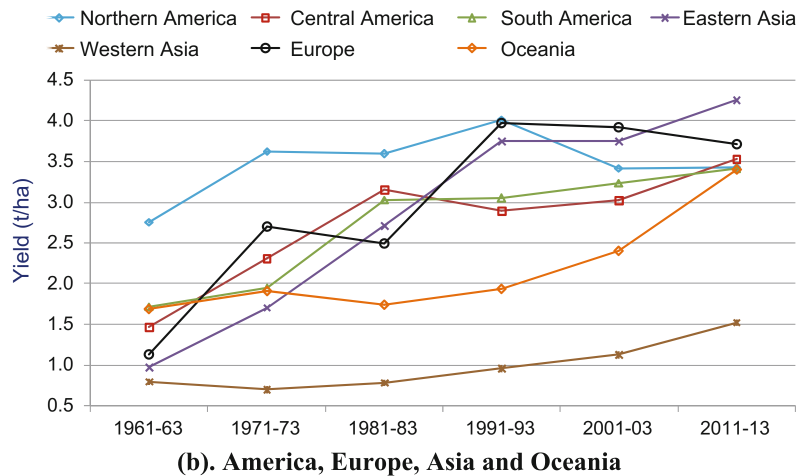


Fig. 3 Global trends in sorghum yield (3-year average, 1961–1963 to 2011–2013)

whereas it was insignificant in the post-rainy season.

6 International Trade

World trade in sorghum is mainly linked to demand for livestock products, which is governed by the feed requirements and prices in developed countries. Only about 6 % of the world sorghum trade is for food use, which is in the form of imports by countries in Africa (ICRISAT & FAO 1996). As the trade is primarily for animal feed, the quantity of sorghum traded depends on the difference in the prices of sorghum and maize and fluctuates considerably.

Most of the sorghum is consumed in the countries where it is produced. However, export volumes have increased from less than 3 m t in 1961 to over 13 m t in the early 1980s, peaking at 1981 with 14.48 m t. Substantial expansion took place in the mid-1960s and early 1970s. During this period within a span of roughly 12 years world trade in sorghum almost tripled. This was in line with the rise in imports of other coarse grains as well. The next sharp rise occurred in the early 1980s, when as a result of an export embargo by the United States, the former USSR started purchasing large quantities of sorghum in the international market (ICRISAT & FAO 1996). Exports started declining from 1985 onwards and remained at around 9 m tons until

Table 2 Sorghum yield levels in top sorghum-producing countries[a]

Country	Yield (kg/ha)					
	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2013
United States	2756	3624	3596	4013	3416	3431
Nigeria	856	637	1624	1043	1119	1294
Mexico	2347	2601	3491	3317	3214	3762
India	490	485	703	845	762	920
Argentina	1718	1953	3332	3636	5014	4380
Ethiopia	793	950	1462	1402	1280	2169
Sudan	936	776	642	621	688	576
Australia	1693	1912	1738	1938	2402	3400
China	971	1710	2715	3755	3785	3809
Brazil	2500	2232	1952	1740	2044	2721
Burkina Faso	482	471	583	985	938	1016
Niger	675	370	322	165	290	379
Cameroon	858	710	659	765	1317	1456
Mali	681	765	847	829	773	998
World	**932**	**1248**	**1498**	**1380**	**1329**	**1443**

[a]Each figure is a 3-year average for the respective period, for example, 1961–1963

the early 1990s, and then dropped further to 6–7 m tons to a current level of 6.4 m tons (2011–2012 average) valued at US $1657.24 m (Table 3). This decline in export volume and value resulted from a number of factors including a sharp reduction of production in the United States, narrowing of the price gap between maize and sorghum that made sorghum less competitive as a feed ingredient, and the lifting of a ban on maize imports by various countries including Colombia, Mexico, and Venezuela (ICRISAT & FAO 1996).

Argentina, Australia, China, and the United States are the major sorghum exporters, together accounting for more than 90 % of global export volume (Table 3). Sorghum production and export from Argentina increased sharply between the early 1960s and early 1980s, during which the harvested area rose from 0.8 m ha to 1.9 m ha. However, following a drop in demand in the second half of the 1980s, exports declined significantly. With diversion of wheat area to sorghum and matching increase in production, Australia entered the export market at the beginning of the 1970s. By the mid-1980s, China became an important exporter. However, with a sharp rise in domestic demand for sorghum as animal feed, its share in the world market dropped gradually. In fact, currently to meet its domestic demand, China is importing a significant quantity of sorghum from the international market. Some of countries such as India began exporting large quantities of grains over the years, particularly since 2002. Before 2002, exports were inconsistent, which peaked once in 1993–1994, when total production was hovering around 11–12 m t. On the other hand in countries such as Mexico, the export volume was very high in the late 1960s but over time decreased substantially. Other countries including Romania and Venezuela began sorghum exports in the early 1980s and 1990s, respectively. Ukraine and Bolivia are the two countries that started exporting sorghum after 2000, and their volumes are increasing. Countries that had steady exports till the 1980s or 1990s are Sudan and South Africa, whereas Argentina, Australia, and the United States had very high levels of export almost throughout the whole period.

Table 3 Major sorghum exporters by region ('000 tonnes)[a]

	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2012
Africa	**173.25**	**261.45**	**337.59**	**250.55**	**22.37**	**57.32**
Western Africa		**0.17**	**10.90**	**0.03**	**3.57**	**4.58**
Northern Africa	**90.15**	**67.99**	**288.48**	**204.52**	**11.32**	**16.62**
Morocco	8.88	1.08	0.01	0.09		
Sudan (former)	80.80	66.57	288.42	204.43	10.84	27.24
Eastern Africa	**1.83**	**2.99**	**3.23**	**44.31**	**4.80**	**35.01**
Ethiopia		2.98			0.91	16.34
Kenya			1.24	40.28	0.41	0.40
Southern Africa	**81.27**	**190.30**	**34.98**	**1.68**	**2.68**	**1.11**
South Africa	75.93	190.30	34.87	0.86	1.48	0.78
Asia	**0.77**	**137.55**	**286.73**	**441.17**	**57.55**	**149.20**
Eastern Asia		**0.08**	**2.14**	**384.61**	**50.84**	**53.28**
China			2.13	383.66	50.82	53.24
Southern Asia	**0.21**	**7.39**	**0.14**	**7.86**	**5.33**	**94.14**
India	0.01		0.03	7.86	5.07	93.47
Southeastern Asia		**129.16**	**284.39**	**48.54**	**1.28**	**1.77**
Thailand		129.16	245.88	48.15	1.22	1.35
Americas	3048.22	5797.62	11728.21	7808.21	6206.64	4979.77
North America (United States)	**2634.91**	**4111.70**	**6469.56**	**6634.86**	**5583.04**	**2661.66**
Central America	**2.60**	**25.84**	**10.02**	**14.21**	**0.48**	**7.34**
Mexico	0.06	19.50	3.42	13.61	0.01	0.34
South America	**410.71**	**1660.08**	**5248.63**	**1159.15**	**623.12**	**2310.76**
Argentina	410.71	1646.12	5188.72	1138.34	499.82	2282.46
Bolivia			0.21		0.28	12.62
Brazil		13.69	14.69	0.04	104.19	0.23
Uruguay		0.00	45.01	0.32	15.10	15.39
Europe	**62.27**	**201.54**	**237.16**	**265.63**	**238.87**	**221.29**
France	1.00	133.96	192.40	246.99	212.41	81.07
Italy	0.18	0.06	0.14	0.07	6.10	4.53
Ukraine					0.59	89.27
Germany	6.24	0.09	0.05	0.13	0.78	12.27
Netherlands	23.46	54.26	3.14	5.15	11.06	5.22
Hungary			6.47	8.25	1.17	4.95
Belgium	22.50	7.37	26.69	4.73	4.49	1.70
Oceania	**25.61**	**748.59**	**726.20**	**187.33**	**402.04**	**987.96**
Australia	25.61	748.59	726.20	187.33	402.04	987.96
World	**3310.12**	**7146.74**	**13315.89**	**8952.89**	**6927.48**	**6395.54**
World Export Value (million US$)	**140.76**	**480.18**	**1726.21**	**993.38**	**738.55**	**1657.24**

[a]Each figure is a 3-year average for the respective period, for example, 1961–1963, except 2011–2012

Among the African regions the biggest sorghum importer is Eastern Africa followed by Northern Africa (Table 4). In the Americas, it is Central America followed by South America, whereas on the Asian continent Eastern Asia has the largest sorghum importers compared to other regions. Analysis of imports by the different countries indicates that although several countries import sorghum, the bulk of the quantity is concentrated in a few countries such as Japan and Mexico, which account for nearly 75 % of international imports. Another significant importer is the European Union with the Netherlands, Spain, and Belgium importing sizeable quantities. Countries including the United Kingdom, Denmark, Poland, and Germany, which used to import significant quantities have reduced imports over the years. Similarly, countries such as Venezuela, Senegal, Swaziland, Sweden, Yemen, and Zambia, which used to import sorghum in small to medium quantities in the 1960s or 1970s, now only import sorghum in some years. Total sorghum imports worldwide have been falling since the mid-1980s and continue to fall (Table 4). Some of the countries without continuous imports but with very high quantity at certain points of time are Benin (1980–1990), Cyprus (1975–1985), Indonesia (1982–1988), Ireland (1966–1981, 2007–2008), Mali (1988–1994), Singapore (1980–1988), Turkey (1989–1991, 1996–1997), Iran (1975–1979, 1982–1989), and Iraq (1984, 1989–1992). India imported sorghum till 1986 and thereafter it has been almost nil. Some of the countries that started importing in the recent past or post-1980s are China, the Philippines, Morocco, Sudan, Switzerland, Thailand, Tunisia, and Uruguay.

7 Sorghum Grain Composition

The composition of sorghum grain is similar to that of maize or other cereal grains. However, the perceived poor nutritional and processing quality of sorghum is because of the presence of tannins and poor protein digestibility, which affects its use in food and feed. In general, sorghum varieties with a pigmented testa have condensed tannins (Dykes and Rooney 2006). Depending on the presence of tannins or other phytochemicals, sorghum is classified into different types. Sorghums without a testa are generally white, and are preferred for direct food uses, as these sorghums contain the lowest amount of phytochemicals. Other types of sorghum contain a testa but do not have tannins, and may be called yellow, although most have a red-colored appearance. Brown-colored sorghums contain a pigmented testa and condensed tannins, and are generally bird-resistant. Red or brown sorghums have the best nutraceutical potential as they have high antioxidant capacity imparted by phenolics, anthocyanins, and tannins (Serna-Saldivar and Rooney 1995; Rooney and Serna-Saldivar 2000). Sorghums are free of tannin, having nearly the same levels of phytin and phytic acid as maize and other cereals, but digestibility is found to be slightly reduced compared to maize (Rooney 2003). The proximate composition and nutritional aspects of grain sorghum have been extensively reviewed by Hulse et al. (1980), and Subramanian and Jambunathan (1980). The grains are particularly rich in starch (56–75 % of the total dry matter) and soluble sugar, pentosans, cellulose, and hemicellulose are low. Sorghum endosperm contains 23–30 % amylose and 70–77 % amylopectin, but waxy varieties contain less than 5 % amylose (Leder 2004). The soluble sugars range from 0.7 to 4.2 % and the reducing sugars from 0.05 to 0.53 %. Crude protein content in sorghum grain ranges from 9 to 13 % of dry matter and is slightly higher than that of maize. Fat content is slightly lower in sorghum grain (2.1–7.6 %) than in maize. Crude fiber ranges from 1.0 to 3.4 % and ash from 1.3 to 3.3 %. Sorghum grain is devoid of xanthophyllin and 70 % of its phosphorus is bound in phytate (Sauvant et al. 2004).

A comparison of nutrients in various cereals is presented in Table 5. The protein quality of sorghum grain is poor because of the low content of essential amino acids such as lysine (1.06–3.64 %), tryptophan, and threonine (Badi et al. 1990). Sorghum is poorly digested by infants (MacLean et al. 1981), but if it is supplemented with foods high in lysine, can be a satisfactory

Table 4 Major sorghum importers by region ('000 tonnes)[a]

	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2012
Africa	**63.37**	**113.62**	**258.88**	**534.81**	**356.31**	**751.19**
Western Africa	**13.86**	**61.17**	**112.49**	**92.38**	**14.74**	**50.77**
Burkina Faso		0.64	11.15	17.77	0.07	
Mauritania			13.68	5.86	1.30	
Niger	0.03	19.22	24.38	21.67	0.77	27.27
Senegal	13.83	41.31	36.00	17.61	0.01	4.95
Northern Africa	**9.77**		**0.14**	**228.25**	**184.67**	**219.73**
Egypt	9.77		0.01	37.03		30.46
Morocco			0.10	0.26	0.11	38.63
Sudan (former)				189.00	41.40	290.27
Central Africa		**11.67**	**89.91**	**12.31**	**0.14**	**48.26**
Chad		11.67	85.00	5.67		45.99
Eastern Africa	**8.68**	**12.47**	**15.07**	**102.22**	**74.38**	**251.57**
Eritrea				14.57	29.11	27.00
Ethiopia		0.94	4.05	51.55	14.31	39.64
Kenya				17.75	0.16	63.86
Mozambique	1.67			10.30	0.09	1.91
Rwanda			0.07	1.40	0.16	18.50
Somalia	1.00	3.94	7.42	20.58	14.42	28.35
Zimbabwe		5.67	0.00	17.13	7.21	13.71
Southern Africa	**31.07**	**28.31**	**41.26**	**99.64**	**82.39**	**180.86**
Botswana	22.33	15.67	23.54	10.16	40.97	84.07
South Africa	1.57	2.88	11.06	85.81	36.58	91.94
Asia	**614.40**	**4687.00**	**4778.75**	**3655.20**	**1875.15**	**1714.95**
Eastern Asia	**431.96**	**3708.89**	**4160.04**	**3374.44**	**1781.40**	**1632.71**
Republic of Korea	0.09	0.04	213.19	79.64	4.40	5.51
Taiwan		23.24	705.55	58.05	50.46	98.07
Japan	431.87	3685.61	3228.48	3229.15	1724.37	1485.80
China		23.24	718.36	61.18	52.62	141.40
Southern Asia	**3.41**	**395.99**	**86.16**	**4.95**	**10.11**	**0.74**
Iran	0.00	0.04	73.18		10.03	
India	3.28	395.95	11.07			
Southeastern Asia			**54.42**	**3.02**	**6.08**	**21.38**
Philippines				0.37	4.76	15.40
Singapore			34.01	0.46	0.05	0.01
Indonesia			15.80		0.30	4.59
Western Asia	**179.03**	**582.13**	**478.13**	**272.79**	**77.55**	**60.11**
Israel	175.97	576.12	369.96	170.33	71.48	43.81
Americas	**84.17**	**480.73**	**3545.24**	**3988.78**	**4477.99**	**3348.74**

(continued)

Table 4 (continued)

	1961–1963	1971–1973	1981–1983	1991–1993	2001–2003	2011–2012
Northern America		**0.18**	**0.60**	**2.08**	**5.76**	**19.34**
United States		0.18	0.60	0.72	0.22	15.44
Central America	**78.54**	**102.93**	**2884.38**	**3892.22**	**4377.79**	**2059.72**
Mexico	75.95	92.34	2880.04	3890.75	4376.75	2053.25
Caribbean	**0.22**	**0.29**	**0.04**	**2.34**	**1.91**	**0.07**
South America	**5.41**	**377.33**	**660.22**	**92.14**	**92.53**	**1269.61**
Colombia			89.02	17.88	27.62	550.02
Ecuador			3.33	21.23		22.67
Venezuela	0.76	361.42	558.65	4.68	0.47	0.01
Chile		7.62	6.27	29.23	51.74	616.65
Peru	0.28	5.11	0.30	9.16	0.13	70.80
Europe	**2255.42**	**1177.80**	**4230.75**	**551.64**	**557.26**	**505.85**
France	13.69	3.04	2.41	1.42	1.43	41.97
Italy	0.60	20.37	4.57	93.82	98.92	47.49
Spain	0.76	313.75	732.82	298.53	324.82	235.39
United Kingdom	408.25	108.96	4.12	2.41	5.16	10.14
Netherlands	701.06	195.98	57.37	31.86	23.14	23.99
Belgium	514.15	263.46	171.14	92.33	44.39	48.69
Denmark	224.78	1.26	0.13	0.17	0.61	1.85
Norway	62.48	37.43	134.91	0.02	32.08	2.68
Portugal		78.08	189.47	1.76	11.60	14.30
Poland	163.57	21.80	0.00	12.29	1.33	7.69
Germany	136.66	57.64	11.39	8.58	8.96	65.11
Oceania			**10.95**	**10.96**	**58.83**	**82.88**
New Zealand			1.50	0.01	26.43	63.24
Papua New Guinea			9.44	5.67	32.14	19.00
World	**3017.36**	**6459.15**	**12824.57**	**8741.38**	**7325.54**	**6403.61**
World Import Value (million US$)	**162.00**	**504.37**	**1820.75**	**1088.72**	**945.86**	**1896.80**

[a]Each figure is a 3-year average for the respective period, for example, 1961–1963, except 2011–2012

weaning food (Badi et al. 1990). Sorghum proteins become less digestible after cooking (Actell et al. 1981; Eggum et al. 1983; Duodu et al. 2003) due to change in the structure of kafirin present in grain protein. Sorghum is a good source of minerals and B vitamins such as thiamin, riboflavin, vitamin B6, biotin, and niacin, but refining leads to losses of all B vitamins (Hegedus et al. 1985). The chief minerals present in sorghum grain are potassium and phosphorus, whereas calcium is low (Khalil et al. 1984).

Sorghum is a rich source of various phytochemicals including phenolic compounds, anthocyanins, phytosterols, and policosanols that are secondary plant metabolites or integral cellular components (Awika and Rooney 2004). Phenolic compounds can be classified as phenolic acids, flavonoids, and condensed polymeric phenols (flavan-3-ols) known as tannins. Condensed tannins decrease the nutritional value of the sorghum grain because they bind to dietary proteins, digestive enzymes, minerals such as

Table 5 Comparison of nutrient composition in 100-g edible portions of various cereals at 12 % moisture

Cereal	Protein* (g)	Fat (g)	Carb. (g)	Crude Fiber (g)	Ash (g)	Energy (kcal)	Calcium (mg)	Iron (mg)	Thiamin (mg)	Niacin (mg)	Riboflavin (mg)
Wheat	11.6	2.0	71.0	2.0	1.6	348	30	3.5	0.4	5.1	0.1
Rice (brown)	7.9	2.7	76.0	1.0	1.3	362	33	1.8	0.4	4.3	0.0
Maize	9.2	4.6	73.0	2.8	1.2	358	26	2.7	0.4	3.6	0.2
Sorghum	10.4	3.1	70.7	2.0	1.6	329	25	5.4	0.4	4.3	0.2
Pearl millet	11.8	4.8	67.0	2.3	2.2	363	42	11.0	0.4	2.8	0.2
Finger millet	7.7	1.5	72.6	3.6	2.6	336	350	3.9	0.4	1.1	0.2
Foxtail millet	11.2	4.0	63.2	6.7	3.3	351	31	2.8	0.6	3.2	0.1
Proso millet	12.5	3.5	63.8	5.2	3.1	354	8	2.9	0.4	4.5	0.3
Little millet	9.7	5.2	60.9	7.6	5.4	329	17	9.3	0.3	3.2	0.1
Barnyard millet	11.0	3.9	55.0	13.6	4.5	300	22	18.6	0.3	4.2	0.1

*(N $\times$ 6.25)
Source Hulse et al. (1980), United States National Research Council/National Academy of Sciences (1982), USDA/HNIS (1984)

iron, and B vitamins including thiamin and vitamin B6 (Wang and Kies 1991) and hence are considered to be antinutritional. However, these phytochemicals have the potential to have a significant impact on human health through high antioxidant activity against different free radicals in vitro (Awika and Rooney 2004). Tannins also provide some resistance against bird damage.

8 Sorghum Utilization

Sorghum is used for two major purposes: human food and animal feed. In the early 1960s a significant portion of the sorghum output was used directly as human food. However, consumption of sorghum for food purposes has been declining since then, whereas consumption of sorghum as animal feed has more than doubled. The volume of total food use has remained unchanged or has slightly declined. In North and Central America, South America, and Oceania most of the sorghum produced is used for animal feed. Globally, the demand for sorghum as feed has been the main driving force in increasing production and international trade since the early 1960s (FAO 1995).

8.1 Food Use Across the World

Sorghum is one of the chief cereal grains consumed in many traditional foods in Asia and Africa (Rooney and Serna-Saldivar 2000). Globally, nearly 50 % of all sorghum produced is used as food. In many parts of the developing world especially in the drier and more marginal areas of the semi-arid tropics, it is a key staple. Though total food consumption of all cereals has risen considerably during the past 50 years worldwide, consumption of sorghum has remained stagnant or gradually falling. A shift in consumer preferences as a result of rapid urbanization and increase in economic status has resulted in a decline in per capita consumption in many countries. Furthermore, the drudgery associated with preparation of food products and unavailability of processed sorghum has added to the decline in per capita usage. Per capita food consumption of sorghum in rural areas remained stable and usually higher compared to urban regions (ICRISAT & FAO 1996). Within the rural areas consumption is higher in the poorest and most food-insecure section of society. However, the scenario is fast changing in many growing economies such as India and China because of enhanced purchasing power and changes in national policy. For example, in India between 1972–1973 and 2009–2010 per capita consumption of sorghum sharply declined from 8.5 to 2 kg in urban areas and 19.2 to 3.5 kg in rural areas (Nagaraj et al. 2013).

Sorghum is consumed in a number of forms depending on the part of the world concerned. Generally, whole grain is processed into flour, from which traditional meals are prepared. Main sorghum-based foods are: unleavened bread prepared from fermented or unfermented dough in Asia and parts of Africa; thin or thick fermented or unfermented porridge, mainly consumed in Africa; and boiled products similar to those prepared from rice or maize grits. In India, sorghum is utilized in the preparation of many traditional foods and in bakery products such as bread, cakes, and biscuits. The grain is mainly consumed in the form of unleavened flat bread or *roti* or *bhakri* prepared from the flour. In certain parts of India, especially in the South, sorghum is traditionally boiled after dehulling. A type of thick or thin porridge is also prepared and is known by different local names. Food preparations of sorghum in various parts of Africa include porridge, sweets, and savories known by different names as *to*, *bogobe*, *ugali*, *ogi*, *injera*, *kisra*, *tortilla*, and *couscous*. Porridge made out of either fermented or unfermented sorghum is consumed with vegetables or meat. Porridges can be thin or thick (Anglani 1998). *Ogi*, which is a fermented porridge, is the most important weaning food for babies. Sorghum grain is mixed into dough and baked as flat unleavened bread to produce tortillas. Couscous is a steamed agglomerated food made from the flour of decorticated cereals such as durum wheat, maize, sorghum, and pearl millet and is usually consumed with milk or buttermilk. It is a major food

staple in North Africa (Kaup and Walker 1986). In the Middle East countries, sorghum grain is used for the preparation of soups, porridges, cakes, and other bakery products. It is also used for the preparation of leavened bread in combination with other flours. In China, sorghum is the main ingredient for production of distilled beverages such as *Maotai* and *Erguotou*. In many countries, especially in Africa, sorghum is being used as a substitute for other grains in the production of gluten-free beer.

In the recent past, sorghum has been processed to ensure the availability of sorghum-based food products throughout the year and across the country in a safe and convenient form. Several processed and value-added food preparations, either ready-to-eat or ready-to-cook, such as cookies, bars, cakes and pancakes, deep-fried food products, flakes, or healthy and convenient products such as multigrain flour, *suji*, and the like have been developed in India (Dayakar Rao et al. 2014). In recent years, contemporary food processing technologies such as extrusion cooking for ready-to-eat food and breakfast cereals, pasta, and vermicelli noodles have been made from sorghum (Malleshi 2015).

8.2 Feed Use

Sorghum and maize are major ingredients in pig, poultry, and cattle feeds in the western hemisphere (Bramel-Cox et al. 1995). The demand for sorghum for feed is concentrated in the developed countries, where animal feed accounts for about 97 % of total use, and in some higher-income developing countries, especially in Latin America where 80 % of all sorghum is utilized as animal feed (FAO 1995). The United States, Mexico, and Japan were the main consuming countries, followed by Argentina, the former USSR, and Venezuela, which together accounted for over 80 % of world use of sorghum as animal feed in the late 1980s (ICRISAT & FAO 1996). However, currently continent-wise the maximum sorghum is consumed in Africa (42.4 % of 62 million tons), next in Asia (19.0 %), and South America (11.2 %). However, countrywise, Mexico is the highest consumer of world sorghum (14.2 %) followed by Nigeria (11.9 %), India (11.2 %), Sudan (7.9 %), the United States (6.7 %), Ethiopia (5.8 %), and others (Table 6). In Mexico 97.7 % is consumed as feed, whereas in Nigeria, India, and Sudan sorghum is used substantially for food purposes. In the United States, 51.2 % sorghum is processed and 43.3 % is used as feed. Worldwide use of sorghum as feed and food remains at 43.1 and 40.4 %, respectively.

Sorghum has been increasingly used as feed over the years in many developed countries as well. For example, in China out of the total global production during 2008–2009, livestock and feed consumption accounted for 42.6 % whereas food consumption accounted for only 41 % (Rao et al. 2010). Sorghum is used primarily as poultry feed and can be fed as the main or only grain in poultry diets, and secondarily as cattle feed. Broilers can be fed up to 70 % low-tannin sorghum in combination with soybean meal, minerals, and vitamins. Low-tannin sorghum has a metabolizable energy comparable to or higher than that of maize (Sauvant et al. 2004), and can replace maize grain to a great extent (Subramanian and Metta 2000). In India, broiler production is expected to grow at 15–20 % per annum and layers at 10–15 % per annum, leading to a corresponding expansion in demand for feed (Parthasarathy Rao and Birthal 2008). In the United States, due to its similar nutritional value, sorghum grains are used primarily as a substitute for maize in livestock feed. Often feed sorghum hybrids containing a higher concentration of tannins and phenolic compounds call for the need for additional processing to allow the grain to be digested by cattle. As tannins are known to reduce growth rate, egg production, and protein utilization in poultry, increasing protein in the diet may alleviate the deleterious effects of tannins. In the case of pigs, feed efficiency may be reduced by 5–10 % between high-tannin sorghum and low-tannin

Table 6 Demand and supply of sorghum in different countries and continents as of 2011

Countries/Continents	Supply ('000 tonnes)				Consumption ('000 tonnes)			
	Production	Import	Export	Domestic supply	Feed	Food	Processing	Other
Mexico	6429	2380		8809	8604			205
Nigeria	6897	14		7411	1112	5179	163	957
India	7003		38	6965	70	6222	0	673
Sudan	4605	290	27	4868	500	3165	168	1035
United States	5447	1	3363	4162	1803	208	2133	18
Ethiopia	3951	53	22	3583		2249		1334
Argentina	4458		1848	2611	2504			108
China	2054	98	69	2082	1000	954		128
Australia	1935		76	1959	1913		41	5
Brazil	1931			1931	1881			50
Rest of world	13,151	1242	219	14,738	4381	7071	1719	1572
Africa	**25,004**	**763**	**113**	**26,281**	**2994**	**16,768**	**1946**	**4573**
Western Africa	11,343	31	5	12,360	1517	8382	876	1586
Southern Africa	215	66	1	330	55	139	116	20
Middle Africa	1842	49		1891	265	1254	104	268
Eastern Africa	6150	218	77	5909	100	3540	682	1586
Asia	**10,292**	**1595**	**110**	**11,767**	**2991**	**7902**	**0**	**876**
Europe	**928**	**770**	**170**	**1517**	**1494**	**1**		**22**
Northern America	5447	5	3363	4166	1807	208	2133	18
South America	7598	1206	1849	6955	6592			365
World	**58,093**	**6772**	**5691**	**61,960**	**26,697**	**25,053**	**4120**	**6095**

Source http://faostat.fao.org/ accessed on July 02, 2016

sorghum (Cousins et al. 1981). In ruminants, the effects are less negative because condensed tannins are complexed and precipitated by ruminal microflora (Reed 1995).

Apart from grains, sorghum stover is an increasingly important feed in the livestock sector in India and some other parts of Asia for dairy animals. Sorghum fodder serves as an excellent cattle feed in the dry seasons when other fodder resources are not available. Although certain varieties are grown solely for grain purposes, there are some varieties that have been developed for forage production. Sorghum is preferred as a forage crop because of its quick growth, high yielding ability, high dry matter content, leafiness, wider adaptability, and drought tolerance. Some of the sorghums specifically grown for fodder purposes can regenerate after cutting the stalks for fodder (multicut fodder sorghum) and harvesting the grain, and hence have much higher biomass yield potential per unit area compared to many other forage crops. Sorghum is suitable for silage and hay making and thus supplements the nutritious supply in lean seasons. Sweet stalked sorghums are suitable for feeding to animals as forage, silage, and hay, apart from industrial uses.

8.3 Industrial Use

Alternative uses of sorghum involve utilization of the grain and sweet stalk in food and nonfood sectors for production of commercially valued products such as alcohol (potable and industrial grade), syrups (natural and high fructose),

glucose (liquid and powder), modified starches, maltodextrins, *jaggery*, sorbitol, and citric acid (CFC & ICRISAT 2004). Industrial utilization of sorghum for starch and ethanol production is on the rise and the potable alcohol sector is the fastest growing sector in India (Kleih et al. 2000). Additionally, new processed and value-added food products for human consumption such as popped sorghum, porridge, *rava*, and the like are emerging, which need to be scaled up, and are likely to be significant avenues for diversifying industrial utilization of sorghum (Parthasarathy Rao et al. 2010). Other technologies such as production of glucose, maltodextrins, high fructose syrup, and cakes from sorghum are in the nascent stages. Presently, industrial use other than livestock feed accounts for about 10 % of world sorghum production (Rao et al. 2010).

Because of its high biomass production and adaptation across semi-arid tropical environments sorghum is widely believed to be a model biofuel feedstock. Sorghum biomass yield as high as 40 t/ha has been reported (Rooney et al. 2007). Sweet stalked sorghum has a long history of cultivation in Asia, Europe, and America. Because of the rapid increase in crude oil prices from the 1970s, sweet sorghum has been investigated as a potential source of fermentable sugars from the juice for ethanol (biofuel) production. The sweet sorghum with its juicy sweet stalk can be used as a bioenergy crop. Other sweet sorghum products such as syrup and *jaggery* have received attention in the production of sweets and ready-to-serve foods (Reddy et al. 2012). In the United States, sorghum syrup is used as an alternative to sweetener for the preparation of bakery items such as biscuits and cakes.

Industrial applications where inexpensive starch is needed to keep the cost of production low (e.g., in production of low-cost adhesives, binders for casting metals, etc.), whole or partially decorticated acid-modified sorghum flour is often used. Packaging materials can also be produced from sorghum (Rooney and Waniska 2000). Excellent quality wallboard and biodegradable packaging material can be produced from sorghum stem fibers. In addition, it is also used as a source of pulp for the paper industry (Belayachi and Delmas 1997). Another potential use of the bagasse is as a fuel source for processing plants (Bennett and Annex 2009). In addition, processes for conversion of lignocellulosic material to ethanol are becoming more economically viable, making sweet sorghum bagasse a possible source of feedstock for such a process. Studies have demonstrated that a large portion of the insoluble carbohydrate (cellulose and hemicellulose) from sorghum can be readily converted to ethanol (Sipos et al. 2009). In India, the majority of the rainy season produce makes inroads for industrial use, mostly for feed and alcohol, because of poor quality of the grains owing to the problem of grain mold.

9 Production Constraints

The majority of smallholding farmers, especially in the semi-arid tropics, grow sorghum as a subsistence or semi-subsistence crop. Sorghum is rarely grown with any external inputs such as chemical fertilizers and plant protection measures. This leads to smaller returns to these marginal farmers as compared to other crops. As sorghum is grown on marginal land, the seed supply chain is largely poor in the majority of sorghum growing regions except India and China. This results in nonavailability of seeds of improved cultivars and repeated cultivation of less productive traditional cultivars or landraces. In some areas production is constrained by bird damage during the grain filling stage. To control this, bird scaring increases labor costs for farm operations. Another important factor throughout Asia and in urban areas of Africa is changing food habits and consumer preferences. With the rise in economic status of most rural traditional consumers in these regions, the preference for coarse cereals has fallen. Moreover, the difficulty in preparation of traditional food items from sorghum compared to fine cereals, as well as urbanization have influenced consumer preference for wheat or rice. Added to this, the government policies to support poor people by

distributing fine cereals at somewhat lower prices through public distribution systems like that in India have also led to the disappearance of sorghum from traditional households in the major consumption regions.

A variety of biotic and abiotic yield-limiting factors also affects the sorghum cultivation in many developing countries. Grain mold causes significant losses in both grain yield and quality, where the rainy season extends beyond the normal duration or the improved cultivars mature early resulting in increased susceptibility of developing grains. Grain mold is one of the principal reasons behind loss of area under rainy season sorghum in India. Other important diseases include downy mildew, anthracnose, leaf blight, ergot, and charcoal rot. Among the insect pests, shoot-fly causes substantial losses in late and off-season crops in many growing countries in Asia and Africa. Stem borers are endemic in all sorghum growing areas. Head bugs, shoot-bugs, midges, mites, and sugarcane aphids limit the crop yield in varying intensity depending on the location, season, and other prevailing climatic and edaphic factors. Another major constraint to sorghum production in many of the African countries is *Striga*, an obligate, root-parasitic flowering plant that draws its moisture and nutrients from sorghum roots, inhibiting plant growth and reducing yield. Important species occurring in Africa include *Striga hermonthica*, *S. asiatica*, *S. aspera*, and *S. forbesii*, and *S. asiatica* used to be a major constraint limiting yield in Asia (Reddy et al. 2012).

In many of the semi-arid tropical regions where sorghum is grown as rainfed crop, varying and irregular rainfall leads to large fluctuations in production. This becomes more relevant under a changing climate (Reynolds et al. 2016). In good years, farm prices are lower and in the absence of adequate storage facilities the farmers are forced to sell the produce at less remunerative prices. In many developing countries except India and China, the grain market is also not well established. Lack of substantial economic return discourages sorghum growers from investing in commercial sorghum production and slowly they divert to more remunerative options. Furthermore, inadequate policy support from the government such as subsidies that favor fine cereals and procurement prices also hinder adoption of sorghum farming in Asia and Africa.

10 Conclusion

The ironical situation with sorghum is that yields have increased over time in most of the growing countries. However, considerable area has been lost to other competitive crops, and this is particularly the case in Asia and the United States. In African countries with traditional reliance on sorghum, although area has increased significantly with expansion of sorghum cultivation to more area to meet the demand from increased population, average yield levels in some areas have gone down. More area is expected to be sown to sorghum in these countries to meet food demand in the future as well. Other countries including Australia, Mexico, and Brazil are also likely to grow sorghum on additional land to meet their feed demand. The international trade is expected to grow with the United States, Japan, Mexico, China, Chile, Spain, and others resorting to large-scale imports to meet the growing feed demand. Needless to say, the strengthening of the sorghum economy is connected to its contribution towards food security in Africa and other regions. Under a changing climate with predicted water stress, sorghum and other millet crops assume renewed importance. Moreover, being C4 plants they hold wider promise in mitigating elevated carbon dioxide levels. Productivity has improved, but more needs to be done, particularly in Africa. Sorghum production needs to be linked to industries to add higher returns to the sorghum farmers. With favorable policy support and accompanying breeding effort, this crop is likely to regain its lost area. There is an urgent need to shift the focus from the big cereals to this marginalized crop to ensure food and nutrition security in a sustainable manner.

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2 Botany, Taxonomy and Breeding

Are Ashok Kumar

Abstract

Sorghum is one of the most important cereal crops grown in the semi-arid tropics (SAT) of Asia, Africa, and the Americas for its food, feed, fodder, and fuel value. Sorghum production is constrained by several biotic and abiotic stresses. Genetic enhancement of sorghum for grain and stover yield, nutritional quality, and plant defense traits (abiotic and biotic) that stabilize the crop performance requires thorough knowledge of crop botany, diversity, and genetics so as to deploy appropriate crop-breeding strategies. Sorghum is one of the well-understood species in terms of botany, floral biology, and genetic diversity. Both cultivated and wild forms are available in sorghum, which are well distributed in Africa, its center of origin, and in the rest of the world. This chapter describes the botany, floral biology, and classification of sorghum and their implications to the breeding methods to be used. Also this chapter presents how the understanding of botany and taxonomy can be effectively used for improving sorghum yield and nutritional quality traits.

1 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a self-pollinating, diploid ($2n = 2x = 20$) species belonging to the Poaceae family with a genome size of 730 Mb, about 25 % the size of maize. It is a C_4 plant with higher photosynthetic efficiency and higher abiotic stress tolerance (Nagy et al. 1995; Reddy et al. 2009) adapted to a range of environments around the world. Its small genome makes sorghum an attractive model for studying the functional genomics of C_4 grasses. Drought tolerance makes sorghum especially important in dry regions such as northeast Africa (its center of diversity), India, and the southern plains of the United States (Paterson et al. 1995, 2009). Genetic variation for micronutrient concentration and its ability to absorb, translocate, and accumulate higher micronutrients in grain makes it an important model for biofortification research (Ashok Kumar et al. 2012). Its high level of inbreeding makes it an attractive association genetics system.

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Sorghum is among the climate-resilient crops that can better adapt to climate changes (Reddy et al. 2011). This chapter deals with the botany, floral biology, and taxonomy of sorghum and their implications for sorghum improvement methods and their utilization in improving sorghum yield and nutritional quality.

Sorghum is among the top 10 crops that feed the world. It is the dietary staple of more than 500 million people in over 30 countries, primarily in the developing world. It is grown on ~40 m ha in more than 90 countries in Africa, Asia, Oceania, and the Americas. The top 10 sorghum producers globally are the United States, India, Mexico, Nigeria, Sudan, Ethiopia, Australia, Brazil, China, and Burkina Faso (Rakshit et al. 2014). Sorghum accounts for 6 % of the global coarse cereals production in the world and is particularly well suited to hot and dry agroecologies in the world. Global sorghum productivity is low (1.4 t ha^{-1}) with wide variation in different parts of the world (Reddy et al. 2011). Although productivity is high in the Americas, China, and Australia, it is low in India, Nigeria, and Sudan.

Broadly, the world sorghum economy consists of two distinct production systems: a traditional, subsistence, smallholder farming production system where most of the production is consumed directly as food (mainly in Africa and Asia) with limited or no marketable surplus, and a modern, mechanized, high-input, large-scale sector where output is used largely as animal feed (mainly in the developed countries and in Latin America). The future of the sorghum economy is linked with its contribution to food security in Africa, income growth and poverty alleviation in Asia, and the efficient use of water in drought-prone regions in much of the developed world.

Sorghum is one of the cheapest sources of energy and micronutrients, and a vast majority of the population in sub-Saharan Africa and India depend on it for their dietary energy and micronutrient requirement. Sorghum provides more than 50 % of the dietary micronutrients, particularly Fe and Zn, to the low-income group, particularly in rural India where both physical and economic access to nutrient-rich foods is limited (Kumar et al. 2011). Thus, sorghum is a unique crop with multiple uses as food, feed, fodder, fuel, and fiber. Different utilizations of sorghum have been detailed in Chap. 1. Sorghum is generally grown in the rainy season (spring) but in India and in some parts of Africa it is grown in both rainy and post-rainy seasons (Reddy et al. 2009). In some parts of the world sorghum is grown in the summer season particularly for forage production.

2 Taxonomy and Classification

Sorghum exhibits various morphophysiological forms and large variation for floral morphology resulting in classification to various basic and intermediate races. Taxonomically it was first described by Linnaeus in 1753 under the name *Holcus*. Originally he delineated several species of Holcus, some of which have been later moved to the tribe Avenae, where the generic name Holcus now belongs. In 1794, Moench distinguished the genus *Sorghum* from genus *Holcus* (Celarier et al. 1959; Clayton et al. 1961). Subsequently, several authors have discussed the systematics, origin, and evolution of sorghum since Linnaeus (de Wet and Huckabay 1967; de Wet and Harlan 1971; Dahlberg 2000). Sorghum is classified under the family Poaceae, tribe Andropogoneae, subtribe Sorghinae, genus *Sorghum* Moench (Clayton and Renvoize 1986). Some authors further divided the genus into five subgenera: *sorghum*, *chaetosorghum*, *heterosorghum*, *parasorghum*, and *stiposorghum* (Garber 1950; Celarier 1959). Variation within these five subgenera except the subgenera *sorghum* has been described (Celarier 1959). *Sorghum bicolor* subspp. *bicolor* contains all of the cultivated sorghums. Doggett (1988) described them as annual plants, with stout culms up to 5 m tall, often branched, and frequently tillering.

Harlan and de Wet (1972) developed a simplified classification of cultivated sorghum that proved to be of real practical utility for sorghum

researchers. They classified *Sorghum bicolor* (L.) Moench, subspp. *bicolor* into five basic and ten hybrid races as depicted below.

Basic races	Intermediate/hybrid races
1. Race *bicolor* (B) 2. Race *guinea* (G) 3. Race *caudatum* (C) 4. Race *kafir* (K) 5. Race *durra*(D)	6. Race *guinea-bicolor* (GB) 7. Race *caudatum-bicolor* (CB) 8. Race *kafir-bicolor* (KB) 9. Race *durra-bicolor* (DB) 10. Race *guinea-caudatum* (GC) 11. Race *guinea-kafir* (GK) 12. Race *guinea-durra* (GD) 13. Race *kafir-caudatum* (KC) 14. Race *durra-caudatum* (DC) 15. Race *kafir-durra* (KD)

The descriptors for five basic races (Fig. 1) in sorghum are as given below.

1. **Bicolor**: Grain elongate, sometimes slightly obovate, nearly symmetrical dorsoventrally, glumes clasping the grain, which may be completely covered or exposed as much as one-fourth of its length at the tip, spikelets persistent.
2. **Guinea**: Grain flattened dorsoventrally, sublenticular in outline, twisting at maturity nearly 90° between gaping involute glumes that are nearly as long as to longer than the grain. They are easily distinguishable by the presence of open glumes.
3. **Caudatum**: Grain markedly symmetrical, the side next to the lower glume flat or in extreme cases somewhat concave, the opposite side rounded and bulging, the persistent style often at the tip of a beak pointing towards the lower glume, glumes half the length of the grain or less.
4. **Kafir**: Grain approximately symmetrical, more or less spherical, glumes clasping and variable in length.
5. **Durra**: Grain rounded obovate, wedge-shaped at the base and broadest slightly above the middle; glumes very wide, the tip of a different texture from the base and often with a transverse crease across the middle.

All 15 races of cultivated sorghum can be identified by mature spikelets alone, although head type is sometimes helpful. This classification is clear and simple and practically all of the variation in cultivated sorghum can be accounted for by the five basic races and their intermediate combinations. The intermediate races involving guinea, for example, have glumes that open partially and seeds that twist noticeably, but not as much as in pure guinea. Intermediate races involving caudatum have asymmetrical seeds, but the character is not as fully expressed as in pure caudatum. Other intermediate combinations can be recognized in a similar manner. The method is so sensitive that even three-way and possibly four-way combinations can also be recognized, but these are usually products of modern plant breeding and not part of the variation of indigenous varieties. If they occur in significant numbers, they could be best treated as subraces of the main races.

In sorghum the degree of expression of these characteristics and their combinations determine the race, for the most part without equivocation. Identification can be made easily in the field or in the laboratory from head or even spikelet specimens. In addition to the basic and intermediate races described above, some of the commercial grain sorghum types are utilized in sorghum improvement programs, the characteristics of which are given in Table 1. They denote only commercial grain sorghum types and cut across intermediate/basic races. The Biodiversity International (formerly International Plant Genetic Resources Institute, IPGRI) Advisory Committee on Sorghum and Millets Germplasm has accepted this (de Wet and Harlan 1972) classification, and recommended this to describe sorghum germplasm (IBPGR/ICRISAT 1980). The amount of genetic variability available in sorghum is immense. Much of the genetic variability is available in areas of origin of the crop (Africa) and regions of early introduction (Asia). In Africa, genetic variability is available in both cultivated species and wild progenitors of the crop (Gebrekidan 1981). De Wet and Harlan (1972) reported on the distribution of both wild relatives and the major cultivated races of the

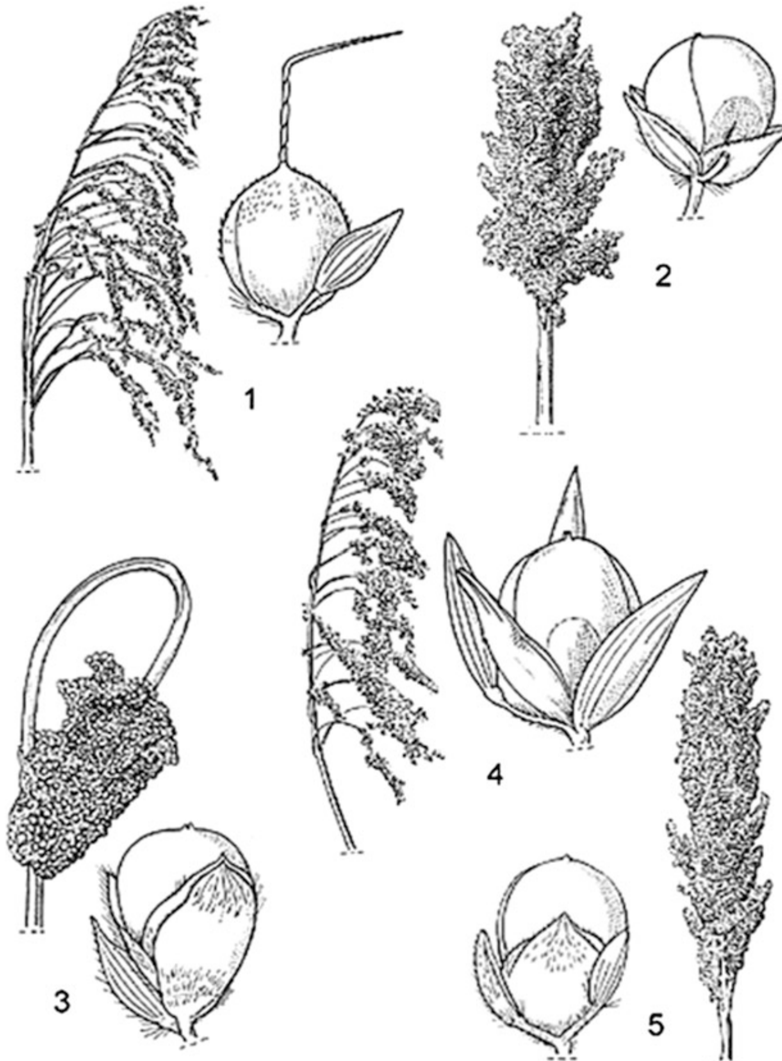

Fig. 1 Panicles and spikelets of the five basic races in sorhgum: (1) bicolor; (2) caudatum; (3) durra; (4) guinea; and (5) kafir. *Source* PROSEA

crop in Africa. However, this natural genetic diversity is subjected to a range of threats from natural selection and destruction of habitats and often merely expedient agricultural practices of mankind. Landraces and wild relatives of cultivated sorghum from the centers of diversity have been rich sources of resistance to new pathogens, insect pests, and other stresses such as high temperature and drought, as well as sources of traits to improve food and fodder quality, animal feed, and industrial products. Preventing the vulnerability of landraces and wild relatives of cultivated sorghum from extinction, following the release of varieties and hybrids, collection and conservation of sorghum germplasm was accelerated about four decades ago. Since then, germplasm collection and conservation have become integral components of several crop improvement programs at both national and international levels (Rosenow and Dalhberg 2000). The gene bank at ICRISAT holds ~38,000 global collections of sorghum germplasm which represent 80 % of the genetic variability in sorghum. All this germplasm is

Table 1 Characteristics of commercial grain sorghum types used in sorghum improvement programs

Grain sorghum type	Brief morphological description	Geographical location
Durra	Hairy rachis, flattened kernels, and dry stalks	Mediterranean, Near East, Middle East
Shallu	Partly pubescent involute glumes, cone-shaped lax panicles, corneous kernels, dry and nonsweet stalks	India, tropical Africa
Guineense	Involute and nearly glabrous glumes and compact panicles	Central and Western Africa
Kafir	Awnless, compact cylindrical panicles and juicy nonsweet stalks	South Africa
Kaoliang	Stiff stalks, thick hard rind, stiff spreading and few panicle branches, and dry and nonsweet stalks	Eastern Asia
Milo	Yellow midrib, transverse wrinkle of the glumes, compact, awned panicles, large round kernels	East Africa
Feterita	Large kernels, brown testa, and dry and nonsweet stalks	Sudan
Hegari	Rounded kernels, brown testa midcompact ellipsoid and branched panicles, and white kernels with a bluish-white appearance	Sudan

well characterized and conserved using short-term and long-term conservation practices. To make the germplasm accessions more useful for crop improvement research, core collections representing ~10 % of the total collections were developed by Dahlberg et al. (2004) and mini-core collections, representing ~1 % of the total collections, were developed by Upadhyaya et al. (2009) in sorghum. Details on the genetic resources of sorghum have been elaborated in Chap. 4.

3 Floral Biology, Reproduction, and Implications for Crop Improvement

Sorghum is an annual/perennial grass adapted to a range of climates spread between 40°S and 40° N latitudes and therefore is widely distributed across various continents. The adaptation features are slightly different for tropical and temperate environments (Vanderlip and Reeves 1972; Rao et al. 2004). The photoperiod insensitivity and dwarfism predominate the temperate adaptation to facilitate mechanized harvesting whereas sorghums grown in the tropics are mostly tall and photoperiod sensitive barring the improved hybrids (Thurber et al. 2013). Sorghum has a well-developed root system and in addition to its subterranean root system, sorghum forms strong aerial roots permeating through the soil and ensuring better stability. The stem is strong, hard, smooth, and divided by nodes and grows up to 1–1.8 m, but sweet sorghums and high biomass sorghums are taller (2.5–4 m). Sorghum leaves are 7 and 28 in number arranged alternating to opposite sides with parallel venation. The leaves are 50–100 mm wide and 0.5–0.8 m long depending upon the genotype. Leaves and stems are often covered with a wax layer which is an adaptation mechanism to tolerate drought and insect attacks. The panicle varies from loose to compact; in some varieties the panicle remains surrounded by sheath, although this is not desirable from an economic yield point of view. In some genotypes the peduncle is recurred resulting in a pendant head referred to as "goose neck". The sorghum panicle consists of spikelets in pairs, sessile and pedicillate types; the sessile is hermaphrodite and fertile and the other pedicillate contains only anthers (Aruna and Audilakshmi 2008). The grain is caryopsis; the endosperm is starchy; the embryo consists of

plumule, coleoptiles, and radical coleorhizae referred to as scutellum.

Sorghum is a short-day plant, and blooming is hastened by short days and long nights. Delayed flowering can be typically observed when winter-season adapted cultivars are grown in the spring season. However, varieties differ in their photoperiod sensitivity (Quinby and Karper 1947) and it has implications in sorghum improvement. In traditional varieties, the reproductive stage is initiated when day lengths return to 12 h. Sorghum follows a predictable pattern of growth from planting through physiological maturity. The duration between growth stages is closely dependent upon the air temperatures and relative maturity of the cultivar. The number of days required for a cultivar to reach maturity depends primarily on location, date of planting, and climatic temperature. For a given cultivar, days to 50 % flowering may vary depending on growing conditions, because daily minimum and maximum temperatures vary from year to year and between locations. The use of cumulative growing degree unit (GDU) gives a better idea from planting to successful growth stages until maturity in sorghum. The GDU (°C) for short-season sorghum cultivars was 1467 whereas for long-season cultivars it may go up to 1849 °C (https://www.uaex.edu/publications/pdf/mp297/MP297.pdf verified on June 20th, 2016). Usually, the floral initial (primary branch primordial differentiation on the floral apex) is 15–30 cm above the ground when the plants are about 50–75 cm tall (House 1980). Floral initiation marks the end of the vegetative phase. The time required for transformation from the vegetative primordial to reproductive primordial is largely influenced by the genotype and the environment. The grand growth period in sorghum follows the formation of a floral bud and consists largely of cell enlargement. In general, sorghum hybrids take less time to reach panicle initiation, more days to expand the panicle, and a longer grain-filling period than their corresponding parents (Maiti 1996). This enabled exploitation of heterosis through hybrids' development and their commercialization.

3.1 Mode of Reproduction and Artificial Hybridization

Sorghum is a self-pollinated crop with the natural cross-pollination from 0.6 to 15 % depending on the genotype, panicle type, and wind direction and velocity (House 1980). The inflorescence is a raceme, consisting of one to several spikelets. The spikelets usually occur in pairs, one being sessile and the second borne on a short pedicel, except the terminal sessile spikelet, which is accompanied by two pedicelled spikelets. The sessile spikelet contains a perfect flower whereas the pedicillate spikelet possesses only anthers but occasionally has a rudimentary ovary and empty glumes.

In sorghum anthesis starts with the exsertion of the complete panicle from the boot leaf. Flowers begin to open two days after complete emergence of the panicle. The sorghum head begins to flower at its tip and anthesis proceeds successively downward. Anthesis takes place first in the sessile spikelets. It takes about 6 days for completion of anthesis in the panicle with maximum flowering at 3 or 4 days after anthesis begins. Anthesis takes place during the morning hours, and frequently occurs just before or just after sunrise, but may be delayed on cloudy damp mornings. Maximum flowering is observed between 06:00 and 09:00 h. Because all heads in a field do not flower at the same time, pollen is usually available for a period of 10–15 days. At the time of flowering (anthesis), the glumes open and all three anthers fall free, while the two stigmas protrude, each on a stiff style. The anthers dehisce when they are dry and pollen is blown into the air. Pollen in the anthers remains viable several hours after shedding. Flowers remain open for 30–90 min. Dehiscence of the anthers for pollen diffusion takes place through the apical pore. The pollen drifts to the stigma, where it germinates; the pollen tube, with two nuclei, grows down the style, to fertilize the egg and form a 2*n* nucleus (Aruna and Audilakshmi 2008). Stigmas get exposed before the anthers dehisce subjecting them to cross-pollination.

Pollination for crossing purposes should start soon after normal pollen shedding is completed during the morning hours.

Sorghum is a breeder-friendly crop as it is amenable for crossing and selfing quite easily. For selfing, after panicle exsertion, bagging should be done by snipping off the flowered florets at the tip. Crossing is done by emasculation of selected panicles and dusting of pollen from identified plants. Hand emasculation is most commonly practiced in sorghum. Because of this ease in crossing, hybridization is most commonly followed in sorghum for trait improvement. For effective results in artificial hybridization the pollen is collected in pollen bags and thoroughly dusted on the emasculated or male-sterile panicles.

4 Sorghum Improvement Methods

The crop improvement methods depend on the pollination control mechanisms and cultivar options. As mentioned earlier, sorghum is a breeder-friendly crop. One can employ the breeding methods that can be used to improve both self- and cross-pollinated crops with ease in sorghum. This is the reason why one can find sorghum pure line varieties, hybrids, and populations as cultivar options in different parts of the world. However, sorghum hybrids are superior to pure lines and populations for yield and other important agronomic traits. The discovery of cytoplasmic-nuclear male sterility in sorghum helped to produce hybrid seeds on a mass scale using a three-line system (A, B, and R) for commercial cultivation of hybrids (Stephens and Holland 1954a, b).

4.1 Pure Line Selection

Pure line selection is the most common method of crop breeding particularly in self-pollinated crops. Pure line selection is practiced under two situations: (i) when there is a need to develop a variety from a landrace population, and (ii) while developing a variety from a segregating population. For example, in sorghum, for post-rainy season adaptation in India, the local landraces from the state of Maharashtra were collected and single plant selections were made for a couple of generations and the performance for grain and stover yields of the selected lines was compared. The line showing better performance than the check variety for yield traits across locations was released for commercial cultivation (Audilakshmi and Aruna 2008). In the case of segregating populations, the individual plants are heterozygous in the beginning as they are the products of crossing between two homozygotes and attain homozygosity in successive generations upon self-pollination. Individual plant selections have to be carried out for at least five to six generations to achieve the desired level of homozygosity of a pure line. A higher number of plants (3000–10,000) of segregating population are evaluated and selection is practiced to obtain the desired plants.

4.2 Mass Selection

Mass selection differs from pure line selection, wherein a number of desirable plants (instead of only one) are selected and compositing is done on the harvested seed to produce the next generation (Allard 1960). This method has a few drawbacks. It is not known whether the plants being grouped are homogeneous and some of them if heterogeneous would segregate further in following generations, and repeated selection would be required (Sharma 1988). Mass selection is generally practiced to purify a variety. A large number of single plants are selected from an impure variety population, each line progeny tested, and similar type progeny bulked to form the pure seed lot. The success of the method depends upon high heritability, that is, the presence of additive gene action and minimal influence of genotype × environment interaction on the expression of the selected trait. Mass selection is relatively less used in sorghum except for the improvement of plant height or grain size.

4.3 Hybridization-Based Methods

The term hybridization refers to the crossing of two genetically different individuals as it combines the traits of two varieties and provides an opportunity to select plants with desirable features of both parents through recombination in the segregating progeny. As the natural variability for most traits is limited or already exploited, there is a need to create new variability by making artificial hybrids to make any further dent in developing improved varieties through selection in the segregating populations. As most of the traits of interest in sorghum are quantitatively inherited, sorghum breeders generally use the pedigree method of selection in segregating populations. In the pedigree method, the records of the ancestry or pedigree of each progeny is maintained and it is easy to trace back the parentage and selection. With the pedigree system, the F_2 generation represents the first and the maximum opportunity for selection. Selection for superiority is based on the vigor and other agronomic features of progeny (families). In F_2, selection is limited to individuals. In F_3 and subsequent generations, until a reasonable level of genetic homozygosity is reached, selection is practiced both within and between families. Of the >700 sorghum female parents (A-/B-pairs) developed by ICRISAT for various traits of global importance (Table 2), more than 600 parents are used in crossing to develop them using the pedigree method (Reddy et al. 2007).

Bulk population breeding is an economic method of obtaining homozygous lines in self-fertilized crops. However, it is not widely used in sorghum. The backcross method is widely used in sorghum improvement particularly for disease resistance, transferring male sterility to the identified maintainer lines by test crossing. Similarly, it is the most sought-after method for transferring quantitative trait loci (QTLs) for shoot-fly resistance and stay-green traits (Kumar et al. 2011).

The choice of parents for hybridization programs is crucial for their success and requires careful and critical evaluation of potential parents for various attributes such as yielding ability, disease resistance, adaptation, quality of the produce, and morphological features relevant to crop management practices. Inasmuch as new strains are intended to have superior yield potential to the existing varieties, one of the parents used in the crossing program is invariably the adapted variety of the area. The other parent is primarily chosen for complementing the specific weakness of the variety, which needs to be replaced. The general combining ability of a parent is likely to be reflected adequately in the parental performance of the trait. In addition to selection of the parents on the yield performance and general and specific combining abilities in the partial diallel crosses or line × tester crosses, it is desirable to analyze the potential parents for important traits such as panicle length, number of primary/secondary branches, grain per primary branch, and grain size (Audilakshmi and Aruna 2008).

Population improvement is another important method for sorghum improvement, which includes: (i) the development of broad genetic-based gene pools, and (ii) its improvement through recurrent selection methods. In sorghum a single gene in recessive homozygous condition confers male sterility and eight different genes reported in sorghum are involved in control of genetic male sterility. Using these genes, population improvement methods can be successfully deployed in sorghum which provides a long-term breeding strategy to derive diverse and broad genetic-based superior varieties/hybrid parents (Reddy et al. 2008). More than 50 sorghum hybrid parents (A-/B-pairs) at ICRISAT were developed using population improvement methods.

Heterosis breeding is most important as hybrids are the cultivar options in sorghum wherever they are available. Although heterosis was demonstrated as early as 1927 in sorghum (Conner and Karper 1927), its commercial exploitation was possible only after the discovery of a stable and heritable cytoplasmic-nuclear male sterility (CMS) mechanism (Stephens and Holland 1954a, b). This CMS system has been designated as A1 (milo). Since then a large number of hybrids have been developed and

Table 2 Details of the sorghum trait-specific (milo) and non-milo hybrid parents (A-/B-pairs) developed at ICRISAT–Patancheru using diverse sorghum basic and intermediate races

ICSA numbers	Traits	No. of lines
1–103	High yielding	77*
88001–88026	”	15*
89001–89004	”	4
90001–90004	”	4
91001–91010	”	10
94001–94012	”	12
201–259	Downy mildew resistant	59
260–295	Anthracnose resistant	36
296–328	Leaf blight resistant	33
329–350	Rust resistant	22
351–408	Grain mold resistant	58
409–436	Shoot-fly resistant (rainy)	28
437–463	Shoot-fly resistant (postrainy)	27
464–474	Stem borer resistant (rainy)	11
475–487	Stem borer resistant (postrainy)	13
488–545	Midge resistant	58
546–565	Head bug resistant	20
566–599	Striga resistant	34
600–614	Acid soil tolerant lines	15
615–637	Early-maturity lines	23
638–670	Durra (large grain) lines	33
671–674	Tillering lines	4
675–687	Stay-green lines	13
688–738	Non-milo (A_2) cytoplasmic lines	51
739–755	Non-milo (A_3) cytoplasmic lines	17
756–767	Non-milo (A_4) cytoplasmic lines	12
24001–24005	High yielding	5
25001–25005	High yielding	5
28001–28006	High yielding	6
29001–29006 and 29017	Shoot-fly resistant (rainy)	7
29007–29016	Grain mold resistant	10
11001–11040	High yielding	40
13001–13030	High yielding	30
14001–14035	Sweet sorghum	35
14036–14039	Shoot-fly resistant (rainy)	4
14040–14042	Grain mold resistant	3
14043–14044	High yielding	2
Total		**836**

*The number of lines being maintained

released/marketed for commercial cultivation in Asia, the Americas, Australia, and Africa. The hybrids have contributed significantly to the increased grain and forage yields in several countries. Grain productivity increased by 47 % in China and by 50 % in India from the 1960s to the 1990s (FAO 1960–1996), which corresponds well with the adoption of hybrids in these countries. Adoption of the first commercial hybrid (CSH 1) in India over much of the rainy season sorghum area while local varieties were confined to fairly narrow specific environmental niches stands testimony to the wide adaptability of hybrids over varieties (House et al. 1997). Currently, over 95 % of the sorghum area is planted to the hybrids in the United States, Australia, and China. In India, over 85 % of the rainy season sorghum area is planted to hybrids (Reddy et al. 2006).

5 Taxonomy and Sorghum Improvement

Sorghum improvement deals with production of new crop cultivars that are superior to existing cultivars for traits of interest. Availability of genetic variability for these traits, knowledge about their heritability and inheritance, and availability of effective phenotyping methodologies are fundamental for success of any crop improvement program. In fact, the efficiency of phenotyping and its robustness decides the success of the crop improvement program in terms of producing a tangible product or technology. As indicated above, in sorghum, a large collection of germplasm is available at ICRISAT (∼38,000 accessions) and other places with characterization information on various morphological, agronomic, and adaptive traits. These germplasm lines predominantly consist of the intermediate races of sorghum with a good number of basic races. Inheritance of major traits is well studied and phenotyping techniques developed for efficient selection/screening for major traits of interest. There is continuous exchange of material and information across research groups. As a result, a large number of sorghum cultivars were developed and commercialized around the world for traits of interest. For example, during the period 1976–2010, a total of 242 sorghum cultivars were released in 44 countries using the ICRISAT-bred sorghum material by private and public sector organizations (Kumar et al. 2011), which increased to 268 cultivars by 2016. The list is quite exhaustive if we consider cultivars developed by the national programs of all sorghum-growing countries. Focused sorghum improvement programs backed by the germplasm sources, information on heritability and gene action for traits of interest, phenotyping tools, established selection procedures, massive adaptive trials in partners' locations, and, above all, collaborative research contributed to the large-scale development and commercialization of improved cultivars. In the majority of cases of cultivar development conventional methods were used. Various basic races and the phenotyping tools employed in sorghum improvement programs for various traits of global importance are discussed hereunder. Cultivated sorghum can be grouped differently based on adaptation, usages, and so on.

5.1 Adaptation

Sorghum is produced in the rainy (hot) season in most parts of the world for various uses—food, feed, fodder, and industrial starch, among others—whereas in India it is grown in both rainy and post-rainy (cold) seasons. Limited sorghum area (mostly forages) is grown in summer seasons but it is very small compared to the global area of 40 m ha.

5.1.1 Rainy Season Sorghum

This is the most important adaptation globally spanning from May/June to August/September with more than 30 m ha sorghum area across continents. A variety of sorghums belonging to different races (basic or hybrid/intermediate), different cultivar types (mostly hybrids and varieties), and different grain color (red, brown, white, etc.) types are grown for a variety of end uses in more than 90 sorghum-growing countries. For a plant breeder, the target materials and criteria for selection depend on the prevailing seed systems and the utilization pattern of the crop and consumer preference. For example, medium tall (1.5–1.8 m) dual-purpose hybrids with bold white grains are preferred in India for both food and feed use whereas grain types with red pericarp are preferred for food and brewing purposes in East Africa, and tall, long-duration photoperiod-sensitive guinea sorghums are preferred in West Africa for food. In contrast, short (0.8–1.3 m) hybrids are preferred in the United States, South America, and Australia for mechanical harvesting of sorghum for use as animal feed. Plant height, pigmentation, time to flowering, crop duration, panicle exsertion, panicle size, glume coverage, grain number, grain size and color, and grain thresh ability are major selection criteria in addition to the grain yield. In dual-purpose types, apart from grain yield, stover yield and quality are also important selection criteria. A plant breeder needs to select appropriate germplasm and breeding methods keeping the end product in mind; the maturity duration of the cultivar should correspond with the length of the growing period in the target area with the grain development stage preferably coinciding with the dry period to get the best quality grain. The important biotic constraints in rainy season sorghum include shoot-fly, stem borer, midge, grain mold, striga, and among abiotic constraints, drought predominates (Reddy et al. 2010). An overview of broad adaptation of basic races showed that the *guinea* race and intermediate races involving the *guinea* race are predominant in Western and Central Africa; the *caudatum* race per se and in combination with others as intermediate races in North Eastern Nigeria, Chad, Sudan, Uganda, Western Ethiopia, and rainy season adaptation in India; and the *durra* race along with intermediate races in Ethiopia and other Eastern and Southern Africa including post-rainy season adaptation in India and the *kafirs* in Southern Africa, Tanzania, and Northern Nigeria (Upadhyaya et al. 2014).

5.1.2 Post-rainy Season Sorghum

It is a unique adaptation to India with approximately 4.5 m ha where the crop is grown from September/October to January/February taking advantage of residual and receding moisture in black soils. The Deccan plateau in India encompassing the states of Maharashtra, Karnataka, Andhra Pradesh, and Telangana is the major post-rainy sorghum growing region. The post-rainy sorghum grain is preferred for food use in India owing to its bold globular lustrous nature. However, no differences were observed between the flatbreads made from rainy (but matured under rain-free condition) and post-rainy sorghums in a sensory evaluation involving traditional sorghum-eating populations (ST Borikar, personal communication). The stover from the post-rainy crop is the most important animal feed particularly in the dry periods. In addition to the traits mentioned under rainy season adaptation, photoperiod sensitivity, temperature insensitivity, and grain luster are the major selection criterion. Varieties are the cultivar choice but there is good scope for hybrid development using the white-grained rainy season adapted lines as female parents and landrace restorers as

pollinators. Although terminal drought is the major production constraint, shoot-fly, aphids, and charcoal rot play havoc with post-rainy season production (Kumar et al. 2011). The *durras* possess the typical grain traits preferred by post-rainy sorghum farmers; that is why almost all major cultivars in post-rainy sorghums belong to this race, with characteristic low diversity.

5.2 Yield and Yield Attributes

Grain yield obviously is the most important trait in sorghum breeding as in other major food crops; however, stover yield is equally important in sorghum particularly in countries like India. Breeding for grain yield improvement is carried out by selecting genotypes directly for grain yield and for component traits. For higher yield, genotypes with a plant height of around 150 cm are desirable, which are amenable to mechanical harvesting with medium maturity duration (100–120 days). Longer duration types give higher yields but the length of the growing period (LGP) in most sorghum growing areas does not allow for breeding long duration types, with the exception of West Africa. If we reduce the crop duration, it is likely that the yield goes down. Therefore, the breeder first has to fix the plant height and maturity duration for a given environment. However, in the context of climate change, longer duration types need to be maintained in the breeding program considering the fact that when temperatures increase by 2 °C, the longer duration types behave as medium duration types and produce higher yields than other types (Reddy et al. 2011). Another important consideration is photoperiod sensitivity. Photoperiod insensitivity is the ability of a genotype to mature at any given period in the calendar year irrespective of its planting date. It is feasible to identify the photoperiod-sensitive genotypes by planting them at different dates (at 15- or 30-day intervals) and recording the days for 50 % blooming in the genotypes. The genotypes that take less time for flowering when planted late can be considered photoperiod sensitive. In sorghum improvement programs in West Africa and in post-rainy sorghum improvement in India, photoperiod sensitivity is a key trait, although *guinea* types are predominant in West Africa and *durras* predominate in post-rainy sorghum in India. Among the component traits, long panicles, higher number of primary and secondary branches, bold grains, large number of grains per panicle, and higher 100-seed weight contribute to higher grain yield and most of these traits have high heritability enabling the plant breeder to improve for these traits through selection. The gap between flag leaf sheath and panicle base should be minimal to have good grain filling and the glume coverage on grains is to be less for higher threshability. Grain size can be visually judged and grain color can be selected as per the consumer/market preference in the given adaptation (Reddy et al. 2009; Rosenow and Dalhberg 2000). If there is no stringent grain quality preference, use of *caudatums* gives the best yields owing to their high per se performance and higher combining ability in hybrid combinations.

5.2.1 Grain and Stover Yield

In addition to sorghum grain, its stover has high feed value. In areas where sorghum stover is important as animal feed, breeding dual-purpose types is the best choice. Heterosis for grain and stover yield is high in sorghum and therefore hybrid development should be targeted. A heterosis of 30–40 % for grain yield is reported compared to the best varieties (Kumar et al. 2011). Development of hybrid parents is critical for exploiting heterosis and therefore genetic and cytoplasmic diversification of hybrid parents is a major breeding objective. Population improvement is also being followed for improving the

grain and stover yields. In general, *caudatums* show higher yield and higher combining ability and therefore are widely exploited in developing hybrid parents in sorghum.

Quality of grain and stover is as important as grain yield in sorghum. This is more so in the case of post-rainy season sorghum where consumers prefer bold, lustrous white-grain types, which is generally available only in landrace varieties (Reddy et al. 2009). The grain luster is visually scored on a scale 1–3 where 1 = lustrous and 3 = dull among the white-grained types. The genetic base of these *durra* landraces is narrow and therefore it is more challenging to improve for post-rainy season adaptation. Similarly, heterosis is low when both parents are derived from landraces (*durra* × *durra*). A more practical method for developing post-rainy season hybrids is by using rainy season adapted lines (mostly *caudatum* types) as females and landrace varieties (*durras*) as pollinators. While improving the stover yield, one has to keep in the mind the stover digestibility and protein content in addition to the stover yields. The stover yields have to be recorded on oven-dried samples after harvesting the grains and for stover quality; indirect selection for stover digestibility using near-infrared reflectance spectroscopy (NIRS) is the most practical method for assessing feed quality of stovers.

5.2.2 Plant Height and Maturity

Plant height is a major consideration in sorghum improvement and in fact it is one the criteria for classifying sorghums as grain sorghums, dual-purpose sorghums, forage sorghums, and high biomass sorghums. In sorghum, four loci are known to be involved in the control of plant height. These genes are assigned the symbols *Dw1*, *Dw2*, *Dw3*, and *Dw4* (House 1980). Tallness is partially dominant to dwarfness. The zero dwarf type (dominant [*DW*-] at all loci) may reach a height of 4 m. The change from four to three dominant genes may result in a height change of 50 cm or more. If genes at one or more of the loci are recessive, the difference in height resulting from the recessive condition at an additional locus may have a smaller effect in reducing plant height. The difference between a 3-dwarf (recessive genes [*dw dw*] at three loci) and a 4-dwarf type may be only 10 or 15 cm (Rosenow and Dalhberg 2000). Breeders have to keep in mind these facts while selecting genotypes with appropriate height. For example, farmers in India prefer to use two gene dwarfs that result in a height of 1.7–2.0 m as they wish to have fodder along with grain and the plant height is directly proportional to the height (Quinby 1974). The plant height is always recorded from the base of the plant to the tip of the panicle. Plant height and days to flowering data give an idea about the genotype in terms of suitability for various uses. In practical sorghum breeding, the plant height should be less than 150 cm in grain sorghums, up to 170 cm in dual-type sorghums, more than 200 cm in forages, and 300 cm or more in the case of high biomass sorghums.

In sorghum, Quinby (1974) identified factors at four loci that influence maturity, *Ma1*, *Ma2*, *Ma3*, and *Ma4*. Generally, tropical types are dominant (*Ma*-) at all four loci, and a recessive condition (*mama*) at any one of them will result in more temperate zone adaptation that takes more time for maturity. Most sorghum improvement programs target medium maturity types (crop duration less than 120 days) as they yield high; however, the targeted maturity is to be decided based on the length of the growing period of the target area. In general, sorghum takes 35–40 days from flowering to maturity. The grain is to be harvested at physiological

maturity stage. The hilum turns dark at physiological maturity and this is an important criterion for harvesting (Rosenow and Dalhberg 2000). There are some racial differences in maturity of the crop. Certain *guinea* race sorghums take up to 180 days for maturity. However, one can find large variation for maturity in any given race.

5.3 Nutritional Quality Traits

Sorghum is one of the major food crops in the world and has a predominant role in meeting the dietary energy and micronutrient requirements particularly in the low-income group populations; improving sorghum nutrition quality is of paramount importance (Parthasarathy Rao et al. 2006). There is a conscious focus on improving the nutritional quality of sorghum.

5.3.1 Protein and β-Carotene Contents

Protein content is more intensively studied in sorghum wherein high genetic variability is reported. Gains in protein content were reported by various authors (Virupaksha and Sastry 1968; Ramesh and Hudda 1994; De Mesa-Stonestreet et al. 2010). The best method for measuring protein content is through the Micro-Kjeldahl method or Technicon autoanalyzer (TAA) method (Johnson and Craney 1971; Jambunathan 1983). Sorghum is a good source of protein vis-à-vis other cereals and there exists large variability for protein content in sorghum although there is no correlation between protein content and races in sorghum. Sorghum is not a good source of β-carotene, like other cereal staples. In a study on a limited number of germplasm lines, hybrid parents in sorghum did not show appreciable variability for β-carotene content in sorghum (Reddy et al. 2005). A similar case with yellow endosperm lines is reported wherein the β-carotene content did not exceed 1.1 ppm (Reddy et al. 2005). For phenotyping this trait, spectrophotometry followed by high-performance liquid chromatography (HPLC) gives more accurate information.

5.3.2 Grain Fe and Zn Concentration

Sorghum is a good source of grain Fe and Zn although we cannot attribute the variability found in sorghum for grain Fe and Zn to any given basic or intermediate races (Table 3). Large-scale screening of sorghum core germplasm accessions, hybrid parents, and commercial hybrids showed high genetic variability for grain Fe and Zn concentrations (7–70 ppm) and most of this variation is heritable (Reddy et al. 2005; Kumar et al. 2012). Significant positive association exists between grain Fe and Zn concentrations ($r^2 = 0.6–0.8$) and it is possible to improve both traits simultaneously (Kumar et al. 2009). Additive gene action plays a significant role in conditioning the grain Zn concentration whereas both nonadditive and additive gene actions condition the grain Fe concentration (Ashok Kumar et al. 2013a, b). The Fe and Zn concentrations can be estimated using inductively coupled plasma optical emission spectrometry or atomic absorption spectrometry (Houk 1986). This is a precise but destructive and laborious method. The most rapid and low-cost method for assessing grain Fe and Zn concentrations is by using the X-ray fluorescence spectrometry (XRF) method which is nondestructive and can be used routinely to screen the breeding materials. There is high correspondence between the values obtained by both methods, indicating that XRF can be used for assessing grain Fe and Zn concentrations particularly for discarding the poor lines in the breeding material (Ashok Kumar et al. 2015).

Table 3 Mean performance of selected sorghum germplasm lines evaluated for grain Fe and Zn concentration at ICRISAT–Patancheru during the 2007 and 2008 post-rainy seasons

IS No./pedigree	Race	Origin	Days to 50 % flowering	Plant height (m)	Glume coverage (%)	Grain yield (t ha^{-1})	Grain size (g 100^{-1})	Iron (mg kg-1)	Rank	Zinc (mg kg-1)
5427	Durra	India	65	2.0	54	2.0	2.8	61	1	57
5514	Guinea-bicolor	India	68	1.7	71	1.4	3.0	56	2	45
55	Durra-caudatum	US	71	1.0	75	1.3	2.6	54	3	38
3760	Caudatum-bicolor	USSR	68	1.9	67	2.2	2.2	53	4	37
3283	Bicolor	US	66	1.8	71	1.9	2.7	50	5	42
17580	Caudatum	Nigeria	66	1.9	79	1.6	2.1	50	6	41
15952	Guinea	Cameroon	81	2.4	38	2.5	3.4	49	7	41
3813	Durra	India	79	2.2	83	1.4	1.7	49	8	38
15266	Caudatum	Cameroon	70	1.4	54	2.7	2.7	49	9	44
2939	Kafir	US	69	2.0	63	3.6	3.9	48	10	37
4159	Durra	India	65	1.9	50	1.5	3.3	48	11	38
3929	Kafir-durra	US	75	2.0	79	2.2	2.1	48	12	40
3443	Guinea-caudatum	Sudan	68	1.7	63	3.3	3.5	47	13	39
3925	Durra-caudatum	US	78	2.0	67	2.4	2.0	47	14	39
5460	Durra-bicolor	India	66	1.5	79	1.4	2.7	47	15	46
12452	Caudatum-bicolor	Sudan	64	1.9	79	3.2	4.3	47	16	33
2801	Caudatum	Zimbabwe	71	1.8	71	2.3	3.1	46	17	45
2536	Kafir-caudatum	US	72	1.8	83	2.3	2.4	45	18	37
5429	Durra	India	66	1.7	79	2.8	3.0	44	19	30
356	Durra	US	84	1.1	46	2.2	2.7	44	20	33
2265	Durra-bicolor	Sudan	75	2.2	71	1.8	1.7	44	21	41
12695	Bicolor	South Africa	68	1.9	100	2.8	2.6	44	22	39
5538	Durra	India	65	1.4	33	1.7	2.2	44	23	37

(continued)

Table 3 (continued)

IS No./pedigree	Race	Origin	Days to 50 % flowering	Plant height (m)	Glume coverage (%)	Grain yield (t ha^{-1})	Grain size (g 100^{-1})	Iron (mg kg-1)	Rank	Zinc (mg kg-1)
5476	Durra	India	69	1.5	75	2.1	2.7	41	24	36
16337	Caudatum	Cameroon	80	1.7	38	2.4	3.0	41	25	34
5853	Guinea-durra	India	65	1.7	29	2.4	5.9	41	26	32
14318	Bicolor	Swaziland	79	2.1	79	2.2	2.3	39	29	38
10674	Durra-caudatum	China	65	2.1	46	1.9	4.0	39	30	38
22215	Durra-bicolor	USSR	80	2.1	71	2.9	2.4	26	31	21
Controls										
ICSR 40			70	1.2	33	3.3	3.2	40	27	24
296B			86	1.1	29	2.7	2.8	40	28	24
Mean			71	1.76	63	2.26	2.9	46		37
SE +			0.96	0.12	7.30	0.26	0.2	3		3
CV (%)			2.32	11.51	20.06	20.12	10.4	10		13
CD (5 %)			2.68	0.33	20.44	0.74	0.5	8		8

6 Conclusion

The botany and taxonomy of the genus *Sorghum* were well studied and exploited in improving the sorghum for various traits of interest. The classification of sorghum and the adaptation of various basic and intermediate races in sorghum to different geographies still hold. However, it is increasingly realized in most of the sorghum improvement programs that the *caudatums* and *durras* were extensively used in hybrid development programs and it is important to bring in the *guineas* to further increase the variability so as to increase the yield. A classic example is that at the ICRISAT gene bank, the largest number of sorghum germplasm accessions (53.21 % of total accessions) belong to races *durra* (21.28 %), *caudatum* (20.19 %), and their intermediate races *durra–caudatums* (11.74 %). Therefore, their utilization is also very high in the ICRISAT sorghum improvement program for hybrid parents' development. However, considering that sorghum yield is reaching a plateau in most breeding programs and that grain mold resistance is low, it is high time to bring *guineas* into the crossing program to break the yield barriers and increase grain mold resistance.

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3 Cytogenetics of Sorghum

Sujay Rakshit, K.N. Ganapathy and K.B.R.S. Visarada

Abstract

Sorghum is an interesting genus having a large number of well-recognized species taxonomically classified into five subgenera. Cytogenetic analysis led to the understanding of the nature of chromosomal variations, origins, and probable relationships based on chromosome morphology. Progress in the science of conventional and molecular cytogenetics, and genomic research provide a detailed insight into the genome organization of an individual or species, leading to enhanced utilization of genetic and physical information towards improvement of the crop. The integration of genetic, physical, and cytomolecular maps of the *Sorghum* genus is useful to scientists working on genomics of grass species. Large-scale molecular karyotyping of grass genomes would facilitate alignment of related chromosomal regions among different grass species and also facilitate genetic and cytogenetic studies of chromosome organization and evolution. As compared to other crop species little is known about the karyomorphology in sorghum mainly due to the small size of its chromosomes. In this chapter efforts have been made to collate the scattered information on karyotype studies, cytotaxonomy, phylogenic relation, numerical and structural variations, genome architecture, and wide introgression in sorghum. Implications of the information on sorghum improvement are discussed.

1 Introduction

Cytogenetic analysis of a crop and its wild relatives yields a wealth of information about their homology and genome compositions, which forms the basis of understanding for the breeders and researchers about the ways and means of genetic manipulations in crop plants. Sorghum is an interesting genus having 25 well-recognized

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species, which are taxonomically classified into five subgenera: Eusorghum, Chaetosorghum, Heterosorghum, Parasorghum, and Stipossorghum. Sorghum is grouped under the family Poaceae, tribe Andropogoneae, subtribe Sorghastrae, and genus *Sorghum*. The genus has diploid chromosome numbers of 10, 20, 30, or 40 (Garber 1950; Lazarides et al. 1991).

Cytogenetic analysis in sorghum by various workers led to the understanding of the nature of chromosomal variations, origins, and probable relationships based on chromosome morphology. Extensive studies on cytological and breeding behavior involving different sorghum species have been carried out by various workers. Huskins and Smith (1932) were the first to report on chromosome morphology in sorghum. With the advent of the science of conventional and molecular cytogenetics, and genomic research, it is now possible not only to organize and integrate the genetic, molecular, and cytological information of the genomic resources, but also to have a detailed insight into the genome organization of an individual or species giving the sequence and chromosomal view of the genome. Cytogenetic analysis of chromosomes also complements and enhances the utilization of genetic and physical information developed from large-scale genotyping and sequencing projects. The integration of genetic, physical, and cytomolecular maps of genus *Sorghum* is useful to scientists working in various disciplines particularly for those involved in genomic investigation of grass species. Large-scale molecular karyotyping of grass genomes would facilitate alignment of related chromosomal regions among different grass species and also facilitate genetic and cytogenetic studies of chromosome organization and evolution.

2 Basic Chromosome Number and Cytogenetic Structure

2.1 Basic Chromosome Number

Researchers proposed different basic chromosome numbers to the members of the tribe Andropogoneae. Huskins and Smith (1934) proposed basic chromosome number, $x = 7$, whereas Sharma and Bhattacharjee (1957) proposed $x = 4$. Garber (1950) and Celarier (1956) considered the base chromosome number of this tribe to be either five or ten. Doggett (1976) concluded that the basic chromosome number of Sorghastrae is $x = 5$. This supports the suggestion of Garber (1950) who observed predominance of genera with chromosome numbers of five and ten. However, Garber (1950) raised the question whether five indicated the endpoint of base chromosome numbers in the order of descending series or whether it is the starting point of a polyploid series. Morakinyo and Olorode (1988) reported that evidence supporting the basic number to be five was not available. Spangler et al. (1999) attempted to resolve the base chromosome number of Andropogoneae using chromosome number information with the phylogenetic tree derived from *ndhF* gene sequence analysis. A wide variety of taxa with $n = 10$ at the base of the tree led them to the understanding that $n = 10$ and not 5 for the tribe. However, the occurrence of $n = 5$ and $n = 10$ species raises questions regarding the base number of this genus and the widely accepted basic chromosome number of sorghum is $x = 5$.

In sorghum, the reported occurrence of bivalents during meiosis of haploids (Brown 1943; Kidd 1952; Endrizzi and Morgan 1955) further supports the idea of its tetraploid origin. Endrizzi

and Morgan (1955) observed translocations in the progeny of haploids and proposed that these originated from recombination between homologous duplicated regions (resulting from polyploidy) in the haploid genome. However, no strong evidence supporting tetraploid origin is observed from meiotic pairing of diploid sorghum (Brown 1943; Endrizzi and Morgan 1955). Celarier (1959) opined that polyploidy was of widespread occurrence in Sorgheae, as he assumed the basic chromosome number to be five. He observed that most of the tetraploid sorghums cytologically behave as diploids except those representing Parasorghum and Stiposorghum, which behave as autotetraploids. But, use of single-copy probes in fluorescent in situ hybridization (FISH) consistently identified two loci on two different chromosomes, which provided evidence that sorghum is not of tetraploid origin (Gomez et al. 1997). However, the basic chromosome number of subtribe Sorghastrae is assumed to be five and therefore it is often believed that sorghum is of tetraploid origin. A large number of complementary genes between them also indicate their tetraploid origin (Rooney 2000).

2.2 Karyotype Analysis

Karyotype analysis is very useful for understanding the origin, chromosomal variation, and genetic relationships at species or genus level. Very little is known about the karyomorphology in sorghum mainly due to the small size of its chromosomes. Huskins and Smith (1932) analyzed the chromosomal structure in various sorghum species and observed that the somatic chromosome length ranged from 1.2 to 3.3 µm, half the length of chromosomes observed from root tips of maize. They reported a chromosome with one long portion having a prominent subterminal attachment constriction and the shorter portion connected with the longer portion by only a fine thread of chromatin. The differences observed in the morphology of chromosomes seem to be small but definite enough to be considered as appropriate for systematic classification. Most of the chromosomes have median or submedian constrictions and often some of them have secondary subterminal constriction attachment. In *S. halepense* they observed the somatic chromosome number of 40, whereas in other species and varieties studied it was 20. The study also gave indication that *S. halepense* is an allotetraploid originated from chromosome doubling of a hybrid.

Huskins and Smith (1934) further studied the cytological structure of the meiotic chromosomes of the genus *Sorghum*. They observed 10 bivalents in all the diploid sorghums. However, quadrivalent and hexavalents were also noticed by them. In *S. halepense* they commonly observed 10–14 bivalents, and the rest of the chromosomes were in quadrivalent or higher associations. In sorghum the chromosome pairing of the normal 10 chromosomes is similar to that of pairing in hexaploid wheat and oats where the 42 chromosomes form 21 bivalents and multivalents are rarely observed. However, asynaptic pairing in sorghum leads to formation of multivalents and is known to occur very commonly (Huskins and Smith 1934). Endrizzi (1957) reported cytological investigations involving sorghum parents and hybrids with $2n = 20$ and $2n = 40$ chromosomes. He reported that the parental and hybrid combinations showed similar chromosome pairing with only 10 bivalents. Hybrids formed with $2n = 20$ and $2n = 40$ generated trivalents in meiosis with either allotetraploids or segmental allopolyploids (Raman and Sankaran 1979). The total chromosome length of the karyotype complement in their study varied from 19.28 µm ($2n = 20$) to 61.69 µm (*S. Purpureo-sericeum*, $2n = 40$).

Sharma and Bhattacharjee (1957) and Celarier (1958) studied karyotypes of 18 species of the genus *Sorghum* in detail. The studies of Sharma and Bhattacharjee (1957) showed the number of chromosomes with secondary constrictions to vary between species, which indicates their role in evolution. They also reported 23 idiograms based on the position of primary and secondary constrictions and the relative size of the

chromosomes made for all species. They reported five pairs of chromosomes in *S. vulgare* to have satellites with one pair of chromosomes at both ends. The chromosome length in *S. halepense* and *S. nitidum* were in the range of 2.31–4.41 and 1.63–3.33 µm, respectively. *S. versicolor* and *S. purpureo-sericeum* possessed chromosomes with longer length. All chromosomes of *S. purpureo-sericeum* had lengths longer than 6.90 µm, whereas those of *versicolor* were in lengths of 5.99–7.42 µm. The chromosome lengths of *S. stipoideum* and *S. intrans* were in the range of 3.72–4.89 and 5.41–6.52 µm, respectively. They grouped sorghum chromosomes as large, medium, and small. The species *S. purpureo-sericeum* and *S. versicolor* were reported to have larger chromosomes, whereas *S. subglabrescens* and *S. durra* recorded both the medium and small category. They observed clear karyotypic differences between different strains or genotypes in the idiograms in terms of number and position of constriction regions. However, the karyotype similarities observed in the study led to inclusion of strains under a common bigger taxonomic unit. From the observation made on chromosome structure they also labeled 10 major chromosomes from A to J. Further based on minor differences of these they were again sub-classified as B, B1, B2, and B3. Sharma and Bhattacharjee (1957) reported six species with chromosomal fragments in their somatic cells. In most cases there were no more than two fragments per cell and none were observed during the meiotic division.

Gu et al. (1984) analyzed the karyomorphology of seven sorghum species, namely *S. bicolor*, *S. halepense*, *S. nitidum*, *S. versicolor*, *S. purpureo-sericeum*, *S. stipoideum*, and *S. intrans*. All the species studied differed in chromosome number, size, position of centromere, secondary and tertiary constrictions, and staining ability of chromosomes. In the cultivated species *S. bicolor*, the chromosome size ranged from 2.7 to 5.6 µm with the most of the chromosomes in the range between 2.5 and 4.0 µm. From staining it was observed that the heterochromatin regions adjacent to the centromere were more stainable than other regions. Other than the longest chromosome, all had a median centromere, and the longest chromosome showed submedian constriction with a tertiary constriction on its long arm. Gu et al. (1984) observed greater variation among chromosomes of the complement of *S. bicolor*. In their study *S. halepense* recorded similarities to *S. bicolor* for arm ratio and relative lengths especially for longer chromosomes. A similar trend was observed between *S. purpureo-sericeum* and *S. versicolor*. Likewise, *S. stipoideum* and *S. intrans* showed resemblances. However, in the mentioned study of Gu et al. (1984) karyographs of Parasorghum and Stiposorghum showed a clear-cut difference from Eusorghum. *S. purpureo-sericeum* recorded the largest somatic chromosome among the seven species studied. In *S. versicolor*, the SAT chromosome is the longest, which supports earlier reports (Schlarbaum and Tsuchiya 1981; Singh and Tsuchiya 1982). A similar phenomenon is reported in *S. nitidum*. Chromosomes of the species in Eusorghum tend to stain differentially in prophase with deep stain only in the regions adjacent to centromeres. Wu (1982) reported the terminal or subterminal position of the nucleolus organizer region (NOR) in meiotic configurations of *S. versicolor*, *S. nitidum*, and *S. purpureo-sericeum*.

Kim et al. (2005a, b) developed a FISH-based karyotype of the sorghum inbred line Btx623. The ordering and nomenclature of the chromosomes were based on chromosome length at metaphase as SBI-01 (longest) to SBI-10 (shortest). The SBI-01 chromosome of BTx623 is exceptionally long. It has eight pairs of metacentric chromosomes from SBI-02, SBI-03, SBI-04, SBI-05, SBI-07, SBI-08, SBI-09, and SBI-10 and one pair of medium-sized submetacentric chromosomes SBI-06. In addition to its long length (5.11 µm), the SBI-01 chromosome is one among the only two submetacentric chromosomes and is the only satellite chromosome. Lengths of chromosomes SBI-02, SBI-03, SBI-04, and SBI-05 were in the size range of 3.87–3.44 µm, and those of SBI-06, SBI-07, SBI-08, and SBI-10 were in the size range between 3.15 and 2.97 µm. The only secondary constriction and NOR observed in BTx623 was

near the centromere in the short arm of the SBI-01 chromosome. NOR in type Combine-kafir60 is located in the center of the fifth longest chromosome (Yu et al. 1991), and NOR of *S. propinquum* is located in the short arm of the smallest chromosome (Magoon and Shambulingappa 1961). Such variation in structure can complicate linkage analysis beyond a particular parental combination. The procedure of unique nomenclature followed in designating BTx623 could be the basis for genetic, breeding, and genomic applications.

2.3 Cytotaxonomy

The first taxonomic report of sorghum and related materials in the name of *Holcus* was given by Linnaeus (1753). Linnaeus added several other species such as *H. lanatus* and *H. laxus*, which were later included in another tribe, *Aveneae*, and have retained the generic name *Holcus*. Use of sorghum as a generic name was first given by Adanson (1763) as a substitute for Linnaeus' *Holcus*. This remained as a base for Moench (1794) to differentiate sorghum from *Holcus* in the materials originally considered by Linnaeus. Taxonomic studies by Stapf (1919), Snowden (1935, 1936, 1955) and Garber (1950) led to the understanding that sorghum is a genus of wide variability. Garber (1944, 1948, 1950) made a detailed cytological investigation in a number of species of this genus and established a large number of facts governing the distribution and taxonomy of the genus. His findings are the most detailed among various research conducted in cytogenetic analysis in sorghum.

Haeckel (1885) grouped sorghum into a broader group *Andropogon sorghum*, which he subdivided into *A. sorghum halepensis* and *A. sorghum sativus*. *A. sorghum halepensis* included wild perennial and annual types whose spikelet is deciduous at maturity, and *A. sorghum sativus*, annual forms having persistent spikelets. Hackel (1889) considered all the subtribe of *Sorgheae* under subgenus *Andropogon*. In contrast, Stapf (1919) considered it distinct from *Andropogon* and grouped them together as *Sorghastrae* under subtribe *Andropogonineae*. Piper (1916) stressed the need for distinction between Johnsongrass (*S. halepense*) and cultivated forms indicating that they are confined to Mediterranean regions and absent in tropical parts of Africa and also the probable center of origin for *A. sorghum sativus*, the cultivated types. Huskins and Smith (1932) indicated that the sorghum genus includes a wide range of forms. The classification in the section *Sorgastrum* is well understood, but within section *Eusorghum*, it includes all the numerous cultivated and wild forms. Keng (1939) proposed a separate subtribe under the name Sorgheae. The genera *Sorghum* Moench, *Sorghastrum* Nash, *Astenochloa* Buse, *Lasiorrhacis* Stapf, *Cleistachne* Benth, *Rhaphis* Lour, *Vetiveria* Bory, and *Chrysopogon* Trin were included in the subtribe Sorgheae. Keng (1939) placed the genus *Pseudosorghum* in the subtribe Sorgheae and shifted the *Chrysopogon*, *Rhaphis*, and *Vetiveria* to the subtribe *Rottboelliinae*. Celarier (1959) later considered *Pseudosorghum, Chrysopogon*, and *Vetiveria* as members of the Sorgheae due to their close resemblance. He opinioned that sorgheae was derived from both riochloeae and also believed that the pseudosorghum occupies an intermediate position.

Celarier (1958) made an extensive review of the cytotaxonomy of the *Andropogoneae* subtribe *sorgheae*, genus *Sorghum*. They used the subdivision of the genus into subgenera as given by Garber (1950).

2.3.1 Various Subgenera Within Sorghum

Eusorghum

Eusorghum is also referred to as true sorghum. Snowden (1955) classified the section into two subsections, Arundinacea and Halepensia, and further into series and subseries. The subsection Arundinacea has series Spontanea and Sativa. Sativa was further subclassified into six subseries: Drummondii, Guineense, Nervosa, Bicoloria, Caffra, and Durra. Hybridization among different species and subseries are feasible and there are no barriers for gene exchange

between any of these species (Karper and Chisholm 1936). The detailed studies on taxa in series Sativa was studied by Laubscher (1945). The cross between *S. drummondii* and *S. caffrorum*, two partners within Eusorghum, was cytologically analyzed by Endrizzi (1957) and the F_1s were regular. Under the subsection Arundinacea in series Spontanea Snowden (1955) identified 17 species, which are grasses in nature. Of these, 16 are African and two are Indian species. The African species were found on the eastern half of the continent and three in West Africa. Two species, *S. verticilliflorum* Stapf and *S. sudanense* Stapf, have been introduced extensively and become naturalized in many tropical countries. Endrizzi (1957) analyzed the chromosome behavior in the F_1 of *S. verticelliflorum* × *S. sudanense*, *S. arundinacea* × *S. caffrorum*, and *S. verticelliflorum* × *S. dochna* var. *technicum*. All the crosses studied had 10 bivalents in the F_1s; however, in some instances the chiasma frequency observed was lower than the parents.

Under subsection Halepensia six species were included by Snowden (1955): *S. halepense* (L.) Pers, *S. miliaceum* (Roxb.) Snowden, *S. controversum* (Steud.) Snowden, *S. almum* Parodi, *S. randolphianum* Parodi, and *S. propinquum* (Kunth) Hitchc. Several species of this subsection were crossed with various species of subsection Arundinancea and hybrids were cytologically studied. Crosses with $2n = 20$ species with the Arundinancea subsection have proven fertile with regular meiotic divisions (Celarier 1958). From crosses with the $2n = 40$ species two types of F_1s are produced. A normal F_1 with $2n = 30$ is infrequent and many of the F_1s that have been studied are $2n = 40$.

Parasorghum

This subgenus was first proposed by Snowden (1935), which includes all sorghum species having bearded nodes and simple panicle primary branches. Both cytological and morphological differences observed in this subgenus were observed in subgenera *parasorghum* and *stiposorghum* as well but these were isolated geographically (Celarier 1958). Five species were included in *Parasorghum*: *S. purpureo-sericeum* Aschers. and Schweinf., *S. versicolor* JN Anderss, *S. nitidum* Pers., *S. leiocladum* CE Hubb, *S. australiense* Garber and Snyder. *S. purpureo-sericeum* is an extremely variable species with several varieties and distributed in East Africa and West India. Most accessions were reported to have 10 somatic chromosomes and completely regular meiotic divisions. *S. versicolor* is in many respects similar to *S. purpureo-sericeum*. The natural distribution of the species is spread towards Southeast Africa. *S. nitidum* has the largest geographical distribution among *parasorghums* and is spread mostly in Southeast Asia, Indonesia, and Australia. Ayyanger and Ponnaiya (1941) first reported this species as $2n = 10$ from a collection in western India. *S. leiocladum* is an Australian species and was studied cytologically by Garber (1950, 1954). It is a tetraploid with $2n = 20$. At diakinesis and metaphase I it showed univalents, bivalents, trivalents, and quadrivalents. *S. australiense* is an annual species from Australia. It is similar to *S. trichocladurn* with $2n = 20$ (Garber and Snyder 1951). Detailed studies by Garber (1954) showed that it is similar to *S. leiocladum* in meiotic chromosome behavior and is also considered to be an autotetraploid.

Stiposorghum

This subgenus is mainly found in Northern Australia and includes six species: *S. intrans* F. Muell, *S. stipoideum* Gardner and Hubbard, *S. brevicallosum* Garber, *S. matarankense* Garber and Snyder, *S. plumosum* Beauv, and *S. timorense* Buese. *S. intrans* was previously studied by Garber (1948, 1950) who observed $2n = 10$ with regular meiosis. Attempts were made to cross this species with *S. stipoideum* and *S. brevicallosum* but no seeds could be obtained (Garber 1950). *S. stipoideum* was studied by Garber (1950) from collections from the Northern Territory of Australia. All accessions studied were diploid with regular meiotic behavior. Attempts made to cross this species with *S. intrans* and *S. brevicallosum* resulted in no seed formation (Garber 1950). All the accessions of *S. brevicallosum* as studied by Garber (1950) were found to be diploid with regular meiotic behavior. He

also attempted to cross this species with *S. intrans* and *S. brevicallosum* but no seeds were produced. *S. matarankense* represents diploid species in the *S. intrans–S. stipoideum–S. brevicallosum* complex (Garber and Snyder 1951). *S. plumosum* has widest distribution in the subgenus and is the only perennial in this subgenus. Both tetraploid and hexaploid types are known (Garber 1954) and both have high frequency of multivalents. He analyzed two tetraploid accessions and found that they were similar to the *Parasorghum* species, *S. leiocladum*, and *S. australiense* in meiotic behavior. *S. timorense* is morphologically very similar to *S. plumosum* and possibly should only be considered as a variety of that species.

Chaetosorghum

The species *S. macrospermum* Garber was accommodated in this subgenus by Garber (1950). Though they are glabrous noded, they would not fit in either Eusorghum or Sorghastrum. Garber (1950) analyzed the meiotic behavior of the species and found that it had 20 bivalents at metaphase I. However, he observed one plant that had a high frequency of univalents which he explained as partial desynapsis.

Heterosorghum

This is another subgenus that was mentioned by Garber (1950) to include one species that will not fit easily into other subgenera. The species, *S. laxiflorum* FM Bailey, had very extensive distribution and is found in Australia, New Guinea, and the Philippine Islands. Garber (1950) analyzed one accession from northern and another from southern Queensland. In both the cases the $2n$ number was 40, and meiosis observed was regular with 20 bivalents at the diakinesis state and metaphase I stage.

2.3.2 Phylogenetic Relationship Within Sorghum Subgenera

Celarier (1958) detailed the phylogenetic relationship within sorghum subgenera (Fig. 1). In *Parasorghum*, *Stiposorghum*, and *Eusorghum*, the pedicellate spikelets are staminate type, whereas in *Chaetosorghum* and *Heterosorghum* only glumes are seen. The entire pedicellate spikelet is lost in the genus *Sorghastrum*. Likewise, panicle branching is significant in phylogenetic relationships. The open divided type is observed in *Eusorghum* and *Heterosorghum*, which are primitive type. Simple branching is observed in *Parasorghum*, *Stiposorghum*, and *Chaetosorghum*, which are the advanced types. Cytologically, the chromosome number and size vary among the subgenus. Chromosomes of *Parasorghum* and *Stiposorghum* are quite large whereas *Eusorghum* and *Heterosorghum* are much smaller. In *Parasorghum* and *Stiposorghum* five is the lowest basic chromosome number and examples of polyploidy suggest autopolyploidy of building units by 10 (i.e., $2n = 10$, 20, 30). In *Eusorghum*, the lowest number is 10, and allopolyploids are built by units of $2n = 20$ and 40. *Chaetosorghum* and *Heterosorghum* are allopolyploids with $2n = 40$. Celarier (1958) opined that *Parasorghum* and *Stiposorghum* are closely related and are to be considered in one subgenus and these genus can be clearly differentiated from *Eusorghum*. Relationships of *Chaetosorghum* and *Heterosorghum* are not clearly understood.

2.3.3 Cytological Relationship Among Various Species of Sorghum

The wild species of sorghum represent a potential diverse source of germplasm for sorghum breeding. Parvatham and Rangaswamy (2004) made a detailed study of the karyomorphological and phylogenetic studies in six cultivated and wild sorghum species, *S. intrans*, *S. Propinquum*, *S. purpureo-sericeum*, *S. halopense, S. sudanense*, and *S. bicolor*. They used relative chromosome length, arm ratio, chromosome index, and nucleolar organizers for characterizing the chromosomes of different species. All the species possessed basic chromosome number, $x = 5$. *S. intrans* possessed one pair of long median and four pairs of submedian chromosomes, whereas the tetraploid species *S. propinquum* ($2n = 20$), *S. purpureo-sericeum* ($2n = 20$), and *S. bicolor*

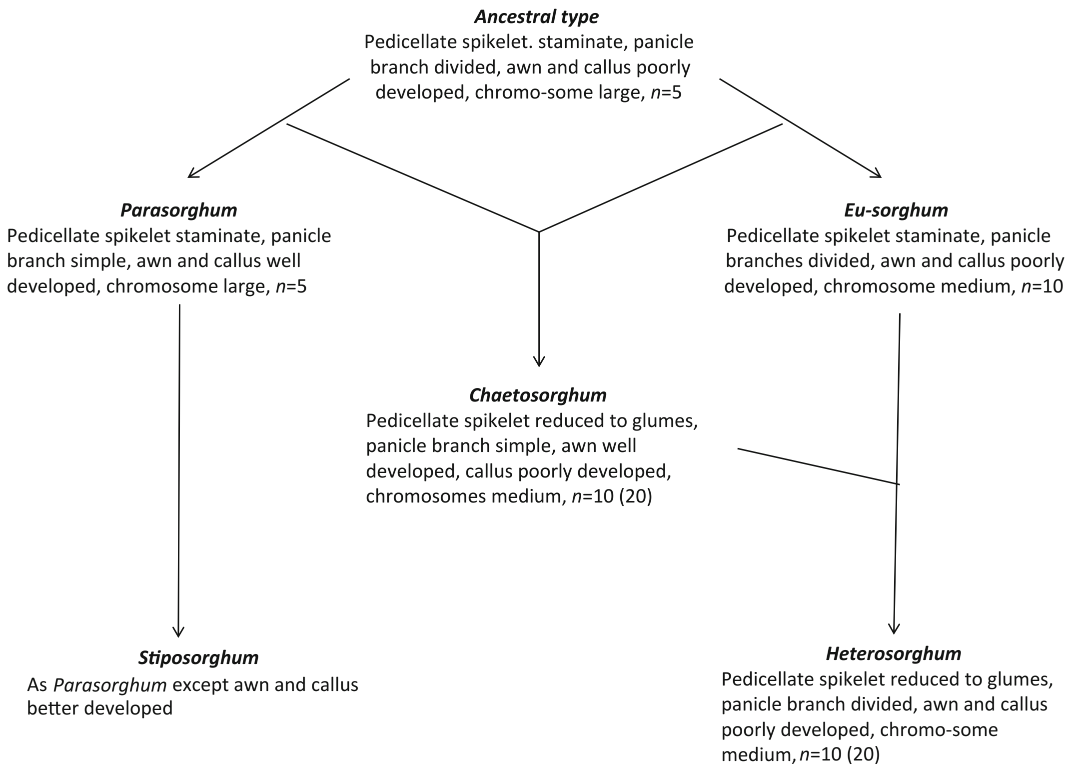

Fig. 1 Phylogenetic relationship between different subgenus in sorghum (adapted from Celarier 1958)

($2n = 20$) were reported to possess median, submedian, and smaller chromosomes. *S. propinquum* and *S. purpureo-sericeum* showed similarity in having seven and three pairs of median and submedian chromosomes, respectively. The hexaploid species, *S. halepense* and *S. sudanense*, possessed comparable chromosome architecture but differed in sizes. One pair of satellites (SAT) each was observed in all the species revealing their role in nucleolar organization. Their study revealed the uniqueness of the SAT regions, occupying only the longest chromosomes except hexaploid species *S. sudanense* where it was observed in the second longest chromosome. Variation in chromosome number of species is attributed to the duplication of the chromosome complements (Stebbins 1950). Those species with lower chromosome number are believed to be primitive as also evidenced from the taxonomic characteristics (Parvatham and Rangaswamy 2004). Vinall (1926) revealed that the wild annual sorghums cross readily with large annual grain sorghum but crosses between Johnsongrass and annual forms is possible only with great difficulty and the hybrids are mostly sterile. He attempted crosses between sorghum and Johnsongrass and could obtain only two successful crosses. The study further revealed that the successful cross could be obtained only when sorghum variety, Black amber sorgo, was used as the pistillate parent. No successful cross was obtained when Johnsongrass was fertilized with the sorghum pollen. However, chromosomal studies did not reveal any major differences from those of other cultivated sorghums. Allopolyploidy could have played a major role in the evolution of species in sorghum (Parvatham and Rangaswamy 2004).

Hadley (1953a, b) attempted crosses between *S. vulgare* ($2n = 20$) and *S. halepense* ($2n = 40$) and they obtained two hybrids with 30 and one with 40 chromosomes. One hybrid having 30

chromosomes set seed when backcrossed with *S. vulgare*, however, the other was sterile. In the backcross progeny the chromosome numbers varied from 20 to 22. Plants having 20 chromosomes appeared regular with some plants sterile. On the other hand, the 40-chromosome hybrid was fertile and set a large number of seeds and in their F_2s the chromosome numbers ranged from 38 to 40.

Hybrids formed using *S. propinquum* and *S. bicolor* are reported to be meiotically regular with 10 bivalents (Doggett 1988). Analysis of meiosis of *S. bicolor* × *S. halepense* hybrids proved that *S. halepense* possesses one genome similar to *S. bicolor*, and another divergent or a rearranged genome. This leads to the understanding that *S. halepense* is an allopolyploid or segmental allopolyploid (Duarra and Stebbins 1952; Tang and Liang 1988).

2.3.4 Molecular Phylogenic Relation Among Sorghum Species

It is evident that *Sorghum* genera are highly heterogeneous. Efforts have been made to decipher the phylogenetic relationship among them at the molecular level. DNA markers have been deployed in establishing the relationship of sorghum to other grasses such as maize (Whitkus et al. 1992). Allozymes (Morden et al. 1990), nuclear genes, for example, *ITS1* (Sun et al. 1994; Dillon et al. 2004; Ng'uni et al. 2010), *ndhF* (Spangler et al. 1999; Dillon et al. 2004, 2007a), *Adh1*(Dillon et al. 2007a), *chitinase-b* (Qin et al. 2008), and chloroplast genes, such as *psb*Z-*trn*G, *trn*Y-*trn*D, *trn*Y-*psb*M, and *trn*T-*trn*L (Ng'uni et al. 2010) have helped in better understanding the phylogenetic relationship within the *Sorghum* genus. Spangler (2003) suggested the existence of three distinct lineages—*Sarga*, *Sorghum*, and *Vacoparis*—representing sorghum. He included the set of species that formerly represented the bulk of the subgenera, *Parasorghum* and *Stiphosorghum*, and placed Australian taxa, *S. macrospermum* and *S. laxiflorum* in a new genus *Vcoparis*. *S. bicolor*, *S. halepense*, and *S. nitidum* were retained in *Sorghum*. However, sequence comparison of *ITS1* and *ndh*F genes showed Australian species *S. laxiflorum* and *S. macrospermum* most closely related to cultivated sorghum (Dillon et al. 2004). The study suggested reduction of subgeneric sections from five to three: *Eusorghum* (unchanged), a combined *Chaetosorghum*/*Heterosorghum* to reflect the very close relationship between these two species, and a combined *Parasorghum*/*Stiphosorghum* section. Findings of Dillon et al. (2007a) further supported this conclusion and contradicted the result of Spangler (2003). Using *ITS* and chloroplast *ndh*F sequences Price et al. (2005a) put *Sorghum* into two lineages, one comprising the $2n = 10$ species with large genomes and their polyploid relatives, and the other with the $2n = 20$ and 40 species with relatively small genomes. Qin et al. (2008) used the *Chi-b* gene to establish molecular phylogeny among 10 sorghum species. Their study placed the 10 species into three sections: the first with *S. halepense*, *S. almum*, and *S. silk*; the second with *S. bicolor*, *S. propinquum*, and *S. arundinaceum*; and the third with *S. nitidum*. A combined sequence analysis with four regions of chloroplast DNA and *ITS* of nuclear DNA by Ng'uni et al. (2010) clearly demonstrated that classifying Australian species *S. macrospermum* and *S. laxiflorum* as a separate section was not well supported. They further found that *S. almum* was closely associated with *S. bicolor*, suggesting the latter to be the maternal parent of the former.

All the reports on molecular phylogeny of sorghum are in agreement that there are two well-supported major clades within sorghum (Kellogg 2013). *S. bicolor* and its close relatives, *S. halepense*, *S. propinquum*, *S. arundinaceum*, *S. almum*, and *S. drummondii* are represented in clade 1. Except *S. propinquum*, which is Asian, all are of African origin. Australian species *S. macrospermum* and *S. laxiflorum* are also in this clade (Dillon et al. 2004; Price et al. 2005b). Seventeen species are included in clade 2. These are Australian species representing subgenera *Stiposorghum* and *Parasorghum*, which are cross-compatible with cultivated sorghum (Price et al. 2006).

3 Ploidy Variations

The number of complete sets of chromosomes in a cell of an organism is referred to as ploidy. Hence ploidy variation deals with differences in terms of chromosome numbers. Ploidy variation is considered to be an important adaptive mechanism leading to evolution and speciation. The preceding discussion has shown that different species within the subtribe Sorghae have considerable ploidy variations. However, such variation has also been reported within cultivated species of a crop and has much cytogenetic significance with anticipated agronomic significance as well. Two types of ploidy variations in crop plants have been reported, namely, euploidy and aneuploidy. Euploids of a species vary in terms of complete chromosome set, whereas aneuploids differ in terms of one or more chromosomes from the normal set.

3.1 Euploidy

Since the 1930s sorghum scientists observed abnormal plants within the experimental materials of sorghum. However, it was Brown (1943) from Texas Agricultural Experiment Station who first recognized them as ploidy variants of cultivated sorghum. Since his report, haploids, triploids, autotetraploids, and higher ploidy variants in sorghum have been published by various authors (reviewed by Murty and Rao 1974).

3.1.1 Haploids

Brown (1943) reported the occurrence of abnormal plants over 10–12 years among grain and forage sorghums grown at Chillocothe, Texas. Observing the characteristics of these abnormal plants he surmised them to be haploid plants and characterized two such plants: one was obtained from the F_3 progeny of a Blackhull kafir × Feterita cross in 1940, and the other was found in a genetic stock in 1941. Haploid plants had relatively slender stalks, narrow leaves with smaller stomatal guard cells, small and highly sterile panicles, and particularly small glumes. Pollen grains of haploids are in general smaller, empty, and collapsed. Brown (1943) observed 4–6 and 3–7 anaphase separation to be most common, whereas the separation ranged from 5–5 to 0–10 in a number of cases. Out of 150 metaphase nuclei he studied in one case, he reported 3 II + 4 I, two cases of 2 II + 6 I, and 13 cases of 1 II + 8I. In addition, in nearly 8 % of the cases he reported anaphase bridge formation among other abnormal disjunctions. Observed bivalents in sorghum haploids supported earlier suggestions that five is the basic chromosome number in sorghums (Karper 1930; Longley 1932; Karper and Chisholm 1936).

Since the report of Brown (1943), haploids in sorghum have been reported by various authors (Kidd 1952; Endrizzi and Morgan 1955; Schertz 1963). Kidd (1952) obtained the haploid as a "twin seedling" from variety Resistant Wheatland (GC 38288). Out of the 55 haploid cells Kidd studied, eight recorded 1 II + 8 I, four 2 II + 6 I, seven had 1 III + 7 I, and the rest had 10 I. Gaines and Aase (1926) and Levan (1941) observed di- and poly-ploid cells in haploid plants of wheat and *Phleum*, respectively, in which univalents remained the rule. Similarly, Kidd (1952) also observed diploid cells in nearly 30 % of the cases. However, in these cases he noticed "stickiness" among the chromatids, whereas Gaines and Aase (1926) and Levan (1941) reported "syncytes". Endrizzi and Morgan (1955) obtained haploids from a cross of *S. vulgare* Pers. cv. "Texas Blackhull kafir" × *S. arundinaceum* (Willd.) Stapf. They recorded 1 II + 8 I in four out of 26 diakinesis or metaphase I cells studied. In the remaining 22 cases, 10 irregularly distributed univalents were observed. Although the haploids were predominantly sterile in nature, they could obtain seeds by pollinating haploid plants with Texas Blackhull kafir pollen.

Schertz (1963) reported 19 haploids from a population of 41,300 plants at Chillicothe, Texas, a ratio of 1 in 2174 plants. The haploids reported by all authors recorded smaller stature, glumes, anthers, and panicles with sparse seed set. Schertz (1963) further added that the pollen stainability varied from 0 to 1.5 %, and seed set

on selfing ranged from 0 to 2 %. They could obtain 394 progeny of haploids by cross-pollination. Cytology of the haploids recorded around 3 % of metaphase cells with 8 Is and an association of two chromosomes. He also recorded unequal chromosome distribution during anaphase I leading to a large nucleus in one secondary sporocyte and no or small nucleus in the other sporocyte. He also observed multinucleate secondary sporocytes, many micronuclei and nuclei of varied sizes. He recorded varied distribution of chromosomes to poles at anaphase.

Murty and Rao (1974) have concluded that the occurrence of bivalents in the haploids indicates the duplicate nature of the genome of grain sorghum. Almost all authors have observed the occasional production of diploid plants from haploids. This suggests that diploid nuclei with full haploid complement as occasionally observed by Brown (1943), Kidd (1952), and others are formed during disjunction leading to normal ovule development.

3.1.2 Triploids

Kidd (1952) obtained a triploid plant from the F_1 of a cross between *ms*#2Dwarf Tan Kafir and Redlan F_1, which was sterile. Because male sterility is a recessive trait, the sterile plant in the F_1 generation itself was diagnosed and investigated. Out of 24 cells investigated eight cells showed 10 III, five had 9 III + 1 II + 1 I, six recorded 8 III + 2 II + 2 I, three had 7 III + 3 II + 3 I, and one each was detected with 6 III + 4 II + 4 I and 4 III + 5 II + 8 I cytological configurations. Subsequently triploids in sorghum have been reported by Price and Ross (1957), Erichsen and Ross (1957), Quinby et al. (1958), Munoz et al. (1963), and Schertz and Stephens (1965). These were obtained from both natural populations and from controlled crosses. Schertz and Stephens (1965) obtained triploids in progeny from plants that had been emasculated with hot water and pollinated subsequently. It is suggested that when hot water is used for emasculation, subsequent pollinations were often delayed or incomplete, which may lead to self-pollination from pedicelled spikelets. This delayed pollination enhances the probability of triploid formation (Schurtz 1966). Hybrid plants with 30 chromosomes from interspecific crosses of *S. vulgare* (2 N = 20) and *S. halepense* ($2n = 40$) have also been reported (Hadley 1953a, b, 1958; Hadley and Mahan 1956; Endrizzi 1957). Triploids can be distinguished from diploids of the same cultivar in terms of fertility and morphology and cytologically (Murty and Rao 1974). However, in most cases, triploids were not morphologically different from tetraploids, except for high levels of sterility (Roony 2000). Triploids are highly sterile and consistently have large stomatal guard cells. Meiotic pairing of chromosomes is irregular with most common meiotic configuration of 9 IIIs (Murty and Rao 1974). Upon backcrossing triploid plants to diploids in general produce diploid and aneuploid stocks (Price and Ross 1957; Schertz and Stephens 1965). Sengupta and Weibel (1971) reported the occurrence of male sterile triploids to the extent of 0–22 % upon crossing diploid sorghum cultivars to tetraploid *S. halepense*.

3.1.3 Tetraploids

Autotetraploids and other polyploids in sorghum have been reported by Salomon (1940), Dusseau (1945), Chin (1946), Casady and Anderson (1952), Butany (1955), Atkinson et al. (1957), Doggett (1957), Krishnaswamy et al. (1958), Narayan (1961), Ross and Chen (1962), Doggett (1962), Magoon and Tayyab (1968), Sengupta and Weibel (1971), and others. Crosses between cultivated sorghum and other *Sorghum* species, particularly *S. halepense* are reported to produce F_1 hybrids with 40 chromosomes along with triploids (Hadley 1953a, b; Endrizzi 1957; Hadley 1958). Schertz and Stephens (1965) suggested that the tetraploids in such interspecific crosses were derived from abnormal eggs with 20 chromosomes. Tetraploids are reported to produce dark foliage with wavy margins, shorter but stouter straw, larger pollen grains and grain

size, higher protein content, longer stomata with a general tendency to flower late and varying degrees of male and female fertility (Murty and Rao 1974; Roony 2000). Chiasmata percentage in the tetraploids is similar to the diploids (Chin 1946). Quadrivalent formation in sorghum tetraploids, particularly of an apomictic culture R 473, is much lower compared to maize (Murty and Rao 1974). Mean quadrivalent frequency per cell in tetraploid sorghum ranges from 0.13 (Krishnaswamy et al. 1958) to 6.22 (Doggett 1964).

Ross and Chen (1962) made a cross between an autotetraploid derived from grain sorghum variety, Experimental 3, and a colchicine-induced mutant *M15* derived from Experimental 3. They observed an increase in fertility from 0.5 % in the autotetraploid to 56.9 % in the hybrid. Among the hybrids a reduced number of quadrivalents and univalents, and a higher number of bivalents were observed at diakinesis. From this they concluded that a mutation that might have occurred in diploid *M15* aided in reduction of univalent formation.

Tetraploids recorded increased seed size, thus there was a renewed interest in the 1960s to develop autotetraploid grain sorghum cultivars for cultivation (Doggett 1962). A lower level of fertility among tetraploids was the main hindrance towards this direction. However, selection for improved fertility led to identification of tetraploids with nearly the fertility level of diploids (Doggett 1962; Luo et al. 1992). Doggett (1962) suggested that certain neutral genes present in the diploid level influence seed set of the autotetraploid. However, with the initial excitement no substantial progress in this direction has been made over subsequent decades.

3.1.4 Higher Polyploids

Octaploids along with tetraploids in sorghum were first reported by Chin (1946) after treatment of grain sorghum with colchicine. Longley (1946, see Murty and Rao 1974) also found octaploid forms of grain sorghum with the use of colchicine. Octaploids are stouter and shorter than diploids, slower in growth, and lower in fertility.

3.1.5 Hypoploidy

Schertz (1962) reported the occurrence of hypoploids with chromosome number of 38–39 from a colchicine-treated population of cultivar SA403. Hypoploids in general had less fertile pollen; lower spikelet number, seed number, and total seed weight; but higher grain weight. They observed more univalents, and fewer secondary sporocytes and microspore quartets free of micronuclei as compared to tetraploids.

3.2 Aneuploidy

Vigor and fertility level are relatively better among aneuploids as compared to euploids, and often play an important role in gene mapping across plant species. Price and Ross (1955, 1957) were the first to report aneuploids in grain sorghum. They made a triploid × diploid cross and obtained nine normal diploids, nine single trisomics, one probable tetratrisomic, one triple trisomic, one tetrasomic quadruple trisomic complex, and one with undetermined chromosome number from a total of 25 plants. They did not find any noticeable increase or decrease in vigor due to extra chromosomes.

3.2.1 Trisomics

Price and Ross (1955, 1957) reported isolation of trisomics in sorghum. Their studies indicated likelihood of failure to identify trisomic plants. This was probably due to possible heterozygosity of their sources leading to segregating population. Although karyotype analysis enabled the successful identification of trisomics in other species, in sorghum this yielded limited success. While handling haploids derived from a wide cross Endrizzi and Morgan (1955) encountered a trisomic and they suggested that it might have originated during irregular meiosis of the haploid. Hadley and Mahan (1956) obtained a male sterile triploid by crossing male sterile (*ms2*) Texas Blackhull Kafir to Johnsongrass.

This sterile line was backcrossed to sweet sudangrass. In the progeny they obtained plants with 21 chromosomes along with normal diploids and other aberrants. They observed the occurrence of 10 II + 1 I as well as 9 II + 1 III. Schertz (1963) recovered five trisomics upon crossing haploids to normal diploids. Pi and Tsai (1965) reported trisomics in Shallu-type sorghum. Poon and Wu later in 1967 studied pachytene chromosomes of seven of them. Heterozygous segregation and associated heterosis often masked the distinguishable phenotypic effect of the extra chromosome (Lin and Ross 1969). To circumvent this Schertz (1966) used a homozygous background of Tx403, an early four-gene dwarf cultivar. They obtained trisomics by crossing triploid Tx403, obtained as a result of hot water emasculation, to diploids of the same cultivar. Based on characteristic features of the derived trisomics, Schertz (1966) grouped them into "Small-glume trisomic", "Stiff-branch trisomic", "Cone trisomic", "Large-glume trisomic", and "Bottle-brush trisomic". These trisomics were distinguishable only after panicle emergence, and became distinct near seed maturity. Using these cytological stocks Venkateswarulu and Reddi (1968) through karyotype analyses linked the bottle brush trisome to chromosome number 9 and large-glume to number 10. Lin and Ross (1969) obtained ten different types of trisomics by selfing a homozygous triploid from the sorghum cultivar, *SD100*, which was derived by selfing a haploid (Erichsen and Ross 1963). Based on the frequency of trivalent types and total number of trivalents all obtained trisomics were grouped into tens, and arranged in order of decreasing vigor compared to the normal diploid. These ten types were all primary trisomics as there was no association of more than three chromosomes or no ring configuration was observed. The Type 9 involved the nucleolar chromosome. The probable relationship between trisomic frequency and chromosome length was postulated by them. They postulated that Types 4 and 5 identified by them were the same as that of bottle brush and large-glume described by Venkateswarulu and Reddi (1968).

Hanna and Schertz (1971) succeeded in uncovering the identity of these trisomics by crossing to translocation stocks. They succeeded in identifying all 10 primary trisomics described by Schertz (1966) and Lin and Ross (1969). They identified these as A—bottle brush, B—sparse branch, C—compact, D—stiff branch, E—large glume, F—asymmetric, G—cone, H—light green, I—small glume, and J—spear. Schertz (1974) further characterized 5 (B, C, F, H, and J) of the 10 primary trisomics discussed above.

The first effort to localize genes using trisomics was made by Hanna and Schertz (1970). Using trisomic stocks they made an effort to localize 24 seedling lethal mutations. F_2 segregation ratios of trisomic × genetic stocks showed that the gene w_{18} was associated with the bottle-brush trisome, and the genes w_{10} and w_{20} with the cone trisome. Coleoptile color-controlling genes (Rs_1 and Rs_2) were associated with the small-glume and stiff-branch trisomes, respectively.

3.2.2 Other Aneuploids

Reports on other aneuploids in sorghum are scanty. Hadley and Mahan (1956) while backcrossing male sterile triploids to sweet sudangrass obtained a series of aneuploids with 22, 30, 33, 41, and 43 chromosomes along with predominant normal diploids and occasional trisomics. All these plants showed reduced fertility and loss of vigor upon selfing. Schertz (1962) suspected the occurrence of hypotetraploidy in his colchicine-induced tetraploid progeny. In the mentioned work of Lin and Ross (1969) they most frequently obtained single trisomics. Among 111 offspring obtained upon selfing of the triploid cultivar, *SD100*, they obtained 40 normal diploids, 58 primary trisomics, 11 double trisomics, one triple trisomic, and one plant with unknown configuration as it did not reach heading. They grouped the double trisomics on morphological bases as Types 1–4, 2–8, 3–6, 4–6, 5–3, 5–6, and 8–9. Similarly the triple trisomic was typed as 4–5–6.

Since the 1970s not many reports on aneuploidic variations in sorghum have been available in the published literature.

4 Structural Variations

Occurrence of structural variants is a common phenomenon among plant species. Such variants are distinguished only in heterozygous conditions. Garber (1948) first reported the occurrence of reciprocal translocation in *S. versicolor*. While handling haploids derived from a cross of *S. vulgare* cv. Texas Blackhull kafir × *S. arundinaceum*, Endrizzi and Morgan (1955) encountered structural variants in sorghum. Like Brown (1943) and Kidd (1952) they also observed occasional bivalents in the haploid. Upon crossing the haploid as female with pollen from Texas Blackhull kafir they obtained three out of 29 F_1s with atypical cytological behavior. In one case they observed a ring or chain of four, typically exemplifying a case of reciprocal translocation. They attributed this occurrence of reciprocal translocation to "crossing over in interstitial duplicate regions of two partially homologous chromosomes during megasporogenesis of the haploid." A second cytologically atypical F_1 indicated occurrence of the "loss of a major portion of the long arm of a heterobrachial chromosomes by crossing over in the duplicate segments of two otherwise non-homologous chromosomes of the hybrids." The third derivative was a trisomic case. Similarly, Schurtz (1963) also reported the occurrence of a translocation derivative in a haploid × diploid cross. Haensel (1960) obtained reciprocal translocations upon gamma irradiation of "colchicin reactive" grain sorghum variety Experimental 3. Huang et al. (1963) studied three such reciprocal translocation stocks, T165, T231, and T396, during meiosis. They recorded approximately 50 % viable pollens, which indicated occurrence of duplications and deficiencies. T396 involved the nucleolar chromosome and a common chromosome pair was found to be involved in T165 and T231. Lessman (1965) used two of these translocation stocks, T231 and T396, for the first time towards gene mapping. He could associate seed color with T396 and midrib color with T231. He concluded that the gene for midrib color is located either distal to the centromere near the breakpoint of one of the interchanged segments, or on one of the opposite arms of the chromosomes involving the centromere. Papathanasiou and Lessman (1969) proposed male sterile CK60 as a standard for chromosome analyses in sorghum and further associated midrib color (*Dd*) and seed coat color (*Yy*) to T231 and T396, respectively. Schertz (1970a, b) developed a set of 11 homozygous translocation stocks through gamma irradiation of cv. Combine 7078. These 11 stocks represent at least two translocations involving each of the 10 chromosomes. Using these translocation stocks Hanna and Schertz (1971) identified all 10 trisomes and associated them with their respective phenotypes (discussed in Sect. 4.2.1). Reciprocal translocations in wild and cultivated sorghum accessions have been reported by Morakinya and Olorode (1988). They observed a ring of up to nine chromosomes with the tenth sticking out of the ring at each pole in a cross, TH2 × IB12. This suggests the occurrence of terminal reciprocal translocations involving all the chromosomes except one in which it is restricted to only one arm. The study suggested the possible origin of sorghum and its wild relatives through segmental allotetraploidy.

5 *B*-Chromosomes

B-chromosomes are extra chromosomes that are dispensable for the cell, either in certain cells within the organism or in certain individuals within the population. The first report on occurrence of the *B*-chromosome in sorghum was made by Janaki-Ammal (1939, 1940). Among 100 plants of wild diploid grass, *S. purpureo-sericeum*, the author reported 40 extra chromosomes, which varied from one to six in number. They found that such extra chromosomes were confined to the shoot system alone and were never in the roots. Garber (1944) observed *B*-chromosomes in 38 % of the plants they studied.

Darlington and Thomas (1941) studied the mechanism of the loss of *B*-chromosomes (subsequently referred to as *B*s) in roots in the same material of Janaki-Ammal (1940). They recorded three types of *B*s. The first types are of similar

length to those of *A*-chromosomes, the second were shorter than the *A*-chromosomes, and the third were very long isochromosomes. They found that the young roots lose *B*s during seed development itself, whereas among shoot cells there is rare loss of *B*s. Furthermore, in growing inflorescence the *B*s are eliminated from those floral parts not involved in germ cell production. Pollen mother cells (PMC) are free from micronuclei and found to contain an invariable number of *B*s, yet other walls rarely contain mucronuclei. It is found that *B*s are always delayed in metaphase orientation and sometimes may fail to congregate on the plate altogether. *B*s may sometimes fail to disjoin, leading to the formation of the micronucleus. Sometimes misdivision of the centromere may lead to formation of telocentric chromosomes. All these anomalies often lead to the loss of *B*s in cell lineages. However, in PMCs *B*-chromosomes occur in regular numbers in all first methaphase cells. They congregate perfectly but pass on to one pole only without division. At second mitosis they divide without any delay. Primary division in the pollen grains is regular irrespective of whether *B*s are present or not, which occurs roughly one week after meiosis. During this division some structural changes may occur. However, it is observed that the vacuole, which generally separates the vegetative and generative nuclei, disappears and the nuclei remain close together. This is followed by certain abnormalities including nonpolarization, subequal division of cytoplasm, and rapid divisions of the vegetative nucleus. The supernumerary division of the vegetative nucleus is quite uncommon in other species. During these rapid supernumerary divisions *B*-chromosomes show somatic defects by lagging on the anaphase spindle. However, they do not get lost from the cell, and mostly pass undivided towards the generative pole. At this point the germ-track nucleus doubles its set of *B*s. The supernumerary divisions during stated polymitosis are believed to be stimulated by the presence of *B*-chromosomes. During sperm formation polymitosis leads to three developments: no sperm, two sperm, and two pairs of sperm of unequal size. Janaki-Ammal (1940) observed much increase in empty grains in *B*-chromosome "plus" plants, and this happens due to increased occurrence of polymitosis during middle pollen grain growth. Both Janaki-Ammal (1940) and Darlington and Thomas (1941) observed that *B*-dosage effect on polymitosis is geometric rather arithmetic. This is a typical disjunction of the *B*-chromosome, which is referred to as "sorghum type disjunction" (Schulz-Schaeffer 1980). Garber (1950) observed that the *B*-chromosome did not influence pollen fertility or seed set until four *B*s are present.

B-chromosomes are heterochromatic with variable behavior in different cells. A varied degree of condensation depending on the activity of the cell and the size of the nucleus may be behind this variable behavior. Variation in the sizes of nucleoli and persistence to anaphase are the characteristic features of polymitotic pollen grains.

Darlington and Thomas (1941) suggested that differential behavior of *B*-chromosomes in sorghum is a special adaptive behavior. They opined that polymitosis is a suicidal action (they also referred to it as "malignant mitosis") and there needs a powerful compensation for this to survive. They found that this is achieved by doubling of the *B*s in the second generative nucleus. Such doubling leads to an increase in the content of *B*s in every generation; opposite physiological selection reduces their frequency to an equal extent. Bosemark (1957) summarized that *B*-chromosomes in *S. purpureo-sericeum* are of various size classes, heterochromatic, observed in anthers and never in root tissues, do not pair with ordinary chromosomes but among themselves and show nondisjunction on the male side. Basic microscopic organizational differences between the centromeres of *A*- and *B*-chromosomes have been observed (Reddy 1958). This structural difference is considered to be the causal factor underlying the abnormal behavior of *B*s at mitosis.

Magoon et al. (1961) observed a different degree of sterility in *S. purpureo-sericeum*, which was dependent on the numbers of *B*-chromosomes. They observed one stunted plant with fewer tillers showing 95–98 % sterility.

Cytologically they found it to contain 10 A + 3 fairly large *B*-chromosomes. In this cytological stock they observed partial pairing at pachytene but pronounced desynapsis to follow during diakinesis, leading to less than 2 bivalents at metaphase I in the majority of cells. Fragmentation predominantly of B^{II} type was also observed in nearly 50 % of the cells in metaphase I. In addition, they reported other meiotic abnormalities as well. This further supports the observation of Janaki-Ammal (1940) and Darlington and Thomas (1941) that *B*-chromosome numbers have a geometric effect on pollen viability. The work of Reddy (1958) suggested that the marked heterochromatic nature of *B*s' rather inadequate centromeres likely causes nondisjunction of *B*s at the second pollen mitosis.

B-chromosomes have not been reported from any other species of Parasorghum or Eusorghum (Murty and Rao 1974). Wu reported the occurrence of *B*-chromosomes in three other species of sorghum: *S. nitidum* (Wu 1980), *S. purpureo-sericeum* (Wu 1984), and *S. stipoideum* (Wu 1992). The findings in these reports are in unison with earlier observations. At meiosis a more stable and regular fashion of behavior by *B*-bivalents was reported than the *B*-univalents, particularly in male mitosis. These reports suggested mosaicism of *B*-chromosomes in microsporocytes and tapetal cells, and total elimination from stems and leaves.

6 Molecular Cytogenetic Maps and Genome Architecture

6.1 Molecular Cytogenetic Map

Advances in molecular tools have aided in understanding the genome of plant species more precisely, often making conventional cytological tools obsolete. Draye et al. (2001) discussed a technique for generating synaptonemal complex (SC) spreads from sorghum cv. BTx623, in which all 10 bivalents could be differentiated based on relative lengths and arm ratio. They observed distinct kinetochores in some SC sets, which are otherwise not common in sorghum. SC sets are six to seven times longer than mitotic metaphase chromosome sets. The longest SC is often found to be associated with remnants of nucleolus. It was further observed that the sixth longest SC often was closely associated with an amorphous structure of unknown identity. They suggested the possibility of use of linkage group specific bacterial artificial chromosomes (BAC) in fluorescence in situ hybridization (FISH)-based "chromosome paint," which is also referred to as "cytomolecular mapping." Efforts towards construction of a robust physical map of sorghum were initiated towards the end of the last millennium. The BAC library of cultivated sorghum and wild species *S. propinquum* was created (Woo et al. 1994; Lin et al. 1999; Klein et al. 2000).

Perhaps the first deployment of FISH in sorghum genome analysis was made by Gomez et al. (1997) who showed that a sequence complementary to maize *sh2* cDNA is located at one end of a midsize metacentric sorghum chromosome. For this purpose they used a marker-selected 205-kb sorghum BAC clone and a sorghum plant containing an extra copy of one arm of the sorghum chromosome arbitrarily designated with the letter D. The results suggested an homology between one arm of chromosome D in sorghum and the long arm of chromosome 3 in maize. Zwick et al. (1998) analyzed the liguleless (*lg-1*) linkage group in sorghum and compared it with rice and maize taking advantage of the available sorghum BAC library of Woo et al. (1994). Using six liguleless-associated rice RLFP markers they selected 16 homologous sorghum BACs to map an *lg-1* linkage group to chromosome I physically. This was confirmed using sorghum cytogenetic stock, trisomic for chromosome I. Using FISH Sang and Liang (2000) reported that 18S-5.8S-26S rDNA sequences were located at two sites on the chromosomes in *S. bicolor* and *S. versicolor* ($2n = 10$), but at

four sites on the chromosomes of *S. halepense* ($2n = 40$) and the tetraploid *S. versicolor* ($2n = 20$). The rDNA sequence is found on the sorghum chromosome 1. With this study they excluded *S. versicolor* as the possible progenitor of *S. bicolor*.

Multiple molecular cytogenetic probes using BAC clones containing molecular markers mapped across sorghum linkage groups were developed by Kim et al. (2002). Using 17 such BAC clones they could successfully identify all 10 chromosomes. Islam-Faridi et al. (2002) further using 19 BAC clones harboring markers for linkage group (LG) 1 could physically map different markers to the short and long arm of chromosome 1. Following a similar strategy Kim et al. (2005b) painted LG-02 and LG-08 using 21 and 19 BAC clones, respectively. They successfully localized *Rf1* gene to the ~0.4 Mbp euchromatic region of LG-08. FISH painting of all 10 SB chromosomes was completed by Kim et al. (2005a). They found that euchromatic DNA spans ~50 % of the sorghum genome, with the maximum (~60 %) in chromosome 1 (SB-01) and the minimum in chromosome 7 (SB-07). Heterochromatic regions (~411 Mbp) are characterized by a ~34-fold lower rate of recombination and ~3-fold lower gene density. Their study also revealed macrocolinearity between sorghum and rice chromosomes even though the sorghum genome is ~2-fold bigger than that of rice.

6.2 Integrated Physical and Genetic Map

Towards development of an integrated physical and genetic map in sorghum, Klein et al. (2000) adopted a strategy to use arbitrary primer PCR-based amplified fragment length polymorphism (AFLP) fingerprinting of complex DNA populations resulting from pooling of low-coverage BAC libraries. The robustness of physical maps was further improved by merging probe-to-BAC hybridization data with DNA fingerprint data and using the restriction fragment matching method using pools of BAC DNA (Lin et al. 2000). This aided in resolving the chromosomal origin of BAC clones (Draye et al. 2001). Bowers et al. (2005) integrated genetic markers, BAC fingerprints, and BAC hybridization data to develop an integrated physical map of sorghum. It largely represented a genomic component containing 80 % of the single-copy genes. It showed conserved microsynteny with rice. Combining this integrated physical map, detailed genetic map (Chittenden et al. 1994; Bowers et al. 2003), and a whole-genome shotgun sequence (Gardner et al. 1981) approach, Paterson et al. (2009) released the genome sequence of sorghum cv BTx623. It was found that ~75 % of DNA in the sorghum genome was mostly heterochromatic. Euchromatic regions that represent gene-rich regions are nearly 252 Mb in size. One third of the sorghum genome is recombination rich and the rest is assumed to be recombination poor.

6.3 Molecular Architecture of the Centromere

The centromere plays the most important role in the functioning of chromosomes. Centromeres remain highly heterochromatic throughout the cell cycle and are found to possess very intriguing features. The presence of a repetitive sequence is one of the most characteristic features of the centromere across species (Melters et al. 2013). Jiang et al. (1996a, b) isolated a 745-bp repetitive DNA clone, *pSau3A9* from the sorghum BAC clone 13I16 (renamed from 52A4). Through FISH they demonstrated that this element is located in the centromeric regions of all sorghum chromosomes. Such an element could be obtained from other cereal species, including rice, maize, wheat, barley, rye, and oats. The *pSau3A9* family is associated with the centromere function of cereal chromosomes, and its absence in dicot suggests a faster divergence

of centromere-related sequences compared to the telomere-related sequences in plants. Miller et al. (1998) isolated another 823-bp repetitive DNA element from the sorghum BAC clone, 13I16, which was named *pSau3A10*. Sequence analysis showed that *pSau3A10* consists of six copies of an approximately 137-bp monomer. This six monomers are organized into three dimers. They further found that the monomers within the dimers shared 62–72 % homology and the dimers were 79–82 % homologous with each other. Long stretches of *Sau3A10* sequences are interrupted by other centromeric DNA elements. The *Sau3A10* sequence is one of the most abundant families located in sorghum centromeres. It is conserved in closely related sorghum species but not in other grass species, including rice, sugarcane, bamboo, rye, and wheat. The study suggested that the *Sau3A10* family is probably an important part of sorghum centromeres.

Gomez et al. (1998) discovered a 45-kb sorghum BAC (22B2) that differentially hybridizes to centromere regions of 10 of the 20 chromosomes of sorghum. Furthermore, hybridization of this BAC to trisomics for chromosomes E, H, and I displayed 11 signals, which provided strong evidence for the tetraploid origin of sorghum and existence of two subgenomes of five chromosomes each in the *S. bicolor*. A subclone, *pCEN38*, which is a 1047-bp insert was soon isolated from the reported BAC 22B2 (Zwick et al. 2000). This sequence produced the strongest signal near the centromeres of half of the chromosome sets as reported by Gomez et al. (1998). *pCEN38* showed narrow taxonomic distribution across 21 crop plants tested. DNA sequence analysis revealed that the *pCEN38* fragment contained three tandem dimer (280 bp) repeats of the same sequence family found in sorghum clone *pSau3A10*. Furthermore, each dimer consisted of two divergent monomers (140 bp). Sequence homology between the *pCEN38* monomers and the *SCEN* 140-bp tandem repeat family of sugarcane was striking. With these findings Zwick et al. (2000) suggested that sugarcane and sorghum share at least one ancestor harboring elements similar to *pCEN38* and *SCEN* and that each species had an ancestor in which the repetitive element was weakly present or lacking. In a recent study of candidate centromere tandem repeat sequence comparison of 282 animal and plant species did not display readily apparent conserved characteristics (Melters et al. 2013). This study showed that the closely related *Sorghum* and *Miscanthus* species have similar 137-bp repeats, but interestingly, no sequence similarity was found between the closely related *Zea* and *Sorghum* species or between *Oryza* species and *Brachypodium*, *Aegilops*, or *Hordeum*. Organization of the centromere and its significance in plant phylogeny is still an active area of research.

7 Wide Introgression Program

7.1 Wild Relatives of Sorghum: A Treasure-Trove of Valuable Traits

Wild sorghum species have greater adaptability due to continuous exposure to harsh climates, and have resistance to many biotic and abiotic stresses that affect sorghum grain production. Introgression of genes from undomesticated *Sorghum* species into cultivated sorghum is the first step towards accessing these unique unexploited genes for both biotic and abiotic stresses and agronomic traits. However, genes from wild sorghums are underutilized in crop improvement programs. Based on the extent of crossability of the wild species with a cultivated gene pool, wild species are classified into three gene pools: (i) cross-compatible wild species that produce fertile F_1 plants categorized into the *primary gene pool*; (ii) the *secondary gene pool* consisting of distant wild species that produce partially sterile hybrids; and (iii) the *tertiary gene pool* consisting of far distant wild species that have

difficulty producing F_1 hybrids (Harlan and de Wet 1972). The primary gene pool contains all three subspecies of *S. bicolor*: subsp. *arundicum*, *bicolor*, and *drumondii* (Cox 1983; de Wet et al. 1976). The two species in *Eusorghum*, *S. propinquum* and *S. halepense*, constitute the secondary gene pool. *Sorghum bicolor* and *S. propinquum* crosses are easily made, in which the meiosis is normal and progeny are fertile. However, there has been negligible use of this germplasm in applied sorghum improvement (Wooten 2001). Sorghum and *S. halepense* hybrids are possible with difficulty. Major efforts to utilize *S. halepense* are directed towards developing perennial grain crops (Piper and Kulakow 1994; Cox et al. 2002; Dweikat 2005). The tertiary gene pool contains the remaining 17 species within the four other sections. Most of the desirable traits for cultivated sorghum are contained in the tertiary gene pool (Harlan 1965), but this gene pool has remained more or less inaccessible as successful hybrids could not be recovered despite numerous efforts (Kuhlman et al. 2010). Regarding genetic improvements of sorghum, most common genetic variations from within the primary gene pool have been used.

As early as 1967, Magoon et al. observed irregular chromosome pairing in the two interspecific hybrids between the nonrhizomatous subsect, Arundinacea, the *S. propinquum* and rhizomatous subsect, Halepensia species, based on which they suggested alienating *S. propinquum* from the subsect Arundnacea. Other taxa of sorghum were crossable with cultivated types leading to formation of different races. Introgression of genes from *arundinaceum* into the early cultivated types produced the *guinea* phenotype and permitted extension of grain sorghum cultivation into forest areas (de Wet et al. 1976). Race *virgatum* crosses with *durra* sorghums; race *verticilliflorum* is widespread and crosses with cultivated types; and race *aethiopicum* crosses with cultivated races *caudatum* and *durra*. *S. halepense* or Johnsongrass, is one among 10 worst weeds of the world but it has tremendous vigor and adaptation. Several desirable traits from Johnsongrass, including resistance to greenbug and chinch bug, and adaptability to cold temperatures, can be transferred to the hybrid progeny.

Sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* Weston and Uppal (Shaw) is a serious disease of sorghum. Out of 16,000 world sorghum collections screened at ICRISAT only about 130 accessions were resistant against the SDM pathogen (Pande et al. 1997). Kamala et al. (2002) reported 36 potential sources of resistance against this fungus from wild species of sorghum. Members of the tertiary genepool, representing Chaeto-, Hetero-, Stipo-sorghum, and the Australian Parasorghum, are reported to be immune to the disease. Although in the study they found *S. versicolor*, *S. purpureo-sericeum*, and *S. nitidum* to be highly resistant, these accessions showed susceptibility in other reports (Bonde and Freytag 1979; Bonman et al. 1983). It has been found that the majority of wild Eusorghum are highly susceptible to SDM with the exception of two accessions (IS 18821 and IS 18882) and a weedy accession of *S. halepense* (IS 33712).

Striga is an important parasitic weed of sorghum, with much economic significance in Africa. *S. versicolor* was reported to be highly resistant to *striga* (Deodikar 1951). Rich et al. (2004), while screening 55 wild accessions and 20 sorghum cultivars, reported the presence of various mechanisms towards *Striga* resistance among wild species. These include low germination stimulant production, germination inhibition, and low haustorial initiation activity among others. PQ-434 and IS18803 and others were found to be potential sources of *Striga* resistance. Wild species harbor sources of resistance against insects as well. Kamala et al. (2009) identified 32 accessions from Para-, Stipo- and Hetero-sorghum with near immune response to shoot-fly (*Atherigona soccata* Rond.) under field conditions. They further identified one accession each from Heterosorghum (*S. laxiflorum*) and Chaetosorghum (*S. macrospermum*) with very low shoot-fly damage. Wild species, *S. versicolor* and *S. purpureo-sericeum,* were reported to be immune to shoot-fly infestation in

India (Bapat and Mote 1983). Sources of resistance against spotted stem borer (*Chilo partellus*) were also identified in wild species of Heterosorghum, Parasorghum, and Stiposorghum (Kamala et al. 2012). The stem borers were reported to develop on Sudan grass (Khan et al. 2000) and *S. arundinaceum* (Muyekho et al. 2005). Sources of resistance against sorghum midge (*Stenodiplosis sorghicola*) have been reported from Australian sorghum species (Harris 1976; Sharma and Franzmann 2001). *S. macrospermum* ($2n = 40$), the only member of the section Chaetosorghum, does not possess any agronomically desirable traits, but was reported to have significant resistance against sorghum midge (Franzmann and Hardy 1996; Sharma and Franzmann 2001). It is also resistant to sorghum downy mildew (Kamala et al. 2002) and has high tolerance to shoot-fly (Sharma et al. 2005). Resistance against green bug [*Schizaphis graminum* (Rondani)] biotype C and E is found in the race *virgatum* of *S. bicolor* ssp. *verticilliflorum* and in *S. halepense*, respectively (Duncan et al. 1991). Johnson- and sudangrass possess allelopathic properties by reducing the growth of weeds (Kamala et al. 2015). However, this is likely to affect growth of subsequent plants as well (Weston et al. 2013).

Australian native *Sorghum* species are reported to possess good grain starch and nutrition properties (Dillon et al. 2007b). Increased protein in the starchy endosperm of the wild species has been reported that influences the digestibility of sorghum.

7.2 Utilization of Wild Species in Sorghum Improvement and Application of *Iap* Allele

Wild species of sorghum are rich sources for various traits, however, there has been only limited exploitation of wild species in secondary and tertiary gene pools due to cross incompatibility (Rao et al. 2003). Using three-generation backcross (BC_3) lines and hybrids with *S. propinquum* Wooten (2001) attempted to improve cultivated grain sorghum with limited success. Dweikat (2005) succeeded in obtaining an interspecific hybrid with $2n = 20$ chromosomes between a nuclear male sterile sorghum and *S. halepense*. The hybrid plant was fertile and segregated normally in the F_2 generation. Hadley and Mahan (1956) derived F_1 progeny from kafir Johnsongrass, *S. halepense*, which were triploids. However, backcross progeny resulted in 20 chromosome plants with large morphological variation.

Huelgas et al. (1996) pollinated *S. bicolor* with four wild species from the tertiary gene pool —*S. macrospermum*, *S. timorense, S. matarankense*. and *S. stipoideum*—without success. Hybridization with an Australian species, *S. macrospermum* and *S. bicolor*, demonstrated partial compatibility among themselves (Kuhlman 2007). Hodnett et al. (2005) determined that pollen–pistil incompatibilities are the main cause of reproductive isolation between sorghum and the tertiary gene pool. Incompatible reaction leading to no pollen tube growth of wild species in the stigma and style leads to unsuccessful fertilization (Dhillon et al. 2007a, b).

Laurie and Bennett (1989) screened *S. bicolor* accessions using maize pollens. They discovered a sorghum accession (Nr481) from China in which the maize pollen germinated and pollen tubes grew into the styles of some sorghum plants. It was found that a recessive allele, *iap* (*inhibitor of alien pollen*), when present in the pistillate sorghum plant in the homozygous state, allows the maize pollen tubes to grow through the sorghum pistils, whereas the dominant allele *Iap* did not. The authors demonstrated that the recessive *iap* allele circumvents pollen–pistil incompatibilities and permits hybrids to be made between *S. bicolor* and species of the tertiary gene pool. Using this allele, hybrids were obtained between *S. bicolor* and *S. macrospermum* (Price et al. 2005a). The hybrids were phenotypically intermediate between the parents. These hybrids revealed moderate levels of allosyndetic recombination (2.6 II per PMC), indicating that introgression through genetic recombination is possible. The interspecific hybrids obtained by Price et al. (2005a) were derived by crossing CMS sorghum lines with

pollens from *S. macrospermum*. The efficiency of hybrid production was improved dramatically with the use of a sorghum genotype homozygous for the *iap* allele. Using a similar strategy Price et al. (2006) recovered hybrids between sorghum and *S. macrospermum*, *S. nitidum*, and *S. angustum*, though only hybrids with *S. macrospermum* survived till maturity. Promising sorghum lines with fodder attributes were developed through interspecific hybridization between cultivated sorghum CO 27 ($2n$ = 20) and *S. halepense* ($2n$ = 40) (Raveendran et al. 2000).

Discovery of the *iap* mutant and its transfer in homozygous condition to an improved parental line has opened up the introduction of variation from incompatible sources into cultivated sorghum. Identification of the *iap* allele in germplasm and transfer of the allele to a cultivated background was carried out by Mullet et al. (2010). Kuhlman and Rooney (2011) transferred the *iap* allele and *ms3* into an agronomically superior germplasm line, Tx3361. This new line was released by the Texas Agrilife Research sorghum breeding program in January 2010 (Reg. No. GP-661, PI 659454). Bartek (2010) and Bartek et al. (2012) recorded high levels of pollen tube growth into the ovaries of this line even when pollens from *Pennisetum ciliare* and four accessions of maize were used. This germplasm is unique because it lacks a key factor that represses alien pollen growth on the stigma. This line is restricted for use in breeding programs through patents (Publication No. EP2312935 A1). Lower humidity was reported to maximize maize pollen adhesion and germination on the stigmas of Tx3361 (Gill et al. 2014). At 45 % humidity multiple maize pollen tubes were observed in the sorghum style and ovary. Thus, intergeneric crosses with *iap* sorghum should be performed at low humidity levels. Using this genetic stock as a female parent, interspecific crosses with wild species of sorghum and intergeneric crosses with sugarcane were obtained (Hodnett et al. 2005, 2010; Price et al. 2006; Kuhlman et al. 2008, 2010). The intergeneric hybrid between sorghum and sugarcane was called Sorcane. Introgression of wild genes from an Australian species *S. macrospermum* was successful after repeated backcrosses of the progeny (Kuhlman et al. 2010). In BC_2F_1 relatively stable lines with alien addition and substitution were obtained. Interspecific hybrids between cultivated sorghum (*iap/iap*) and three *S. halepense* accessions were successfully made by Whitmire (2011). The *Iap* gene is localized to a 48-kb region on the short arm of chromosome 2 (Gill et al. 2014). This has opened up the possibility of transfer of this allele to other germplasm.

7.3 Sorghum × Sugarcane Crosses

Crosses between sorghum and sugarcane were taken up to introduce resistance to shoot-fly in sorghum. Intergeneric hybrids between the sorghum male sterile line, ICSA56, and *Saccharum officinarum* (IJ76-316) were obtained and the hybridity was demonstrated through cytological evidence (Nair 1999). Four out of five hybrid seedlings obtained from 3670 sorghum florets could survive. The number of hybrids obtained was higher when sugarcane was used as the female parent and lower when sorghum was the female parent. These hybrids more resembled *S. officinarum* in gross morphology with a few sorghum characters such as soft, narrow, and drooping leaves. The somatic chromosome number of the hybrids ranged from 62 to 66. Sorghum × sugarcane hybrids in general lacked vigor and were slow in growth and establishment. Though the hybrids were phenotypically close to the sugarcane parent, random amplified polymorphic DNA (RAPD) markers could precisely identify the true hybridity (Nair et al. 2006). These hybrids did not set seeds at Coimbatore, India (where sugarcane sets seed) or at Hyderabad, India. At the ICAR-Indian Institute of Millets Research, Hyderabad, India, these are maintained vegetatively. They flower during the winter season in November and December. The panicles resemble sugarcane. Pollen fertility in these florets was 10–15 %.

8 Male and Female Sterility

8.1 Male Sterility

Cytoplasmic-genic male sterility, a potential system for hybrid seed production, results from the interaction between the cytoplasm and nuclear genetic factors. Commercial hybrid development and cultivation in sorghum became possible with the discovery of cytoplasmic-nuclear male sterility (CMS) designated as A1 (milo) developed from the progeny of a cross between cultivars, dwarf yellow sooner milo (female) and Texas blackhull kafir (male; Stephens and Holland 1954). From then onwards, CMS-based hybrids have been developed and commercialized worldwide. However, these are principally A1 CMS system-based (Reddy et al. 2007) with infrequent establishment of A2-based hybrids (Qing Shan et al. 2000). Subsequently several non-milo CMS systems, namely A2, A3, A4, IndianA4 (A4 M, A4VZM, A4G), A5, A6, 9E, and KS were reported in sorghum (Webster and Singh 1964; Ross and Hackerott 1972; Schertz and Ritchey 1978; Quinby 1981; Worstell et al. 1984; Schertz 1994).

Several genes have been reported for male sterility in sorghum resulting in failure of pollen production or pollen abortion. Three independent genes *ms1*, *ms2*, and *ms3* were identified and described by Ayyangar and Ponniaya (1937), Stephens (1937), and Webster (1965). The pollens are nonfunctional but anthers are normal in these male sterile plants. Ayyangar (1942) reported male sterility with the absence of pollen. A male sterility source with the absence of anthers was reported by Karper and Stephens (1936). Barabas (1962) in his study used mutagenic treatment to induce male sterility and the resultant plant showed very small anthers without pollen inside. Singh and Hadley (1961) attempted to study the reason for pollen abortion in sorghum. They revealed that the meiosis towards development of anthers is normal in both fertile and sterile plants. However, the post-meiotic abnormal tapetum behavior in the MS plant caused pollen abortion. The tapetum of sterile plants was thicker especially at later stages and varied greatly from the fertile plants. Damon (1961) and Warmke and Overman (1972a, b) attributed this to the destruction and regeneration of the callose walls of the microsporocytes in cytoplasmic male sterile plants. Erichsen and Ross (1963) also observed similar irregularities in colchicine-induced male sterile mutants. They further described abnormal chromosome numbers in male sterile plants at microsporogenesis resulting from failure of breakdown of the cell walls of microsporocytes during meiosis prophase I, followed by fusion of their cytoplasm.

8.2 Female Sterility

Female sterility in sorghum was first reported by Casady et al. (1960) resulting in the development of rudimentary stigma, ovary, and style. The sterility was reported to be governed by two genes (*Fs1* and *Fs2*) with dominant complementary interaction. However, male fertility was not affected in the presence of these genes. The sterility was governed by the dosage of alleles. Heterozygous plants at two loci (*Fs1fs1Fs2fs2*) resulted in female sterility, whereas plants with three heterozygous loci resulted in dwarf plants with no heading. A third locus, *Fs3* was reported by Malm (1967) also governing female sterility.

8.3 Fertility Restoration

The inheritance of fertility restoration is dependent on the type of cytoplasm and nuclear genes. A single gene is responsible for fertility restoration in A1 cytoplasm but is controlled by two or more genes when the same nuclear genotype interacts with a different cytoplasm (Schertz

1994). Ashok Kumar et al. (2008) reviewed different genes restoring fertility in different CMS backgrounds. Progeny segregating with A1 cytoplasm in the F_2 generation showed that a single gene was responsible for fertility restoration in A1 cytoplasm (Murty 1986; Murthy and Gangadhar 1990). Other results on A1 cytoplasm have described that one or two genes (Qian 1990) or even up to three genes (Lonkar and Borikar 1994) are involved in controlling fertility restoration. Murthy (1986) described at least three genes controlling the fertility restoration of A_2 cytoplasm. Lonkar and Borikar (1994) described that two to four genes are required, but three genes were optimal for the fertility restoration in backcross generations in A2 cytoplasm. In another study, Murthy and Gangadhar (1990) concluded that two complementary genes are required for fertility restoration in A2 cytoplasm. ICRISAT studies showed that the recovery of fertile plants' frequency were less on A3 than A4, A2, and A1 indicating a higher number of genes are involved in governing fertility restoration on A3 than the other CMS systems (Reddy and Prasad Rao 1992).

9 Future Prospects

Undomesticated wild species of sorghum are found to be the repository for resistance to biotic and abiotic stresses and these are underexploited in sorghum breeding programs. Breaking the pre-fertilization barriers and introducing the wild traits through backcrossing can open a new avenue to increasing sorghum production. With the genome sequence available in sorghum, and a large number of genotypes being resequenced, it is high time that promising wild species with proven traits of importance are to be sequenced so that genes or alleles can be discovered and deployed in breeding programs. With the advent of molecular genomics, conventional cytogenetic tools might have become redundant but are needed to bring together the plethora of cytological information generated over this period with the genomic information being generated at a rapid pace.

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4 Sorghum Germplasm Resources Characterization and Trait Mapping

Hari D. Upadhyaya, Mani Vetriventhan and Santosh Deshpande

Abstract

Sorghum is the fifth most important cereal crop mostly grown for food, feed, fodder, and bioenergy purposes, and a staple for over 500 million resource-poor people in marginal environments. Globally, over 236,000 sorghum germplasm accessions have been conserved in genebanks, of which the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India and the Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, University of Georgia, USDA-ARS, together conserve about 32 % of the total global sorghum collections. Germplasm diversity representative subsets such as core and mini core collections and a genotyping-based reference set have been established in sorghum providing access to large diversity. The sorghum mini core collection established at the ICRISAT is being widely used for identification of sources for resistance to various biotic and abiotic stresses, and for agronomic and grain nutritional traits. Large genetic and genomic resources are available in sorghum, and resequencing of diverse germplasm resources including the mini core collection and wild and weedy relatives will provide researchers opportunities to relate sequence variations with phenotypic traits of interest and their utilization in sorghum improvement. Genomewide association mapping studies have

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identified genomic regions that are associated with important agronomic traits and resistance to biotic and abiotic stresses. High-throughput phenotyping platforms/technologies are required for precise phenotyping to attain greater genetic gains. The current status of germplasm, its characterization and utilization has been summarized in this chapter.

1 Introduction

Global food production has to increase by 70 % to feed over 9 billion people by 2050 in a background of uncertainties associated with climate change, shrinking land, and water available for agriculture (IAASTD 2009; http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf). Crop losses due to extremes in the environment have risen steadily over the past several decades and climate models predict an increased incidence of floods, droughts, and extreme temperatures (Dwivedi et al. 2013; Mickelbart et al. 2015).

Sorghum [*Sorghum bicolor* (L.) Moench.] is a multipurpose crop cultivated for food, feed, fodder, and bioenergy purposes, and a staple for over 500 million resource-poor people in marginal environments. The genus *Sorghum* Moench is subdivided into five subgenera or sections: *Chaetosorghum, Heterosorghum*, *Parasorghum*, *Stiposorghum*, and *Sorghum.* Section *Sorghum* has three species: two wild perennials, *S. halepense* (L.) Pers. ($2n = 40$), *S. propinquum* (Kunth) Hitchcock ($2n = 20$), and an annual *S. bicolor* (L.) Moench. ($2n = 20$). The *S. bicolor* contains three subspecies: (i) ssp. *bicolor* (all domesticated sorghum); (ii) ssp. *drummondii* (Steud.) de Wet comb. nov, derivatives of hybridization among cultivated sorghums and their closest wild relatives; and (iii) ssp. *verticilliflorum* (Steud.) (earlier subsp. *arundinaceum* (Desv.) de Wet et Harlan), the wild progenitors of cultivated sorghums. Cultivated sorghums (*S. bicolor* ssp. *bicolor*) can be classified into five races (*bicolor, guinea, caudatum, kafir*, and *durra*) and ten intermediate races (*guinea-bicolor, caudatum-bicolor, kafir-bicolor, durra-bicolor, guinea-caudatum, guinea-kafir, guinea-durra*, *kafir-caudatum*, *durra-caudatum*, and *kafir-durra*) based on mature spikelet/panicle morphology (Harlan and de Wet 1972), whereas wild sorghum *S. bicolor* ssp. *verticilliflorum* (Steud.) Piper includes four botanical races/ecotypes: *aethiopicum*, *virgatum*, *arundinaceum*, and *verticilliflorum* (de Wet 1978). Liu et al. (2014) suggested a new subgeneric classification of Sorghum Moench into three distinct subgenera, (i) subg. *Chaetosorghum* E.D Garber with two sections (sect. *Chaetosorghum* (E.D. Garber) Ivanjuk. & Doronina and sect. *Heterosorghum* (E.D. Garber) Ivanjuk. & Doronina), (ii) subg. *Parasorghum* (Snowden) E.D. Garber and (iii) subg. *Sorghum*. Subg. Sorghum includes nine species such as S. *almum* Parodi, *S. arundinaceum* (Desv.) Stapf, *S. bicolor* (L.) Moench, S. x *drummondii* (Nees ex Steud.) Millsp. & Chase, *S. halepense* (L.) Pers., *S. miliaceum* (Roxb.) Swoden, *S. propinquum* (Kunth) Hitchc., *S. sudanense* (Piper) Stapf, and *S. virgatum* (Hack.) Stapf).

Genetic loci that ensure productivity in challenging environments exist within the germplasm of crops; their wild and weedy relatives that are adapted to extreme environments necessitate utilization of germplasm resources more than ever before to develop varieties more tolerant to rapidly changing environmental conditions. Sorghum researchers have access to vast genetic and genomic resources. Globally, 236,617 germplasm accessions have been conserved in genebanks, providing the opportunity to access wide genetic variability. In addition, germplasm diversity representative subsets such as core (10 % of entire collection), mini core (10 % of core or 1 % of entire collection), and composite collections and a genotyping-based reference set are available to sorghum researchers to mine novel genetic variations for use in crop improvement. In recent years, advances in

DNA sequencing technology (next-generation sequencing, NGS) and the development of high-throughput genotyping have drastically reduced the time and cost requirements for sequencing a large number of genebank accessions. There have been substantial efforts in developing high-throughput phenotyping platforms for rapid and accurate assessment of phenotypic traits including tolerance to abiotic stresses. Here we summarized the current status of sorghum germplasm conserved globally, its characterization and utilization in sorghum improvement.

2 Sorghum Germplasm Resources —Global Status

A total of 236,617 sorghum accessions are being conserved in genebanks globally (98.3 % are cultivated and 1.7 % wild and weedy relatives; Table 1), of which the majority are conserved in Asia (39.18 %), the Americas (35.72 %), and Africa (16.40 %). Major genebanks conserving sorghum cultivated and wild germplasm resources are presented in Table 2. Four genebanks such as (i) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India (39,553 accessions, 16.7 % of total germplasm conserved globally), (ii) Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, University of Georgia, USDA-ARS (36,173 accessions, 15.3 % of total germplasm conserved globally), (iii) Institute of Crops Science, Chinese Academy of Agricultural Sciences (ICS-CAAS), China (18,263 accessions, 7.7 % of total germplasm conserved globally), and (iv) National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India (17,466 accessions, 7.4 % of total germplasm conserved globally) are the major genebanks that together conserve about 47 % of the total global sorghum germplasm. The ICRISAT genebank has 39,553 accessions originating from 93 countries and comprises 34,156 landraces, 4836 advanced breeding lines, 100 cultivars, and 461 wild and weedy relatives. The ICRISAT genebank collection is dominated by accessions belonging to *durra* (20.19 %), *caudatum* (19.60 %), *guinea* (12.66 %), *durra-caudatum* (12.17 %), and *guinea-caudatum* (10.80 %), and the remaining races/intermediate races represent <6.5 % of total collection.

3 Germplasm Diversity Representative Subsets

Large collections of sorghum germplasm accessions are available worldwide. However, utilization of these conserved germplasm in breeding is very limited. It is mainly due to the large size of collections with inadequate availability of reliable data on traits of economic interest, in addition to other factors such as linkage load of many undesirable genes and assumed risks, restricted access to the germplasm collections due to limited seed quantities (particularly of wild relatives and unadapted landraces), and regulations governing international exchange (Upadhyaya et al. 2014a). Thus, breeders tend to concentrate only on their working collection consisting mainly of improved materials and a few sources of different traits, and avoid use of wild and weedy relatives and unadapted landraces in their breeding program. Therefore, sampling the available diversity (at species level) for selecting a representative number of germplasms that captures diversity of a particular species is more rewarding for in-depth characterization and their enhanced utilization in breeding programs. Such germplasm diversity representative subsets serve as an entry point for mining novel variations and their utilization in crop improvement. These germplasm diversity subsets are also sufficiently diverse and could serve as a panel for association mapping, for detailed characterization of traits of economic importance to plant breeding programs and for assessment of allelic diversity in genes associated with traits of interest.

3.1 Core Collection

To facilitate germplasm maintenance, assessment, and utilization of germplasm resources in

Table 1 Global status of cultivated and wild sorghum germplasm accessions

Region		Wild	Cultivated	Total
Africa	Central Africa	7	327	334
	Eastern Africa	173	26,172	26,345
	Northern Africa		3341	3341
	Southern Africa	11	1756	1767
	Western Africa		7026	7026
	Total	191	38,622	38,813
America	Central America		12,729	12,729
	Northern America	200	43,560	43,760
	South America		28,035	28,035
	Total	200	84,324	84,524
Asia	Central Asia		1358	1358
	Eastern Asia	13	24,144	24,157
	Southeastern Asia		4117	4117
	Southern Asia	3179	59,441	62,620
	Western Asia	15	442	457
	Total	3207	89,502	92,709
Europe	Eastern Europe	7	6173	6180
	Northern Europe	35		35
	Southern Europe		827	827
	Western Europe	31	9011	9042
	Total	73	16,011	16,084
Oceania	Oceania	346	4141	4487
	Grand Total	4017	232,600	236,617

Source http://apps3.fao.org/wiews accessed on 28 July 2015

crop improvement programs, Frankel (1984) proposed the concept of “core collection”. A *core collection* is a limited set of accessions chosen to represent the genetic spectrum in the whole collection. Under the sampling theory of selectively neutral alleles, Brown (1989) suggested a core collection size of about 10 % of the entire collection. For establishing a core collection of the ICRISAT genebank sorghum collection, the entire ICRISAT sorghum collection was reduced to landraces from a latitude range of 40° N–40° S latitude, with complete passport information and characterization data (Grenier et al. 2001a). This reduced collection consisted of 22,473 landraces from 76 countries, which was 62 % of the entire collection conserved at the ICRISAT genebank, and was stratified into four clusters according to the photoperiod sensitivity (1160 accessions as photoperiod insensitive, 1062 as mildly photoperiod sensitive, 10,630 as photoperiod sensitive, and 9621 as highly photoperiod-sensitive landraces). By following the logarithmic sampling strategy, Grenier et al. (2001b) established a core collection that represented 10 % of the landraces collection (2247 accessions) from the 22,473 accessions conserved at the ICRISAT genebank. Core collections and other subsets such as mini core collections that have been formed in sorghum are presented in Table 3.

3.2 Mini core Collection

In some cases, core collections are too large in size for meaningful and precise evaluation for important economic traits. For example, the

Table 2 Cultivated and wild sorghum germplasm accessions conserved in major genebanks globally

Region	Country	Institute/organization	Wild	Cultivated	Total
Africa	Ethiopia	Institute of Biodiversity Conservation (IBC)		9772	9772
	Kenya	National Genebank of Kenya, Crop Plant Genetic Resources Centre—Muguga (KARI-NGBK)	92	5774	5866
	Zambia	SADC Plant Genetic Resources Centre (SRGB)	27	3692	3719
	Sudan	Plant Breeding Section Agricultural Research Corporation (ARC)		3145	3145
	Mali	Unité des Ressources Génétiques (URG)		2673	2673
	Uganda	Serere Agriculture and Animal Production Research Institute (SAARI)		2635	2635
	Mali	Station de Recherche Agronomique de Cinzana (S.R.A.C)		1836	1836
	Rwanda	Rwanda Agriculture Board (RAB)		1144	1144
America	United States	Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, University of Georgia, USDA-ARS	197	35,976	36,173
	United States	National Center for Genetic Resources Preservation (NCGRP)	2	7535	7537
	Brazil	Embrapa Milho e Sorgo (CNPMS)		7225	7225
	Mexico	Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP)		3990	3990
	Brazil	Embrapa Recursos Genéticos e Biotecnologia (CENARGEN)		3587	3587
	Argentina	Banco Base de Germoplasma, Instituto de Recursos Biológicos, Instituto Nacional de Tecnología Agropecuaria (BBC-INTA)		3249	3249
	Argentina	Banco Activo de Germoplasma de Manfredi (BGMANFREDI)		3200	3200
	Mexico	Programa de Recursos Genéticos, Centro de Investigaciones Forestales y Agropecuarias (CIFAP-MEX)		3000	3000
	Mexico	Estación de Iguala, Instituto Nacional de Investigaciones Agrícolas (INIA-Iguala)		2500	2500
	Venezuela	Fundación para la Investigación Agrícola (DANAC)		2068	2068
	Honduras	Escuela Agrícola Panamericana El Zamorano (EAP)		2000	2000
	Brazil	Empresa Pernambucana de Pesquisa Agropecuária (IPA)		1737	1737
	Argentina	Estación Experimental Agropecuaria Manfredi (EEA INTA)		1384	1384
	Colombia	Centro de Investigación La Selva, Corporación Colombiana de Investigación Agropecuaria (CORPOICA)		1290	1290
	Colombia	Corporación Colombiana de Investigación Agropecuaria Tibaitata, CORPOICA (ICA/REGION1)		1006	1006
Asia	India	International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)	461	39,092	39,553
	China	Institute of Crop Science, Chinese Academy of Agricultural Sciences (ICS-CAAS)		18,263	18,263
	India	National Bureau of Plant Genetic Resources (NBPGR), New Delhi (NBPGR)	2674	14,792	17,466
	Japan	Department of Genetic Resources I, National Institute of Agrobiological Sciences (NIAS)	13	5061	5074

(continued)

Table 2 (continued)

Region	Country	Institute/organization	Wild	Cultivated	Total
	India	All India Coordinated Sorghum Improvement Project-Rajendranagar (AICSIP-Rajendranagar)		2000	2000
	Pakistan	Plant Genetic Resources Program (PGRP)	16	1716	1732
	Thailand	Department of Agronomy, Faculty of Agriculture, University of Kasetsart (AD-KU)		1500	1500
	Thailand	National Corn and Sorghum Research Center, Kasetsart University		1277	1277
	Philippines	Crop Science Cluster-Institute of Plant Breeding, College of Agriculture, University of the Philippines, Los Baños College (CSC-IPB, UPLB-CA)		1190	1190
Europe	Russian Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry VIR (RUS001)		3963	3963
	France	Laboratoire des Ressources Génétiques et Amélioration des Plantes Tropicales, ORSTOM (ORSTOM-MONTP)	27	3562	3589
	France	Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)		2000	2000
	France	CIRAD, Département des Cultures Annuelles (IRCT-CIRAD)		1716	1716
Oceania	Australia	Australian Tropical Crops & Forages Genetic Resources Centre (ATCFA)	346	4141	4487

Source http://apps3.fao.org/wiews accessed on July 28, 2015

Table 3 Sorghum germplasm diversity representative subsets for enhancing genetic base of cultivars and trait discovery

Germplasm subsets	No. of Acc. Used	No. of Acc. in Subset	Remark	Reference
Core		3011	Randomly selected based on countries of origin representing 77 countries	Dahlberg et al. (2004)
Core	2553	352	Core from Sudan collection conserved at US National Plant Germplasm System	Dahlberg et al. (2004)
Core	33,100	3475	Seven morphological traits	Prasada Rao and Ramanth Rao (1995)
Core	22,473	2247	Photoperiod sensitivity grouping and logarithmic random sampling	Grenier et al. (2001b)
Mini core	2246	242	Using 21 morphoagronomic traits and passport information	Upadhyaya et al. (2009)
Composite collection	–	3384	This includes accessions from ICRISAT-India, CIRAD-France, and CAAS-China.	http://www.generationcp.org/issue-59-march-2012/32-research/sorghum/180-sorghum-products
Reference set	3367	383	Using 41 SSR markers	Billot et al. (2013)
Diversity research set	320	107	Using 38 SSR marker	Shehzad et al. (2009)

ICRISAT sorghum core collection consisted of >2000 accessions (Table 3), thus precise evaluation of such a large size of core collection under replicated multilocations/environments would be costly and ultimately not be precise, reducing its utility in a breeding program. Therefore, Upadhyaya and Ortiz (2001) postulated a "mini core" concept (10 % of core or 1 % of entire germplasm accessions of the species). Following this approach, Upadhyaya et al. (2009) developed a sorghum mini core of 242 accessions from the existing core collection of 2247 accessions (Grenier et al. 2001b).

3.3 Composite Collection and Genotyping-Based Reference Set

The Global Composite Germplasm Collection (GCGC) of sorghum, which consists of 3384 cultivated and wild accessions has been established (http://www.generationcp.org/issue-59-march-2012/32-research/sorghum/180-sorghum-products). This included collections from ICRISAT-India, CIRAD-France, and CAAS-China, comprising 280 breeding lines and elite cultivars from public sorghum breeding programs, 68 wild and weedy accessions, and over 3000 landraces from collections held by CIRAD or ICRISAT that were selected either from previously defined core and mini core collections (Grenier et al. 2001b; Upadhyaya et al. 2009), and for resistance to various biotic stresses and/or for variations in agronomic and quality traits. Furthermore, this sorghum GCGC was genotyped with 41 simple sequence repeat (SSR) markers and formed a genotyping-based reference set of 383 accessions, representing 332 landraces, 28 breeding lines, and 23 wild/weedy accessions, representing all 5 races and 10 intermediate races from different geographic origins (Billot et al. 2013). Shehzad et al. (2009) developed a sorghum diversity research set of 107 accessions based on SSR markers' diversity assessment of 320 accessions. These 320 accessions were selected out of >3500 accessions conserved at the National Institute of Agrobiological Science (NIAS), Japan, based on geographic distribution mostly from Asia and Africa.

4 Phenotypic Characterization and Evaluation

4.1 Agronomic Traits

Sorghum germplasm accessions conserved globally have a wide diversity for morphoagronomic and other important economic traits. A wide range of variation was reported for various agronomic traits in sorghum core and mini core collections conserved at the ICRISAT genebank (Table 4) provide an opportunity for identification of sources for various economically important traits. Seetharam (2011) evaluated a sorghum reference set under multilocations in India and identified 20 accessions each as trait-specific sources for early flowering and dwarf plant height, 100-seed weight, panicle weight, and grain yield. Mannai et al. (2011) evaluated a diversity research set of 107 sorghum accessions developed by Shehzad et al. (2009) and reported a wide variation for flowering time ranging from 56 to 133 days and categorized the accessions into three groups based on the number of days to flowering as early (<75 days), medium (>75–95 days), and late flowering (>95 days). Extensive evaluation of these accessions in different locations may be useful to assess the stability of the identified trait-specific accessions and their utilization in sorghum breeding.

4.2 Grain Nutritional Traits

The sorghum mini core collection has large variation for grain Fe (25.8–48.9 mg kg^{-1} seed) and Zn (13.5–42.6 mg kg^{-1} seed) contents and 11 accessions were identified with high Fe (IS# 16382, 23992, 28313, 28389, 28849, 20743, 21645, 21863, 28747, 30508, and 31681; Fe, 40.3–48.6 mg kg^{-1} seed), 14 accessions with high Zn (IS# 30460, 602, 17980, 19859, 28451, 30466, 30536, 5301, 8774, 4951, 25249, 24139, 24175, and 24218; Zn, 32.2–36.4 mg kg^{-1} seed) contents, and 9 accessions with both high Fe and Zn contents (IS# 1219, 1233, 30450, 30507, 1212, 27786, 30383, 31651, and 24503; Fe, 40.8–48.9 mg kg^{-1} seed; Zn, 32.8–42.6 mg

Table 4 Range and mean of quantitative traits in core and mini core collections of sorghum

Agronomic traits	Range		Mean	
	Core	Mini core	Core	Mini core
Days to 50 % flowering	47.79–117.62	50.36–117.36	82.2	82.6
Basal tillers	01–10	01–08	2.1	2.2
Plant height, cm	84.32–393.29	118.28–393.29	228.5	234.7
Panicle exsertion, cm	3.27–40.15	3.27–40.15	18.3	18.8
Panicle length, cm	8.98–39.01	9.72–37.51	21.1	21.5
Panicle width, cm	1.71–42.35	2.66–40.59	7.5	7.7
Yield per plant, g	15.26–29.48	16.94–29.48	21.3	21.4
Plot yield (kg ha^{-1})	751.24–2172.82	853.95–2172.82	1206.4	1221.5
100 seed weight, g	1.72–5.71	1.75–5.71	2.9	2.9
Seed size, mm	2.12–3.96	2.15–3.89	3	3

Source Upadhyaya et al. (2009)

kg^{-1} seed) over controls (Fe, 29.6–34.1 mg kg^{-1} seed; Zn, 23.9–25.7 mg kg^{-1} seed; Upadhyaya et al. 2016a). Six (IS# 1004, 23514, 23579, 23590, 28141, and 31706) and four (IS# 1004, 27034, 28141, and 31706) accessions, respectively, showed 8–39 % and 9–38 % greater Fe and Zn contents over control IS 33844 and produced grain yields similar to that of IS 33844 (Upadhyaya et al. 2016a).

4.3 Biotic Stress Resistance

4.3.1 Diseases

Diseases such as downy mildew, grain molds, anthracnose, leaf blight, and rust are the important and widespread diseases in tropical and subtropical regions of the world that can cause severe epidemics, resulting in considerable yield losses in sorghum. Germplasm sources for resistance to these diseases have been reported, for example, for downy mildew (Karunakar et al. 1994a; Prom et al. 2007, 2015; Sharma et al. 2010), grain molds (Bandyopadhyay et al. 1988; Sharma et al. 2010; Prom and Erpelding 2009; Cuevas et al. 2016; Thakur et al. 2008), anthracnose (Prom et al. 2007, 2012; Cuevas et al. 2016; Sharma et al. 2012; Erpelding 2012), leaf blight (Sharma et al. 2012; Singh and Singh 2014), and rust (Sharma et al. 2012; Cuevas et al. 2012). Wild and weedy relatives of sorghums as sources for downy mildew resistance have been reported (Karunakar et al. 1994b; Kamala et al. 2002). Karunakar et al. (1994b) identified 29 wild and weedy accessions of sorghum that were free from downy mildew. Kamala et al. (2002) reported 45 wild accessions comprising 15 species from four sections, *Parasorghum*, *Heterosorghum* (*S. laxiflorum* Bailey), *Chaetosorghum* (*S. macrospermum* Garber), and *Stiposorghum* (*S. angustum* S.T. Blake, *S. ecarinatum* Lazarides, *S. extans* Lazarides, *S. intrans* F. Muell. ex Benth., *S. interjectum* Lazarides, and *S. stipoideum* [Ewart & Jean White; C. Gardener & C.E. Hubb]) including all accessions from Australia to exhibit immunity to downy mildew. Cultivated types and wild races of section *Eusorghum* showed the greatest susceptibility, whereas accessions of *S. halapense* (L.) Pers. were comparatively less susceptible. Two wild accessions from the primary genepool, IS 18821 (*aethiopicum*) and IS 18882 (*arundinaceum*) were free from downy mildew and cross-compatible with cultivated sorghum. These may be used directly to develop downy mildew-resistant cultivars.

The sorghum mini core collection (Upadhyaya et al. 2009) has been extensively screened against several diseases and sources for disease resistance have been identified for use in breeding programs (Table 5).

Table 5 Sources for disease resistance identified in sorghum mini core collection accessions

Trait	Mini core accession	Reference
Downy mildew resistance	IS# 28747, 31714, 23992, 27697, 28449, and 30400	Sharma et al. (2010)
Grain mold resistance	IS# 602, 603, 608, 1233, 2413, 3121,12697, 12804, 20727, 20740, 20743, 20816, 30562, 31681, 2379, 2864, 12302, 13971, 17941, 19389, 23992, 26694, 29335, 21512, 21645, 12945, 22294, 995, 2426, 12706, 16151, 24453, 26701, 29326, 30383, 30533, 30536, 20956, 29314, 30092, 10969, 23590, 29187, 29269, 473, 29304, 1212, 13893, 29241, 29568	Sharma et al. (2010)
Anthracnose resistance	IS# 473, 5301, 6354, 7679, 10302, 16382, 19153, 20632, 20956, 23521, 23684, 24218, 24939	Sharma et al. (2012)
Leaf blight resistance	IS# 473, 2382, 7131, 9108, 9177, 9745, 12937, 12945, 14861, 19445, 20743, 21083, 23521, 23644, 23684, 24175, 24503, 24939, 24953, 26694, 26749, 28614, 29187, 29233, 29714, 31557, 33353,	Sharma et al. (2012)
Rust resistance	IS# 473, 23521, 23684, 24503, 26737, 33023	Sharma et al. (2012)
Multiple disease resistance	IS# 473 for grain mold, anthracnose, leaf blight and rust; IS# 23684 and 23521 for anthracnose, leaf blight and rust; IS 24939 for anthracnose and leaf blight; IS 23992 for grain molds and downy mildew; IS# 12945, 26694, 29187 for grain mold and leaf blight; IS 20956 for grain mold and anthracnose	Sharma et al. (2010, 2012)

4.3.2 Insect Pests

Sorghum is damaged by over 150 insect species, of which sorghum shoot-fly (*Atherigona soccata*), stem borers (*Chilo partellus*), aphids (*Melanaphis sacchari*), greenbug (*Schizaphis graminum*), sorghum midge (*Stenodiplosis sorghicola*), and head bugs (*Calocoris angustatus* and *Eurystylus oldi*) are the most important insect pests worldwide (Sharma et al. 2003). An extensive screening of sorghum germplasm accessions conserved at the ICRISAT genebank led to identified stable sources of resistance to key insect pests such as shoot-fly, stem borer, midge, and head bug (Sharma et al. 2003). Forty germplasm accessions have been identified as resistant to sorghum shoot-fly, of which IS# 1054, 1071, 2394, 5484, 18368, 2123, 2195, 4664, and 18551 have shown stable resistance to shoot-fly damage; 71 accessions identified as resistant to spotted stem borer, of which IS# 2205, 1044, 5470, 5604, 8320, and 1853 were stable across seasons and locations; 50 accessions identified as resistant to sorghum midge, of which DJ 6514, TAM 2566, AF 28, IS 10712, IS 8891, and IS 7005 were stable and diverse sources of resistance; and 35 accessions have been identified as resistant to head bugs, of which IS# 17610, 17618, 17645, 20740, and 20664 were highly resistant (Sharma et al. 2003). The sorghum mini core collection (Upadhyaya et al. 2009) has been evaluated extensively and sources for resistance to shoot-fly, stem borer, and aphid were identified (ICRISAT unpublished). Resistance responses of wild and weedy sorghum germplasm accessions to shoot-fly and spotted stem borer have been reported (Kamala et al. 2009, 2012).

4.4 Abiotic Stress Resistance

Sorghum, in general, has great adaptation potential to various abiotic stresses; however, genotypes/cultivars show large variability for adaptation to various abiotic stresses. For example, in the sorghum reference set, grain yield varied significantly between genotypes under drought-stressed (mean 20.6 g plant^{-1}, range 0.3–36.6 g plant^{-1}) and well-watered (mean 42.0 g plant^{-1}, range 2.1–82.8 g plant^{-1}) conditions; overall the mean grain yield decreased about ~50 % under the drought-stressed condition as compared to under the well-watered condition (Vadez et al. 2011). This

large genetic variation for drought adaptation traits offers great breeding opportunities. Therefore, identification of genetic loci from the diverse germplasm including wild and weedy relatives adapted to extreme environments that ensure productivity in challenging environments are prerequisite for developing varieties more tolerant to rapidly changing environmental conditions. Vadez et al. (2011) evaluated 149 accessions from the sorghum reference set using a lysimetric system under terminal drought stress and fully irrigated conditions. They found differential response of races and intermediate races to drought stress, for example, accessions from the race *durra* had the highest water extraction capacity, whereas *caudatum-bicolor* and *durra-caudatum* had poor water extraction ability; accessions from *durra*, *caudatum*, and *guinea-caudatum* recorded the highest transpiration efficiency (TE), whereas the *guinea* race had the lowest TE. Seetharam (2011) evaluated the sorghum reference set (384 accessions) under well-watered and drought-stressed conditions and identified drought-tolerant accessions based on drought-tolerance indices and SPAD chlorophyll meter readings (SCMR). The accessions IS 8882 (*Caudatum*, Uganda), IS 13845 (*Kafir*, South Africa), IS 22334 (*Kafir*, Botswana), and IS 29872 (*Kafir*, Zimbabwe) were found to have high drought-tolerance indices and high SCMR. The accessions identified as tolerant to drought are dominated by the race *caudatum* and by the intermediate race *guinea-caudatum*. Kapanigowda et al. (2013) identified genotypes such as PI 510898, IS 1212, and PI 533946 as high yielding under drought conditions with 57, 38, and 38 %, respectively, increase over the check BTx642. Reddy et al. (2008) reported 18 accessions (IS# 164, 237, 707, 1045, 1049, 1052, 1069, 1087, 1178, 1232, 1243, 1261, 1263, 1328, 1366, 1568, 19604, and 29789) tolerant to salinity. Fernandez et al. (2014) identified 8 accessions (PI# 76408, 90271, 408822, 408824, 408816, 550608, 563923, and 619672) for cold tolerance. Evaluation of sorghum mini core collection (Upadhyaya et al. 2009) under low temperature (at seedling stage) (Upadhyaya et al. 2016b) and post-flowering drought stress (Upadhyaya et al. 2017) conditions resulted in identification of accessions that has higher percentage of seedling vigor (IS# 1212, 14779, 15170, 22986, 7305, and 7310), and germinability (IS# 602, 1233, 7305, 10302, and 20956) under low temperature stress, and tolerance to post-flowering drought stress (IS# 14779, 23891, 31714, 4515, 5094, 9108, and 15466). These identified accessions for various abiotic stresses could be utilized in developing cultivars for adaptation to diverse climate conditions.

4.5 Bioenergy Traits

Sweet sorghum is an important food, feed, and biofuel crop worldwide. It can be grown under limited inputs (water and fertilizer) under diverse environmental conditions. Sweet sorghum accumulates fermentable sugars (10–20 %) in the stalk and thus has an advantage of producing grain for food and bioethanol from stalk juice without compromising food security (Reddy et al. 2005). The environments and their interaction with genotypes have a strong influence on the cultivar's adaption. Upadhyaya et al. (2014b) studied the response of a sorghum mini core germplasm collection for stalk sugar content (Brix %) under well-watered and drought-stress conditions. They reported that drought stress in comparison to the irrigated control significantly increased the mean Brix % in accessions that flowered <60–90 days after sowing, with percent increase ranging from 12.11 to 26.76 %. However, late-flowering accessions (flowering >90 days after sowing) did not show a significant difference for Brix %. Mini core accessions showed different responses for Brix % under drought stress. For example, the mean Brix % increased under drought in 169 accessions, decreased in one accession, and the remaining accessions were not affected. Upadhyaya et al. (2014b) identified sorghum mini core accessions such as IS# 13294, 13549, 23216, 23684, 24139, 24939, and 24953 with significantly greater mean Brix (14.0–15.2 %) as compared to the best control, IS 33844 (12.4 %).

However, these accessions had lower yields and lower 100-seed weight. In contrast, IS# 1004, 4698, 23891, and 28141 significantly out-yielded IS 33844 by 11.7–22.7 % and had almost the same Brix content (~13 %). Cuevas et al. (2014) identified two accessions (PI# 653616 and 455286) as superior for both Brix content (>10 %) and dry matter weight (112 g $plant^{-1}$) and nine accessions (PI# 653617, 144335, 155518, 648080, 643003, 648098, 648091, 155555, and 562267) for higher biofuel potential (Brix > 10 % and dry matter yield > 60 g $plant^{-1}$). These identified accessions could be utilized in a breeding program for developing dual-purpose sorghum cultivars.

5 Next-Generation Phenotyping

More accurate and precise phenotyping strategies are required to associate phenotypic variations with high-resolution sequence variations to achieve maximum genetic gains. Rapid developments are taking place in the field of nondestructive, image-based phenotyping that allow characterization of plant traits in high-throughput enabling researchers to develop crops with the ability to perform well under diverse environmental conditions (Topp et al. 2013; Araus and Cairns 2014; Honsdorf et al. 2014; Neilson et al. 2015; Walter et al. 2015). High-throughput phenotyping is being used in sorghum. For example, Neilson et al. (2015) investigated the growth and phenotypic response of sorghum under water-limited conditions and different levels of fertilizer using "The Plant Accelerator" at Adelaide, Australia. They showed that imaging sorghum using a high-throughput system can accurately identify and differentiate between growth and specific phenotypic traits. For example, diurnal leaf curling and leaf area index correlated with an improved tolerance to water stress. Color images revealed that leaf greenness correlated with foliar nitrogen and chlorophyll content, whereas near-infrared reflectance analysis proved to be a good predictor for water content, and leaf thickness correlated well with plant moisture content (Neilson et al. 2015). Recently, Batz et al. (2016) demonstrated imaging for high-throughput phenotyping in energy sorghum. At ICRISAT, a high-throughput phenotyping platform called the "LeasyScan" facility has been established to measure leaf area quicker so as to access the dynamics of leaf development and leaf conductance, traits that are the focus for plant drought adaptation in ICRISAT mandate crops including sorghum. It is based on a novel 3D scanning technique to capture leaf area development continuously, a scanner-to-plant concept to increase imaging throughput and analytical scales to combine gravimetric transpiration measurements (Vadez et al. 2015). The combination of the multifunctional phenotyping tools and genomewide sequencing provides deep insights into the genetic architecture of important traits as demonstrated in rice (Yang et al. 2014).

6 Next-Generation Sequencing and Diversity Assessment

Advances in DNA sequencing technologies have enabled rapid high-throughput genotyping and also reduced time and cost required. Next-generation sequencing technologies are being used for whole genome sequencing for a wide range of crop species, and they also support germplasm management and enhance utilization of germplasm in crop improvement programs (van Treuren and van Hintum 2014). NGS data could also be used to monitor the regeneration of accessions in order to ensure the maintenance of genetic integrity by comparing sequence data of samples before and after regeneration. When combined with precise phenotyping methods, NGS technologies provide a powerful and rapid tool for identifying the genetic basis of agriculturally important traits and for predicting the breeding value of individuals in a plant breeding population (Varshney et al. 2014).

Understanding genetic diversity in sorghum germplasm collections assists in mining novel alleles/genes associated with important traits and enhances the use of germplasm in breeding programs. Currently the sorghum research

community has access to numerous genomic resources, including DNA markers (SSR, DArT, SNPs; Mace et al. 2008, 2013b; Li et al. 2009; Nelson et al. 2011; Bouchet et al. 2012; Billot et al. 2012; Evans et al. 2013), the high-density genetic maps (Bowers et al. 2003; Mace et al. 2008; Kong et al. 2013), and the sequenced genomes (Paterson et al. 2009; Mace et al. 2013a). In addition, a Web-based large-scale genome variation database called SorGSD has been developed (Luo et al. 2016). It contains ~62.9 million single nucleotide polymorphisms (SNPs) identified from the resequencing data of 48 sorghum lines (landraces, improved breeding inbreds, and weedy and wild relatives) mapped to the reference genome of BTx623, which serves as a valuable resource for researchers to perform genetic and breeding studies.

Considerable numbers of sorghum germplasm accessions have been sequenced through the genotyping-by-sequencing approach (GBS) to investigate population structure and diversity (Morris et al. 2013; Wang et al. 2013b). These studies revealed that the phylogenetic relatedness and patterns of sorghum diversity are structured according to geographic regions and races within a region. For example, the ICRISAT's sorghum mini core collection (242 accessions) genotyped using a GBS approach (Wang et al. 2013b) indicated that accessions are structured along both geographic origin and sorghum races. Accessions of different races from southern Africa tended to be more similar to each other, as were those from East Asia. Race *caudatum* accessions from widespread geographical distributions were found to be clustered, which was the strongest example of population structured based on race. *Guineas* from West Africa and *durras* from India were clustered by race and origin. Race *bicolor* clustered among other races and formed only one clear *bicolor*-centric cluster. Similarly, Morris et al. (2013) characterized 971 accessions of worldwide sorghum collections including the mini core collection, that have adapted to diverse agroclimatic conditions using ~265,000 SNPs. They showed structured populations along both morphological type and geographic origin: the *kafir* types that predominate in southern Africa showed the strongest pattern of population subdivision as compared to other races; *durra* types found in semi-arid or warm desert climates of the Horn of Africa, Sahel, Arabian peninsula, and west central India, formed a distinct cluster that was further differentiated according to geographic origin; *bicolor* types were not notably clustered, except those from China which formed a distinct subgroup and showed genetic similarity to *durra* types, particularly those from Yemen; *caudatum* types, which are primarily found in tropical savanna climates of central Africa, are diverse and showed only modest clustering according to geographical distribution; and *guinea* types, which are widely distributed in tropical savanna climates and showed five distinct subgroups, four of which clustered according to their geographic origin (far west Africa, west Africa, eastern Africa, and India) (Morris et al. 2013). The phylogenetic relationship of five main sorghum races indicated that the race *bicolor* is the more primitive race, and showed a close phylogenetic relationship with wild types (Zhang et al. 2015). Population differentiation (i.e., fixation index (F_{ST}) between wild sorghums with those of five primary races) revealed that the race *bicolor* (F_{ST} = 0.04) had a closer genetic relationship with wild sorghums than did those of the other four primary races (F_{ST} between populations recorded are: 0.11 for *guinea*-wild, 0.20 for *durra*-wild, 0.33 for *kafir*-wild, 0.14 for caudatum-wild), with *guinea* and *caudatum* apparently representing early derivatives. Races *caudatum*, *durra*, and *kafir* showed clustering patterns that are substantially distinct from one another and showed a relatively high level of population differentiation (F_{ST} between populations: 0.26 for *durra-caudatum*, 0.46 for *durra-kafir*, 0.33 for *caudatum-kafir*) (Zhang et al. 2015). Cultivated sorghums harbor lower diversity as compared to wild and weedy relatives, indicating domestication of sorghum to be accompanied by a genetic bottleneck (Mutegi et al. 2011; Mace et al. 2013a). Mace et al. (2013a) resequenced 44 genotypes of sorghum including landraces, improved breeding inbreds and weedy and wild relatives, and observed strong racial structure. The study revealed a lower level of diversity in the improved inbreds as compared to both landraces

and wild and weedy genotypes. The proportion of wild-specific alleles was highest (34 %) as compared to improved inbred specific alleles (8 %) and landrace- specific alleles (18 %). These results provide critical evidence of lower diversity of improved lines and the rich diversity existing in wild and weedy lines as well as in landraces that could be used to diversify the cultivated genepool. The wild species belonging to the primary genepool, *S. propinquum* ($2n = 2x = 20$) is divergent from other sorghums with 22 % of *S. propinquum* reading unmapped to *S. bicolor* and remaining underutilized in sorghum improvement (Mace et al. 2013a).

7 Mining Crop Diversity and Trait Mapping

Advances in sequencing technologies have enabled large-scale genotyping of germplasm collections. The long history of recombination events captured in germplasm collections, when combined with dense marker coverage permit increased genetic resolution sometimes to a level that allows causative sequence variants to be identified. Researchers have used the association mapping approach in sorghum to dissect sequence variations associated with phenotypic traits of interest using diverse germplasm accessions, for example, plant architecture (Mantilla Perez et al. 2014), photoperiod sensitivity (flowering time; Bhosale et al. 2012), plant height and Brix % (Murray et al. 2009), grain yield under drought (Besufekad and Bantte 2013), and grain yield under phosphorus stress condition (Leiser et al. 2014). In addition, germplasm diversity representative subsets such as core and mini core collections are being used as association mapping panels. The detailed status of association mapping in sorghum is given in Chap. 7. Some significant findings are briefed here. The ICRISAT's sorghum mini core collection consisting of 242 accessions (Upadhyaya et al. 2009) has been extensively used as an association mapping panel to identify marker-trait associations for plant height and maturity (Wang et al. 2012; Upadhyaya et al. 2012b, 2013a), kernel weight and tiller number (Upadhyaya et al. 2012a), anthracnose resistance (Upadhyaya et al. 2013b), leaf rust and grain mold resistance (Upadhyaya et al. 2013c), germinability and seedling vigor under low temperature (Upadhyaya et al. 2016b), and saccharification yield (Wang et al. 2011, 2013a). Putative candidate genes have been identified using the sorghum mini core collection as an association mapping panel. Upadhyaya et al. (2013a) identified putative candidate genes including a sugar transporter (*SbSUC9*), an auxin response factor (*SbARF3*), an *FLC* and *FT* regulator (*SbMED12*) and a photoperiod response gene (*SbPPR1*) for maturity and peroxidase 53, and an auxin transporter (*SbLAX4*) for plant height. Further SNPs associated with anthracnose resistance and grain mold and rust resistance have also been reported using the minicore collection. Wang et al. (2011) identified two significant markers for saccharification yield that are close to *ß-glucanase* (*Bg*) and steroid binding protein (*Sbp*) genes. *Bg* is critical for cell wall assembly and degradation, but *Sbp* can suppress the expression of *Bg* as demonstrated in *Arabidopsis* (Yang et al. 2005). Also these markers are close to the genes encoding plant cell wall synthesis enzymes such as xyloglucan fucosyltransferase and UDP-D-glucose 4-epimerase (Wang et al. 2011). Further using a large number of SNPs and the mini core collection, Wang et al. (2013a) identified seven loci significantly associated with saccharification yield, and identified possible candidate genes, the most promising candidates being ß-tubulin that determines the orientation of cellulose microfibrils in plant secondary cell walls, and NST1, a master transcription factor controlling secondary cell wall biosynthesis in fibers. These candidate genes and markers identified for several economically important traits need to be validated and developed into molecular tools for genetic improvement of sorghum.

In addition, use of multiparent mapping populations such as nested association mapping (NAM), backcross-nested association mapping (BC-NAM), and multiparent advanced generation intercross (MAGIC) populations offer ways

to enhance mapping resolution of quantitative trait loci (QTLs). Morris (2015) reported a grain sorghum NAM developed using the US breeding line RTx430 as a common parent and 10 diverse founders that represent all major botanical races of sorghum, and the NAM population captured about 75 % of the global genetic diversity of sorghum in 2500 lines (10 recombinant inbred line families). Jordan et al. (2011) developed a BC-NAM and demonstrated it as an effective way to introduce new alleles from unadapted sorghum germplasm into elite breeding material. Mace et al. (2013b) used a BC-NAM population and diversity array technology (DArT) markers and identified 40 significant associations for flowering time, 24 of which were colocated with previously identified loci for flowering time in sorghum and 16 were novel.

8 Future Prospects

Sorghum is an important multipurpose crop, widely used for food, feed, and bioenergy purposes. The wide genetic variants conserved in genebanks provides a reservoir of genes for crop improvement. Sorghum wild and weedy relatives have potential genes for adaptation to biotic and abiotic stresses and have not been fully utilized. Also, representation of wild and weedy relatives of sorghum is very low in genebanks, therefore, systematic collection and conservation of wild and weedy relatives are essential to uncover the huge potential of these resources in sorghum improvement. The development and use of effective field-based high-throughput phenotyping platforms are required in sorghum in order to dissect the genetics of quantitative traits, particularly those related to yield and stress tolerance (e.g., yield potential as well as increased drought, heat tolerance, and nutrient efficiency, etc.). The sorghum research community has access to large genetic (germplasm with unique traits) and genomic (SSRs, SNPs, high-density genetic maps, genome sequence) resources, and many QTLs/candidate genes associated with agronomic traits are known in sorghum. Markers and candidate genes identified in sorghum need to be validated and developed into molecular tools for sorghum improvement. Resequencing of diverse germplasm resources includes the mini core collection; wild and weedy relatives provide researchers opportunities for related sequence variations with phenotypic traits of interest and their utilization in sorghum improvement. Multiparent mapping populations (MAGIC, NAM, and BC-NAM), TILLING, and Eco-TILLING approaches are being used to a limited extent, and need to be accelerated to allow fast genomewide identification of QTL for sorghum improvement.

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5 Sorghum Genome Mapping and Its Impact Generated Through Public and Private Efforts

Sylvester Elikana Anami, Hong Luo, Yan Xia and Hai-Chun Jing

Abstract

In 2009, the sequence and annotation of a sorghum whole genome was reported by a team of international collaborators, followed by resequencing and complete annotation of the sorghum transcriptome and methylome together with the identification of genomewide structural variations through national initiatives. The genome, structural variations, quantitative trait loci (QTLs), genes, and alternative splicing (AS) events encode useful agronomic information that needs to be decoded for sorghum improvement through genetic manipulations of key metabolic pathways. Here, we review the background history of the national and international sorghum genome initiatives, public and private partners involved, sorghum genome databases for robust computational methods for sequence analysis, and the impact of the genome information on sorghum improvement.

1 Introduction

Sorghum [*Sorghum bicolor* L. (Moench)] is an African grass with its intrinsic abilities to withstand limited water conditions and heat stress. These qualities of the crop have made it suitable to thrive in arid and semi-arid regions of the world. Sorghum has a relatively small genome size of 730 Mb (Paterson et al. 2009). Excellent genetic and germplasm resources are available in sorghum in various national and international programs (Dillon et al. 2007). It has a close evolutionary relationship with other important crop species including maize, rice, and sugarcane (Aitken et al. 2014; Jiao et al. 2014). All these make the sorghum genome an important resource for application in comparative genomics, which in turn facilitates discovery of novel agronomical alleles for crop improvement through molecular breeding. First domesticated in Ethiopia and Sudan about 8000 years ago (Wendorf et al. 1992), sorghum is the only globally important cereal of African origin. Currently sorghum is the fifth

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most important grain crop grown in the world based on tonnage, after maize, wheat, rice, and barley (www.fao.org). About 500 million poor people in sub-Saharan Africa depend on sorghum as their primary source of food, in addition to a substantial mass of the population from the Indian subcontinent.

Since the 1950s maize genetics has advanced substantially as compared to other crops in the Gramineae family. For instance, mobile DNA elements, *Dissociator* (*Ds*) and *Activator* (*Ac*) in the maize genome were described (McClintock 1950). Genetically mapped and cloned maize DNA fragments that could detect polymorphism became available (Coe Jr et al. 1988; Helentjaris et al. 1986; Burr et al. 1988), accelerating the generation of a maize genetic map (Coe Jr et al. 1988), estimation of evolutionary relationships between related species (Springer et al. 1989), and calculation of the genetic distance between various maize breeding stocks. At that point, it became evident that the maize genetic technology could be applied to the sorghum crop that is ideal for environments prone to drought and extreme heat.

2 Sorghum Genetic Maps

The construction of sorghum genetic maps began in earnest in the 1990s. DNA fragments characterized in maize were found to hybridize strongly to DNA from sorghum and other related crops including foxtail millet, Johnsongrass, and sugarcane, suggesting that polymorphic loci in maize and sorghum are conserved (Hulbert et al. 1990). In addition, maize restriction fragment length polymorphism (RFLP) probes were utilized to construct a sorghum linkage map and the probes were found to be similar to those of sorghum (Binelli et al. 1992; Whitkus et al. 1992; Berhan et al. 1993). This allowed the study of comparative genetics between related species and opened the possibility of examining horizontal gene transfer from sorghum to other species and vice versa. A high-density RFLP linkage map of sorghum spanning 1789 cMs consisting of 190 loci was constructed by Xu et al. (1994). The maps were grouped into 14 linkage groups and the markers were principally detected with sorghum low-copy-number nuclear DNA clones. This map was constructed in an F_2 mapping population composed of 50 plants derived from a cross of IS3620C and BTx623 inbred lines. The second high-density RFLP linkage map of sorghum consisted of 276 RFLP loci as detected by *Pst*I-digested *S. bicolor* genomic probes (Chittenden et al. 1994). The map had 10 linkage groups and it was built in a segregating 56 F_2 progeny of a cross between *S. bicolor* and *S. propinquum*. An F_2 population derived from a cross between *S. bicolor* ssp. *bicolor* (CK60) and *S. bicolor* ssp. *drummondii* (PI229828) was used to develop an RFLP genetic linkage map of sorghum for comparative mapping in maize. The map consisted of 201 loci distributed among 10 linkage groups covering a map distance of 1530 cm, with an average 8 cm between adjacent loci (Pereira et al. 1994). Ragab et al. (1994) used both sorghum and maize probes to construct an RFLP map based on an F_2 population from a cross between sorghum lines BSC 35 and BTX 631, improving on the limitation to the use of heterologous maize clones for sorghum map constructions. An interspecific sorghum F_2 population was used as a reference to reveal correspondence among quantitative trait loci (QTLs) affecting height and/or flowering in maize, rice, wheat, and barley populations (Lin et al. 1995). To facilitate the mapping of related complex polyploid species such as sugarcane, a sorghum composite linkage map was constructed using RFLP markers with two recombinant inbred line (RIL) populations of 110 and 91 individuals derived from two interspecific crosses with *S. bicolor* ssp. *bicolor* (IS2807 × 379 and IS2807 × 249; Dufour et al. 1997). Subudhi and Nguyen (2000) aligned five major RFLP maps of Boivin et al. (1999), Chittenden et al. (1994), Pereira et al. (1994), Ragab et al. (1994), and Xu et al. (1994) and integrated with 10 linkage groups using a RIL population revealing important QTLs, but the map lacked higher marker density for positional cloning of genes, genetic anchoring of bacterial artificial chromosome (BAC)-based physical maps, or assembly of a genomic shotgun sequence. To facilitate anchoring of BAC-based physical maps

of both *S. bicolor* and *S. propinquum*, rapid gene isolation by map-based cloning and provision of landmarks for eventual genomic sequence assembly, Bowers et al. (2003) integrated much of the aforementioned sorghum genetic linkage maps and constructed a high-density genetic recombination map of sequence-tagged sites (STS) for sorghum, fostering comparative genomics of sorghum with sugarcane, maize, rice, millet, buffelgrass, the Triticeae (wheat, barley, oat, rye), and *Arabidopsis*.

Random simplified polymorphic DNA (RAPD) markers have had limited utility in the construction of genetic maps in sorghum when compared to other markers such as RFLP. Nevertheless, Tuinstra et al. (1996) used a population of 98 RI lines derived from two genotypes with contrasting drought reactions to generate a sorghum linkage map using 150 RAPD markers that mapped to 17 linkage groups. In addition, Agrama et al. (2002) employed 75 RAPDs and Knoll et al. (2008) used 67 RAPDs markers to generate sorghum linkage maps. RAPD markers exhibit a dominant mode of inheritance and are associated with lack of reproducibility. Thus, their use in sorghum genome mapping is limited.

Due to the robustness of the amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) in genetic mapping, it was widely employed in saturation of sorghum linkage maps constructed earlier using RFLP and RAPD markers. One hundred and thirty seven AFLP markers were combined with RFLP markers described by Dufour et al. (1997) to construct a sorghum genetic linkage map of 443 loci extending the map length from 977 to 1899 cm (Boivin et al. 1999). A combined sorghum linkage map from two RI populations was constructed using AFLP, SSR, RFLP, and RAPD markers, of which 249 markers were AFLP on 11 linkage groups and had a length of 1424 cm (Haussmann et al. 2002). Subsequent sorghum linkage maps have included AFLP markers for QTL identification and for map saturations (McIntyre et al. 2005; Mace et al. 2008; Murray et al. 2008a; Ritter et al. 2008; Ramu et al. 2009; Shiringani and Friedt 2011). However, the use of AFLPs has become less popular due to difficulty in marker-assisted breeding and map-based cloning applications. In addition, the technique is costly, and requires a considerable amount of time to achieve results. Therefore, there was a need to develop techniques for plant genotyping that are robust and inexpensive.

Simple sequence repeat (SSR) markers with a high degree of polymorphism became much more preferred over RAPD and AFLP markers for plant genotyping because of their low costs, technical simplicity, throughput level, and automation (Varshney et al. 2005), and as such they have contributed to the molecular dissection of agriculturally important traits in different crops including sorghum (Das and Rao 2015; Kumar et al. 2015; Murray et al. 2008b). Screening of sorghum genomic AG-enriched libraries with labeled poly(AG)/poly(CT) combined with database searches enabled the characterization of 13 SSR markers in sorghum (Taramino et al. 1997). These markers revealed a high degree of polymorphism in nine sorghum inbred lines and seven of these SSR markers were mapped on five linkage groups on the RFLP map of Pereira et al. (1994). Tao et al. (1998, 2000) assigned eight new SSR markers to an RFLP linkage map of cross QL39 × OL41. Kong et al. (2000) and Bhattramakki et al. (2000) used a BTx623 × IS3620C population and mapped 31 and 113 new SSR markers, respectively, on a RFLP linkage map of Peng et al. (1999). The combination of mapped SSRs and AFLP markers permitted the construction of a high-density genetic map (Menz et al. 2002). Feltus et al. (2006a, b) added 15 additional new SSRs, which were used as bridge markers for map alignment among different genetic maps (Bhattramakki et al. 2000; Kong et al. 2000; Menz et al. 2002; Bowers et al. 2003). Bhattramakki et al. (2000) reported an integrated SSR and RFLP linkage map of sorghum using a large number of SSR and RFLP loci of sorghum. A sorghum linkage map developed has a map length of 997.5 cm with an average marker distance of 8.8 cm that included 38 novel SSR markers (Wu and Huang 2007).

The difficulty in the de novo development of the SSR markers was overcome by the

generation of larger numbers of expressed sequence tag (EST) libraries (Pratt et al. 2005) and the availability of the complete sorghum genome sequence (Paterson et al. 2009). Srinivas et al. (2008) developed 50 genic microsatellite markers targeting the stay-green QTLs. Using the RIL population of 296B × IS18551, Srinivas et al. (2009) employed genomic and genic microsatellite markers to construct a sorghum linkage map. Here, map positions of 28 drought EST-SSRs and seven SSRs were determined. Taking advantage of the rice-sorghum synteny, Ramu et al. (2009) developed 600 EST-SSRs in which 55 were mapped on an N13-E36-1 genetic map (Haussman et al. 2002).

The development of EST-derived microsatellites (SSRs) was confronted with the problem of sequence redundancy resulting in multiple sets of markers at the same locus. Unigenes were then developed with the advantage of having unique identity and positions in the transcribed regions of the genome. For instance, Reddy et al. (2012) identified 1519 unigene SSRs and constructed a linkage map with 228 SSRs in sorghum.

The availability of the complete sorghum genome sequence enabled the identification of 1758 new genomic SSR primers amplifying polymorphism from screening a panel of eight sorghum lines (Li et al. 2009). Using *in silico* mapping, 1692 of the SSRs were mapped onto the 10 sorghum chromosomes. Compared to conventional genetic mapping, the in silico mapping seems convenient and low cost, and it saves labor and serves as a reference map for genetic map saturations and comparative genetic mapping.

The use of RFLP, RAPD, AFLP, and SSR markers has been characterized by a low level of genome coverage and reproducibility. Diversity array technology (DArT) markers have the potential of high-throughput and whole-genome profiling based on hybridization-based technology and can overcome the above limitations. A sorghum genetic linkage map was developed consisting of 358 DArT markers in addition to 47 genomic-SSR and 188 AFLP markers distributed over the 10 chromosomes spanning a genetic distance of 1431.6 cm (Mace et al. 2008). A sorghum consensus map based on six mapping populations was developed consisting of 1190 DArT and 839 non-DArT markers (Mace et al. 2009). A cross of BR007 × SC283 was used to construct a sorghum genetic map consisting of 255 DArT markers, 83 SSRs, 5 sequence-tagged sites (STS), and 1 RFLP (Sabadin et al. 2012). These genetic maps are resources for various genetic studies and for the integration of DArT markers with other genomic resources.

Single-nucleotide polymorphisms (SNPs) uncovered by next-generation sequencing (NGS) are currently the marker of choice for plant genetic research and plant breeding due to their large numbers in virtually all populations. Genetic applications such as linkage mapping, population structure, association studies, map-based cloning, marker-assisted plant breeding, and functional genomics continue to be enabled by access to large collections of SNPs (Kumar et al. 2012). The development of the ultra-high-density linkage map based on high-quality SNPs generated from low coverage sequences involving 244 RILs of sorghum crosses was enabled through resequencing, establishing the utility of resequencing in sorghum (Zou et al. 2012). The map consisted of 3418 bin markers and spanned 1591.1 cm of the genome size with an average distance of 0.5 cm between adjacent bins. This made it easier to anchor the genetic bins to the physical positions of the SNPs, facilitating the identification of potential genes conditioning various agronomic traits in the target regions.

3 Sorghum Physical Maps

The construction of genetic maps was closely followed by the construction of physical maps as well. Bacterial artificial chromosome libraries are a useful resource for physical mapping and map-based cloning applications. Such libraries provide an important tool for structural, functional, and comparative genomic studies of eukaryotic genomes. Towards this, a BAC library with 13,440 clones was developed in

sorghum variety BTx623 by Woo et al. (1994). The library was screened with six sorghum probes and three maize probes. All but one sorghum probe hybridized to at least one BAC clone in the library. On the other hand, Cot-based sequence discovery represents a powerful means to selectively fraction, clone, and characterize low-copy and repetitive sequences. Following this strategy, Woo et al. (1994) and Peterson et al. (2002) produced HRCot, MRCot, and SLCot genomic libraries in the sorghum variety, BTx623. The Cot analysis suggested that the sorghum genome is approximately 700 Mb and that highly repetitive, moderately repetitive, and single-/low-copy components comprise 15, 41, and 24 % of sorghum DNA, respectively. Recently, binary BAC (BIBAC) libraries for sorghum landrace Nengsi-1 have been constructed, facilitating transfer of large intact DNA inserts from BAC clones to the BIBAC vector and to test functional complementation of large DNA fragments (Wang et al. 2013). Bowers et al. (2005) described two genetically anchored sorghum physical maps, anchoring thousands of genetically mapped STSs. This helped to study microsynteny among related species and wild relatives of sorghum. The BAC and Cot clones, physical map, and genome information obtained from the BTx623 genotype were used for functional complementation studies and positional or homology-based cloning of genes for translational genomics. These became fundamental resources to accelerate the genetic, molecular, and genomic studies of sorghum including whole-genome sequencing.

4 Sorghum Genome Sequencing and Beyond

4.1 Sequencing Initiatives

With the aforesaid key developments in sorghum genomic analysis, and the need to increase sorghum production dramatically to sustain human populations in many regions, an international meeting was organized in St. Louis, Missouri on November 9, 2004. The meeting proposed to perform 8× whole-genome shotgun coverage of the *S. bicolor* genome for public release (Kresovich et al. 2005; Paterson et al. 2005b; Messing 2009). Five years after this announcement, the initial analysis of the 730-megabase sorghum cultivar BTx623 whole genome was presented (Paterson et al. 2009).

The whole-genome shotgun (WGS) approach was employed to generate a high-quality genome sequence of sorghum genotype BTx623 with an error probability of 1 error per 10 kb, and coverage of 98.46 %. This approach was based on isolation of total DNA, then fragmenting it into small sizes using restriction enzymes, purifying them by cloning, and defining the start of sequencing with a short oligonucleotide. As the process of genome fragmentation produces overlapping fragments, sequences can be concatenated easily by overlapping sequences, thereby reconstructing contiguous sequences (contigs; Messing 2009). Prior to the release of the sorghum genome sequence by Paterson et al. (2009), WGS sequencing of cultivar ATx623 was carried out by means of methylation filtration technology. With this, exons, introns, promoters, microRNAs, and SSRs were preferentially captured. Thus, 96 % of the sorghum genes could be tagged (Bedell et al. 2005). This provided a robust view of the functional parts of the sorghum genome. The sorghum genome sequence project identified ~71,000 SSRs in the genome that lacked information on the SSR motifs and availability of primers (Paterson et al. 2009). Yonemaru et al. (2009) identified 5599 nonredundant SSR markers in sorghum through WGS sequencing of sorghum cultivar ATx623. The SSR marker information included regions flanking the SSRs. They found $(AT/TA)_n$ repeats constitute 26.1 % of all SSRs, followed by $(AG/TC)_n$ representing 20.5 %, $(AC/TG)_n$ 13.7 %, and $(CG/GC)_n$ 11.8 % of identified SSRs.

The WGS has facilitated the identification of molecular mechanisms controlling diverse traits in sorghum. This has created new opportunities for crop improvements. The genetic basis of plant architecture (root structure, leaf architecture, stem composition, plant height, and

tillering), flowering time, biomass bioconversion efficiency, abiotic (leaf stay-green and drought, cold stress, salt stress, aluminum toxicity) and biotic (insect pests, diseases, and *Striga* parasitism) stress resistance, and grain yield in sorghum have been resolved (Anami et al. 2015a). These QTLs are likely to accelerate the progress of the use of molecular markers for gene isolation, map-based cloning, and marker-assisted selection aimed at improving sorghum cultivars (Mohamed et al. 2014; Ejeta and Knoll 2007; Leiser et al. 2014).

4.2 Public and Private Partners in Sorghum Genome Sequencing

The US Department of Energy and Joint Genome Institute united the expertise of seven laboratories, namely Plant Genome Mapping Laboratory, University of Georgia; International Crops Research Institute for the Semi-Arid Tropics, India; Institute for Genomic Diversity, Cornell University; Waksman Institute for Microbiology, Rutgers University; Mississippi Genome Exploration Laboratory, Mississippi State University; DOE Joint Genome Institute, Walnut Creek and Department of Plant and Microbial Biology, University of California, Berkeley, along with 14 other laboratories (Table 1) for integrated high-throughput whole-genome sequencing and computational analysis of the genome of the BTx623 sorghum cultivar. Additional funding for this project was provided by the US National Science Foundation, German Federal Ministry of Education GABI initiative, and from private partners including the International Consortium for Sugarcane Biotechnology, National Sorghum Producers, and John Simon Guggenheim Foundation. Whole-sorghum genome sequencing and resequencing initiatives by both public and private partners and the countries that were involved in the initiatives are summarized in Table 1.

It may be observed that the biggest initiative was by Paterson et al. (2009), which generated the framework sequence for sorghum. This, in turn, became the basis of all subsequent resequencing projects. It is the joint and combined effort of public sector organizations and private partners that played a synergistic role in completing this daunting task. Other efforts have mostly remained within public organizations but the impact made by these efforts is really significant.

4.3 Next-Generation Sequencing

The next-generation sequencing approach involving a whole-genome shotgun strategy and Illumina Genome Analyzer sequencing technology combined with highly efficient alignment software was applied to resequence additional sorghum lines to discover large numbers of SNPs and structural variants in previously sequenced genomes (Nelson et al. 2011; Zheng et al. 2011; Bekele et al. 2013; Mace et al. 2013; Morris et al. 2013). The structural variations in genomes include SNP, insertions-deletions (in-dels), duplications, inversions, translocations, copy number variations (CNV), and presence/absence variants (PAV; Saxena et al. 2014). The updated status of different sequencing/resequencing projects is summarized in Table 2. Sorghum has a well-assembled and well-annotated genome enabling the utility of restriction-site-associated DNA tags (RAD-seq) that are spread at a high density throughout the genome. This relies on genome complexity reduction for SNP discovery and high-resolution genotyping. Nelson et al. (2011) employed the RAD-seq sequencing approach to identify 283,000 SNPs from eight sorghum accessions (BTx623, BTx430, P898012, Segaolane, SC35, SC265, PI653737 [*S. propinquum*], and 12–26 [*S. bicolor* ssp. *Verticilliflorum*]). Structural variations in genomes have been implicated as playing a role in evolutionary and biological processes and studies need to be designed to correlate genetic variations with plant performance. Bekele et al. (2013) resequenced five genetically diverse sorghum genotypes, including three sweet sorghums and two grain sorghums cultivars. They identified and validated 2620 robust and polymorphic sorghum SNPs. In another study, approximately 265,000 SNPs were characterized

Table 1 Sorghum genome sequencing national and international participants

Public participants	Private participants	Countries involved	Reference
Stanford Human Genome Center, Stanford University	International Consortium for Sugarcane Biotechnology; National Sorghum Producers; John Simon Guggenheim Foundation	United States, China, Germany, India, Pakistan, Switzerland	Paterson et al. (2009)
MIPS/IBIS, Helmholtz Zentrum München			
Center for Integrative Genomics, University of California, Berkeley			
College of Sciences, Hebei Polytechnic University			
Institute of Plant Biology, University of Zurich			
Department of Genetics and Biochemistry, Clemson University, Clemson			
Institut fur Entwicklungs und Molekularbiologie der Pflanzen, Heinrich-Heine-Universitat			
Cold Spring Harbor Laboratory, Cold Spring Harbor			
Department of Biological Sciences, Purdue University			
Department of Botany and Plant Pathology, Purdue University			
Department of Horticulture and Institute for Plant Genomics and Biotechnology, Texas A&M University			
Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana			
National Institute for Biotechnology & Genetic Engineering (NIBGE)			
USDA NAA Robert Holley Center for Agriculture and Health, Ithaca			
US National Science Foundation			
German Federal Ministry of Education GABI Initiative			
US Department of Agriculture-Agricultural Research Service			
Department of Agronomy, Kansas State University, Manhattan		United States of America	Nelson et al. (2011)
Department of Plant Pathology, Kansas State University, 4024 Throckmorton Plant Sciences Center, Manhattan			
Institute of Botany, Chinese Academy of Sciences, Beijing		China	Zheng et al. (2011)
Shenzhen Key Laboratory of Transomics Biotechnologies, BGI-Shenzhen			
Temasek Life Sciences Laboratory Limited, 1 Research Link National University of Singapore			
Chinese Academy of Sciences			

(continued)

Table 1 (continued)

Public participants	Private participants	Countries involved	Reference
Department of Plant Breeding, Justus Liebig University, Giessen, Germany	Service XS, Leiden, Netherlands; CLC Genomics, Cologne, Germany	China, Germany	Bekele et al. (2013)
KWS Saat AG, Einbeck, Germany			
German Federal Ministry of Education and Research (BMBF)			
Federal Ministry for Consumer Affairs, Nutrition and Agriculture (BMVEL)			
Max-Planck Institute for Plant Breeding Research, Cologne, Germany			
Department of Agriculture, Fisheries and Forestry Queensland (DAFFQ), Warwick		Australia, China, Denmark	Mace et al. (2013)
Shenzhen Key Laboratory of Transomics Biotechnologies, BGI-Shenzhen			
The University of Queensland, School of Agriculture and Food Sciences, Brisbane			
Science and Engineering Faculty, Queensland University of Technology, Brisbane			
DAFFQ, Cooper's Plains, Brisbane			
Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Warwick			
Department of Biology, University of Copenhagen			
The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen			
Grains Research and Development Corporation (GRDC), Australia			
Australian Research Council (ARC)			
Beijing Genomics Institute			

using genotyping by sequencing 971 worldwide accessions that have adapted to diverse agroclimatic conditions (Morris et al. 2013). A high coverage (16–45×) resequencing of genomes of 44 diverse sorghum lines was taken up by Mace et al. (2013). The study material included 18 landraces, 17 improved inbreds, and 7 wild and weedy sorghums. They identified 8 million high-quality SNPs, 1.9 million in-dels, and specific gene losses. A 12× coverage resequencing of two sweet (Keller, E-Tian) and one grain (Ji2731) sorghum inbred line identified 3234 CNVs associated with 2600 genes and 16,487 PAVs associated with 1416 genes (Zheng et al. 2011; Saxena et al. 2014). These were found to be associated with phenotypic variation of disease resistance. In addition, 1,057,018 SNPs, 99,948 in-dels of 1–10 bp in length and 16,487 PAVs as well as 17,111 copy number variations were uncovered. Zhang et al. (2014a) characterized in detail 51 larger size PAVs (>30 kb) identified in the previous study. They found the PAVs to vary considerably for repeat sequences and mobile elements with DNA transposons as the major components. The PAVs

Table 2 Sorghum lines sequenced thus far and total single nucleotide polymorphism (SNP) present in each line (http://sorgsd.big.ac.cn/snp/pages/help/data_statistics.jsp)

Cultivar category	Sorghum cultivar	Country of origin	Total SNP
Improved varieties	BTx642	Ethiopia	1,517,831
Improved varieties	PI525695	Mali	1,939,649
Improved varieties	PI586430	Sierra Leone	1,916,335
S. propinquum	*S. propinquum 369-1*	–	5,145,808
S. propinquum	*S. propinquum 369-2*	–	4,805,314
Improved varieties	Ai4	China breeding program	1,148,341
Improved varieties	B923296	Australia breeding program	648,022
Improved varieties	ICSV745	India breeding program	899,003
Improved varieties	Karper 669	US breeding program	1,112,491
Improved varieties	KS115	US breeding program	928,408
Improved varieties	LR9198	China breeding program	1,245,014
Improved varieties	Macia	Mozambique	772,165
Improved varieties	Malisor 84-7	Mali	345,479
Improved varieties	QL12	Australia breeding program	1,028,161
Improved varieties	R931945-2-2	Australia breeding program	1,234,614
Improved varieties	Rio	US breeding program	817,774
Improved varieties	RTx7000	US breeding program	1,122,424
Improved varieties	B963676	Australia breeding program	643,809
Improved varieties	PI563516	Mali	1,008,016
Improved varieties	Ji_2731	China breeding program	546,841
Improved varieties	Keller	US breeding program	334,453
Improved varieties	E-Tian	China breeding program	441,887
Landraces	Early Hegari	Sudan	900,247
Landraces	IS3614-2	Nigeria	1,301,792
Landraces	IS8525	Ethiopia	936,298
Landraces	IS9710	Sudan	952,704
Landraces	M35-1	India breeding program	793,507
Landraces	SC103-14E	South Africa	792,792
Landraces	SC108C	Ethiopia	729,488
Landraces	SC170-6-8	Ethiopia	823,103
Landraces	SC23	Ethiopia	1,341,925
Landraces	SC237-14E	Sudan	854,065
Landraces	SC326-6	United States/Ethiopia	849,759
Landraces	SC35C	Ethiopia	1,217,473
Landraces	SC56-14E	Sudan	956,926
Landraces	SC62C	Kenya	988,328
Landraces	PI585749	Mali	1,434,636
Landraces	IBC/E-38432	Ethiopia	1,691,268
Landraces	Yik.solate (IBC/E-339)	Ethiopia	1,098,136

(continued)

Table 2 (continued)

Cultivar category	Sorghum cultivar	Country of origin	Total SNP
Landraces	Cherekit (IBC/E-460)	Ethiopia	922,336
Landraces	SS79	Limpopo, South Africa	1,286,223
Wild and weedy	Greenleaf	US breeding program	1,505,551
Wild and weedy	*S. bicolor* ssp. *drummondii* (PI330272)	Ethiopia	1,487,442
Wild and weedy	*S.bicolor* ssp. *verticilliflorum*	South Africa	2,965,815
Wild and weedy	*S. bicolor* (PI226096)	Kenya	1,942,266
Wild and weedy	*S. bicolor* ssp *verticilliflorum*	Australia	1,975,326
Wild and weedy	Kilo (IBC/E-382)	Ethiopia	1,248,859
Wild and weedy	Zengada (IBC/E-308)	Ethiopia	1,560,232

differed substantially in terms of frequency and distribution across a large number of sorghum inbred lines. Shen et al. (2015) characterized 5511 genic small-size PAVs (40 bp–10 kb).

Various approaches addressing sequencing of defined regions of the genome have emerged. One of these is whole-exome sequencing (WES or exome-seq), in which only the coding regions of the genome are sequenced (Hodges et al. 2007). This approach has been applied in pine (Neves et al. 2013) and barley (Mascher et al. 2013, 2014). Application of such techniques in sorghum may help us better understand the genetic basis of resistance or susceptibility to biotic stress such as *Striga* parasitism. Amplicon sequencing, in which selected genome regions (tagged amplicons) are amplified by PCR, can facilitate analysis of variations within genes in the genomes. This assists in answering many questions in population genetics and systematics that rely on sequencing specific genes of known function or diversity levels (Bybee et al. 2011). Sequencing of multiplexed tagged amplicons followed by bioinformatics processing identified single nucleotide polymorphisms and allelic sequences of 10 candidate genes related to drought/osmotic stress from *Quercus* spp. (Homolka et al. 2012). This example suggests that application of amplicon sequencing might likely identify molecular markers defining drought stress tolerance in sorghum given that drought stress tolerance is the major objective of sorghum breeding.

4.4 Genomewide Association Study

A combination of molecular markers spread across the genome and phenotyping of large unrelated germplasm, referred to as a genome-wide association study (GWAS), has been used to identify candidate genes controlling traits in sorghum. These include traits such as polyphenol concentrations, vegetative branching, high grain yield, tolerance to aluminum toxicity, plant height components, and inflorescence architecture (Morris et al. 2013; Kong et al. 2014; Leiser et al. 2014; Rhodes et al. 2014). However, in this context identification of novel genes underlying complex traits such as abiotic and biotic stress resistance and genes with small to modest effect still remains a challenge. Adequate sample size and higher marker density for GWAS in plants are still limited and yet are necessary in identification of novel genes with small effects. Detailed progress in GWAS in sorghum is elaborated in Chap. 7.

4.5 Alternate Splicing

Genome sequence has also enabled the identification of alternate splicing (AS) events in plants. AS occurs in many forms including exon skipping, alternative 5′ and 3′ splice sites, intron retention, mutually exclusive exons, alternative promoters/5′ transcription start sites (TSSs), and alternative 3′ poly(A) sites (Grabowski 2002). All these events affect transcript levels and cause transcript degradation via nonsense-mediated decay, potentially reducing the level of gene expression (Barbazuk et al. 2008). AS produces multiple transcripts from a single gene by using different splice sites, offering the possibilities of generating different protein isoforms with altered functions related to developmental and physiological processes (Syed et al. 2012; Staiger and Brown 2013). Olson et al. (2013) reported 7112 genes with multiple splice variants in sorghum. Of these, 3949 (56 %) contained a splice variant within 20 % of the length of the canonical transcript (the longest transcript with the longest translation), and of these, 3446 (87 %) encoded an altered coding region. In *Arabidopsis*, the AS profile at the genomic level is regulated by phytochrome (Shikata et al. 2014). Using RNA-seq data for sorghum genome annotation, Olson et al. (2013) reported that 33 % of sorghum genes contained two or more exons producing two or more transcript-isoforms and that intron retention was the most common isoform whereas alternative 5′ and 3′ acceptor sites were observed in 671 and 1211 isoforms. In another study, 2137 complex and intron retention AS events were identified in sorghum, highlighting the prevalence of splicing site recognition for definition of introns in sorghum (Panahi et al. 2014).

4.6 Dissection of Biological Functions Through RNA Sequencing

Sequence information is also useful in expression profiling for dissecting biological functions of genes. The upregulation or downregulation of gene expression is sufficient for re-creating phenotypic differences (Jiang et al. 2013b). Pasini et al. (2014) employed microarrays to identify differentially regulated genes and microRNAs upon drought stress in sorghum cultivar IS19453. In the study, 1205 genes were found to be upregulated. Identified differentially expressed genes were mostly involved in regulation of transcription (*bZIPs*, *MYBs*, *HOXs*), signal transduction (phosphoesterases, kinases, and phosphatases), carbon metabolism (*NADP-ME*), detoxification (*CYPs*, *GST*, *AKRs*), osmoprotection mechanisms (*P5CS*), and stability of protein membranes (*DHN1*, *LEA*, *HSPs*). Using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR), a differential expression profiling of sucrose phosphate synthase, sucrose synthase, and soluble acid invertase in intergeneric hybrids involving sugarcane and sorghum was identified (Ramalashmi et al. 2014). This indicated the possibility for production of novel hybrids with improved sucrose content and early maturity. Next-generation sequencing has been applied to generate a more complete sorghum transcriptome and methylome using RNA-sequencing (RNA-seq; Olson et al. 2013; Makita et al. 2015). Computational methods designed for RNA-seq data are capable of discovering new transcripts and alternative spliced forms far beyond the scope of a DNA-microarray (Roberts et al. 2011). These can be of great use in identifying breeding targets for genetic improvement of sorghum. In addition, RNA-seq can be utilized in the study of noncoding RNAs, small RNAs, transcriptional structure of genes, and their post-transcriptional modifications.

4.7 Transposable Elements and Genome Size Variation

Sorghum and rice genomes are found to have similar quantities of highly hypomethylated euchromatin chromosome arms (Kim et al. 2005; Olson et al. 2013). However, the expansion of the sorghum genome relative to rice is largely pericentromeric-localized heterochromatin occupying at least 460 Mb, far more than in rice with 63 Mb (Kim et al. 2005; Paterson et al. 2009).

Such net size expansion of the sorghum genome relative to rice could be due to transposons, organelle nuclear DNA insertions, and to a larger extent, genome duplication.

Mobile DNA elements in plants, also referred to as transposable elements, were first described by Barbara McClintock in 1950 in maize and were responsible for chromosomal rearrangements (McClintock 1950). These mobile elements are fundamental agents for genome size enlargement and evolution (Feschotte et al. 2002) and are grouped either as retrotransposons or Class I elements, and DNA transposons or Class II elements (Wicker et al. 2007). Long terminal repeat (LTR) retrotransposons are Class I elements and are transposed via RNA intermediates. These are characterized by two long terminal repeats, starting with "TG" and terminating with "CA", containing a primer binding site, polypurine tract, and several genes related to the transcription and replication process (Du et al. 2010). With the availability of genomic data from sequencing experiments, it has become clear that a larger proportion of DNA in plants is made up of LTR retrotransposons arising from unequal homologous recombination (UR) and illegitimate recombination (IR; Devos et al. 2002; Tian et al. 2009). For instance, the net size expansion of the sorghum genome relative to rice largely involved 55 % LTR retrotransposon sequences enriched in gene-poor heterochromatic regions (Paterson et al. 2009). Jiang and Ramachandran (2013) identified a total of 23,915 full-length LTR retrotransposons in sorghum. They found that unlike rice, sorghum full-length LTR retrotransposons were not enriched in centromere regions. A total of 1343 genes were captured by these elements in sorghum. It was found that a higher percentage of LTR-captured sorghum genes were under purifying selection and 72.4 % of them were functional. In addition, sorghum has a higher ratio of gypsy-like (Ty3-gypsy, Metaviridae) to copia-like (Ty1-copia, Pseudoviridae) elements (3.7–1 and 4.9–1) than maize (1.6–1; Paterson et al. 2009). Indeed, 24 families of copia-like elements and two families of gypsy-like elements were found in sorghum genomic DNA with the potential for use as transposon tagging systems in callus protoplasts (Muthukumar and Bennetzen 2004). Centromeric and telomeric repetitive DNAs could also explain the sorghum genome size variability with other plant species. Fluorescence in situ hybridization (FISH) identified a 745-bp repetitive DNA clone, *pSau3A9*, located in the centromeric regions of all sorghum chromosomes (Jiang et al. 1996). In addition, an 823-bp *Sau3AI* fragment (*pSau3A10/pCEN38*) was subcloned from a sorghum BAC clone, 13I16, that contains DNA sequences specific to the centromeres of grass species (Miller et al. 1998). Telomeric DNA repeats have also been identified in sorghum such that 15 out of 20 chromosome ends terminated in telomeric repeats (Paterson et al. 2009). Small LTR retrotransposons (SMARTs) of 292 bp (FRetro129), with 85-bp direct terminal repeats, distributed throughout the genomes and often located within or near genes with insertion patterns similar to MITEs, have recently been reported in *Oryza brachyantha*. Elements homologous to FRetro129 were found in the sorghum genome (Gao et al. 2012). These elements have the potential to play an important role in genome evolution and genic innovation and may provide a valuable tool for gene tagging systems in grass. For instance, insertions of SMARTs into or near genes can, in a few instances, alter both gene structures and gene expression. In addition, SMART-specific small RNAs (sRNAs) were identified that may be involved in gene regulation (Gao et al. 2012).

Class 2 transposable elements (TEs) are the predominant elements in and around plant genes generating significant allelic diversity. In sorghum, 7.5 % of the genome constitutes DNA transposons, which is intermediate between maize (2.7 %) and rice (13.7 %; Paterson et al. 2009). Helitrons belong to this class, which are DNA transposable elements and are widely present in the genomes of diverse eukaryotic taxa. These elements occupy a relatively high proportion of a species' genome, approximately 2.2 % of the B73 maize genome (Du et al. 2009), 2 % of the *A. thaliana* genome (Hollister and Gaut 2007), 2.09 % of the rice genome (Kapitonov and Jurka 2001), and 0.8 % of the sorghum genome (Paterson et al. 2009). These elements prefer to insert near other helitrons and into AT-rich regions (Yang and Bennetzen

2009). They are distinct from other transposons in their ability to capture gene fragments within the genome and their rolling-replication mechanism. Helitrons play a role in exon shuffling and gene duplication to create new proteins intrinsic in their ability to capture and move gene fragments (Brunner et al. 2005; Morgante et al. 2005). Helitrons can also disrupt microsatellites and affect their flanking sequence (Coates et al. 2010). The movement of genes or gene fragments captured by helitrons disrupts the genetic collinearity (Lai et al. 2005; Xu and Messing 2006) and can induce promoter shuffling to generate new regulatory networks (Cultrone et al. 2007). Therefore, helitrons may alter host genome organization, regulate gene structure and expression, mediate structural diversity (Wang and Dooner 2006), and shape the evolution of host genomes (Lai et al. 2005). Gene fragments captured during the transposition of many helitrons in maize happen in a stepwise manner, with multiple gene fragments within one helitron resulting from several sequential transpositions (Dong et al. 2011).

Miniature inverted-repeat transposable elements (MITEs) are ubiquitous, nonautonomous class II transposable elements characterized by small size (mostly <600 bp), high terminal inverted repeats (TIRs), and target site duplication (TSD), sequence similarity, and a potential for very high copy number and no coding capacity (Wessler et al. 1995). Such elements are AT-rich (>50–65 %) (Turcotte et al. 2001), and preferentially inserted into intergenic, near-genic, or intronic regions where they can alter or disturb gene structure, expression, and/or function. These elements usually avoid exonic regions (Sampath et al. 2014). MITEs are a major fraction of plant genomes, up to 10 % in rice, 8 % in Medicago, 4 % in *B. rapa*, 0.71 % in *A. thaliana* (Chen et al. 2014), and 1.7 % in sorghum (Paterson et al. 2009). The predominant sorghum DNA transposons are CACTA-like elements that occupy 4.7 % of the genome. Out of the 13,775 CACTA-like elements identified, 200 encode no transposon proteins but contain at least one cellular gene fragment. These elements seem to relocate genes and gene fragments. Many sorghum CACTA elements are nonautonomous deletion derivatives in which transposon genes have been replaced with nontransposon DNA including exons from one or more cellular genes (Paterson et al. 2009). Organellar DNA insertion contributed only 0.085 % to the sorghum nuclear genome, far less than the 0.53 % of rice (Du et al. 2009).

Whole-genome duplication (WGD) or polyploidy refers to a situation where an organism possesses more than a diploid complement of chromosomes. Evolutionary processes such as rapid and substantial genome reorganization, transgressive gene expression alterations, gene fractionation, gene conversion, genome downsizing, and sub- and neofictionalization of duplicate genes impact the polyploid genome. Often these genomic changes are accompanied by heterosis, robustness, and the improvement of crop yield, relative to closely related diploids, thereby driving phenotypic diversification (Soltis et al. 2009). Sorghum and other grasses shared at least two rounds of ancient WGDs earlier than 70 million years ago, before the divergence of modern grass lineages (Tang et al. 2010). Genomewide signatures of collinearity suggest a process of WGD rather than local tandem or segmental duplication. Indeed, in sorghum a total of 19,929 sorghum gene models were found to be in blocks collinear with rice (Paterson et al. 2005a). After whole-genome duplication, gene features such as evolutionary rate, structural complexity, and GC3 content significantly contribute to gene retention (Jiang et al. 2013a). As such in sorghum, these features are likely to have contributed to one copy retention for 13,667 collinear genes with 13,526 being orthologous in rice–sorghum and the retention of an additional copy of 4912 genes (Paterson et al. 2009). One copy of 1070 genes was pseudogenized after whole-genome duplication in sorghum. Some genes are able to duplicate iteratively, whereas others are consistently restored to singleton status along divergent lineages (Seoighe and Gehring 2004). The genes retained in duplicates are not evenly distributed among different functional categories. However, a total of 2 and 10 protein functional domains showed enrichment for

duplicates and singletons, respectively, in sorghum but not rice, suggesting duplicated genes are functionally biased (Seoighe and Gehring 2004; Freeling 2009; Paterson et al. 2009; Schnable et al. 2009). Syntenic duplication blocks in sorghum and rice, in contrast to *Arabidopsis*, maize, poplar, and soybean, are classified into one major age group in addition to containing several recent segmentally duplicated blocks that are greatly influenced by gene conversions (Jiang et al. 2013a; Paterson et al. 2009). Tandem duplication events in sorghum might have contributed to the expansion of micro-RNA families derived from repetitive elements and duplication events at the genome scale (Sun et al. 2012).

4.8 Synteny with Other Species and Sequences

To gain an understanding of the genetic architecture of other crops where genomic sequence is lacking, powerful comparative genomic studies have been undertaken (Paterson et al. 2009; Salse et al. 2009). Comparative mapping between sugarcane and sorghum using DArT, SNP, and EST-SSR markers revealed 98–100 % collinearity between four sugarcane homology groups (HGs) to four sorghum chromosomes (Aitken et al. 2014). In addition to these, they identified four major chromosome rearrangements between the other four sugarcane HGs of sorghum. Two cases experienced condensations of chromosomes leading to reduction of basic chromosome number of sugarcane from $x = 10$ to $x = 8$. Use of maize DNA probes to produce the sorghum linkage map shows that most sorghum linkage groups are composed of loci that map to two maize chromosomes and that the amount of recombination in these conserved linkage groups is roughly equivalent in maize and sorghum (Whitkus et al. 1992). The genomewide comparison of repetitive sequences indicated that the repeats in *Coix aquatica* were more similar to those in sorghum than maize (Cai et al. 2014).

Since the first WGD took place about 700 million years ago (Paterson et al. 2004), rice and sorghum have not experienced further genome duplications and subsequent diploidization. Synteny conservation spans large genomic regions covering hundreds to thousands of orthologous genes (Paterson et al. 2009). Integrating synteny and phylogenomic comparisons of genomes of the monocots (viz., rice, sorghum, oil palm, and banana) and eudicot (viz., grape and sacred lotus) suggests that the majority (~80 %) of ancestral loci had been differentially retained in the lineage leading to cereals, and is still syntenic in present-day rice and sorghum genomes (Angiosperm Phylogeny Group 2009). In addition, about 3.51 % of sorghum genes have no orthologues in rice, oil palm, and banana (Jiao et al. 2014). Detailed syntenic comparison has been elaborated in Chap. 6.

5 Genomic Resources

5.1 Sorghum Genome Databases

With the sorghum genome sequence available in addition to an increasing number of sequenced plant genomes and the introduction of third-generation single-molecule sequencing methods over the coming years, it is necessary to develop robust computational methods for sequence analysis. A number of databases for sequence analysis in sorghum are available including SorGSD, the sorghum genome SNP database, available at http://sorgsd.big.ac.cn/snp/index.jsp. It contains 62 million SNPs from a diverse panel of 48 sorghum accessions divided into four groups, for example, improved inbreds, landraces, wild/weedy sorghums, and a wild relative, *S. propinquums* (Table 2). SorGSD allows users to query the SNP information and their relevant annotations for each sorghum line. The search results can either be visualized

graphically in a genome browser or displayed in formatted tables. Users can also perform comparisons of these data between two or among several sorghum accessions. dbSNP (available at http://www.ncbi.nlm.nih.gov/snp/?term=sorghum) helps in identification of SNPs in sorghum genes. The Database of Genomic Variants archive (DGVa) available at http://www.ebi.ac.uk/dgva provides archiving, accessioning, and distribution of publicly available genomic structural variants, in all species including sorghum. The EnsemblPlants database (http://plants.ensembl.org/Sorghum_bicolor) provides information on sorghum genome assembly, comparative genomics, gene annotations, and short sequence variants and longer structural variants. The MOROKOSHI Sorghum transcriptome database (http://sorghum.riken.jp/morokoshi/Home.html) provides full-length cDNA clone and RNA-Seq data on *Sorghum bicolor* (Makita et al. 2015).

Sweet fuel (http://www.sweetfuel-project.eu/) is a consortium composed of partners from research academia and industry, based in Europe, India, Brazil, South Africa, and Mexico with an objective of optimizing yields in temperate, semi-arid, and subtropical regions by genetic enhancement and improvement of agricultural practices of sweet sorghum. Information on sweet sorghum cultivars with the ability to tolerate low temperature, drought, and low-fertility-prone environments can be accessed at http://www.sweetfuel-project.eu/completed_deliverables. Additional information on sorghum as a promising alternative energy crop can also be accessed by researchers involved in developing sweet sorghum as a dedicated biofuel crop (http://www.sweetfuel-project.eu/general_information/links).

The UniProt database (http://www.uniprot.org) provides the scientific community with a comprehensive, high-quality, and freely accessible resource of protein sequence and functional information. A joint project of the Department of Energy's Joint Genome Institute and the Center for Integrative Genomics (Phytozome) (http://www.phytozome.net/ [is now https://phytozome.jgi.doe.gov/pz/portal.html]) facilitates comparative genomic studies among green plants. In addition, the Gramene database (http://www.gramene.org/) and MIPSPlantsDB-plant database (Nussbaumer et al. 2013) are resources for integrative and comparative plant genome research. The PIGD database (http://pigd.ahau.edu.cn/) is a database to study intronless genes in the Poaceae including sorghum, representing an important resource to study the evolution of gene architecture (Yan et al. 2014). The CSGRqtl (http://helos.pgml.uga.edu/qtl/) database utilizes the sorghum genome sequence as a central reference to provide both for practical needs of crop improvement by serving as a toolbox for QTL visualization and manipulation. It also facilitates investigation of fundamental questions about similarities and differences in the genetic control of traits across paleoduplicated "subgenomes" and across the genomes of divergent taxa (Zhang et al. 2011, Zheng et al. 2014a). A next-generation sequencing-derived gene expression network (GEN) repository for eight plant species (*Arabidopsis thaliana*, *Oryza sativa*, *Solanum lycopersicum*, *S. bicolor*, *Vitis vinifera*, *Solanum tuberosum*, *Medicago truncatula*, and *Glycine max*) referred to as the Plant Omics Data Center (http://bioinf.mind.meiji.ac.jp/podc/) has been constructed, providing a comprehensive integration of large-scale genomes, transcriptomes, and metabolomes resources for the scientific community giving deeper insights into broader aspects of molecular biology (Ohyanagi et al. 2015).

5.2 Sorghum Gene Annotation

The systematic sequencing of the sorghum genome was completed in 2009 and 34,496 gene models (Sbi1.4, version 1.4 of sorghum gene annotations) were identified. Around 27,640 of these genes were considered bona fide, or high-confidence, protein-coding genes (Paterson et al. 2009). The remaining ~6850 sorghum gene models predicted from the genome sequence were considered low-confidence genes

(Paterson et al. 2009). A comprehensive annotation of the sorghum genome and the development of functional genomic resources are key to enabling the discovery and deployment of regulatory and metabolic genes and gene networks for crop improvement (Shakoor et al. 2014). In this study, tissue and genotype-specific expression patterns for all identified sorghum exons and untranslated regions were identified. In all major tissue types analyzed, there was a lack of shared tissue-specific genes and small RNA across genotypes. These indicate the significance of intraspecies variation in sorghum and the importance of selecting the appropriate genotype for targeted changes to gene expression via transgenic and breeding approaches. By analyzing RNA-seq data in conjunction with published transcriptome analysis in *Arabidopsis*, maize, and rice, 34,144 genes of the 34,496 gene models of Paterson et al. (2009) were resolved by Dugas et al. (2011). In the study, gene networks regulated by osmotic stress and hormonal treatment could be resolved in addition to the discovery of more than 50 differentially expressed, drought-responsive gene orthologues for which no function had been previously ascribed. Olson et al. (2013) developed a more complete annotation of the sorghum transcriptome by extending the untranslated region annotations (18,105) with some showing longer protein-coding regions (5096) or previously unannotated alternative transcripts (6493). Thus, the new annotations resolved 50 % of split gene models and included 30 % of conserved genes missing from the Sbi1.4 annotation. It identified a large set of 34,276 novel potentially functional transcribed regions that include protein-coding genes, non-coding RNAs, and other classes of gene products. Makita et al. (2015) constructed a full-length cDNA (FL-cDNA) library from eight growth stages of aerial tissues of sorghum and isolated 37,607 clones. Analysis of 38,981 high-quality expressed sequence tags (ESTs) from these clones led to annotation of 272 novel genes, 323 antisense transcripts, and 1672 candidate isoforms. Through expression profile analysis they confirmed the expression of 70.6 % of the newly identified genes. The sorghum genome annotation is still under improvement and the current improved *S. bicolor* v3.1 includes ~344.4 Mb of essentially finished sorghum sequence integrated into the original V1.0 assembly (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Sbicolor).

6 Perspectives

The Food and Agriculture Organization (FAO) of the United Nations estimated that about 805 million people were chronically undernourished during 2012–2014, with 13.5 % of them residing in developing countries. Sub-Saharan Africa is the region with the highest prevalence (percentage of population) of hunger (http://www.fao.org/publications/sofi/en/). It is the origin of sorghum, a staple food for the resource-poor people in marginal environments. In addition to the food crisis, climate change and shifting weather patterns threaten food production through increased unpredictability of precipitation and a warming atmosphere. These changes aid the spread of pests and diseases to other parts of the world (http://www.unep.org/climatechange/). An integrated approach is, therefore, required to confront the nexus of food crisis and climate change. The vital genetic resources held by sorghum and other C_4 grasses uncovered through whole-genome shotgun and next-generation sequencing platforms is expected to be one of the tools in mitigating food insecurity and the impact of climate changes. The identification of high-quality SNPs, insertions, deletions, PAV, CNV, duplications, translocations, and alternative splicing events in diverse sorghum genotypes are unmatched resources to respond to these challenges and maximize the contribution of C_4 grasses to the food security of the world's most vulnerable and more efficient feed and fuel production (Mace et al. 2013).

Sequence data generation in sorghum has advanced increasingly through the application of high-throughput technologies including whole genome and next-generation sequencing. However, next-generation sequencing is limited in the DNA fragmentation step by generating millions

of short DNA sequence reads of length typically between 25 and 400 bp and also in the amplification step with the possibility to be biased in GC content and repeat sequences. The ability to produce vast numbers of long sequence reads is likely to provide further opportunities for crop genomics. To this end, the third-generation sequencing referred to as single molecule sequencing undertakes sequencing without the requirement for DNA amplification and promises to produce several giga-base pairs (Gbp) of relatively long reads (>1 kbp; Eid et al. 2009). In addition, because of long reads and lack of bias in coverage of AT-rich regions, the use of single-nucleotide sequencing technology has great promise for highly accurate finished genomes, and has already been successful in the de novo assembly of chloroplast genomes (Chen et al. 2014; Li 2014; Siewert et al. 2014; Wu et al. 2014; Zhang et al. 2014b). This enables detection of sensitive SNPs, comparative analysis, and application for chloroplast genome evolutionary genetics and genomics in land plants. Already, several different single-molecule DNA sequencing techniques have been developed or are currently in development including gated single-molecule sequencing-by-synthesis (Lister et al. 2008), single-molecule, real-time DNA sequencing, nanopore-based single-molecule sequencing, and microscopy-based single-molecule sequencing and are being commercialized (Horn et al. 2016; Li et al. 2016). With the availability of genomic sequences generated by the NGS-based platforms on the few sorghum genotypes that have thus far been sequenced, genomes of additional diverse cultivars would need to be sequenced so that genetic and genomic diversity in sorghum can be examined in much more detail to localize alleles of agronomically important complex traits such as stay-green drought resistance, insect resistance, disease resistance, resistance to *Striga* parasitism, grain size and grain quality, and exploitation of structural variations in the sorghum genome associated with favorable variants. The sorghum genome-sequencing information, just like other genomes, has facilitated the development of molecular tools for functional studies including efficient *Agrobacterium*-mediated transformation, sequence-specific nucleases for genome editing that will play an important role in future genetic improvement and breeding of sorghum ideotypes (Anami et al. 2015a) adaptable to specific agroclimatic regions.

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Synteny with Allied and Model Genomes

6

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Abstract

Analysis of synteny is an integral part of comparative genomics inasmuch as it helps us to understand the structures and functions of genes in the related genomes in relation to genome evolution and their roles in gene expression. The identification of synteny blocks, the basic unit of genome synteny, may provide insights into the gene structure and regulation that are essential for biological processes. During earlier days, synteny blocks were identified through ad hoc methods that were slow, lacked reproducibility, ignored the conservation of gene order and orientation, and were not suitable for general applications. However, during the last decade, concerted efforts by several researchers have led to the development of a large volume of genomic data as well as computational resources that allow comparative genomic analysis between genomes of interest with high resolution. Comparative analysis of map-based genomic sequences led to the identification of shared intragenomic duplications, which provide important clues on the evolution of crop genomes from common ancestors. Recent studies in synteny analysis involve transcriptomic synteny to understand the functional conservation of orthologous genes and paleogenomic synteny to understand the role of whole-genome duplications on genome evolution. This chapter discusses the role of synteny analysis in comparative genomics; various computational tools employed for synteny analysis; synteny of sorghum with allied and model genomes with reference to molecular maps, markers, and the whole genome; emerging trends in synteny analysis; and future prospects.

1 Introduction

Analysis of synteny among two species is an important aspect of comparative genomics inasmuch as it helps us to understand the gene order and functions of a set of genes in the genomes

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and their roles in gene expression. The term *synteny* was first mentioned as it is understood today by Renwick (1971), where it referred to "presence together on the same chromosome, whether or not the loci have been shown to be linked." However, according to Nadeau and Taylor (1984), synteny refers to the presence of two or more pairs of homologous genes with conserved function on the same chromosomal segment. The presence of synteny blocks or segments in the genome may be due to the selection pressure of that region during the divergence from their recent common ancestor (Vergara et al. 2009). Earlier, synteny referred to the colocalization of genes in corresponding chromosomes of different species. However, in recent times, synteny indicated the conservation of such colocalized genes in the same order between various genomes (Vergara and Chen 2010). Therefore, the information on the relative order of genes from one species can provide vital clues to their presence and also function in other species. Even though plant genomes are of highly variable sizes and complex, the gene content and gene order are highly conserved among themselves. Moreover, the functions of homologous genes are similar (Bennetzen 2000). After the sequencing of the first plant genome, *Arabidopsis thaliana*, several comparative genomics studies have been performed by various research groups (Klein et al. 2003; Zhu et al. 2003; Choi et al. 2004; Salse et al. 2004; Timms et al. 2006; Kumar et al. 2009) to understand the synteny among different plant species. These include synteny analysis between *Arabidopsis* and tomato (Ku et al. 2001), *Arabidopsis* and rice (Salse et al. 2002), soybean and *Arabidopsis* (Shultz et al. 2007), and between common bean and soybean (McClean et al. 2010), to name a few.

Understanding sorghum synteny is important because it belongs to grasses, whose genomes generally exhibit more collinearity (Moore et al. 1995) and are the plant family that is extensively sampled through whole-genome sequencing (Schnable and Lyons 2011). Sorghum, being a tropical grass with characteristic C_4 photosynthesis, is considered as a logical complement to rice with C_3 photosynthesis, a characteristic of temperate grasses. Therefore, the sorghum genome has an evolutionary link to that of maize as well as sugarcane genomes. This is confirmed by its relatively more shared common ancestry with sugarcane (5 million years ago, Mya) and maize (12 Mya) than with rice (42–47 Mya). Analysis of synteny between two genomes or multiple genomes involves either a comparison at the gene level or whole-genome level. This relies heavily on the computational tools associated mostly with sequence alignments and visualization, which allows the researchers to compare the genes or genomes of interest intuitively. This chapter discusses in detail the importance of synteny analysis, computational tools employed, synteny of sorghum with allied and model genomes at the level of molecular maps, markers, and whole genome with critical analysis and future prospects.

2 Comparative Genomics and Synteny

Comparative genomics helps in understanding the structural and functional features of genomes by translating knowledge gained about some genomes to the object of study. Prior to the availability of whole-genome sequences, pairwise comparisons for understanding the synteny were usually performed on relatively short sequences up to a few thousand nucleotides. However, the availability of whole-genome sequences offers excellent opportunity to compare entire genomes in order to have a better understanding of the large-scale structural and functional features of genomes and the evolutionary forces such as duplications, recombination, and rearrangements that have impacted these features. Currently, the availability of whole genomes of many plant species and the enormity of sequence lengths has pushed researchers to perform multiple comparisons to get maximum information. But, the comparison of two genomes with several billion nucleotides itself is a major challenge computationally coupled with the poor understanding of the

sequences and lack of a suitable evolutionary model. To overcome this constraint, the computational scientists defined conserved sequence markers (well-understood coding regions of the genome) to form large-scale patterns that provide alternative representations of the genomes. Synteny blocks are the simplest representation that indicates conserved large sequence blocks across the species as revealed by common markers and similarity of patterns. According to Ghiurcuta and Moret (2014), synteny blocks (i) confer robustness across individuals and sources of error, (ii) reduce the dependence on an accepted sequence evolution model, (iii) reduce the complexity of the analysis, (iv) provide superior features for additional evolutionary studies, and (v) identify specific target regions for detailed studies.

Before proceeding further, it is necessary to understand clearly the meaning of some important terms associated with synteny analysis. The term "synteny" is derived from the Greek word *syntainia* meaning "on the same ribbon," which means genomic segments located on the same linkage group/chromosome. These segments may be genes or markers or any other nucleic acid or protein sequence. Synteny blocks are the segments in the genome that extend up to several kilobases to a few megabases and consist of an orthologous set of genes sharing the same gene order or collinearity between two species (Zeng et al. 2008). The genes within a synteny block share common functions and are often coregulated. Synteny or syntenic segment refers to a synteny block where the order of genes or markers is preserved. In addition, a qualitative distinction is sometimes drawn between macrosynteny and microsynteny. *Macrosynteny* refers to the preservation of synteny in large chromosomal segments (50 cM/Mb or less) whereas *microsynteny* refers to the preservation of synteny in small chromosomal segments (0.5 cM/Mb or less) or only a few genes at a time in two different species.

Synteny blocks may be complex when they include different functional clusters and topological arrangements. They may be classified into conserved and nonconserved blocks, referring to the block of genes with preserved gene order devoid of mismatch within the block and with a mismatch within the block, respectively. Synteny blocks enable comparisons across broad ranges of genomes because they filter out much of the individual variability, specify target regions for detailed analysis, and assist comparisons of the whole genome through visualization tools. According to Wei et al. (2002), the key criteria of the syntenic regions include: (i) the length of syntenic regions and proportion of sequence similarity between conserved syntenic regions; (ii) the proportion of genomic sequences constituting the syntenic regions; (iii) the genomewide distribution of these regions; (iv) the gene content, gene density, and gene order of such regions; and (v) repeat content. Therefore, identification of synteny blocks is vital to comparative genomics because it may provide clues for genes and regulatory element arrangements involved in biological processes (Zeng et al. 2008; Vergara et al. 2009; Vergara and Chen 2010). Synteny blocks were identified earlier using ad hoc methods, which were slow, not reproducible, ignored conservation of gene order and orientation, and were not suitable for general applications. Therefore, computational approaches gained popularity because they are more effective and rapid in the identification of synteny blocks through the implementation of efficient algorithms. The identification of markers (highly conserved short sequences) is essential for the detection of synteny blocks as well as for the alignment of complete genomes. The construction of synteny blocks uses subsets of markers, mostly genes, for the detection of synteny blocks whereas genome alignment employs the markers as anchors, especially scaffolds, maximum unique matches, genes, and assembly contigs (Ghiurcuta and Moret 2014).

3 Computational Tools Employed for Synteny Analysis

The growing availability of completed as well as assembled genome sequences of several plant species has led to the development of algorithms and tools for comparative genome analysis, for example, pairwise and multiple alignment of assembled and whole genomes. Visualization of

sequence alignment is very important to identify functional elements such as exons (reviewed in Ureta-Vida et al. 2003; Freeling and Subramaniam 2009), study genomewide rearrangements and genome evolution (Drosophila 12 Genomes Consortium 2007), and align draft and reference genomes (Richter et al. 2007), which have to be represented graphically. Alignment is followed by the identification of conserved synteny, which is defined as the conserved genomic location of several genes (Gregory et al. 2002) representing potential functional regions. Among the various representations available for the graphic depiction of synteny at the whole-genome level, two-dimensional "dot plots" are generally used in the depiction of local conservation. Dot plots are useful in the identification of genome rearrangement and duplication as off-diagonal lines and horizontally or vertically stacked identical lines, respectively. In addition to analyzing the synteny between completed genomes, dot plots are useful in genome assembly and finishing. Pill-shaped ideograms of chromosomes reveal bands that are color-coded to represent the aligned region of chromosomes on the reference genome. Even though synteny depicted by ideogram does not reveal the physical location of aligned regions on the reference genome, the aspect of color-coding offers easy visualization revealing the redistribution of the compared genome across the reference genome. However, the most attractive and visually pleasing way of representing genomic synteny was introduced in the tool, Circos (Krzywinski et al. 2009), which represents two or more genomes as arcs in a circle, avoiding the visual confusion that would result from linear representation. Many tracks can be aligned as inner circles along the genomes. Table 1 gives details about various databases that are useful for performing synteny analysis of sorghum with other related crop species and Table 2 gives the details about the software/computational tools used for synteny analysis.

A comparison of multiple "subgenomes" derived from genome duplications as in the case of plants needs a shift from the more conventional one-to-one syntenic comparisons used in

Table 1 Comparative genomics databases useful for sorghum

Database	Description	Availability
Gramene	Comparative Resource for Plants	http://www.gramene.org/
Phytozome	The Plant Genomics Resource	http://www.phytozome.net/
GreenPhylDB	Web Resource for Comparative and Functional Genomics in Plants	http://www.greenphyl.org/cgi-bin/index.cgi/
CoGE	Accelerating Comparative Genomics	https://genomevolution.org/CoGe/
PLAZA	Comparative Genomics in Plants	http://bioinformatics.psb.ugent.be/plaza/
CSGRqtl	Comparative QTL Database for Saccharinae	http://helos.pgml.uga.edu/qtl/
$(GIGA)^n$ DB	Genome data from sweet and grain sorghum	http://gigadb.org/dataset/100012
PIP	Potential Intron Polymorphism Database	http://ibi.zju.edu.cn/pgl/pip/
MOROKOSHI Sorghum Transcriptome Database	RIKEN full-length cDNA clone and RNA-Seq data in *Sorghum bicolor*	http://sorghum.riken.jp/morokoshi/Home.html
GRASSIUS	Grass Regulatory Information Services	http://grassius.org/
MiRBase	The microRNA Database	http://www.mirbase.org/
PMRD	Plant microRNA Database	http://bioinformatics.cau.edu.cn/PMRD/
NRDR	Noncoding RNA Databases	http://www.ncrnadatabases.org/

Table 2 Important computational tools useful for comparative genomics and synteny analysis

Tool	Features	Reference	Availability
DAGchainer	Identifies chains of gene pairs that share conserved order representing segmental duplications within a genome or syntenic regions between related genomes	Haas et al. (2004)	http://dagchainer.sourceforge.net/
ACT: Artemis Comparison Tool	Displays pairwise comparisons between two or more genomes and associated annotations. Useful to identify and analyze regions of similarity, insertions, and rearrangements between genomes at the whole-genome to base-pair level	Carver et al. (2005)	http://www.sanger.ac.uk/science/tools/artemis-comparison-tool-act
SynBrowse	Useful for visualization and analysis of genome alignments both within and between species and studying macro-, microsynteny, and homologous genes	Pan et al. (2005)	–
AutoGRAPH	Identifies conserved segments and breakpoint regions, analyzes marker/gene order conservation, constructs synteny maps, and detects chromosomal rearrangements	Derrien et al. (2007)	http://autograph.genouest.org/
Cinteny	Useful for synteny analysis with different sets of markers and levels of synteny blocks. Computes reversal distances by the comparison of multiple genomes automatically and detects synteny blocks	Sinha and Meller (2007)	http://cinteny.cchmc.org/
SynMap	Generates a syntenic dot plot between two genomes, identifies syntenic regions, detects putative genes or regions of homology, and sets of collinear genes or sequences exhibiting similarity	Lyons et al. (2008)	https://genomevolution.org/CoGe/SynMap.pl
OrthoCluster	Identifies the complete set of synteny blocks using annotated gene sets of candidate genomes and pairwise orthologous relationships. Useful in the detection of genome rearrangements as well as segmental duplication in a genome	Zeng et al. (2008)	http://genome.sfu.ca/projects/orthocluster
EDGAR	Analyzes large groups of related genomes and identifies orthologous genes, classifies them as core genes or singletons	Blom et al. (2009)	https://www.uni-giessen.de/fbz/fb08/Inst/bioinformatik/software/EDGAR
MizBee	Explores the relationship types such as proximity/location, size, orientation and similarity/strength, and the four scales of genome, chromosome, block, and genomic feature	Meyer et al. (2009)	http://www.cs.utah.edu/~miriah/mizbee/Overview.html
MicroSyn	Useful in the detection of microsynteny in genomic regions surrounding genes in gene families by searching for conserved, flanking collinear gene pairs between two genomic fragments	Cai et al. (2011)	http://fcsb.njau.edu.cn:8044/microsyn/
SyDiG	Identifies microsyntenic regions, extension of homologous boundaries, and reconstruction of synteny blocks	Jean and Nikolski (2011)	–
GSV	Useful in interactive visualization of the synteny between two genomes by uploading the files that contain synteny regions. The users can upload annotation files for the visualization of annotated regions	Revanna et al. (2011)	http://cas-bioinfo.cas.unt.edu/gsv/homepage.php

(continued)

Table 2 (continued)

Tool	Features	Reference	Availability
SyMAP v3.4	The original SyMAP (Synteny Mapping and Analysis Program) was developed to detect synteny blocks between a genome and a FPC map. In SyMAP v3.4, this has been extended to detect synteny blocks between pairs of sequenced genomes.	Soderlund et al. (2011)	http://www.agcol.arizona.edu/software/symap/
MapSynteny	A macro in MS Excel® useful for generating images revealing the relationship between genetic maps and large sequences such as scaffolds, chromosomes, BACs, and so on	Fernandez et al. (2012)	–
MCScanX	Analyzes chromosome structural changes and exposes the evolution of gene family expansions that might be involved in the adaptation of lineages and taxa	Wang et al. (2012)	http://chibba.pgml.uga.edu/mcscan2/
SynChro	Reconstructs synteny blocks between pairwise comparisons of multiple genomes, providing graphical outputs such as dot plots, chromosome paintings, and detailed synteny maps	Drillon et al. (2014)	http://www.lgm.upmc.fr/CHROnicle/SynChro.html
Crowsnest	A comparative map viewer useful for investigating genomewide chromosome organization as well as synteny between two or more plant genomes, designed to study synteny among genetic, physical, and BAC/BES-based maps	Nussbaumer (2014)	http://pgsb.helmholtz-muenchen.de/plant/crowsNest/index.jsp

vertebrate genomes because the synteny blocks between two plant genomes can be one-to-many or many-to-many based on the time of occurrence of whole-genome duplications (WGD) in the evolutionary history. The majority of software used for the identification of synteny blocks employs chaining or clustering of putative homologous gene pairs (Bowers et al. 2003; Haas et al. 2004; Soderlund et al. 2006; Simillion et al. 2008) and the synteny blocks thus identified are not differentiated based on their evolutionary origin and time, which is critical for further analysis downstream. This leads to ambiguous identification of syntenic orthologues inasmuch as they identify some blocks from false syntenic regions as well as those derived from shared ancient WGD. The QUOTA-ALIGN algorithm developed by Tang et al. (2011) helps in the identification of a subset of synteny blocks relevant to a particular evolutionary event by employing the expected quota known a priori by considering the evolutionary origin and time of past WGD events.

The general approach to study local synteny involves the alignment of "conservation tracks" to quickly identify conservation within an individual genome. However, the researcher cannot view the features within the reference genome as well as the genomic regions compared. To overcome this constraint, several tools were developed that involve stacking of track-like representations of a reference with the compared genomic regions and indicating the synteny by drawing lines between them. Features such as gene models or expressed sequence tags (ESTs) may be added to the aligned regions. In this approach, users can visually browse an alignment without disturbing the genomic annotations and conserved regions can be linked based on alignment, gene orthology, protein cluster (Crabtree et al. 2007), or even gene model (Wang et al. 2006; Brendel et al. 2007).

4 Synteny of Sorghum with Related and Model Genomes

4.1 Synteny Based on Molecular Maps and Markers

Among the grasses, rice, maize, and sorghum have become important model species for various genetic studies and for understanding the genome organization in the grasses. Prior to the availability of the complete genome sequences and the information on genome annotations, several studies focused on the analysis of synteny through the comparison of genetic maps with different marker systems. A comparison of the genetic maps of maize and sorghum has revealed large regions of collinearity between them (Hulbert et al. 1990; Binelli et al. 1992; Whitkus et al. 1992; Melake-Berhan et al. 1993; Pereira et al. 1994; Paterson et al. 1995; Dufour et al. 1996; Peng et al. 1999). Similarly, a comparison of sorghum and maize maps involving 201 restriction fragment length polymorphism (RFLP) loci revealed high correspondence of loci order and genetic distance between these two genomes. The majority of the loci showed linkage in maize (45 of 55) as well as sorghum with the presence of both conserved and rearranged locus orders (Pereira et al. 1994). Comparative genetic mapping of *Saccharum* with sorghum by Ming et al. (1998) revealed that one inter- and two intrachromosomal rearrangements differentiated sorghum from *Saccharum officinarum* as well as *S. spontaneum*. Comparison of the sorghum RFLP map with that of maize, rice, and oat revealed important sorghum–maize linkage group (LG) rearrangements and homologies such as LG-5 of maize and LG-G of sorghum possess more homologous genetic material than reported earlier, a segment of maize 5L and 5S are homologous to LG-A of sorghum, maize LG-4 and LG-6 are partly homologous to LG-E of sorghum, LG-4 and the short arm of LG-2 in maize are partly homologous to LG-G and LG-F of sorghum, respectively, and a short segment of LG-1 of maize is homologous to that of LG-I in sorghum (Peng et al. 1999). A comparison of genetic linkage maps of sorghum and rice based on a common set of 123 RFLP probes revealed that 69 % of the loci mapped were syntenic between rice and sorghum, of which, 84 % exhibited collinearity (Ventelon et al. 2001).

The majority of the markers on LG-1 of rice are present on the LG-G of sorghum and most of the markers in LG-A of sorghum are present on LG-8 of rice. The collinear linkage groups between sorghum and rice are LG-A = LG-8; LG-B = LG-4; LG-C = LG-3, LG-10, LG-7; LG-D = LG-2; LG-E = LG-5; LG-F = LG-7, LG-9; LG-G = LG-1; LG-H = LG-6; LG-I = LG-12; LG-J = LG-11, which suggested extensive conservation of gene order between these two genomes (Mahalakshmi and Ortiz 2001). Similarity searches of the reported RFLP markers for the four stay-green quantitative trait locus (QTL) regions (*Stg1*, *Stg2*, *Stg3*, and *Stg4*) against the rice genome resulted in the identification of syntenous regions for *Stg1* and *Stg2* on chromosome 1, for *Stg3* on chromosome 9, and for *Stg4* on chromosome 11 of rice. The synteny of these QTL regions with that of rice was experimentally established by the comparative mapping of 10 polymorphic genic-microsatellite markers of sorghum to the stay-green QTL positions (Srinivas et al. 2008). A new set of EST-derived simple sequence repeat (SSR) markers developed from 600 SSR-containing ESTs of sorghum selected based on their homology with 12 rice chromosomes were mapped across sorghum linkage groups, mostly to regions exhibiting synteny between rice and sorghum. This demonstrates the use of rice–sorghum synteny information for the targeted development of markers to saturate the linkage maps of related species (Ramu et al. 2009).

Comparative mapping of 327 eSSR markers revealed substantial amounts of sequence orthology and synteny between foxtail millet and sorghum (~68 %), maize (~61 %), and rice (~42 %) chromosomes, suggesting the occurrence of the nested chromosome fusion, a characteristic of grass genome evolution (Kumari et al. 2013). Similarly, the synteny relationships among the genomes of foxtail millet and other related species was established by the comparative *in silico* mapping of 15,573 SSR markers of foxtail millet against the mapping data of

sorghum, maize, and rice (Pandey et al. 2013). The presence of greater synteny of certain chromosomes of sugarcane to sorghum than others was revealed by the comparative mapping study by Aitken et al. (2014) in which diversity array technology (DArT), single nucleotide polymorphism (SNP), and EST-SSR markers were used. Good collinearity was observed in four out of eight homology groups (HGs), whereas four major chromosome rearrangements were observed between the other four HGs, two of which were chromosomal condensations leading to the reduction in the basic chromosome number of sugarcane from $x = 10$ to $x = 8$.

In addition to the use of genetic maps for understanding the synteny between sorghum and other related crop species, the information on the mapped QTL or genes was also used in several studies. One such example is the aluminum tolerance (*AltSB*) in sorghum. Even though a significant macrosynteny was observed for DNA markers associated with putative orthologous Al tolerance loci in the group 4 chromosomes between Triticeae (wheat, barley, and rye) and sorghum, the *AltSB* locus was mapped towards the end of chromosome 3 in sorghum and was more likely to be analogous to the Al tolerance QTL on chromosome 1 of rice as revealed by intertribal map comparisons (Magalhaes et al. 2004). Another study by McIntyre et al. (2005) identified several clusters of resistant gene analogues (RGAs) that were syntenic between sugarcane and sorghum, the prominent ones being three RGAs associated with brown rust that were mapped to the same linkage group in sorghum. Again, while comparing sorghum with sugarcane, Ritter (2007) concluded that the sorghum chromosomes (SBI-01, SBI-03, and SBI-05) possessing QTL for sugar-related traits, also contained sugarcane SSRs from three homology groups (2, 3, and 4), which possess a robust QTL for sugar-related traits. This resulted in the identification of similar loci in sorghum and sugarcane that are associated with sugar-related traits. A comparison of rice and sorghum revealed an inverse synteny relationship for a majority of the Ca^{2+} transporter genes present on Chr 1 and Chr 3 and these transporter proteins are highly conserved between these two species (Goel et al. 2011).

Three heterotic trait loci (HTL) with synergistic intralocus effects for grain yield heterosis were identified in sorghum by Ben-Israel et al. (2012). Among these, two exhibited synteny with an overdominant QTL for grain yield reported earlier in maize. Resistance to grain shattering in *Sorghum propinquum*, a wild relative of sorghum is conferred by a WRKY transcription factor (*SpWRKY*), and the *YABBY* locus confers shattering resistance in sorghum and other cereals. However, the shattering allele (*SpWRKY)* might have evolved recently in the wild relative and lies only 300 kb apart from the *YABBY* locus (Tang et al. 2013). Synteny analysis of 19 branching-related genes of rice revealed eight homologues in sorghum present in syntenic regions within the identified branching-related QTL regions on chromosomes 1, 3, 7, and 8 (Kong et al. 2014). Another synteny analysis by de Setta et al. (2014) revealed that the larger size of the sugarcane genome relative to the sorghum genome is mainly due to the accumulation of transposable elements and other uncharacterized intronic and intergenic regions.

4.2 Synteny Based on Whole Genome

Model genomes are the genomes of organisms possessing a wealth of biological data, which makes them examples for the study of other species and/or natural phenomena that are otherwise difficult to study directly. *Arabidopsis thaliana* (mouse-ear cress) is a dicot plant and a popular model organism in plant biology and also the first plant genome to be sequenced (The Arabidopsis Genome Initiative 2000) and extensively used for dissecting the genetic, cellular, and molecular biology of flowering plants. The Poaceae is an important family of agricultural importance inasmuch as it includes staple food crops such as rice, maize, wheat, barley, and sorghum, as well as industrially important grasses such as switchgrass and *Miscanthus* that are useful for lignocellulose biomass production. Rice is a model species of monocot plant,

whereas maize is a model organism for basic and fundamental research as well as for studying domestication, epigenetics, evolution, chromosome structure, and transposable elements. Sorghum, being a representative of Saccharinae, is a model organism for biomass plants for starch, sugar, and cellulose. *Brachypodium* is emerging as a model plant for Triticeae and also for understanding biomass production systems in grass species. Since the sequencing of the rice genome (International Rice Genome Sequencing Project 2005), complete genome sequences of sorghum (Paterson et al. 2009), maize (Schnable et al. 2009), *Brachypodium* (The International Brachypodium Initiative 2010), barley (The International Barley Genome Sequencing Consortium 2012), and wheat (The International Wheat Genome Sequencing Consortium 2014) have been sequenced. Generally, grasses are an attractive system for comparative genomic studies due to high levels of genome collinearity (Moore et al. 1995) and the plant family with extensively sampled whole-genome sequences (Schnable and Lyons 2011).

Being a tropical grass with C_4 photosynthesis, sorghum is considered an important model for studying the structure, function, and evolution of cereal genomes. Moreover, it is an attractive model for functional genomics studies due to its lower level of gene duplication as compared to cereals such as rice and maize. In spite of the low level of gene duplications, sorghum is much more closely related to complex genomes of maize and sugarcane than rice. Sorghum and maize diverged from a common ancestor ~12 Mya (Gaut et al. 1997; Swigonova et al. 2004), whereas rice and the maize/sorghum lineage dates back to ~42 Mya (Paterson et al. 2004). Sugarcane is closer to sorghum having similar gene order (Ming et al. 1998) and the lineage dates back to ~5 Mya (Sobral et al. 1994). Whole-genome duplication (WGD), commonly known as polyploidy, is a vital component of evolutionary novelties (Ohno 1970; Lynch and Conery 2000; Adams and Wendel 2005; Soltis et al. 2009) and found commonly in the evolution of flowering plants, which have experienced paleopolyploidies (Paterson et al. 2009; Schnable et al. 2009; The International Brachypodium Initiative 2010; Wang et al. 2011; D'Hont et al. 2012; Singh et al. 2013). In Poaceae, genomes of rice and sorghum diverged with the first WGD in the ancestral lineage and subsequent diploidization, throwing conservation of synteny to large stretches of genome involving thousands of orthologous genes (Paterson et al. 2009). Subsequently, since its divergence from sorghum, maize has experienced one WGD (Swigonova et al. 2004) whereas sugarcane has experienced at least two WGDs (Ming et al. 1998).

4.2.1 Sorghum–Rice Synteny

Rice has a unique distinction of being the first monocot plant species whose genome has been sequenced. Being a model crop and belonging to the family Panicoideae of which sorghum is also a member, it will be interesting to study the extent of synteny between these two crop species. Initially, extreme collinearity of sorghum was observed with seven linkage groups of rice in addition to major translocations, which is a characteristic of Panicoideae (Devos and Gale 1997). The conserved sorghum–rice microsynteny was distributed in chromosomal regions excluding heterochromatic (Feltus et al. 2004) and pericentromeric regions (Cheng et al. 2001; Jiao et al. 2005). This lack of conserved synteny in pericentromeric regions of rice and sorghum is due to increased recombination and physical length of chromosomes in sorghum (Bowers et al. 2005). About 19,929 gene models of sorghum in the blocks were collinear with rice whereas only a single copy was retained for 13,667 collinear genes after the shared WGD. However, both these plant species retained copies of 4912 genes and one copy of 1070 genes was lost in sorghum (Paterson et al. 2009). In the same study, a duplicated segment was identified on chromosomes 5 and 8 of sorghum, which was also found in the corresponding segmental duplication on chromosomes 11 and 12 of rice (The Rice Chromosomes 11 and 12 Sequencing Consortia 2005; Wang et al. 2005; Yu et al. 2005). According to Bowers et al. (2005), the sorghum genetic maps represent about 80 % of single-copy genes that exhibited

conserved microsynteny with rice, corresponding to the cytologically demarcated "euchromatin" region. Greater microcollinearity was observed in euchromatic regions as compared to heterochromatic regions and the distribution of conserved microsynteny between these two crop species does not include heterochromatin (Feltus et al. 2004) and pericentromeric regions (Cheng et al. 2001; Jiao et al. 2005). The conservation in gene order also corresponded to ancient WGD regions (Kishimoto et al. 1994; Nagamura et al. 1995; Goff et al. 2002; Paterson et al. 2004; Wang et al. 2005) with 57 % of duplicated regions in rice exhibiting conserved synteny with sorghum contigs (Paterson et al. 2004).

The sequencing of the sorghum genome by Paterson et al. (2009) revealed the conservation of specific domains between sorghum and rice, as follows. First, the frequency of nucleotide-binding-site–leucine-rich-repeat (NBS-LRR) containing proteins in sorghum was only about half that of rice, most of them coding for the CC type of N-terminal domains. However, two sorghum genes (Sb02g005860 and Sb02g036630) possessed the TIR domain whereas the NBS domain was not present in these genes. The abundance and conservation of NBS-LRR genes were observed on sorghum chromosome 5, and its homologue in rice chromosome 11. Second, the C_4 photosynthesis in sorghum was evolved as a result of the reorientation of progenitor genes of the C_3 system and also due to the inclusion and functional divergence of gene duplication of ancient as well as recent origin, which was confirmed by the fact that the solitary C_4 *pyruvate orthophosphate dikinase (ppdk)* and the *phosphenolpyruvate carboxylase kinase (ppck)* genes along with two isoforms in sorghum have only a single orthologue in rice. The cell wall biogenesis genes such as *CslF* and *CslH* are conserved between sorghum and rice. Last, the conservation of one miRNA and many gene families and their expansion in sorghum as compared to rice may be one of the important factors associated with the drought adaptation in sorghum. This is emphasized by the presence of five homologues of rice miRNA 169g, associated with drought stress, in sorghum. Abundance of Cytochrome P450 domain-containing genes in sorghum as opposed to rice (326 vs. 228) and also the increased number of copies of gene coding for the enzyme class, Expansins in sorghum than in rice (82 vs. 58) further support this hypothesis.

4.2.2 Sorghum–Maize Synteny

Maize is considered as an excellent model system for fundamental research to understand the inheritance of genes, the gene to chromosome linkage, the mechanism relating cytological crossovers and recombination, and the characteristics of telomeres, epigenetics, and transposition (Bennetzen 2009). Several rounds of genome duplication and an additional WGD event (Blanc and Wolfe 2004; Swigonova et al. 2004) differentiate maize from sorghum, its close relative. However, the gene orders were largely conserved between these two plant species with a limited number of rearrangements (Devos and Gale 1997). A draft genome sequence of the maize genotype B73 published by Schnable et al. (2009) revealed that the size of exons of maize genes were comparable to their orthologous counterparts in sorghum, even though the former possessed large introns due to insertion of repeats (Wei et al. 2009; Haberer et al. 2005). They also found that the genome of sorghum and maize contained similar numbers of gene families, of which, 405 were shared between these two crop species. Visual comparisons revealed a greater extent of coalignment between sorghum and maize, even in centromeres harboring undermethylated repeats as compared to the heterochromatin surrounding them (Palmer et al. 2003; Zhang et al. 2008). Numerous chromosomal disruptions and fusions were associated with the return of the maize genome to a genetically diploid state after the WGD. However, since its divergence from rice, the chromosomal constitution of sorghum resembles two ancestral subgenomes of maize (Wei et al. 2007) due to the occurrence of comparatively few interchromosomal rearrangements (Swigonova et al. 2004) of two subgenomes of maize. The duplicate regions

in maize were explained by the cosynteny of its genes with the reference genes shared by sorghum and rice.

According to Abrouk et al. (2010), the genomes of maize and sorghum evolved from an intermediate ancestor with 12 chromosomes through two chromosomal fusions resulting in a Panicoideae ancestor ($n = 10$). Maize and sorghum subsequently evolved independently from this ancestor. Although the sorghum genome organization was similar to the ancestral genome ($n = 10$), the maize genome underwent a WGD event, resulting in an intermediate genome ($n = 20$) followed by numerous chromosomal fusions leading to the current genome structure ($n = 10$). The sequencing of the maize genome also helped in the identification of the origin of the duplicated regions in comparison with sorghum, which also diverged from the common ancestor. The original duplicated regions in the maize genome were reconstructed based on its orthology to the sorghum chromosomes by Schnable et al. (2011) using whole-genome dot plots. Because inversions and intrachromosomal rearrangements are considered to occur more commonly between different chromosomes, the maize chromosomal segments orthologous to the same sorghum chromosome might have been acquired from the same chromosome in the tetraploid ancestor. Both full ancestral copies in the maize genome were reconstructed for the five sorghum chromosomes but only one full ancestral copy was reconstructed for the remaining five chromosomes of sorghum.

4.2.3 Sorghum–Sugarcane Synteny

Sugarcane is an important biofuel crop belonging to the Saccharinae subtribe of which sorghum is also an important member, exhibiting remarkable characteristics associated with bioenergy production. Comparative mapping of 17 out of 31 sugarcane RGAs in sorghum revealed synteny of several RGA clusters (McIntyre et al. 2005). Three sugarcane RGAs associated with brown rust resistance are mapped to the same linkage group in sorghum: one is mapped to a region containing a major QTL for rust resistance in sorghum, and the remaining two are mapped to another region. A comparison of 454 pyrosequences of 20 bacterial artificial chromosomes (BACs) of sugarcane selected by the hybridization of 1961 single-copy sorghum probes with sorghum sequences revealed that a mean of 95.2 % sequence identity was shared by the genic regions of the sugarcane BACs, and about 53.1 % of them were aligned with the sorghum sequence (Wang et al. 2010).

Alignment of 378 bidirectional BAC end sequences (BESs) corresponding to 42 BACs of sugarcane to the sorghum genome sequence by Figueira et al. (2012) revealed that about 84 BESs were aligned with at least one of the chromosomes of the sorghum genome in a concordant manner (Class I), confirming the presence of sugarcane/sorghum synteny. However, a set of 88 BESs were aligned to the same sorghum chromosomes in a discordant manner in the same orientation, indicating that these sugarcane genomic regions might have been inverted, expanded, or reduced during the divergence of sugarcane/sorghum. Sugarcane chromosomes exhibited varied levels of synteny with sorghum (Aitken et al. 2014). They noticed a good collinearity for four out of the eight homology groups (HGs) between these two crop species, and four major chromosomal rearrangements were observed between the remaining HGs of sugarcane and sorghum. Among these, two were condensations resulting in the reduction of the basic chromosome number (x) of sugarcane from 10 to 8. Among the sugarcane HGs, HG1, HG3, HG4, and HG8 were highly syntenic to Sb4, Sb3, Sb1, and Sb8, respectively.

A total of 349 unique genes and 228 intergenic regions were identified in sorghum by de Setta et al. (2014) from 122 synteny blocks from 98 BACs of sugarcane. Even though the number of bases of exons was similar in sugarcane (0.446 Mb) and sorghum (0.449 Mb), the synteny blocks were 3.533 and 1.990 Mb long in these crops, respectively. This indicated that introns, the promoter, and intergenic regions may be the reason behind the 1.543 Mb larger syntenic region in sugarcane as compared to sorghum. In addition, the repeat content in sugarcane was more than double that of

sorghum, which accounted for the expansion of the sugarcane genome, especially transposable elements. The de novo transcriptome assembly of six genotypes of sugarcane used in biparental crosses by Cardoso-Silva et al. (2014) resulted in 72,269 unigenes, which exhibited significant similarity to >28,788 proteins with sorghum. This unigene set also included 5272 unigenes, the majority of them probably the genes that are not described in sugarcane.

4.2.4 Sorghum–Foxtail Millet Synteny

A draft genome sequence of foxtail millet published by Zhang et al. (2012) revealed that about 32,701 genes among the 38,801 genes predicted in the draft genome using an integrated annotation pipeline, exhibited homology with that of sorghum, which accounted for 84.3 %. A total of 19 large collinear blocks was identified between foxtail millet and sorghum genomes, which indicated the presence of 72.1 % collinearity between them. The presence of such a high conserved collinearity confirmed a close evolutionary relationship between sorghum and foxtail millet. With respect to the three nested chromosome fusions in foxtail millet in relation to rice, chromosomes 2, 3, and 9 of foxtail millet were collinear with chromosomes 7 and 9, 5 and 12, and 3 and 10 of rice, respectively. Among these, two were also involved in the evolution of sorghum, indicating that these two fusion events might have occurred prior to the divergence of sorghum and foxtail millet. However, the fusion event involving chromosomes 8 and 9 of sorghum with chromosome 3 of foxtail millet might have occurred after divergence of foxtail millet from sorghum. The phylogenetic analysis among the grasses revealed that the foxtail millet diverged from sorghum and maize ~27 Mya and the WGD in foxtail millet preceded this event. About 24,722 (65.2 %) genes of foxtail millet were in collinear blocks with the genes of sorghum. Analysis of the carbonic anhydrase genes involved in the CO_2-concentrating process of C_4 plants revealed that a gene cluster was formed by the CAα and CAβ genes on chromosome 5 (~27 Mb) in a collinear block with their homologous counterparts in chromosome 3 of sorghum (~57.3 Mb). In the low-recombination regions, a clustered 155-bp repeat unit was identified, which exhibited sequence similarity to the 140-bp centromere elements in sorghum (Murat et al. 2010). This repeat unit is likely to be a constitutive, centromere-related element.

4.2.5 Sorghum–Brachypodium Synteny

Brachypodium, is a wild annual grass belonging to the third most economically important subfamily of Poaceae, namely, Pooideae. The availability of a complete genome sequence is a valuable functional genomics resource for the grasses, which also allows the whole-genome comparisons of the members of the other two economically important subfamilies of Poaceae: rice, maize, sorghum, sugarcane, and millet. A comparison of the whole-genome sequence of *Brachypodium* (The International Brachypodium Initiative 2010) to that of the sorghum genome resulted in revealing the valuable information on the extent of synteny between these two plant species. Phylogenetic trees constructed using 62 gene families from sorghum revealed that the distribution of genes in *Brachypodium* was similar to that of sorghum, highlighting the importance of *Brachypodium* for the functional genomics studies in grasses. The evolutionary relationship between *Brachypodium* and sorghum determined using the mean synonymous substitution rates of gene pairs that were orthologous indicated that *Brachypodium* diverged from sorghum 45–60 Mya. Fourteen major syntenic interruptions were identified between *Brachypodium* and rice/sorghum, which might have occurred due to the nested whole-chromosome insertions in centromeric regions (Kellogg 2001; Srinivasachary et al. 2007; Luo et al. 2009). Similar nested insertions were also observed in sorghum (Luo et al. 2009). Four of the relatively large gene families of *Brachypodium* exhibited greater synteny with sorghum although two (NBS-LRR and F-box) were not in syntenic order, consistent with the rapid diversification of these two gene families (Meyers et al. 2003). The Bd5S portion of the *Brachypodium* genome acquires retrotransposons by

replication, loses few by recombination, and this along with Sb6S also houses the fewest possible collinear genes.

4.2.6 Sorghum–Bamboo Synteny

Bamboos are woody plants belonging to the family Poaceae (subfamily Bambusoideae), which also includes crops such as rice, maize, sorghum, and other cereals. Bamboos are considered as a unique member of the grass family inasmuch as they are woody plants and flower only once. Greater synteny was noticed between the bamboo BAC sequence GQ252886 and an orthologous region on sorghum chromosome 4 by Gui et al. (2010). Even though the putative kinase gene (Sb04g030710) in sorghum exhibited high similarity with Gene 6 of bamboo, only the 3′ region was retained in sorghum and the 5′ region was found to be pseudo due to the presence of in-dels/mutations. Similarly, a ~4 Kb potential insertion was observed in the gene of sorghum (Sb07g027320) that is syntenic to the bamboo gene (Gene 1 in GQ252869). Apart from rice, which is closely related to bamboo and considered to be the best reference genome for the comparative genomic studies in bamboo, the availability of complete genome sequences of sorghum (Paterson et al. 2009) offers an additional reference genome for bamboo.

5 Emerging Trends in Synteny Analysis

Even though many genomes of grass species belonging to the Poaceae family have been sequenced, a comparative analysis of their transcriptomes has been lacking, thereby limiting the information on the evolution and regulation of the core transcriptome of this family as well as the genes that are specific to this lineage. The recent emergence of high-throughput next-generation transcriptome sequencing technology is superior over earlier adopted expression profiling methods and provides better opportunities for the complete analysis of global gene expression patterns (Marioni et al. 2008; Nagalakshmi et al. 2008; Wang et al. 2009; Costa et al. 2010; Davidson et al. 2011).

In a first of its kind study on comparative transcriptomics of three Poaceae species, Davidson et al. (2012) identified a large number of syntenic gene pairs between rice and sorghum (13,033) compared to *Brachypodium* and rice (10,805) and *Brachypodium* and sorghum (9404). However, very few synteny blocks and largest average block size were observed between rice and sorghum, compared to *Brachypodium* and rice, and *Brachypodium* and sorghum. These observations highlight the karyotypic changes in the lineages of *Brachypodium* and sorghum involving multiple chromosome fusions and genome rearrangements compared to the 12-chromosome ancestral monocot genome, similar to rice (Salse et al. 2009; The International Brachypodium Initiative 2010). The study also revealed that orthologous genes upregulated in the physiologically similar tissues (leaves, anthers, and embryo) are more highly conserved than those in compound tissues (whole flowers and seeds). They observed that relatively small proportions of lineage-specific genes contribute to reproductive expression phenotypes compared with multitaxa genes, and that significant proportions of orthologous genes have been recruited to perform lineage-specific functions. The study further illustrated that genomic collinearity and conservation of expression patterns among orthologous gene pairs between species are correlated.

The genetic, phenotypic, and ecological diversity among plants is presumed to be due to the repeated polyploidization followed by diploidization through genomewide fractionation and structural rearrangements (Bowers et al. 2003; Blanc and Wolfe 2004; Adams and Wendel 2005; Freeling and Thomas 2006; Fawcett et al. 2009; Freeling 2009; Schnable et al. 2009; Van de Peer et al. 2009; Paterson et al. 2010;). Earlier, the analysis of WGD was phylogenetic and mostly based on the distribution of synonymous substitution rates (Ks) between duplicated gene pairs (Lynch and Conery 2000; Blanc and Wolfe 2004) or gene family tree topology

(Bowers et al. 2003). In this approach, the dating of WGD based on discriminating duplication and speciation tree topologies (Bowers et al. 2003) was affected by limited EST sampling data and variation in lineage evolutionary rate (Tang et al. 2008a). The more recent phylogenomic approach considers temporal signals whereas the synteny approach considers spatial signals (conservation of gene position and order) to identify WGD (Vandepoele et al. 2002; Haas et al. 2004; Tang et al. 2008b). However, integration of temporal and spatial signals is considered as it also integrates studies of genome structure and molecular evolution.

6 Future Prospects

The majority of the computational resources available for synteny analysis use genes as conserved sequence markers. However, the rapid sequencing of the plant genomes through next-generation sequencing offers excellent opportunity to compare complete genomes at once to achieve a better understanding of the large-scale structural features of genomes brought about by evolutionary events such as duplications, rearrangements, recombination, etc. This approach uses the maximum available data for the analysis. Compared to synteny block-based analysis, whole-genome-based synteny analysis is computationally challenging and warrants refinement in the existing alignment and visualization tools. Stringent sequence alignment and statistical validation are extremely crucial to evaluate accurately whether the identified association between two or more genes of the same order on different chromosomes is by chance or due to conserved collinearity. Even though many algorithms and effective visualization tools are available in the public databases, these tools lack statistical testing in confirming the robustness of the relationships revealed by aligned sequences, which will immensely help in the tracking of the evolutionary mechanisms.

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7 Association Mapping and Genomic Selection—Where Does Sorghum Stand?

Pawan L. Kulwal

Abstract

Sorghum is cultivated as a staple cereal crop in the semi-arid regions of the world. Because of its drought-tolerance ability and high biomass production, it is preferred over other crops for fodder production. In addition to its use as fodder, it is also being used as a bioenergy crop as well as a sugar crop in sweet sorghum. In the last few years, because of advances in genomic techniques, large numbers of molecular markers have been developed in sorghum, which has enabled identification of several quantitative trait loci (QTLs) for various traits using different biparental mapping populations. However, deployment of these identified QTLs in a sorghum improvement program is still lacking as the genetic background in which they were identified does not always represent the breeding program. In recent years the newer approaches of quantitative genetics including association mapping (AM) and genomic selection (GS) has not only facilitated identification of QTLs in diverse genetic backgrounds but also the utilization of all these QTLs in genomic prediction in many crops. This has become possible due to the advancements in the area of computational biology. However, despite the availability of large genomic resources, the progress made in terms of numbers of studies related to AM and GS in sorghum are few, which is in contrast to the situation in other crops such as maize, wheat, and rice. This offers the opportunity for the application of these techniques in a sorghum improvement program. The progress made thus far and the scope available for the use of these two approaches in sorghum is discussed in detail in this chapter.

1 Introduction

Over the years, identification of loci controlling quantitative traits (QTLs) in a biparental mapping population and deployment of the QTL-linked marker(s) for use in marker-assisted

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selection (MAS) has been a common practice in crop improvement programs in crops including sorghum. Large numbers of QTLs for various traits have been identified in sorghum in different genetic backgrounds using this approach (Zou et al. 2013; Reddy et al. 2014; Wang et al. 2014) and some of these major QTLs and linked markers have also been used successfully in sorghum improvement (Franks and Mayor 2014; Mohamed et al. 2014). Further details about QTL mapping are available in earlier chapters. However, in the era of genomics the newer approaches of quantitative genetics including association mapping (AM) and genomic selection (GS) are becoming an integral part not only of animal breeding, but also in plants as well (Kulwal et al. 2012a). This has been made possible with the availability of a large number of molecular markers and the advances in the high-throughput and cost-effective genotyping technologies as well as progress in the area of computational analysis. The progress of these techniques in different crops has been unprecedented. Both these approaches of AM and GS are being used in many cereals including wheat, maize, rice, barley, and many other crops and have offered new insights into the genetics of different traits of economic importance (Crossa et al. 2010; Kulwal et al. 2012b; Poland et al. 2012; Gupta et al. 2013; Bentey et al. 2014; Xu et al. 2014; Sallam et al. 2015). Although the draft genome sequence of this important crop was published in 2009 (Paterson et al. 2009) and also despite the availability of genomic resources (Mace et al. 2013), the pace of studies including AM in general and GS in particular did not progress much in this crop. This is in contrast to other cereals such as maize and wheat. With its relatively smaller, diploid, and completely sequenced genome, sorghum is highly amenable to genomics-based breeding approaches (Bekele et al. 2013). In this chapter, the status of the AM and GS studies in sorghum is reviewed and the future possibilities for use of these techniques are also discussed.

2 Association Mapping

Association mapping, also known as linkage disequilibrium (LD) mapping, is a method of finding marker–trait (phenotype–genotype) associations (MTA) based on historic recombinations. Because of its ability to use the natural diversity and to search for functional variation in a broader germplasm, AM is becoming popular over the commonly used biparental QTL mapping and has specific advantages over the latter. These advantages have been discussed and explained in greater detail in several review articles (Zhu et al. 2008; Gupta et al. 2014). Although well-planned AM studies have been carried out in many crop plants, the majority being in maize, there are not very many reports of these studies in a crop such as sorghum. The pace with which these studies are being conducted has gained momentum only in the last 5 years and several aspects of sorghum breeding including morphological traits, grain quality parameters, biochemical factors, and biotic and abiotic stress tolerance have been studied. Details of these studies are given in Table 1. The initial studies were mostly aimed at finding population structure and the genetic diversity in a set of sorghum germplasm (Caniato et al. 2011; Bouchet et al. 2012). Association mapping can be carried out following either the approach of whole-genome AM or the candidate gene (CG)-based AM. The choice between these approaches depends upon the objective of the study as well as the genetic information available about the trait under study (Gupta et al. 2014). Both types of studies have been conducted in sorghum (Table 1).

2.1 Genomewide Association Mapping

Genomewide AM, also commonly called genomewide association study (GWAS), is often utilized when one is interested in finding all

Table 1 Details of genomewide association mapping studies carried out in sorghum

Sr. No.	Trait	Lines used	Marker type	No. of markers used	Reference
Genomewide studies					
1.	Eight morphological traits	377 diverse lines representing all major cultivated races	SSR	47	Casa et al. (2008)
2.	Brix, plant height	Diverse panel of 125 sorghums (mostly sweet)	SSRs + SNPs	47 + 322	Murray et al. (2009)
3.	26 morphological traits	107 accessions from 27 countries	SSR	98	Shehzad et al. (2009)
4.	Saccharification yield	202 accessions from the mini-core collection	SSR	703	Wang et al. (2011)
5.	Plant height, maturity	Mini-core collection of 242 accessions	SSR	703	Upadhyaya et al. (2012a)
6.	Kernel weight, tiller number	Mini-core collection	SSR	43	Upadhyaya et al. (2012b)
7.	Plant height	216 accessions of the mini-core collection	SSR	703	Wang et al. (2012)
8.	Drought tolerance	151 accessions	SSR	39	Besufekad and Bantte (2013)
9.	Sugar yield related traits	Synthetic population of 119 genotypes	SSR	51	Lv et al. (2013)
10.	Plant height, maturity	Mini-core collection	SNPs	14,739	Upadhyaya et al. (2013a)
11.	Anthracnose resistance	Mini-core collection	SNPs	14,739	Upadhyaya et al. (2013b)
12.	Saccharification yield	Mini-core collection	SNPs	14,739	Wang et al. (2013)
13.	Plant height components, inflorescence architecture	971 worldwide accessions	SNPs	265,487	Morris et al. (2013a)
14.	Flavonoid pigmentation traits	336 diverse accessions	SNPs (GBS)	265,487	Morris et al. (2013b)
15.	Grain polyphenol concentration	Global sorghum diversity panel of 381 genotypes	SNPs	404,628	Rhodes et al. (2014)

(continued)

Table 1 (continued)

Sr. No.	Trait	Lines used	Marker type	No. of markers used	Reference
Candidate gene-based studies					
16.	Photoperiod flowering time	219 inbred accessions mainly of the guinea race	SNPs	In the region of 6 genes	Bhosale et al. (2012)
17.	Kernel hardness, kernel weight, kernel diameter, crude protein, fat, P, acid detergent fiber, starch, and total digestible nutrients	Diverse panel of 300 sorghum accessions	Genomewide SNPs	1290 (genome wide) + 333 (candidate gene based)	Sukumaran et al. (2012)
18.	Aluminum tolerance	209 accessions from the landrace + 45 inbred lines	SSR	38	Caniato et al. (2014)
19.	Brassinosteroid and plant architecture	Diverse sorghum collection of 315 accessions	SNPs	263	Mantilla Perez et al. (2014)

genomic regions that may be involved in controlling the trait of interest (Gupta et al. 2014). Ideally, in any AM study, a set of diverse genotypes is chosen and genotyped with few unlinked markers (e.g., simple sequence repeat, SSR) from across the genome to find the existing population structure in the set of genotypes used. Knowledge of the underlying population structure can greatly help in reducing the number of spurious associations. In order to increase the suitability and efficiency of the set of genotypes representing diversity at the genotypic and phenotypic levels, community resources and mini-core collections have also been developed in sorghum for AM.

2.1.1 Community Resources and Mini-core Collection for Association Mapping in Sorghum

In one of the first attempts to develop a community resource for AM in sorghum, Casa et al. (2008) assembled a set of 377 sorghum accessions representing all major cultivated races comprising tropical lines from diverse geographic and climatic regions, and important US breeding lines and their progenitors showing substantial morphological variation. Based on the genotyping using 47 SSR marker loci, they reported that models accounting for population structure and kinship performed better than the model that did not (naïve approach). One of the interesting observations made in this study was that the optimal number of subpopulations used to correct for population structure was trait dependent. Similarly, an integrated genomic resource in the form of a nested association mapping (NAM) population was developed in sorghum by developing the backcross populations (BC_1F_4) derived from 56 different inbred lines (Jordan et al. 2011). Availability of a community resource as in the case of maize (NAM population) and sorghum as above are important for researchers (Mace et al. 2013) as one can use it to phenotype for any additional trait(s) in different environments and can use the available genotypic data to find the MTAs for the trait of interest. Although such community resources are valuable, they do not always represent the global diversity.

A total of more than 37,000 accessions are available in the ICRISAT sorghum genebank,

which offers the choice to select for the desired genotypes for analysis but at the same time makes it difficult to choose appropriately among them. To harness the maximum diversity among this huge set of genotypes, a core and a mini-core collection have also been developed (Upadhyaya et al. 2009). This mini-core collection (1 % of the entire collection) comprising 242 genotypes has been used extensively in the AM studies for various traits (Table 1). It is apparent that the same mini-core collection panel has been phenotyped for various traits and genotyped using different sets of markers, SSRs, and single nucleotide polymorphisms (SNPs) for performing the analysis (see later). Earlier, Dahlberg et al. (2004) also developed a core collection of 3011 accessions from a stock of over 40,000 sorghum accessions maintained at the US National Plant Germplasm System (NPGS). From this lot, a country-specific core collection of 352 accessions representing Sudan was also developed (Dahlberg et al. 2004).

2.1.2 Genomewide Association Mapping Using Mini-Core Collection

Wang et al. (2011) used 202 accessions from the mini-core collection to find significant MTAs for saccharification yield using the pool-based method wherein 703 SSR markers were first tested on the two contrasting pools (high and low) made by mixing DNA from eight genotypes having extreme values for the trait. The polymorphic markers between the pools were only used for genotyping the entire panel. Two markers, one each from chromosomes 2 and 4, were found associated with the trait. These two markers were located in the vicinity of the genes β-glucanase (*Bg*) and steroid-binding protein (*Sbp*). Saccharification is an important process in the production of biofuel and identification of significant markers associated with this trait is an important step towards improvement of this trait.

This pool-based method of AM has also been used in another study comprising the entire mini-core collection of 242 sorghum accessions along with the same set of 703 SSR markers as reported by Wang et al. (2011). Using these data, Upadhyaya et al. (2012a) identified a total of five markers associated with plant height and maturity following the pool-based method. The significant markers identified by them were previously reported to be linked with these two traits (plant height and maturity) in different genetic backgrounds. As sorghum is preferred for its fodder, which in turn is positively correlated to plant height and maturity, the results offer the possibility for utilization of these markers in a fodder improvement program. This pool-based two-stage method is simpler and faster and is a form of commonly used bulk segregant analysis (Michelmore et al. 1991). Using a much smaller set of 43 SSR markers and the mini-core collection, Upadhyaya et al. (2012b) identified one marker linked to kernel weight and two markers linked to tiller number in sorghum. Using the same set of 703 SSR markers and a total of 216 accessions of the mini-core collection as used by Upadhyaya et al. (2012a) for plant height, Wang et al. (2012) identified four markers associated with plant height on chromosomes 2, 6, and 9. Only two of the markers identified in this study are the same as reported by Upadhyaya et al. (2012a).

Making use of the same mini-core collection, Upadhyaya et al. (2013a) conducted another AM study for plant height and maturity using a total of 14,739 SNPs. In this study using six different models to evaluate the effects of population structure, a total of six loci linked to height and ten linked to maturity in at least two environments with at least two SNPs in each locus were identified. Of these 16 loci, 14 were present in close proximity to previously mapped height/maturity QTL in sorghum. Using the same genotypic data, Upadhyaya et al. (2013b) performed AM for anthracnose resistance. Using the mixed linear model (MLM) approach, a total of eight loci linked to resistance for anthracnose were identified, seven of which were found in the vicinity of the disease-resistance genes including the NB-ARC class of *R*-genes on chromosome 10, hypersensitive response-related genes (autophagy-related protein 3 on chromosome 1 and 4 harpin-induced 1 (*Hin1*) homologues on chromosome 8), and a RAV

transcription factor (part of *R*-gene pathway), in addition to an oxysterol-binding protein that functions in the nonspecific host resistance, and homologues of menthone:neomethol reductase (*MNR*) that catalyzes a menthone reduction to produce the antimicrobial neomenthol. Anthracnose is considered to be one of the important destructive diseases of sorghum and the significant markers identified in this study can potentially be useful in offering anthracnose resistance in sorghum.

Later, Wang et al. (2013) used the same AM panel and the marker dataset as above for association analysis of saccharification yield in sorghum and identified seven marker loci that were associated with the trait. Five of these identified loci were syntenic with regions in the maize genome that contain QTLs underlying saccharification yield and cell wall component traits.

In almost all these whole-genome studies based on the mini-core collection, the identified significant SSR markers colocalized with the earlier identified QTLs as well as with the known candidate gene. Although the same mini-core collection with a varying number of genotypes was used in several studies, different numbers of QTLs were identified for the same trait (Table 1). This also explains the reason why AM is rather sensitive so that addition or exclusion of few genotypes from the mini-core collection results in identification of different significant markers. However, the markers, which were found to be consistently associated with the traits in different studies, can be used in the crop improvement program.

2.1.3 Genomewide Association Mapping Using Diverse Collection

In addition to mini-core collection, diverse collections have also been used for AM in sorghum. Sorghum is cultivated for its diversified uses including ethanol production. In order to confirm the major QTL for total stem sugar (Brix), or any of the QTLs for height previously identified in other genetic backgrounds, Murray et al. (2009) performed AM in a diverse panel of 125 sorghums, mostly comprising sweet sorghums. This diverse panel was genotyped with 47 SSRs and 322 SNPs. Three significant associations for height and one for Brix were detected. They also identified three major groupings within the sweet sorghum, each with multiple subgroupings. In another such attempt to identify QTLs for 26 different morphological traits, Shehzad et al. (2009) used a sorghum diversity research set of 107 sorghum accessions from Asia and Africa. Using 98 SSR markers and three different models of association analysis, a total of 14 significant loci were identified to be associated with 12 different traits. Use of different models/criteria to identify QTLs can be an efficient way to detect reliable associations in the genomewide association studies (Gupta et al. 2014).

Drought is considered to be an important yield-limiting factor in sorghum. In order to identify the chromosomal regions associated with drought tolerance, Besufekad and Bantte (2013) conducted an association study in a set of 151 sorghum accessions using 39 SSR markers. A total of four SSR markers were found to be consistently associated with days to 50 % flowering, panicle exsertion, and grain weight per panicle. All four markers were reported to be associated with these traits in other studies. However, details regarding how the drought condition was imposed and how these loci influence drought stress have not been described in this study. Similarly, using a smaller set of a population of 119 genotypes comprising sweet and grain sorghum from China, the United States, and India and a set of 51 SSR markers, Lv et al. (2013) performed AM for three sugar yield-related traits including stalk sugar concentration, fresh stalk weight, and stalk juice weight. Different degrees of LD were observed in different regions, which ranged up to 7.31 cM. They reported four loci to be associated with these three traits, two of which were common for fresh stalk weight and stalk juice weight. However, the method used by them was the general linear model (GLM) which has its own limitations and there are chances that some of these significant associations can be false positive.

A comprehensive study on whole-genome AM for plant height components and inflorescence architecture was published by Morris et al.

(2013a), wherein a larger set of 971 worldwide accessions were genotyped using 265,487 SNPs generated following genotyping by sequencing (GBS). Several important loci for plant height and candidate gene for inflorescence architecture were mapped in the study. It was observed that sorghum has a complex population structure because of extensive ancient crop diffusion and can pose a challenge for conducting GWAS of agronomic traits. Using the subset of 336 accessions, another study was carried out on flavonoid pigmentation traits with the same set of markers (Morris et al. 2013b). It was found that for this trait GLMs could not precisely map *tan1-a*, a known loss-of-function allele of the *Tannin1* gene, with either a small (n = 142) or large association panel (n = 336). It was also found that controlling for population structure either using the Q matrix in GLM or using MLM can identify the association. It was also observed that a compressed MLM performs worse than the naïve GLM (Morris et al. 2013b).

In a rather interesting study, Rhodes et al. (2014) quantified the natural variation of health-promoting compounds including total phenols, proanthocyanidins, and 3-deoxyanthocyanidins in a global sorghum diversity panel of 381 genotypes. Of these, 308 accessions were from the Sorghum Association Panel (SAP) and an additional 73 accessions were selected based on the presence of pigmented testa using the US National Plant Germplasm System's Germplasm Resources Information Network. The GWAS study with 404,628 SNP markers identified novel QTLs (24 significant associations) for these traits, some of which colocalized with homologues of flavonoid pathway genes from other plants. This study is a comprehensive one as a large number of SNP markers were used and the identified QTLs were colocalized with the known genes.

From the whole-genome AM studies carried out in sorghum thus far (Table 1), it is observed that in the majority of these studies, either the number of markers used or the number of genotypes studied or both are very low, except for the studies employing a few thousand SNP markers. Moreover, in the majority of these studies, fewer SSR markers were used for analysis. Use of fewer genotypes and markers definitely influences the results of any AM study.

2.2 Candidate Gene-Based Association Mapping

In contrast to the whole-genome AM, where markers from across the genome are used to find the significant MTAs, candidate gene-based AM focuses on a particular gene to find the causal polymorphism. However, the major limitation with this approach is that in order to choose a potential candidate gene for study, researchers must already have an understanding of the mechanisms underlying the trait of interest. In recent years, candidate gene-based AM studies are preferred over the whole-genome studies because of their associated advantages (Gupta et al. 2014). Few candidate gene-based AM studies have been carried out in sorghum (Table 1).

Flowering time is a very important adaptive trait in any crop. In order to study the effect of polymorphisms within the genes putatively related to variation in flowering time on photoperiod-sensitive flowering in sorghum, a total of six genes were studied in a set of 219 sorghum accessions mainly of the guinea race from West and Central Africa (Bhosale et al. 2012). These six genes include *CRYPTOCHROME1* and *2* (*Cry1* and *Cry 2*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *GIGANTEA* (*GI*), *HEADING DATE 6* (*HD6*) and *Dwarf8* (*SbD8*). Significant associations between several SNPs with photoperiod response index for the genes *CRY1-b1* and *GI* were detected in this study. These significant markers could be utilized for development of functional markers for MAS in sorghum aimed at identifying genotypes having specific sensitivities to photoperiod.

Using the diverse panel of 300 sorghum accessions which included 251 lines from the sorghum conversion program as described by

Casa et al. (2008) and 49 important breeding lines and their progenitor from the United States, Sukumaran et al. (2012) conducted both whole genome as well as candidate gene-based AM study for 10 different grain quality traits. Of the 333 SNPs from the candidate gene regions used, a total of eight significant MTAs were detected including one SNP each in genes, namely starch synthase *IIa* (*SSIIa*) and starch synthase *IIb* (*SSIIb*) for kernel hardness (KH) and starch content, respectively, whereas one SNP in loci *pSB1120* was associated with starch content.

Sorghum is primarily cultivated in the tropical and subtropical regions of the world and the soils of these regions are known to be acidic and have a higher concentration of aluminum (Al) causing aluminum toxicity. Therefore Al toxicity represents a major constraint for crop production worldwide leading to significant yield losses (Caniato et al. 2011). Using a set of 252 accessions comprising 209 landraces and 45 inbred lines that are frequently used in breeding programs in the United States and Brazil, Caniato et al. (2014) performed the CG-based AM in the 24.6 kb region of chromosome 3 where the Alt_{SB} locus conferring Al tolerance is located. The result suggested that LD in the Alt_{SB} locus decreased much faster than the one reported in earlier studies in sorghum. The analysis further suggested the recent origin of Al tolerance mutations within Alt_{SB}. Similarly, Mantilla Perez et al. (2014) used the candidate gene approach in a diverse sorghum collection of 315 accessions to assess MTAs between brassinosteroid (BR) biosynthesis and signaling genes and six plant architecture traits. Out of the total of 263 SNPs from 26 BR genes that were tested, 73 SNPs were associated significantly with the phenotypes of interest and 18 of these were associated with more than one trait.

Although candidate gene-based AM studies have been conducted in sorghum and offered information about the key genes involved in controlling these traits, more such studies for various other traits are needed to unravel the genetics of these traits for their further utilization in allele mining programs.

3 Genomic Selection

The identification of specific genomic regions for the trait of interest using any of the QTL mapping approaches (either biparental or GWAS) and the use of the marker, which is linked to the desired QTL, is an important part of a MAS program. However, over the years it was found that the majority of the identified QTLs either through biparental mapping population or in a set of germplasm explained a relatively small proportion of the genetic variation for a quantitative trait. This little variation for the trait of interest was not expected to result in a significant increase in the rate of genetic improvement (Nicholas 2006) and therefore MAS has been less effective than was initially thought in the crop and animal improvement programs (Hayes and Goddard 2010). The use of MAS in plant breeding mostly has been constrained to simple monogenic traits (Xu and Crouch 2008) and the current MAS methods are better suited for manipulating a few major-effect genes rather than many small-effect genes (Dekkers and Hospital 2002). However, in nature, the majority of the traits of economic importance including yield are polygenic in nature and show poor heritability.

The two important points that limit the successful application of MAS are (i) QTLs that are detected using biparental populations through interval mapping are relevant only for those breeding programs which involve parents that differ for the QTL thus detected and therefore their application in the breeding program involving broader germplasm is mostly limited (Gupta et al. 2014); and (ii) statistical methods used to identify target loci and implement MAS have been inadequate for improving polygenic traits (Heffner et al. 2009). Moreover, for identifying a QTL with sufficiently larger effect by way of increasing the size of the population is not always feasible owing to the higher cost of phenotyping. This makes it necessary to use an alternative approach where the information from a large number of genetic markers is used simultaneously in the breeding programs. This

potential application of genomewide genetic marker information was originally proposed by Meuwissen et al. (2001) for use in animal breeding.

Genomic selection (GS) is basically a form of MAS, albeit on a larger scale wherein effects of the marker across the entire genome are estimated simultaneously to calculate genomic-estimated breeding values (GEBVs; Meuwissen et al. 2001). This is in contrast to the conventional MAS in which a relatively small number of genetic markers linked to the QTL are used in genetic evaluations. The GS involves calculating genomic-estimated breeding values (GEBVs) for a "breeding population" having only genotypic data based on a model that was trained from a "training population," having both genotypic and phenotypic data (Meuwissen et al. 2001). As the individuals from the breeding population are advanced based on the GEBVs, it is necessary to maximize the accuracy of the prediction, which in turn is positively influenced by the association of the training population with that of the selection candidates.

The technique of GS, which was originally proposed for use in livestock breeding (Meuwissen et al. 2001), does not depend on the prior knowledge of QTL effects. In other words, GS is different from MAS as in the latter case, only selected QTLs and the linked markers are used in the selection program. As all the markers are treated as QTLs, and the effect of all the markers is taken for calculating GEBVs, GS offers more promise than the MAS in plant breeding due to increased prediction accuracy of the models. The efficiency of predicting superior lines using GS is dependent upon several factors including the relationship between the training and the breeding populations, the number of generations that separate them, heritability of the trait(s), type and number of markers used, and the accuracy of phenotyping (Varshney et al. 2014).

The availability of thousands of genomewide molecular markers has made possible the use of GS for prediction of genetic values (GEBVs) in plants (e.g., Bernardo and Yu 2007; Piepho 2009; Crossa et al. 2010; Jannink et al. 2010). The results from the GS studies carried out thus far in different crops indicate that it can be an effective strategy for selecting among lines whose phenotypes have yet to be observed in the plant breeding programs. Based on these findings and the choice among different GS models, one can use the appropriate model for increasing the efficiency of the GS in sorghum breeding.

Empirical studies on GS carried out in important cereals such as maize and wheat have greatly facilitated the refinement of this technique for its successful application in crop improvement programs (Poland et al. 2012; Crossa et al. 2014; Lado et al. 2014). Although a lot of progress has been made in terms of genomic resources, computational tools involving the choice of appropriate model, reduction in the computational complexity of the analyses, minimum size of the training population, and other factors influencing GS (Desta and Ortiz 2014; Hickey et al. 2014), the progress of GS studies in sorghum is not encouraging. In fact there is no full-length published report thus far on GS in sorghum. This offers an opportunity for the use of GS in sorghum improvement, both in the public and private sectors. The approach of GS can potentially reshape the way plant breeding is being done by way of increasing genetic gains through appropriate prediction models thereby reducing the time required for the development of new cultivars through early selection. However, efforts are going on and some of them include: (i) in a recent study on GS for biomass traits a global collection of 976 sorghum accessions were genotyped using 0.72 million SNPs generated using GBS. Of this total collection, 300 most representative accessions were phenotyped for biomass-related traits and prediction models were established to predict the biomass-related traits of the remaining untested germplasm (Yu et al. 2014). As sorghum is cultivated for alternate uses including ethanol production, the study of biomass-related traits is an important aspect. More such studies are expected to yield better understanding of the prediction models to be used in the GS program in sorghum. There is no doubt that GS is a promising technique for crop improvement in the

years to come and can revolutionize the way plant breeding is being practiced. This will be benefited immensely by the availability of the high-throughput phenomics platforms.

4 Conclusions

Sorghum is an important crop for the resource-poor farmers of the tropical and sub-tropical regions of the world. Because of its drought-tolerance ability and varying uses, it is preferred over several other crops by marginal farmers. A huge wealth of germplasm accessions is available in this crop and in order to harness the potential of maximum diversity, a set of core and mini-core collections as well as community resources are also available. The advances in the genomics approaches and the constantly decreasing genotyping costs accompanied with new computational tools have offered immense scope and opened new avenues for AM and GS to be practiced in sorghum in the coming years. Successful utilization of these techniques in other crops including maize and wheat offers a lot of scope for their use in public as well as private sector sorghum improvement programs. This will be benefited with the high-throughput phenotyping platforms. As sorghum is used for diversified purposes, there is no doubt that AM and GS are going to be used a great deal in the sorghum improvement program.

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8 Genomics Approaches to Biotic Stress Resistance

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Abstract

Sorghum productivity around the world is influenced by a wide array of biotic and abiotic constraints, some of which cause significant economic losses. Great efforts have been made in the past to improve plant resistance to biotic stresses such as insect pests, diseases, and parasitic weeds through breeding and genetic engineering. However, limited success has been achieved owing to the genetic complexity of stress responses. The application of marker-assisted breeding and transgenics will help to address this challenge more effectively. Genomic approaches such as high-throughput sequencing and large-scale genotyping technologies have been used in germplasm diversity analysis, genetic linkage mapping, association studies, and marker-assisted selection to improve sorghum biotic stress resistance. This chapter presents genomics-assisted methods that have helped in understanding diversity in sorghum germplasm resources and resistance against major insect pests, diseases, and parasitic weeds. Furthermore, the chapter deals with the progress in identification and deployment of gene and quantitative trait locus (QTL) for biotic stress resistance.

1 Introduction

Sorghum is affected by various biotic factors including insect pests, diseases, and parasitic weeds. Nearly 150 species of insect pests have been reported in sorghum, of which sorghum shoot-fly (*Atherigona soccata*), aphids (*Melanaphis sacchari*, *Melanaphis sacchari*, and *Rhopalosiphum maidis*), and sorghum midge (*Stenodiplosis sorghicola*) are the major pests worldwide. The pest management systems rely on an array of different control approaches:

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genetic, physical, chemical, cultural, and biological, among others. Diseases either alone or in combination cause significant economic losses annually (Thakur et al. 2011). The most important diseases affecting sorghum productivity around the world are the grain molds, downy mildew (*Peronosclerospora sorghi*), and sorghum rust (*Puccinia purpurea*). Among the parasitic weeds, *Striga* spp. is the most limiting biotic constraint for sorghum production throughout most of Africa. Yield losses due to *Striga* might reach up to 100 % in heavily infested soils. *Striga* management is difficult, although a number of control measures such as crop rotation, intercropping, transplanting, soil and water management, and hand weeding have been suggested. Host-plant resistance has been an effective means to manage the biotic constraints reducing yield losses in sorghum.

2 Host-Plant Resistance to Insect Pests

2.1 Aphids

The family Aphididae comprises more than 4300 species, all of which are specialized to feed on phloem sap. Aphids feed specifically from the sieve element and cause damage by draining plant nutrients. They also are major vectors of plant viruses. They are crucial agricultural pests injuring nearly all cultivated crops, and almost every major crop is a host for at least one aphid species, some of which are among the world's most serious agricultural pests (Goggin 2007). Greenbug (*Schizaphis graminum*), sorghum aphid (*Melanaphis sacchari*), and corn leaf aphid (*Rhopalosiphum maidis*) are the predominant aphid species damaging sorghum growth.

Greenbug is the most predominant insect pest in the sorghum fields and causes significant loss of the crop worldwide. It sucks juice from and injects toxins into sorghum plant tissues and consequently causes damage to the plants. As a result of adaptation, various biotypes of greenbugs have evolved, and four greenbug biotypes (C, E, I, and K) have been identified as damaging sorghum (Katsar et al. 2002). Among these, biotype I is of economic importance because it causes significant losses in sorghum yield (Harvey et al. 1991; Teetes and Pendleton 2000). Host-plant resistance has been proven as one of the most effective means of managing greenbug. Considerable progress has been made in screening for resistance to greenbugs. Some germplasm materials resistant to greenbug have been identified, of which 43 were resistant to biotype I (Andrews et al. 1993; Katsar et al. 2002; Huang 2006; Wu et al. 2006; Dogramaci et al. 2007; Peterson et al. 2009), 15 were resistant to biotype E (Johnson et al. 1981; Porter et al. 1982; Dixon et al. 1990; Dogramaci et al. 2007), 11 to biotype C (Schuster and Starks 1973; Araya 2001), and one to biotype K (Katsar et al. 2002) with different resistance mechanisms including antixenosis, antibiosis, and tolerance. Recently, the development of new genomic tools such as genome sequencing, quantitative trait locus (QTL) mapping, DNA microarrays, RNA-sequencing, real-time PCR, protein expression profiling, and bioinformatics allow more indepth knowledge about the genetics of host defense and mechanisms involved in host-plant resistance to insect pests (Huang 2014). To date, six independent QTL mapping experiments have been conducted in sorghum to identify greenbug resistance to four different biotypes (Table 1; Agrama et al. 2002; Katsar et al. 2002; Nagaraj et al. 2005; Wu et al. 2007; Wu and Huang 2008; Punnuri et al. 2013). Some major QTLs from different resistance sources accounting for approximately or above 30 % of the phenotypic variation for the resistance to greenbug have been identified. Of these, a QTL designated as *SbAGA01* from resistant source GBIK accounted for 29.1 and 38.4 % of the observed genetic variation in sorghum resistant to greenbug biotypes I and K, respectively (Agrama et al. 2002). Katsar et al. (2002) reported one to three QTL(s) expressing resistance to greenbug biotype E in each resistant source Tx2783. The QTLs on SBI-09 and SBI-08 accounted for 41 and 49 % of the variation, respectively. PI 550607, another strong resistance source, contained a QTL on SBI-09 accounting for 26 % of the total resistance to greenbug biotype E. A QTL derived from the sorghum accession PI

Table 1 Identified QTLs resistant to greenbug

Combination	Population and size	Biotype	Gene symbol	Chr.	Linked marker	PVE (%)	Reference
PI550610/Westland A	F_2, 277	I	*QSsgr-09-01*	SBI-09	*Xtxp289*, *Xtxp358*	54.5–80.3	Wu and Huang (2008)
			QSsgr-09-02	SBI-09	*Xtxp67–Xtxp230*	1.3–5.9	
GBIK/Redlan	RIL, 93	I, K		SBI-01	*B18-885*	5.6–38.4	Agrama et al. (2002)
				SBI-02	*OPC01-880*		
				SBI-03	*Sb5-214*		
				SBI-04	*Sb1-10*		
				SBI-09	*SbAGB03*		
				SBI-09	*Sb6-84*		
				SBI-10	*SBAGA01*		
				SBI-05	*OPA08-1150*		
				SBI-05	*OPB12-795*		
96-4121/Redlan	RILs, 88	I, K	*VIS-GBK1*	SBI-04	*Xtxp12*	9–19.6	Nagaraj et al. (2005)
			VIS-GBK2	SBI-04	*Xcup20*		
			VIS-GBK5	SBI-05	*Xtxp335*		
			VIS-GBI8	SBI-04	*Sb1-10*		
			SPA-I2	SBI-05	*Xtxp43*		
			SPA-I2	SBI-05	*Xtxp85*		
			SPA-K2	SBI-05	*Xtxp335*		
			SPA-K2	SBI-05	*Xtxp204*		
BTx623/S. propinquum	F_3, 370	N/A	*Ssg1*	SBI-09	*pSB347*	4.58–78.74	Katsar et al. (2002)
Tx2737/BTx623	F_3, 489	C	*Ssg2*	SBI-08	*CSU030*		
Tx2783/IS7173C	F_3, 203	C, E	*Ssg3*	SBI-05	*pSB089*, *pSB262*		
RTx430/PI550607	F_3, 195	C, E, I, K	*Ssg4*	SBI-03	*pSB443*		
			Ssg5	SBI-04	*pSB107*		
			Ssg6	SBI-10	*RZ144*		
			Ssg7	SBI-06	*pSB314*		
			Ssg8	SBI-07	*BCD98*		
			Ssg9	SBI-08	*CDO580*		
PI550610/Weatland A	$F_{2:3}$, 233	E		SBI-09	*Xtxp358, Xtxp289*	58.5–84.8	Wu et al. (2007)
PI607900/BTx623	F_2, 371	I	*Qstsgr-sbi09ii*	SBI-09	*Starssbem286–Starssbnm93*	39.8	Punnuri et al. (2013)
			Qstsgr-sbi09iii	SBI-09	*Starssbem298–Starssbnm102*	34.7	
			Qstsgr-sbi09i	SBI-09	*Starssbnm93–Starssbem296*	64.7–82.4	
			Qstsgr-sbi09iv	SBI-09	*Starssbnm78–Starssbnm81*	17.3–17.6	
				SBI03B	*Xtxp16–Starssbem162*	1	
				SBI03B	*Starssbem162–Starssbem265*	1.3	

550610, *QSsgr-09-01*, accounting for approximately 55–80 % of the phenotypic variation for the resistance to greenbug biotype I was identified by Wu and Huang (2008). A major QTL derived from PI 550610 (resistant to greenbug biotype E) on SBI-09 has been identified. The major QTL accounted for 58.5, 78.1, 83.8, and 84.8 % of the observed variation in resistance to greenbug biotype E measured at 7, 12, 17, and 21 days after infestation, respectively (Wu et al. 2007). Four major QTLs on chromosome 9 from PI 607900 resistant to greenbug biotype I were identified. These QTLs collectively accounted for 34.7–82.4 % of the phenotypic variance in greenbug resistance. Among them *Qstsgr-sbi09i* could explain 64.7, 67.0, and 82.4 % of the resistance variation at 10, 14, and 21 days, respectively. Another two major QTLs, *sbi09ii* and *sbi09iii*, could explain 39.8 and 34.7 % phenotypic variance, respectively, after seven days infestation (Punnuri et al. 2013). The above *R*-QTL linked markers are useful to screen more resistant genotypes. Furthermore, the markers tagged to QTL regions can be used to enhance the sorghum breeding program for greenbug resistance through marker-assisted selection (MAS) and map-based cloning (MBC).

Sorghum aphid or sugarcane aphid is another major insect pest causing serious damage to sorghum and sugarcane (Van den Berg 2002; Singh et al. 2004; Wei et al. 2016). Various sources of *M. sacchari* resistance in sorghum have been identified including germplasm accessions (38), parental A/B-lines (27) and R-lines (29), and agronomic elite lines/experimental varieties (69) from all over the world (Singh et al. 2004). Approximately 5000 varieties were screened by artificial inoculation for aphid tolerance in China. Five accessions, 5–27, Niangaoliang, Daluochui gaoliang, Jinsuigaoliang, and Hongkesanma showed some resistance to aphids (Lu and Dahlberg 2001). Recently, Armstrong et al. (2015) reported some newly identified resistant sources that could be utilized in breeding programs that develop agronomically acceptable sorghums. Unlike the biotype differentiation of greenbug, *M. sacchari* is distributed worldwide, and has one of the lowest known rates of genetic diversity. There is no significant differentiation identified in China (Li et al. 2014) and worldwide (Nibouche et al. 2014).

Recently a dominant gene (*RMES1*) conferring resistance to *M. sacchari* has been found in a grain sorghum variety Henong 16 (HN16). It resided between two markers *Sb6m2650* and *Sb6rj2776* on SBI-06, which delimited a chromosomal region of about 126 kb containing five predicted genes (Wang et al. 2013). The nearest flanking markers (*Sb6rj2776* and *Sb6m2650*) being 1 and 2 cM, respectively, away from *RMES1*, could accelerate the use of this important gene in MAS for breeding *M. sacchari* resistance in sorghum and should finally lead to gene isolation by MBC.

Corn leaf aphid, *R. maidis*, is a frequent problem on sorghum at the harvesting stage, a time when chemical control is very difficult and uneconomical. Both nymphs and adults suck the plant sap and cause yellowish mottling of the leaves. A high population of aphids covering the panicle and surrounding leaves can reduce grain filling. Additionally, corn leaf aphid can transmit damaging plant mosaic and dwarf viruses that can cause yield losses (Singh and Grewal 1999; Hemmati and Abbasi 2000). Sorghum genotypes with resistance to aphids would be an extremely valuable way to control this pest. To date, 47 sorghum germplasm accessions resistant to corn leaf aphid have been identified from Sudan, Iran, Argentina, the United States, and India (Pathak and Painter 1958; Gahukar 1993; Singh and Grewal 1999; Hemmati and Abbasi 2000; Fonseca et al. 2005).

Thus far there has been no systemic study to investigate the genetic model or molecular mechanism underlying corn leaf aphid resistance in sorghum. In maize, some studies revealed that aphid resistance was recessive. Two resistance loci, *aph* on maize chromosome 10 (Coe et al. 1988; So et al. 2010) and *aph2* on the short arm of chromosome 2 (Lu and Brewbaker 1999), have been identified. Recently two QTLs have been found on maize chromosomes 4 and 6, respectively, and at least two separate defense mechanisms accounting for the higher level of

corn leaf aphid resistance were proposed (Betsiashvili et al. 2015).

2.2 Shoot-Fly

Sorghum shoot-fly, *Atherigona soccata*, is one of the major insect pests of sorghum grown in Africa, Asia, and Mediterranean Europe. In India, the losses due to damage by the sorghum shoot-fly have been estimated to be as high as 90 % of grain and 45 % of fodder yield (Sukhani and Jotwani 1980). Shoot-fly infests sorghum seedlings from 7 days after emergence (DAE) to 30 DAE. Female shoot-fly has just a 30-day life span and lays eggs on the third to sixth basal leaves parallel to the leafmidrib. The egg hatches into a maggot in 1–2 days; then the maggot enters the central leafwhorl of the seedling where it makes an incision on the central leaf. The maggot cuts the seedling growing point, and feeds on the decaying tissue of the central whorl, which causes desiccation and death of the whorl leaf, and forms a typical deadheart (Riyazaddin et al. 2015).

Shoot-fly resistance in sorghum was classified into three components including antixenosis for oviposition, antibiosis, and tolerance. Some morphological, biochemical, and genetic factors including seedling leafblade glossiness, seedling leafblade trichome density, seedling vigor, and leafsheath pigmentation are positively associated with shoot-fly resistance (Tarumoto 2005). Deadhearts, plants with eggs, leaf glossiness, trichomes on the abaxial surface of the leaf, and leafsheath pigmentation can be used as marker traits to select for resistance to shoot-fly in sorghum (Dhillon et al. 2005). The marker traits of a number of sorghum germplasm materials have been evaluated and some shoot-fly resistant sources identified, such as ICSH 705, ICSV 702, ICSV 705, ICSV 708, IS 923, IS 1054, IS 1057, IS 1071, IS 1082, IS 1096, IS 2146, IS 2205, IS 2312, IS 2394, IS 4663, IS 4664, IS 5072, IS 5470, IS 5636, IS 18369, IS 18551, PS 21318, PS 30715-1, PS 35805, SFCR 125, and SFCR 151. Some of them are being used for shoot-fly resistance breeding programs (Dhillon et al. 2005; Chamarthi et al. 2011; Kumar et al. 2014).

Studies on the genetics of shoot-fly resistance suggested that resistance is complex and polygenically inherited (Halalli et al. 1982) with predominantly additive gene effects (Madhusudhana 2015). QTLs associated with the above traits showing shoot-fly resistance were identified from resistant line IS 18551 on chromosomes SBI-01, SBI-03, SBI-04, SBI-05, SBI-06, SBI-07, SBI-09, and SBI-10 (Sajjanar 2002; Folkertsma et al. 2003; Deshpande 2005; Mehtre 2006; Satish et al. 2009, 2012b). Using another shoot-fly resistance source IS2122, Aruna et al. (2011) mapped QTLs on chromosomes SBI-01, SBI-02, SBI-03, SBI-04, SBI-06, SBI-07, SBI-09, and SBI-10. Thus shoot-fly resistance loci were found on all 10 chromosomes of sorghum except SBI-08 (Table 2). The key QTLs on SBI-05 (for leaf glossiness) and SBI-10 (trichome density, oviposition, deadhearts, and seedling leafblade glossiness) have been further saturated with new markers, and several putative candidate gene-based markers have been identified (Satish et al. 2012b; Kiranmayee et al. 2015). An orthologous insect resistance gene Cysteine protease-*Mir1* (XnhsbmSFC34/SBI-10) was significantly associated with major QTLs for all traits (except seedling vigor) explaining 22.1 % of the phenotypic variation for deadhearts percentage. Similarly, a nucleotide binding site-leucine-rich repeat (NBS–LRR) gene (XnhsbmSFCILP2/SBI-10), involved in rice brown planthopper resistance, was associated with deadhearts percentage and number of eggs per plant. β-1,3-glucanase (XnhsbmSFC4/SBI-10), involved in aphid and brown planthopper resistance, was associated with deadhearts percentage and leaf glossiness (Satish et al. 2012b). Recently, average physical map positions for glossiness and trichome density QTLs on SBI-10 from earlier studies were reduced to 2 Mb and 800 kb. Candidate genes *Glossy15* (Sb10g025053) and ethylene zinc finger protein (Sb10g027550) falling in support

Table 2 Details of identified shoot-fly resistance loci in sorghum

Trait	Combination	Population	Gene symbol	Chromosome	Linked marker	PVE %	Reference
Leaf glossiness	IS18551/296B	RIL, 254		SBI-01	*Xtxp248–Xtxp316*	3.8	Apotikar et al. (2011)
	296B/IS18551	RIL, 168	*QGs.dsr-3*	SBI-03	*Xtxp59–Xtxp336*	10.1	Satish et al. (2009)
			QGs.dsr-5	SBI-05	*Xtxp65–Xtxp30*	14	
			QGs.dsr-6	SBI-06	*GlumeT–Mrco*	8.4	
			QGs.dsr-10	SBI-10	*Xnhsbm1043–Xgap1*	7.6	
	27B/IS2122	RIL, 210	*QGs.dsr-1*	SBI-01	*Xtxp357–Xtxp32*	5.9	Aruna et al. (2011)
			QGs.dsr-2	SBI-02	*Xisp10336–Xtxp1*	7.6	
			QGs.dsr-4.1	SBI-04	*Xtxp24–Xtxp41*	5.7	
			QGs.dsr-4.2	SBI-04	*Xcup20–Xtxp343*	7.5	
			QGs.dsr-10	SBI-10	*Xtxp320–Xcup16*	14.7	
Seedling vigor	296B/IS18551	RIL, 168	*QSv.dsr-3*	SBI-03	*Xtxp59–Xtxp336*	11.8	Satish et al. (2009)
			QSv.dsr-6.1	SBI-06	*Dsenhsbm2–Xtxp6*	8.6	
			QSv.dsr-6.2	SBI-06	*Xtxp145–Xtxp274*	7.4	
			QSv.dsr-10	SBI-10	*Xnhsbm1011–Xgap1*	7.7	
	27B/IS2122	RIL, 210	*QSv.dsr-1.1*	SBI-01	*gpsb3–Xtxp357*	7.9	Aruna et al. (2011)
			QSv.dsr-1.2	SBI-01	*Xcup6–Xtxp302*	4.9	
			QSv.dsr-2	SBI-02	*Xisep1145–Xtxp197*	5.1	
			QSv.dsr-9	SBI-09	*Xisp102271–Xtxp101*	7.1	
			QSv.dsr-10	SBI-10	*Xisep0625–Xgap1*	5.9	
Oviposition	IS18551/296B	RIL, 254		SBI-01	*Xtxp248–Xtxp316*	6	Apotikar et al. (2011)
	296B/IS18551	RIL, 168	*QEg21.dsr-1*	SBI-01	*Xcup24–Dsenhsbm64*	8.3	Satish et al. (2009)
			QEg21.dsr-7	SBI-07	*Xtxp36–Xtxp312*	6.7	
			QEg21.dsr-9	SBI-09	*Xcup02–Xtxp355*	5	
			QEg21.dsr-10	SBI-10	*Xnhsbm1044–Xnhsbm1013*	19	
			QEg28.dsr-5	SBI-05	*Xtxp65–Xtxp30*	7.2	
			QEg28.dsr-7	SBI-07	*Xtxp36–Xtxp312*	6.7	
			QEg28.dsr-10	SBI-10	*Xnhsbm1044–Xnhsbm1013*	16.1	

(continued)

Table 2 (continued)

Trait	Combination	Population	Gene symbol	Chromosome	Linked marker	PVE %	Reference
Deadheart	IS18551/296B	RIL, 254		SBI-01	*Xtxp248–Xtxp316*	6.3	Apotikar et al. (2011)
	296B/IS18551	RIL, 168	*QDh.dsr-5*	SBI-05	*Xtxp65–Xtxp30*	6	Satish et al. (2009)
			QDh.dsr-9	SBI-09	*Xcup02–Xtxp355*	8	
			QDh.dsr-10.1	SBI-10	*Xtxp217–SvPEPcA*	8.8	
			QDh.dsr-10.2	SBI-10	*Xnhsbm1033–Xcup16*	11.4	
			QDh.dsr-10.3	SBI-10	*Xnhsbm1044–Xnhsbm1013*	15	
			QDh.dsr-10.4	SBI-10	*Xnhsbm1043–Xgap1*	5.5	
	27B/IS2122	RIL, 210	*QDh.dsr-1.1*	SBI-01	*Xisp10322–Xtxp316*	8	Aruna et al. (2011)
			QDh.dsr-1.2	SBI-01	*Xisp10314–Xtxp20*	5	
			QDh.dsr-2	SBI-02	*Xisp10336–Xtxp1*	6.4	
			QDh.dsr-6.1	SBI-06	*Xisep0432–Xtxp45*	5.4	
			QDh.dsr-6.2	SBI-06	*Xisp10347–gpsb18*	7.5	
			QDh.dsr-7.1	SBI-07	*Xcup70–Xgap342*	7.1	
			QDh.dsr-7.2	SBI-07	*Xtxp278–Xisp10233*	9.8	
			QDh.dsr-9	SBI-09	*Xtxp101–Xcup21*	6.4	
			QDh.dsr-10.1	SBI-10	*Xtxp320–Trit*	12.8	
			QDh.dsr-10.2	SBI-10	*Xisep0625–Xgap1*	9.4	
Adaxial trichome density	296B/IS18551	RIL, 168	*QTdu.dsr-10.1*	SBI-10	*Xnhsbm1044–Xnhsbm1013*	15.7	Satish et al. (2009)
			QTdu.dsr-10.2	SBI-10	*Xnhsbm1043–Xgap1*	33	
			QTdl.dsr-1.1	SBI-01	*Xtxp32–Xtxp88*	8.8	
			QTdl.dsr-1.2	SBI-01	*Dsenhsbm80–Xtxp302*	5.3	
			QTdl.dsr-4	SBI-04	*Xcup48–Ungnhsbm32*	8.5	
			QTdl.dsr-6	SBI-06	*Xtxp317–Xtxp274*	5.2	
			QTdl.dsr-10.1	SBI-10	*Xnhsbm1048–Xnhsbm1013*	15.1	
			QTdl.dsr-10.2	SBI-10	*Xnhsbm1043–Xgap1*	22.7	
	27B/IS2122	RIL, 210	*QTdu.dsr-7*	SBI-07	*Xcup57–Xtxp99*	4.3	Aruna et al. (2011)
			QTdu.dsr-10	SBI-10	*Xtxp320–Xcup16*	44.1	
			QTdl.dsr-3	SBI-03	*Xtxp70–Xtxp48*	5	
			QTdl.dsr-10.1	SBI-10	*Xgap1–Xtxp320*	24.1	
			QTdl.dsr-10.2	SBI-10	*Xtxp320–Trit*	12.7	

intervals for glossiness and trichome density QTLs have been identified (Kiranmayee et al. 2015).

With the identification of resistance trait linked markers, marker-assisted introgression of such important shoot-fly resistance QTLs for trichome density, nonpreference, and deadhearts using SSR markers *Xnhsbm1044* and *Xnhsbm1013* on SBI-10 and for leaf glossiness on SBI-05 using *Xtxp65* and *Xtxp30* SSRs into commercially important but shoot-fly susceptible seed parents, 296B and 27B from the resistance donor parent IS18551, have been successfully initiated (Madhusudhana et al. 2012).

2.3 Midge

Sorghum midge, *Stenodiplosis sorghicola*, is the most damaging pest of grain sorghum widely distributed in Asia, Australia, Africa, the Americas, and Mediterranean Europe (Madhusudhana 2015). At flowering time, female midges oviposit into spikelets, and the larvae feed on the ovary during the following two weeks, resulting in failure of kernel development and production of empty or chaffy spikelets. The damaged panicles present a blasted appearance.

Host-plant resistance is an effective means of keeping midge populations below economic threshold levels. Nonpreference and antibiosis are the major components of host-plant resistance to sorghum midge. Resistance to midge is governed largely by additive gene action, although nonadditive gene actions may also be involved (Sharma and Vidyasagar 1994). A number of midge-resistant sources have been identified in the wild relatives of sorghum including *S. amplum*, *S. bulbosum*, and *S. angustumthe*, and in the cultivated germplasm (Sharma et al. 1993; Sharma and Franzmann 2001; Sharma et al. 2003). ICSV 197, ICSV 745, ICSV 735, ICSV 758, and ICSV 88032 have high yield potential and at the same time high midge resistance (Agrawal et al. 1987, 1996; Sharma and Vidyasagar 1994; Agrawal et al. 2005; Sharma et al. 2005). Several seed parents, ICSA-/B-488 to 544, with midge resistance have been developed for producing midge-resistant hybrids (Reddy et al. 2007).

QTLs associated with two of the different mechanisms of midge resistance, antixenosis and antibiosis, were identified in a recombinant inbred (RI) population from a cross of sorghum lines ICSV745 and 90562. Two genetic regions located on separate linkage groups were found to be associated with antixenosis and explained 12 and 15 %, respectively, of the total variation in egg numbers/spikelet laid in a cage experiment. One region was significantly associated with antibiosis and explained 34.5 % of the variation of the difference of egg and pupal counts in the RI population (Tao et al. 2003).

3 Host-Plant Resistance to Disease

3.1 Rust

Sorghum rust (*Puccinia purpurea*) is widely distributed and occurs in almost all sorghum-growing areas of the world and reduces forage quality and grain yield. In Australia, the Philippines, and India, yield losses due to rust as high as 13.1, 30, and 50 %, respectively, have been reported (Anon 2002; White et al. 2012). This disease is important because its presence predisposes sorghum to other major disease problems, such as the Fusarium stalk rots, charcoal rot, and grain molding (Frederiksen 1986). Severe rust infection also contributes to lodging by reducing leaf area and increasing plant stress (Ryley et al. 2002). Yield losses associated with rust have been reported in Malawi, Swaziland, Tanzania, Zambia, Zimbabwe, Argentina, and Brazil (Tarr 1962; Anon 2002). Two pathotypes of *P. purpurea* were reported in Hawaii (Bergquist 1974). Recently in Australia four putative pathotypes were tested, of which Pathotypes 1 and 3 were the most common (White et al. 2015).

Availability of adequate genetic variation is a prerequisite for genetic improvement of any crop species. Eight sorghum accessions from either South Africa or Africa were identified to be highly resistant to rust (Wang et al. 2006). Sharma et al. (2012) evaluated 242 germplasm

accessions to identify sources of resistance to foliar diseases, six accessions including IS 473 (guinea-kafir from the US), IS 23521 (guinea-caudatum from Ethiopia), IS 23684 (guinea from Mozambique), IS 24503 (bicolor from South Africa), IS 26737 (kafir from South Africa), and IS 33023 (guinea from Tanzania) showed resistance in both the greenhouse and field screens.

The genetics of host–pathogen interactions for sorghum rust have been investigated in a few studies and genetic analyses from different populations indicated that the resistance was controlled by a simple dominant gene *Pu* (Coleman and Dean 1961), more than one gene (Patil-Kulkarni et al. 1972), three major genes (Rana et al. 1976), or by multiple QTLs with small effects (Tao et al. 1998; Upadhyaya et al. 2013; Wang et al. 2014). By phenotyping 160 random RI lines (RILs) developed from a cross between QL39 and QL41, Tao et al. (1998) determined four QTLs for rust resistance on chromosomes SBI-01, SBI-02, SBI-03, and SBI-08 explaining 16–42 % of trait variation in the population. The major QTL associated with an allele from QL41 on SBI-08 accounting for 42 % of trait variation is believed to be the *Pu* gene (Madhusudhana 2015). This genomic region of sorghum has been shown to be orthologous to the maize chromosome 10S, where *Rp1-D* is located (McIntyre et al. 2005). Conservation of gene function in this genomic region for rust resistance among sorghum, maize, and sugarcane has been established (McIntyre et al. 2005). Because different rust species cause rust in these three plants (*P. sorghi* in maize, *P. purpurea* in sorghum, and *P. melanocephala* in sugarcane), the nature of the conserved function is supposed that the products of resistance genes interact with pathogen avirulence proteins. It is also possible that common avirulence determinants may be recognized in maize, sorghum, and sugarcane rust fungi. Alternatively, *R* (resistance) gene conservation may reflect conserved signaling motifs that activate downstream resistance mechanisms (McIntyre et al. 2005). Recently, an ancestral gene family containing the maize *Rp1-D* gene (GRMZM5G879178), sorghum genes *Rp1-dp3* (Sb03g036450) and *rph1-3* (Sb08g002410) for rust resistance, and the rice *Pi37* (Os01g57310) gene for resistance to rice blast disease was also identified, which had an effector response that conferred resistance to multiple pathogens across species (Mace et al. 2014). In an association mapping analysis, Upadhyaya et al. (2013) identified five SNP loci associated with rust resistance, two of which contained the rust resistance gene homologous to the maize rust resistance gene *Rp1-D* and to the wheat rust resistance gene *Lr1*. The remaining loci contained genes important in defense responses.

A combination of conventional QTL analysis and genomewide association analysis (GWAS) was used to determine the genetic architecture of leaf rust resistance in sorghum using three biparental mapping populations (S2: 119 F_5 RILs developed from the cross between ICSV745 and R890562-1-2; S4: 246 F_5 RILs derived from the IS8525/R931945-2-2; S7: 214 BC_1F_4 RILs derived from crossing *S. bicolor* subsp. *verticilliflorum* and the recurrent parent R931945-2-2) and a set of elite breeding lines (association mapping set: 150 genotypes) phenotyped in hybrid combination with multiple testers (Wang et al. 2014). All populations were phenotyped not only for rust infection response, but also for height and maturity. In population S2, two highly significant and three suggestive rust resistance QTLs have been detected on chromosomes SBI-05, SBI-08-II, SBI-02, SBI-06-I, and SBI-08-I, respectively. Individual QTLs explained between 5.6 and 18.5 % of phenotypic variation in response to rust, with a cumulative total of 53.7 %. In population S4, a total of nine including one highly significant, three significant, and five suggestive QTLs for rust resistance were identified on six chromosomes, SBI-01, SBI-02, SBI-03, SBI-04, SBI-09, and SBI-10. Individual QTLs explained between 2.5 and 10.4 % of phenotypic variation, with a cumulative total of 42.1 %. In population S7, 15 genomic regions were detected with significant marker trait associations on eight chromosomes. Furthermore, four genomic regions were detected with highly significant marker trait associations.

In the association mapping set, 52 genomic regions were identified with suggestive marker trait associations in at least one of the six tester/location combinations, with over half (28) identified as significant in two or more of the tester/location combinations. A total of 64 significant or highly significant QTLs and 21 suggestive QTLs for rust resistance were identified and projected onto a consensus map, in addition to the 11 previously reported QTLs for rust resistance (Tao et al. 1998; Mohan et al. 2010; Upadhyaya et al. 2013), of which 10 are colocated, representing a total of 56 unique regions. Thus rust resistance QTLs were identified on all sorghum chromosomes (Wang et al. 2014). Of the 56 unique genomic regions, 30 were categorized as being unique to rust, whereas 26 were not unique to rust, and colocated with either height, maturity, or stay-green QTLs identified in the same population. Rust QTLs that are not associated with maturity and height are enriched for defense-related gene families. The major nucleotide binding site (NBS) encoding rust resistance gene *Rph1-2*, homologous with the maize *Rp1-D* gene colocated with QTL from the S2 population (*QRustR_S2_8.1*) and the association mapping set (*QRustR_AYT_8.1*). Of the 52 rust resistance QTLs identified in maize, over 80 % (42) collocated with rust QTLs identified in sorghum. Additionally, the wheat rust resistance gene, *Lr34*, previously described by Suenaga et al. (2003), collocated with QTL from the S4 population (*QRustR_S4_1.1*) and the AYT association mapping set (*QRustR_AYT_1.3*) (Wang et al. 2014).

3.2 Grain Mold

Grain mold (GM) is a major disease of sorghum that affects grain production and quality. It is particularly severe to sorghum cultivars maturing during the rainy season in humid tropical and subtropical areas (Thakur et al. 2006). It is caused by a complex of fungal pathogens including more than 40 genera of fungi (Navi et al. 1999). The major fungi associated with early infection events are *Fusarium* spp., *Curvularia lunata*, *Alternaria alternata*, and *Phoma sorghina* (Thakur et al. 2003, 2006). Of these, *F. moniliforme* is the most predominant and most damaging fungal pathogen of sorghum grain worldwide. Production loss ranges from 30 to 100 %, depending on cultivar, time of flowering, and prevailing weather conditions from flowering to harvesting (Singh and Bandyopadhyay 2000).

Bandyopadhyay et al. (1988) reported 156 of the 7132 sorghum accessions screened during the period from 1980 to 1985 at Patancheru, India, to be resistant to grain mold. Fifty grain mold-resistant sorghum accessions from a mini-core, which represents a wide diversity of race types, morphological traits, agronomic desirability, and geographical distribution have been identified (Sharma et al. 2010). These would be desirable sources of resistance for a sorghum breeding program although genetic mechanisms governing grain mold resistance in sorghum are still poorly understood. Among different races constituting the sorghum mini-core, maximum mold resistant accessions were of *bicolor* type (14 of the 15 screened). The resistance in the *bicolor* accessions might be due to loose panicles and dark grain color. None of the 14 *durra*-type accessions (compact panicle) screened had resistance to grain mold (Sharma et al. 2010). It was also reported that the resistance to sorghum GM was associated with a high concentration of phenolic compounds (apigeninidin, flavan-4-ols, and tannin), kernel hardness, and pericarp color (Menkir et al. 1996).

The complexity of pathogens makes the isolation of the resistance gene very difficult. Just a few QTLs and markers were reported to be associated with GM resistance. Klein et al. (2001) reported five QTLs located on chromosomes SBI-04, SBI-06, SBI-07, SBI-09, and SBI-10 in an F_5 mapping population derived from a cross of Sureño and RTx430. Grain mold incidence was affected by five QTLs each accounting for between 10 and 23 % of the phenotypic variance (Table 3). Recently two loci associated with grain mold resistance have been identified in a sorghum mini-core of 242 accessions that had been evaluated for grain mold resistance in the field for 2 years by applying 14,739 SNP markers (Upadhyaya et al. 2013).

Table 3 QTL isolated resistance to grain mold

Linked maker	Chromosome	PVE %	Reference
Xtxp12	SBI-04	12.4	Klein et al. (2001)
Xtxa10062	SBI-07	21.5	
Xtxa10057	SBI-09	11.3	
Xtxa1047	SBI-10	10	
Xtxp95	SBI-06	13.3	
Chr8_8997626	SBI-08	–	Upadhyaya et al. (2013)
Chr2_5600094	SBI-02	–	

Among the two loci linked to grain mold resistance, one contained a NB-ARC-LRR class of *R* gene (Sb02g004900) that shares 37 % identity and 57 % similarity to the nonhost resistance gene of maize, *Rxo1*. Knockout of *Rxo1* in maize abolishes its resistance to the rice pathogen *Xanthomonas oryzae* pv. *oryzicola*, which causes bacterial streak disease, whereas overexpression confers resistance to both *X. oryzae* pv. *oryzicola* and the unrelated pathogen *Burkholderia andropogonis*, which causes bacterial stripe in sorghum and maize (Zhao et al. 2005). This finding suggests that *Rxo1* plays an important role in both host and nonhost resistance which is relevant in defense against multiple pathogens such as in grain mold (Upadhyaya et al. 2013).

3.3 Downy Mildew

Sorghum downy mildew (SDM), caused by *Peronosclerospora sorghi* (Butler 1907), results in serious production losses to sorghum in zones where the disease is prevalent (Odvody and Frederiksen 1984; Pande et al. 1997; Craig 2000; Perumal et al. 2006; Thakur et al. 2007; Perumal et al. 2008). There are some other *Peronosclerospora* species that can attack sorghum, including *P. maydis*, *P. sacchari*, *P. philippinensis* (Bonde and Peterson 1983), and *P. heteropogoni* (Nair et al. 2004). The pathogen was first discovered in Africa and Asia and then probably introduced into America in the late 1950s (Frederiksen 1980). It has been reported that SDM may cause up to 78 % yield losses in India (Thakur and Mathur 2002) and 11.7 % in Africa (Bock et al. 1998). Cultural practices can significantly reduce downy mildew incidence (Frederiksen 1980; Schuh et al. 1987; Pande et al. 1997). Metalaxyl or fosetyl-Al applied as seed treatment can provide effective chemical control of SDM (Odvody and Frederiksen 1984).

Five SDM pathotypes (P1–P5) have been identified (Craig and Frederiksen 1980, 1983; Fernandes and Schaffert 1983; Sifuentes and Frederiksen 1988; Craig and Odvody 1992). These pathotypes can be controlled by metalaxyl seed treatment. However, from 2001, SDM appeared in some sorghum fields in south Texas causing serious yield losses (Isakeit et al. 2003). The new SDM pathotype, pathotype 6 (P6) made the metalaxyl seed treatment ineffective (Isakeit and Jaster 2005). This led to use of germplasms having broad-spectrum disease resistance to SDM.

The resistance to SDM may have regional characteristics. Some African resources showed resistance to SDM in the Americans. Three accessions PI 282860, PI 282864, and PI 563505 from Chad showed high levels of downy mildew resistance in Mexico and Texas, whereas PI 282843, PI 282877, PI 549196, and PI 563438 also from Chad exhibited high levels of resistance to the disease in Texas. Accessions PI 297210, PI 576386, and PI 576395 from Uganda also showed downy mildew resistance in Mexico and Texas. So the sorghum accessions from Chad and Uganda can be utilized for downy mildew resistance breeding in Mexico or Texas (Prom et al. 2010). Accessions PI 609151 and PI 609442 from Mali had high levels of SDM resistance at one Mexican location and two US locations. Malian accession PI 612815 also had a moderate resistant reaction to SDM in two of the

three locations. Accession PI 522108 from Gambia was resistant in Mexico but susceptible in Louise, Texas, United States. The reaction of the 10 lines used as differentials suggested the presence of a pathotype in Mexico that differed from those in the United States (Prom et al. 2015).

A new resistance gene can be found in wild and weedy sorghums. Forty-five of the wild accessions belonging to sections *Heterosorghum*, *Chaetosorghum stiposorghum*, and *Parasorghum* remained downy mildew free, whereas two accessions of *Parasorghum*, IS 18951 (*S. purpureosericeum*) and IS 23177 (*S. versicolor*), developed about 3 % downy mildew. Some new germplasms were identified to be resistant to newly appeared pathotypes. The sorghum germplasm lines Tx3301 through Tx3360 (Reg. No. GP-733 to GP-792, PI 668030 to PI 668089) are resistant to pathotypes 3 and 6 of *Peronosclerospora sorghi* (Rosenow et al. 2014).

The research about the resistance mechanism and resistance gene of SDM was relatively lagging. Gowda et al. (1995) found that it was controlled by a single dominant allele from a population derived from a cross of RT7080 × SC414. The gene from SC414 showed resistance to pathotypes P1, P2, and P3. Two random amplified polymorphic DNA (RAPD) markers (PAL_{360} and $OPQ8_{510}$) were found to be linked to the resistance gene. Oh et al. (1996) showed that SC325 was resistant to pathotypes P1 and P3. The resistance gene was controlled by a single dominant allele which was linked to two RFLP markers, 5.0 cM to *pSbTXS552* and 7.9 cM to *pSbTXS361*.

4 Host-Plant Resistance to Parasitic Weed

4.1 Striga

The witch weed (*Strigas* sp.) is a devastating parasitic weed for various crops in Africa and parts of Asia (Timko et al. 2012). It is the most limiting biotic constraint for sorghum production by small farmers in rainfed agricultural areas of the semi-arid tropics (Satish et al. 2012a). Crop yield losses due to *Striga* might reach up to 100 % in heavily infested soils (Ejeta and Butler 1993).

Striga management is difficult because of the complex host–parasite interactions, the large number of seeds produced by *Striga* plants with prolonged viability, and special germination and development requirements (Pieterse and Pesch 1983; Mohamed 2002). Although a number of *Striga* control measures such as crop rotation, intercropping, transplanting, soil and water management, use of fertilizers, and hand weeding have been suggested, resistant crop cultivars have been recognized as the most cost-effective method and are compatible with low input systems (Joel et al. 2007). To date, some *Striga*-resistant sorghum cultivars have been identified such as N13, SRN39, Framida, 555, ICSVs, SRN39 derivatives (P401–P409), Soumalemba (IS15401), SeguetanaCZ, CMDT45, P9401, P9403, PSL5061, P9401, P9403, and PSL5061 (Teka 2014). Significant progress has also been made to identify molecular markers associated with *Striga* resistance (Table 4; Mohamed et al. 2003; Haussmann et al. 2004; Grenier et al. 2007; Satish et al. 2012a). Details of sorghum SSRs used for foreground selection for marker-assisted backcrossing of *Striga* resistance QTLs are available (Santosh et al. 2013). Among the above QTL studies, Haussmann et al. (2004) conducted a QTL analysis of field resistance to *Striga* using two RIL populations derived from a cross between IS9830, a low germination stimulant producer, and E36-1, a susceptible genotype (RIL-1); and N13 (mechanical resistance) and E36-1 (RIL-2). Composite interval mapping revealed eleven QTLs and nine QTLs in the RIL-1 and RIL-2, respectively. Five QTLs were common to both populations with the resistance alleles deriving from IS 9830 or N13. Satish et al. (2012a) fine-mapped one single recessively inherited gene, *lgs* (low germination stimulant) on sorghum chromosome SBI-05 towards the distal end, which would be an excellent candidate for MAS. The use of molecular markers to breed for *Striga* resistance in sorghum has recently been shown to be successful (Gammar

Table 4 QTLs identified for *Striga* resistance from different sources in sorghum

Resistance source	Gene/QTL	Resistance mechanism	Chromosome	Reference
PQ434 (*S.bicolor* ssp. *drummondii*)	*Lhf*	Low haustorial inducing factors producing	SBI-09	Grenier et al. (2007)
S. arundinaceum	*HR1*	Hypersensitive response	SBI-02	Mohamed et al. (2003)
S. arundinaceum	*HR2*	Hypersensitive response	SBI-05	Grenier et al. (2007)
IS9830	11 QTLs	Low germination stimulant	SBI-01, 02, 03, 04, 06, 09, 10	Haussmann et al. (2004)
N13	9 QTLs	Mechanical resistance	SBI-01, 02, 03, 04, 06, 07	Haussmann et al. (2004)
SRN39	*lgs*	Low germination stimulant	SBI-05	Satish et al. (2012a)

and Mohamed 2013; Mohamed et al. 2014; Yohannes et al. 2015; Ngugi et al. 2016). Two or more major QTLs derived from N13 (*Striga* resistant) have been successfully transferred into three farmer-preferred elite sorghum cultivars: Tabat, Wad Ahmed, and AG-8 (*Striga* susceptible) through MAS. Field evaluation revealed that newly bred lines were *Striga* resistant and agronomically superior with yields ranging from 180 to 298 % higher relative to their recurrent parents, which are the first products of MAS in sorghum released for cultivation by farmers in sub-Saharan Africa (Mohamed et al. 2014).

Understanding of host-plant *Striga* resistance and its genetic basis is an essential prerequisite for breeding programs. It has been suggested that *Striga* resistance in sorghum might be caused by different factors including low germination stimulant activity, low haustorial initiation activity, hypersensitive reactions, and incompatibility (Ejeta 2007). The first resistance gene to *Striga* was identified and cloned in cowpea (Li and Timko 2009). The cowpea cultivar B301 showed resistance to race 3 (SG3) of *S. gesnerioides*, which was characterized by an inability of the parasite to penetrate the endodermis and necrosis of host tissue at the point of attachment. The full-length of the gene sequence predicted a CC-NBS-LRR R protein (named RSG3-301). *R*-genes recognize, directly or indirectly, pathogen effectors (often termed *Avr* genes) to trigger plant defense responses which indicated that plants, like pathogens and some insect pests, may influence plant defense mechanisms in similar ways. Meanwhile, efforts have been made to elucidate the molecular events underlying *Striga* infection using next-generation sequencing and conventional sequencing technology (Spallek et al. 2013). The genome size of *S. hermonthica* was estimated to be 1801 Mbp (±321 Mbp), which is approximately 14 times that of *Arabidopsis* (Yoshida et al. 2010). Also the Parasitic Plant Genome Project (PPGP) is carrying out the comparative functional genomic analysis of parasitic plants in order to discover the genomewide changes that led to the establishment of the parasitic lifestyle and the changes that resulted as a consequence of adoption of the parasitic lifestyle (http://ppgp.huck.psu.edu/).

5 Prospects

Over the last two decades, much progress has been made in the field of sorghum biotechnology both towards transgenic research and DNA marker studies including genomics (Rakshit and Patil 2014). For example, with the help of marker-assisted backcrossing, three to four QTLs were successfully transferred from a donor line N13 into a farmer-preferred sorghum variety for *Striga* resistance (Ngugi et al. 2016). Great progress has been made in the sequencing technologies and bioinformatics at an exponentially reduced cost, which led to a revolution in the field of genotyping technologies. Restriction-associated DNA sequencing (RAD-seq)

and genotyping by sequencing (GBS) have emerged as powerful genotyping platforms, which are capable of identifying, sequencing, and genotyping thousands of markers across almost any genome of interest and number of individuals in a population (Bhatia et al. 2013). The newly developed genomic approaches would rapidly accelerate applications to many different research areas ranging from marker discovery, genetic diversity, and linkage/association mapping to genomic selection, physical mapping, gene discovery, and genomic-assisted breeding to improve biotic stress resistance in sorghum.

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Genomic Approaches for Abiotic Stress Tolerance in Sorghum

9

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Abstract

Although sorghum is a crop grown under harsh environments, its productivity is adversely affected by various abiotic stresses including drought, temperature extremes, low fertility, and mineral toxicity among others. In recent years a large number of genetic and genomic resources have become available in sorghum, which provide researchers opportunities to relate sequence variations with phenotypic traits of interest and their utilization in sorghum improvement programs. The application of the molecular marker and genomic technologies has shown promise for efficient breeding. However, very few successful examples are available in the public domain of research in this direction. Some of these successes specifically related to application of molecular marker technologies for improving abiotic stresses are explained in this chapter. With recent advances in next-generation sequencing technologies and high-throughput phenotyping platforms/technologies, utilizing the new/advanced mapping populations such as nested-association mapping (NAM), backcross-derived NAM has shown great potential. These recent advancements will be the drivers for integration of genomics technologies in routine breeding programs in the immediate future.

1 Introduction

Sorghum [*Sorghum bicolor* L. (Moench)] is vital to the food security of many of the world's poorest people living in fragile agroecological zones. Sorghum is produced by about 100 countries in the world and mainly used as staple food in parts of Asia and Africa, whereas in the United States, Mexico, and Australia it is used as a major feed crop (Rakshit et al. 2014). The genome of sorghum is unique as there is only ~3 % differential sorghum–rice gene loss

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and comparatively less structural rearrangement for sorghum–rice in 50 million years than sorghum–maize rearrangement (with genome duplication events) in ~15 million years in evolutionary lineage (Bowers et al. 2003). The sorghum karyotype shows 70 million years of "abstinence" from genome duplication. Researchers have exploited this great opportunity to study functions of sorghum genes which may still resemble those of the common cereal ancestor. Accordingly, the sequenced ~740 Mb sorghum genome (Paterson et al. 2009) is a logical complement to that of *Oryza* (rice) for grass functional genomics.

In addition to its importance as a model crop, sorghum is the most drought-resistant among the world's top five cereal crops, and an important dual-purpose crop. Sorghum cultivation ranges from the equator (approximately 50° latitude) to elevations of 2500 m (Rao et al. 2015). Sorghum is exceptionally tolerant to low input levels (especially in West Africa), which is an important characteristic for the areas receiving little rainfall. Thus, sorghum plays a vital role in feeding the world's most vulnerable population under the context of increasing demand for limited fresh water supply, increasing use of marginal farmland, and climatic trends. These interesting facts about sorghum along with recent advances in genomics with the advent of the next-generation sequencing (NGS) platforms for genotyping, expression studies, and high-throughput phenotyping facilities make it a model plant species not only to study evolutionary relationships across other grass species but also to help understand the mechanisms and functions of the genome that will lead to better adaptation to climate change, especially for abiotic stresses such as drought, salinity, cold tolerance, and nutrient use efficiency. This is more relevant in the current scenario where global food production has to be increased by 70 % in the background of ever-shrinking cultivable land and water resources (MacIntyre et al. 2009), and increased incidences of environmental extremes such as floods, drought, and extreme temperatures (Mickelbart et al. 2015). Wang et al. (2014a, b) made a detailed review of the status of abiotic stress genomics in sorghum vis-à-vis other cereals, and Anami et al. (2015) reviewed biotic and abiotic stress resistance with specific reference to sweet sorghum. In this chapter, efforts have been made to update the status in this area, keeping the developments in perspective.

2 Stay-Green as a Post-flowering Drought Tolerance Trait

2.1 Importance of Stay-Green

Among the various abiotic stress factors affecting crop growth and productivity, water stress is the single greatest factor and this will be the most important factor under a changed climatic regime (Araus et al. 2002). The impact of drought may largely be addressed through genetic improvement for drought response (Mutava et al. 2011a, b). In sorghum, two distinct drought-stress responses have been identified based on the time of occurrence: a pre-flowering drought response occurring prior to anthesis and a post-flowering drought response during the grain-filling stage (Harris et al. 2007). Even though a number of commonly grown cultivars show tolerance at the pre-anthesis stage, they are not tolerant at post-anthesis stages (Sanchez et al. 2002), which is of more economic consequence. Post-flowering drought susceptibility symptoms are characterized by premature leaf and plant senescence, stalk lodging, charcoal rot, and reduction in seed sizes (Rosenow and Clark 1995). Retention of green leaf area during the grain-filling stage has been found to be associated with post-flowering drought tolerance in sorghum (Rakshit et al. 2016). This capacity of certain genotypes in sorghum is referred as the "stay-green" phenotype, and is one of the most well-characterized and utilized traits in sorghum improvement (Rosenow and Clark 1982; Borrell et al. 2000a, b; Borrell and Hammer 2000; Rakshit et al. 2016). The expression of stay-green has been reported to improve the quality of crop residues (van Oosterom et al. 1996), support the continuation of carbon fixation and supply of starch to the sink (McBee

et al. 1983), prevent premature death and lodging of crop (Rosenow and Clark 1982), sustain grain filling under water stress (Rosenow et al. 1983), and improve grain yield under moisture stress (Borrell and Douglas 1996). Even though some progress has been reported in identification of factors underpinning stay-green expression (Vadez et al. 2011; Kholová et al. 2014), understanding the genetic regulation of the mechanisms that lead to the expression of the stay-green phenotype in sorghum is incomplete. Early works considered the benefit of stay-green in terms of extending the period during which a leaf could actively fix carbon (McBee et al. 1983). Subsequently, it was related to the carbon economy of the plant, addressing the nitrogen status of the plant and, in particular, the balance between nitrogen demand and nitrogen capture (Borrell et al. 2001). Owing to its importance as a most widely characterized drought tolerance component trait in sorghum breeding, several quantitative trait locus (QTL) mapping studies (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Tao et al. 2000; Xu et al. 2000; Kebede et al. 2001; Haussmann et al. 2002; Sanchez et al. 2002; Harris et al. 2007; Srinivas et al. 2009; Habyarimana et al. 2010; Sabadin et al. 2012) for stay-green are reported in sorghum and are well documented (Mace and Jordan 2010; Kiranmayee et al. 2015). There are several well-documented reviews about the progress of research on stay-green in sorghum (Vadez et al. 2013; Wang et al. 2014a, b).

2.2 QTL Analysis for Stay-Green and Associated Root Traits

Overall, seven sources of the stay-green trait have thus far been used for identification of QTLs for this phenotype, and these are B35 (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Xu et al. 2000; Sanchez et al. 2002; Harris et al. 2007), E36-1 (Haussmann et al. 2002), QL41 (Tao et al. 2000), SC56 (Kebede et al. 2001), 296B (Srinivas et al. 2009), SC283 (Sabadin et al. 2012), and SDS 1948-3 (Habyarimana et al. 2010). Among these, the most commonly used source is B35 [BTx642, a BC1 derivative of IS12555, a *durra* sorghum from Ethiopia (Rosenow et al. 1983)]. All studies showed that stay-green is quantitatively inherited, and the QTLs varied across environments and years. However, six major stay-green QTLs, *stgC* (SBI-01), *stg3A* and *stg3B* (on SBI-02), *stg1* and *stg2* (on SBI-03), and *stg4* (on SBI-05) have been detected across several studies (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Tao et al. 2000; Xu et al. 2000; Haussmann et al. 2002; Sanchez et al. 2002; Harris et al. 2007). Although the mapped QTLs are from different mapping populations, their physical positions are consistent across the maps (Wang et al. 2014a, b). A simple sequence repeat (SSR) framework map aligned to sorghum genome assembly and the consensus QTL intervals for stay-green traits from these studies (Mace and Jordan 2011a, b) are integrated (Fig. 1; http://cmap.icrisat.ac.in/cmap/) for selecting additional markers in the intervals, especially to support introgression work. Many studies have attempted to use the identified stay-green QTLs for developing drought-tolerant cultivars through marker-assisted backcrossing (Kassahun et al. 2010; Jordan et al. 2012; Vadez et al. 2013).

Root traits are assumed to play a major role towards improved drought tolerance. (For details refer to Chap. 11.) The nodal root angle in sorghum is hypothesized to influence both horizontal and vertical exploration of roots in the soil (Kato et al. 2006; Hammer et al. 2009; Singh et al. 2010; Mace et al. 2012). A putative association between narrow root angle and moderate to high levels of stay-green was made while studying genetic diversity for nodal root angle in a set of 44 diverse sorghum genotypes (Singh et al. 2011). As sorghum produces only one seminal root and the major root system forms nodal root axes, the root angle measured on nodal roots, the first flush of which appears approximately at the five-leaf stage, is suggested to be the most important parameter (Singh et al. 2010). Thus, small soil-filled root chambers can be used effectively to grow plants for a few

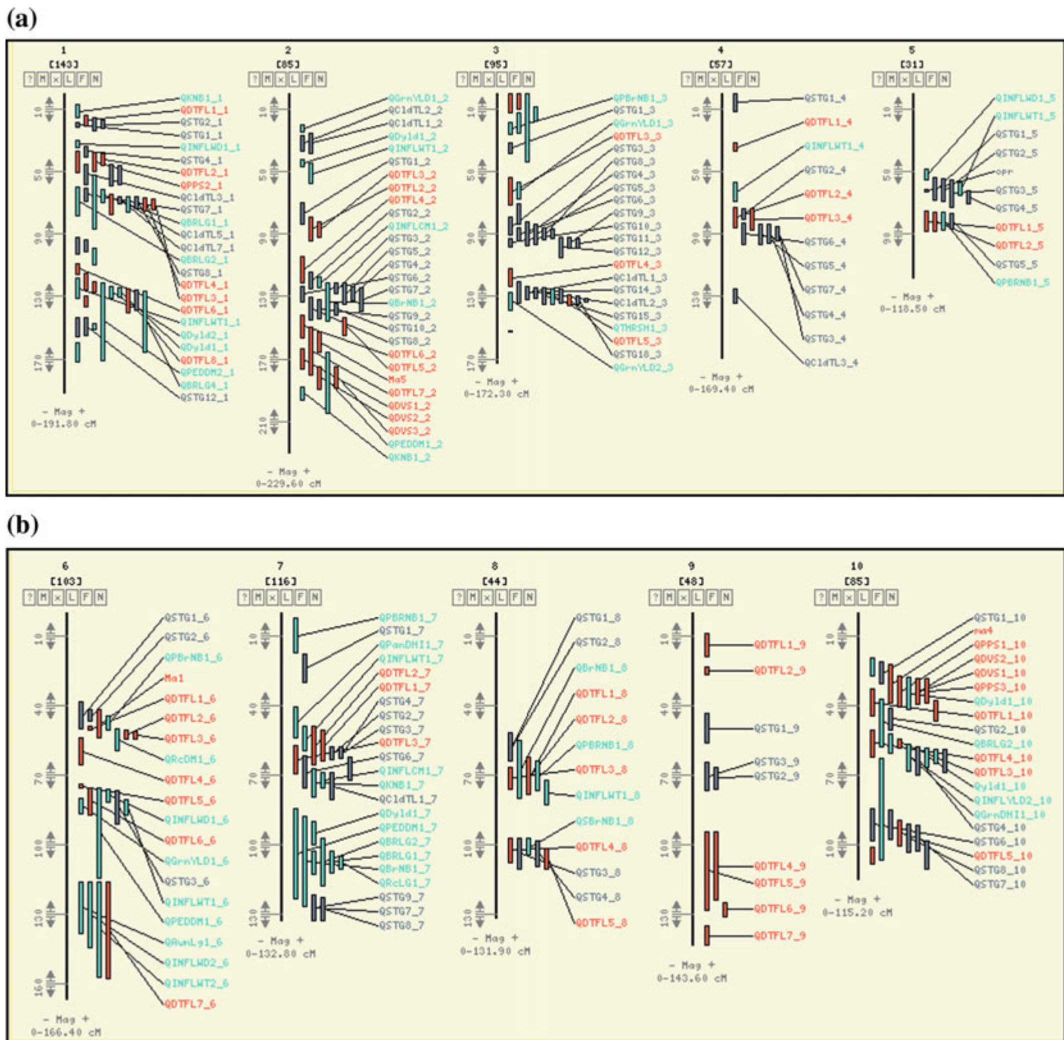

Fig. 1 Consensus QTL intervals for stay-green QTLs from Mace and Jordan (2011a, b) aligned to physically aligned SSRs (vertical bars indicate QTL interval tracks; red tracks for maturity genes/QTLs; aqua for panicle traits, and grey tracks for stay-green). **a** QTL tracks for sorghum chromosome SBI-01 to SBI-05. **b** QTL tracks for sorghum chromosome SBI-06 to SBI-10

weeks to study the nodal root angles (Singh et al. 2010, 2012). A seedling root angle tends to have medium to high heritability (Mace et al. 2012; Singh et al. 2011). Using the rapid root angle screening strategy, Mace et al. (2012) identified four QTLs for nodal root angle in sorghum, explaining 29.78, 11.65, 10.01, and 6.72 % of total phenotypic variation, respectively. These were further confirmed in a backcross-derived nested association mapping population. Three of the four identified QTLs showed homology to previously identified root angle QTL in rice and maize, whereas all four QTLs colocalized with previously identified QTL for stay-green in sorghum. This has opened up the scope to use these reported QTLs towards developing sorghum genotypes with better rooting systems, making them water- and nutrient-efficient genotypes. The

Table 1 SNPs associated with drought-related QTLs (Sujay Rakshit personal communication unpublished data)

Chromosome no.	Trait	QTL	Physical position		No. of SNP
			Starting point	Ending point	
SB01	Shoot dry weight	qSDW1_1[#]	59236915	64917463	75
SB02	Stay-green	Stg3[@]	26010527	28923791	42
	Root dry weight	qRDW1_2[#]	71213786	77627131	124
SB03	Stay-green	Stg2[@]	19344798	22773106	35
	Stay-green	Stg1[@]	25307987	25969626	5
SB05	Stay-green	Stg4[@]	5166739	12984849	111
	Nodal root angle	qRA1_5[#]	13464752	45972507	278
	Root dry weight, Shoot dry weight	qRDW1_5[#]	50053880	51943001	15
	Nodal root angle	qRA2_5[#]	54167643	55807231	28
SB08	Nodal root angle	qRA1_8[#]	8026484	41653341	358
	Root dry weight, Total leaf area	qRDW1_8, qTLA1_8, qTLA2_8[#]	47089939	50926404	66
	Total leaf area	qTLA3_8[#]	54033969	54997392	20
SB10	Bloom	BLMC[$]	56693	11980745	257
	Nodal root angle	qRA1_10[#]	57009865	58763524	25

[$]Burow et al. (2009); [#]Mace et al. (2012); [@]Srinivas et al. (2008)

projection of the six additional significant marker–trait associations for nodal root angle identified in the association mapping study identified that five out of six (83 %) of these new potential QTLs were also found to colocate with regions of the genome containing previously mapped stay-green QTLs (Mace et al. 2012).

In a recent study at the ICAR-Indian Institute of Millets Research, Hyderabad, India, a reference set for drought study comprising 96 genotypes has been developed through a combination of detailed multilocation drought response phenotyping of 258 diverse sorghum genotypes and their molecular diversity analysis using 39 polymorphic SSR markers (Rakshit et al. 2014). Through double digest restriction-site associated DNA ddRAD sequencing of this reference set, 235,009 genomewide single nucleotide polymorphisms (SNPs) have been identified, 1439 of which fall within the coordinates of reported QTLs associated with drought tolerance in sorghum (Table 1; Sujay Rakshit unpublished data).

2.3 Stay-Green QTL Introgression: Successes and Lessons Learned

The initial introgression work carried out at ICRISAT, Patancheru, India, has further dissected the genetic function of the stay-green QTLs. These studies suggest that *stg3A* and *stg3B* QTLs are responsible for transpiration efficiency and vapor pressure deficit response, and are also found to be most stable across several genetic backgrounds and across years (personal communication: Santosh Deshpande). The marker-assisted backcross (MABC) research at ICRISAT till the late 2000s were based on SSRs alone (Vadez et al. 2013), and a very large QTL interval that almost covered the complete chromosome arm was used. These datasets are publicly available (www.icrisat/cmap/ac.in). With progress in the understanding of the genetic regulation of the stay-green phenotype in sorghum, the current introgression of genomic regions involved in expression of the stay-green

phenotype will further lead a well-targeted manipulation of individual components. For instance, it is reported that B35 donor parent alleles at stay-green QTL *stg1* contribute to increased water extraction by the moderately senescent *caudatum* line, S35 (Vadez et al. 2011); however, *stg1* failed to show the same phenomenon in the genetic background of the highly senescent *durra* line, R16 (Vadez et al. 2011). Therefore, it is necessary to identify better donors for each of the component traits of the stay-green phenotype, which may vary with the genetic backgrounds, specific soil, water, and temperature regimes in which improved drought tolerance is desired. Among the investigated six QTLs, *stg3A* and *stg3B* QTLs are more stable across genetic backgrounds and environments. The *stg3A* and *stg3B* QTLs are located next to each other on SBI-02, and the mesocarp gene, *Z*, which governs the grain seed coat color, is located between the two QTL intervals (Mace and Jordan 2010). This poses a problem in selection for grain color coupled with stay-green traits. Similarly, location of the maturity gene Ma_5, just after the *stg3B* interval, also adversely affects fixation of the flowering time (Kim 2003). QTL analysis (Srinivas et al. 2008, 2009) along with in silico studies (Ramu et al. 2010) helped to add several SSRs in the region represented by the mentioned QTLs (Table 2). By exploiting existing backcross generations in ongoing MABC projects, the QTL interval for *stg3A* and

Table 2 A list of SSRs available for stg3A and stg3B QTL region including the original SSRs listed in Vadez et al. (2013)

S. no.	Locus	Stay-green QTL details	Chromosome	Physical position (Mbp)	SSR amplicon size
1	Xtxp013	Stg3A	SBI-02	55.95	119
2	Xiabtp193	Stg3A	SBI-02	56.08	218
3	Dsenhsbm055	Stg3A	SBI-02	56.79	–
4	Dsenhsbm025	Stg3A	SBI-02	56.86	–
5	m13_Xtxp304	Stg3A	SBI-02	57.04	–
6	Xtxp298	Stg3A	SBI-02	57.08	199
7	Stg3a_1	Stg3A	SBI-02	57.17	189
8	Stg3a_2	Stg3A	SBI-02	57.28	442
9	Stg3a_3	Stg3A	SBI-02	57.60	114
10	Stg3a_4	Stg3A	SBI-02	57.70	294
11	Stg3a_5	Stg3A	SBI-02	57.76	197
12	Xisp 280	Stg3A	SBI-02	57.76	–
13	Xisp10278	Stg3A	SBI-02	57.77	–
14	Stg3a_6	Stg3A	SBI-02	57.87	267
15	Stg3a_7	Stg3A	SBI-02	58.13	154
16	XSbAGB03	Stg3A	SBI-02	58.13	144
17	Stg3a_8	Stg3A	SBI-02	58.20	216
18	Stg3a_9	Stg3A	SBI-02	58.33	188
19	Dsenhsbm108	Stg3A	SBI-02	58.43	–
20	Stg3a_10	Stg3A	SBI-02	58.56	212
21	Stg3a_11	Stg3A	SBI-02	58.61	134
22	Xiabtp391	Stg3A	SBI-02	58.87	403

(continued)

Table 2 (continued)

S. no.	Locus	Stay-green QTL details	Chromosome	Physical position (Mbp)	SSR amplicon size
23	Xiabtp265	Stg3A	SBI-02	58.91	246
24	Stg3a_12	Stg3A	SBI-02	58.95	166
25	Stg3a_13	Stg3A	SBI-02	59.07	166
26	Xcup63	Stg3A	SBI-02	59.10	145
27	Xtxp464	Stg3A	SBI-02	59.20	140
28	Xiabtp80	Stg3A	SBI-02	59.20	176
29	Stg3a_14	Stg3A	SBI-02	59.75	153
30	Stg3a_15	Stg3A	SBI-02	59.82	201
31	Stg3a_16	Stg3A	SBI-02	59.88	156
32	Stg3a_17	Stg3A	SBI-02	59.95	253
33	Stg3a_18	Stg3A	SBI-02	59.97	204
34	msbcir339	Stg3A	SBI-02	60.19	176
35	Stg3a_19	Stg3A	SBI-02	60.19	131
36	Stg3a_20	Stg3A	SBI-02	60.21	202
37	Stg3a_21	Stg3A	SBI-02	60.23	227
38	Stg3a_22	Stg3A	SBI-02	60.28	208
39	Stg3a_23	Stg3A	SBI-02	60.28	176
40	Stgnhsbm34	Stg3A	SBI-02	60.44	–
41	Xtxp214	Stg3A	SBI-02	60.44	220
42	Xtxp445	Stg3A	SBI-02	60.45	238
43	Xcup29	Stg3A	SBI-02	60.45	191
44	Stg3a_24	Stg3A	SBI-02	60.46	125
45	Stg3a_25	Stg3A	SBI-02	60.48	224
46	Stg3a_26	Stg3A	SBI-02	60.51	162
47	Xtxp466	Stg3A	SBI-02	60.65	159
48	Xtxp465	Stg3A	SBI-02	60.67	177
49	Xiabtp509	Stg3A	SBI-02	60.82	240
50	Stg3a_27	Stg3A	SBI-02	60.84	103
51	Xtxp430	Stg3A	SBI-02	61.09	158
52	Xtxp430	Stg3A	SBI-02	61.09	158
53	Xsbarslbk2.61	Stg3A	SBI-02	61.09	–
54	Stg3a_28	Stg3A	SBI-02	61.13	221
55	Stg3a_29	Stg3A	SBI-02	61.13	113
56	Xisep0934	Stg3A	SBI-02	61.22	196
57	Stg3a_30	Stg3A	SBI-02	61.26	148
58	Stg3a_31	Stg3A	SBI-02	61.33	169
59	Xtxp001	Stg3A	SBI-02	61.37	211
60	Stg3a_32	Stg3A	SBI-02	61.37	184

(continued)

Table 2 (continued)

S. no.	Locus	Stay-green QTL details	Chromosome	Physical position (Mbp)	SSR amplicon size
61	Stg3a_33	Stg3A	SBI-02	61.47	99
62	Dsenhsbm032	Stg3A	SBI-02	61.47	–
63	Xisep0926	Stg3A	SBI-02	61.54	191
64	Xtxp056	Stg3A	SBI-02	61.57	347
65	Stgnhsbm35	Stg3A	SBI-02	61.66	242
66	Stg3a_34	Stg3A	SBI-02	61.83	268
67	Stg3a_35	Stg3A	SBI-02	61.84	146
68	Stgnhsbm36	Stg3A	SBI-02	61.95	180
69	Stg3a_36	overlap region	SBI-02	62.13	212
70	Stg3a_37	overlap region	SBI-02	62.29	186
71	Xisep0913	overlap region	SBI-02	62.40	208
72	Xisp10336	overlap region	SBI-02	62.69	–
73	Xisp 336	overlap region	SBI-02	62.69	–
74	Xisep0941	overlap region	SBI-02	63.12	188
75	Dsenhsbm045	overlap region	SBI-02	63.13	–
76	Stgnhsbm31	overlap region	SBI-02	63.13	–
77	Xiabtp231	overlap region	SBI-02	63.13	159
78	Stgnhsbm39	overlap region	SBI-02	63.20	–
79	Xtxp286	overlap region	SBI-02	63.39	–
80	Xgap84	overlap region	SBI-02	63.39	–
81	Stgnhsbm40	overlap region	SBI-02	63.44	–
82	Xisep0938	overlap region	SBI-02	63.44	205
83	Xisep0938	overlap region	SBI-02	63.44	–
84	Xiabtp226	overlap region	SBI-02	63.49	283
85	Xcup41	overlap region	SBI-02	63.62	231
86	Stgnhsbm42	overlap region	SBI-02	64.87	–
87	Xisep0849	overlap region	SBI-02	64.91	–
88	Xisp10334	overlap region	SBI-02	65.28	–
89	Xisep0944	overlap region	SBI-02	65.30	–
90	Xisep0939	overlap region	SBI-02	65.48	–
91	Xisep1022	overlap region	SBI-02	65.48	–
92	Xisep0942	overlap region	SBI-02	65.83	–
93	Xisep0910	overlap region	SBI-02	65.95	194
94	Xgpsb128	overlap region	SBI-02	66.17	285
95	Ungnhsbm49	overlap region	SBI-02	67.12	–
96	XmSbCIR187	overlap region	SBI-02	67.19	–
97	Xtxp348	overlap region	SBI-02	67.32	–
98	Xisep0935	overlap region	SBI-02	67.41	–

(continued)

Table 2 (continued)

S. no.	Locus	Stay-green QTL details	Chromosome	Physical position (Mbp)	SSR amplicon size
99	Xisp10200	Stg3B	SBI-02	67.80	–
100	Xiabtp323	Stg3B	SBI-02	68.22	191
101	Dsenhsbm015	Stg3B	SBI-02	68.22	–
102	Xtxp428	Stg3B	SBI-02	68.39	213
103	Xisp10259	Stg3B	SBI-02	68.39	–
104	Xisp 259	Stg3B	SBI-02	68.39	–
105	Xiabtp397	Stg3B	SBI-02	68.69	188
106	Xtxp429	Stg3B	SBI-02	68.85	214
107	Stg3b_1	Stg3B	SBI-02	69.31	152
108	Stg3b_2	Stg3B	SBI-02	69.61	209
109	Xtxp100	Stg3B	SBI-02	69.64	116
110	Stg3b_3	Stg3B	SBI-02	70.09	188
111	Stg3b_4	Stg3B	SBI-02	70.20	164
112	Xtxp207	Stg3B	SBI-02	70.26	184
113	Xtxp007	Stg3B	SBI-02	70.26	230
114	Xcup26	Stg3B	SBI-02	70.26	220
115	Stg3b_5	Stg3B	SBI-02	70.42	188
116	Stg3b_6	Stg3B	SBI-02	70.60	99
117	Stg3b_7	Stg3B	SBI-02	70.66	175
118	Stg3b_8	Stg3B	SBI-02	70.67	151
119	Xisep0733	Stg3B	SBI-02	70.75	330
120	Stg3b_9	Stg3B	SBI-02	70.85	225
121	Xiabtp190	Stg3B	SBI-02	70.89	215
122	Xisep0841	Stg3B	SBI-02	70.89	215
123	Xiabtp388	Stg3B	SBI-02	71.03	129
124	Xtxp296	Stg3B	SBI-02	71.11	168
125	Stg3b_10	Stg3B	SBI-02	71.20	277
126	Stg3b_11	Stg3B	SBI-02	71.43	219
127	Xiabtp484	Stg3B	SBI-02	71.60	117
128	Xiabtp205	Stg3B	SBI-02	71.79	165
129	Xiabtp076	Stg3B	SBI-02	72.17	292
130	Xiabtp317	Stg3B	SBI-02	72.33	236
131	Xcup40	Stg3B	SBI-02	75.36	193
132	Stg3b_1	Stg3B	SBI-02	77.29	140
133	Xtxp019	Stg3B	SBI-02	–	–
134	Xtxp008	Stg3B	SBI-02	–	–

stg3B QTL has been reduced. At ICRISAT additional efforts are currently being made to identify SNPs representing this region of the genome and currently a set of ~70 SNPs from genic regions have already been identified (Santosh Deshpande, personal communication).

These additional SSRs facilitate not only the selection for the stay-green QTLs independently but also for stringent mesocarp color in segregating populations. Similarly, as elaborated earlier, a major nodal root angle QTL distal to *stg3B* (Mace et al. 2012) has provided further opportunities for improvement of drought tolerance by selecting these QTLs simultaneously or in combination. The QTL intervals reported by Vadez et al. (2013) also overlap this root angle QTL within the *stg3B* region. This in turn is an opportunity to evaluate the available introgression lines (Vadez et al. 2011) for the combined effort of stay-green QTL(s) and root angle QTL in different combinations. Recent advances in the area of accurate and precise phenotyping platforms are adding values to dissect complex traits such as stay-green. Vadez et al. (2011) utilized the lysimeter-based estimations for the mechanism responsible for *stg3A* and *stg3B* as water-use efficiency under water-limited conditions and vapor pressure deficit for better transpiration efficiency. These traits help direct trait value measurement rather than stay-green expression. But they are cumbersome to measure for a large breeding or segregating population. At ICRISAT, a high-throughput phenotyping platform called the "LeasyScan" facility has been established to measure leaf area in a quicker way to access the dynamics of leaf development and leaf conductance (Vadez et al. 2015). This further helps to develop an early stage assay for screening the large breeding populations. The technique is based on a novel 3D scanning technique to capture leaf area development continuously, increasing imaging throughput and analytical scales by combining gravimetric transpiration measurements. Utilization of these new phenotyping advances has a great scope for high-throughput trait screening and breeding selections in large breeding populations.

3 Aluminum (Al) Tolerance

3.1 Importance and Mechanism of Al-Tolerance

Soil acidity imposes one of the most severe constraints on crop productivity in the tropics and subtropics (Wang et al. 2014a, b). Many tropical soils are acidic because percolating rainwater leaches the cations such as calcium, magnesium, and potassium, which are replaced by aluminum (Al), manganese, and hydrogen, leading to toxic levels of these elements (Rao et al. 1993). In acidic soils, particularly at pH below 5, Al is solubilized into ionic forms (Al^{3+}) and this leads to phosphorus (P) deficiency as P is fixed as aluminum phosphate (a highly insoluble and unavailable form to plants; Rao et al. 1993). Plants growing in Al toxicity display stunted growth and become susceptible to drought (Marschner 1991; Kochian et al. 2004; Wang et al. 2014a, b). This happens mainly due to inhibition of cell division, cell elongation, or both under low availability of P leading to poor root growth (Delhaize et al. 2004, 2009; Magalhaes et al. 2004). Al toxicity is the single most important factor affecting crop production on 2/3 of the acid soil affected area (Eswaran et al. 1997), and it is a major constraint for sorghum production in tropical and subtropical regions (Doumbia et al. 1993, 1998). Plant adaptation to acid soils is mainly through tolerance and avoidance, of which avoidance occurs more commonly. Avoidance is achieved either through changes in the rhizosphere ecology such as increase in pH through root exudates or release of chelators for Al (such as citrate or malate), or increase in root surface area via mycorrhizae (Marschner 1991).

3.2 Genes for Al-Tolerance in Sorghum

Al tolerance in sorghum is controlled by a single gene (*AltSB/SbMATE*) in a cross between tolerant, SC283, and sensitive, BR007, which has been mapped to sorghum chromosome 3

(Magalhaes et al. 2004). Positional cloning identified a gene coding for an aluminum-activated citrate transporter, which is a member of the multidrug and toxic compound extrusion (MATE) family, the causal component leading Al tolerance (Magalhaes et al. 2007). Markers from this region have been deployed by breeders to introgress favorable *SbMATE* alleles in susceptible sorghum genotypes (Anami et al. 2015). *SbMATE* expression is reported to be induced with time of exposure to Al and the expression is higher in the root apex compared to the rest of the root (Magalhaes et al. 2007). The region 1–3 mm behind the root tip where transition from cell division to cell elongation occurs was reported to be the most sensitive area (Sivaguru et al. 2013). After exposure to Al, sensitive genotypes accumulate several-fold more Al in their root apex compared to Al-tolerant genotypes (Delhaize et al. 1993). The coding region of *SbMATE* is identical between Al-tolerant and Al-sensitive genotypes. However, the second intron of *SbMATE* shows polymorphism between the two types. In the promoter region of *SbMATE*, a tourist-like miniature inverted repeat transposable element (MITE) transposon has been reported, whose repeat numbers are observed to be positively correlated with Al tolerance (Magalhaes et al. 2007). With this it is assumed that the causative mutations underlying aluminum tolerance may have a regulatory nature (Anami et al. 2015).

4 QTL/Marker Analysis for Other Abiotic Stresses

4.1 Early-Season Cold Tolerance

Sorghum, in general, cannot grow well under soil temperature below 15 °C. However, if early-season cold tolerance can be induced in sorghum cultivars, the area under it can be extended to more northern latitudes, and may allow early planting, particularly in the United States (Yu and Tuinstra 2001). Under this situation, improved emergence and early-season vigor are needed, which will ensure establishment of better crop stand, and prevent loss of seedlings due to cold. The "Kaoliangs" sorghum germplasm from temperate regions of China shows better cold tolerance than tropical germplasm (Cisse and Ejeta 2003). Thus, this particular germplasm is a promising source for improvement of cold tolerance (Yu and Tuistra 2001; Franks et al. 2006). However, these germplasms are in general not agronomically promising, and thus require extensive back-crossing to transfer cold tolerance in the breeding program. Gunaratna (2002) found that genetic control of seedling vigor traits under cold stress and under optimal temperatures is similar. In order to identify QTLs for seedling vigor traits under cold stress, Knoll et al. (2008) developed a recombinant inbred line (RIL) population of 153 RILs from a cross between cold-tolerant Chinese Kaoliang "Shan Qui Red" (SQR) and a cold stress-susceptible African *caudatum* SRN39. They could identify two QTLs for germination. One among them on linkage group SBI-03a was significant under cold and optimal temperature. The other, on SBI-07b, was contributed by SQR, and showed higher significance under cold temperatures. They also identified a region on SBI-01a from SQR, favoring seedling emergence and seedling vigor under early and late field plantings. Knoll and Ejeta (2008) further demonstrated favorable effects of SQR alleles towards seedling vigor and/or emergence in two new populations. Subsequently, Burow et al. (2011) reported an additional 16 QTLs for cold germinability, field emergence, and early seedling vigor in a mapping population consisting of 171 RILs derived from the cross between RTx430 (cold-sensitive) and PI610727 (cold-tolerant) using 141 SSR markers. The most promising region for improving field emergence identified in this study is located on SBI-01. Other QTL-rich regions were located on SBI-03, SBI-04, SBI-06, SBI-08, and SBI-09. Utilizing a genetic map based on an F_8 RIL population reported by Shiringani et al. (2010) and by phenotyping a subset of the same population for cold tolerance, Bekele et al. (2013) identified highly interactive epistatic QTL hotspots having a significant effect on prolonged chilling survival. The

major QTL regions on chromosome SBI06 harbor candidate genes that govern tolerance to abiotic stress. They identified several genes conferring maintenance of cell division and growth under early chilling stress within QTL hotspot regions, which can be a potential candidate for breeding cold tolerance.

In sorghum, a higher respiration rate has been correlated with higher germination under cold stress (Balota et al. 2010). Washburn et al. (2013) correlated a rhizome formation trait to overwintering ability of sorghum. The phenomenon of overwintering and rhizomatousness are reported to be controlled by seven QTLs (Paterson et al. 1995; Washburn et al. 2013) that were identified in a mapping population of BTx623/*S. propeinquum*. The ability of sorghum to overwinter and form rhizome has been suggested to be useful for biofuel sorghum production (Anami et al. 2015).

Through a differentially expressed gene (DEG) analysis between contrasting genotypes, HongkeiZi (cold tolerant) and BTx623 (cold sensitive), Chopra et al. (2015) identified 41,603 SNPs. They could validate 89 % of the 114 selected SNPs using endpoint genotyping technology. By combining expression profile data and gene-based SNP information they generated a searchable database, which turned out to be an important resource for sorghum cold stress genomics research (http://www.csrl.ars.usda.gov/psgd/index-sorghum.aspx.html). All these markers are important resources to introgress cold tolerance in sorghum. Recently Upadhyaya et al. (2016) conducted association mapping in a sorghum mini-core collection (Upadhyaya et al. 2009) using 162,177 SNPs and identified only one marker locus (*Locus 7-2*) to be significantly associated with low-temperature germination and none with vigor. The *Locus 7-2* was found to represent field early-emergence QTL flanked by Xtxp278 and Xtxp295 (Burow et al. 2011). The locus was found next to three overlapping emergence QTLs close to sPb-5796 (Fiedler et al. 2012). Furthermore, the syntenic region of this locus colocalizes with two cold-tolerance rice QTLs, and it is found that when its wheat homologue is overexpressed in tobacco, cold-tolerance and germination rate are increased.

4.2 Salinity Tolerance

Salinity is also an emerging problem in sorghum cultivation in different parts of the world. It has been reported that upon Na^+ stress a high-affinity potassium transporter gene family in sorghum, *SbHKT1;4*, is strongly upregulated in salt-tolerant sorghum genotypes, leading to better Na^+/K^+ ratio and optimum plant growth (Wang et al. 2014b). A total of 38 QTLs influencing salt tolerance has been reported from an RIL population comprising 181 lines derived from a cross Shihong137/L-Tian (Wang et al. 2014a). Out of these, six are reported to be major QTLs explaining above 10 % phenotypic variation. Studies suggested that the mechanism of salt tolerance at the germination and seedling stages is different. Further research in this direction is essential before deploying the identified QTLs in MAS.

4.3 Nitrogen Use Efficiency

The demand for nitrogen (N) fertilizer in agriculture across the globe currently stands at ~110 million metric tons per year and is projected to reach up to ~250 million metric tons by the year 2050 (www.fao.org). Because of the high mobility of nitrate in the soil, up to 50 % of applied N is lost by the processes of leaching, runoff, and denitrification. This not only increases the cost of crop production but also adds to the pollution of the groundwater and adversely affects the soil structure. The process has detrimental effects on the environment by increasing greenhouse gases such as nitric oxide in the atmosphere. Hence, developing crop varieties with improved N absorption and utilization can mitigate these problems of modern agriculture. Sorghum is predominantly cultivated under low

fertility conditions, particularly in Africa and Asia. However, in developed countries it is cultivated under high fertility conditions. Significant genotypic differences in terms of nitrogen-use efficiency (NUE) have been reported in sorghum (Maranville et al. 1980; Youngquist et al. 1992). Expression analysis between four low-N tolerant sorghum genotypes (San Chi San, China17, KS78, and high-NUE bulk) and three sensitive genotypes (CK60, BTx623, and low-NUE bulk) under of low-N (LN, 0 kg ha^{-1}) and normal N (NN, 100 kg ha^{-1}) regimes revealed that in sensitive genotypes, N-stress increased the abundance of DEG-transcripts associated with stress responses including oxidative stress, whereas the tolerant genotypes produced greater root mass for efficient uptake of nutrients (Gelli et al. 2014). Higher abundance of transcripts related to high-affinity nitrate transporters (NRT2.2, NRT2.3, NRT2.5, and NRT2.6) and lysine histidine transporter 1 (LHT1) were detected in tolerant genotypes, which indicates possible improved uptake of inorganic and organic forms of nitrogen by these genotypes. Higher abundance of *SEC14* cytosolic factor family protein transcript in tolerant genotypes could lead to increased membrane stability and tolerance to N-stress.

Gelli et al. (2016) sequenced a population of 131 RILs derived from a cross between CK60 (inefficient N user) and China17 (efficient N user) using GbS. Following the composite interval mapping (CIM) technique using 642 polymorphic SNPs they could identify 38 QTLs for 11 agronomic traits under normal (100 kg ha^{-1}) and no N application condition on chromosomes 1, 5, 6, 7, and 9. Phenotypic variation explained by each QTL ranged from 6.2–50.8 %. Using Illumina RNA sequencing on seedling root tissues they could identify 726 differentially expressed transcripts between the parents. Among these 108 were mapped close to the QTL regions. Differentially expressed transcripts were related to nitrogen metabolism (Ferredoxin-nitrate reductase), glycolysis (Phosphofructo-2-kinase), seed storage proteins, plant hormone metabolism, and membrane transport. The study indicated that the differentially expressed transcripts underlying the pleiotropic QTL regions could be potential targets for improving sorghum performance under limited N fertilizer through marker-assisted selection.

5 Molecular Insight into Abiotic Stress Response

5.1 Noncoding RNAs

MicroRNAs (miRNAs) have been associated with regulations of different classes of genes across plant species. Pasini et al. (2014) reported upregulation of miRNA associated with the regulation of transcription, signal transduction, carbon metabolism, detoxification, osmoprotection mechanisms, and stability of protein membranes upon imposition of low moisture stress in four-leaf-old sorghum genotype, IS1945. The study suggested the possible utility of these drought-related genes in identifying drought-tolerant sorghum lines. In fact, miRNA 169 g, as reported to be upregulated under drought stress in rice, has five sorghum homologues (Zhao et al. 2007). Qi et al. (2013) also reported long noncoding RNAs (lncRNAs) from foxtail millet in response to drought stress, having sequence conservation and collinearity with sorghum. Through an in silico study, Ram and Sharma (2013) further indicated a probable role of miRNAs in water stress response in sorghum.

5.2 Role of Genes Governing Auxin and Transcription Factors

Over 50 differentially expressed drought-responsive gene orthologues with enriched ABREs and CGTCA-motifs or motifs responsive to ABA specific to sorghum have been identified (Dugas et al. 2011). A large number of auxin-related gene families including *SbGH3*, *SbLBD*, *SbIAA1*, *SbGH3-13*, *SbLBD32*, and others have been reported that are highly induced by salt or drought stress (Wang et al. 2010). Moisture stress has been observed to trigger upregulation of transcription factor genes of MADS-box, Auxin Responsive Factors, Heme

Activator Protein 2, and so on, specifically in root tissues (Aglawe et al. 2012). Sorghum auxin transporters have been observed to be up- or downregulated in response to abiotic stresses, depending on the class of transporters (Shen et al. 2010).

Over 100 ethylene response factor (ERF) genes have been reported in sorghum (*SbERF*), which have been grouped into 12 classes (A-1 to A-6 and B-1 to B-6) on the basis of sequence homology (Yan et al. 2013). The ERF superfamily plays an important role in both abiotic and biotic stress response in plant systems. In addition to ERF, chloroplast glutathione reductase (*cpGRs*), G-protein complexes, drought response element-binding (*DREB*) proteins, and *SbEST* are reported to play very important roles in abiotic stress responses not only in sorghum but in other plant species as well. In a recent study, Chopra et al. (2015) identified a total of 1910 DEGs under cold and control temperature from cold-tolerant genotype HongkeZi and cold-sensitive genotype BTx623. They could identify TFs including *DREB*, *C-repeat binding factors*, and ERF TFs to be upregulated under cold stress in tolerant genotype HongkeZi. They also identified specific genes such as plant cytochromes, glutathione s-transferases, and heat shock proteins to be differentially regulated under cold stress between cold-tolerant and susceptible genotypes.

5.3 Role of Compatible Solutes

Betaise aldehyde dehydrogenase (*BADH1* and *BADH15*) is reported to be induced in response to moisture deficit leading to accumulation of glycine betaine (Wood et al. 1996). *SbGRRNP* (glycine-rich RNA-binding protein) expression is regulated by salinity and ABA, as well as blue and red light (Aneeta et al. 2002). This suggests that in sorghum a possible crosstalk between abiotic stress and light signal may exist. By engineering higher mannitol synthesis, Maheswari et al. (2010) could induce salinity tolerance in transgenic sorghum cv. SPV462. Studies further suggest that *SbP5CS1* and *SbP5CS2* are upregulated in response to drought, salt, and jasmonic acid treatment (Su et al. 2011). These two are important regulatory genes controlling proline synthesis. Thus, the study suggested a role of proline biosynthesis in imparting abiotic stress tolerance. All the above reports suggest the possibility to develop abiotic stress-tolerant genotypes by modulating the role of these genes, transcription factors, or miRNAs either through the transgenic route or through genome editing.

6 Next-Generation Sequencing (NGS) Tools and Next-Generation Populations

When combined with high-throughput and precise phenotyping platforms developed at ICRISAT (Vadez et al. 2015), NGS technologies provide a powerful and rapid tool for identifying the genetic basis of agriculturally important traits and for predicting the breeding value of individuals in a plant breeding population (Varshney et al. 2014). Next-generation populations such as nested-association mapping (NAM) populations (Yu et al. 2008; Buckler et al. 2009) and backcross-derived NAM (BCNAM) populations (Jordan et al. 2012) are two most potent next-generation populations to dissect genomics of complex traits. All these new tools play a major role in advancing breeding efficiencies by simultaneously aiding genetic studies and also providing access to new variability in comparatively elite backgrounds. These new-generation populations are required for linking phenotypic variations with sequence variations at high resolution. The BCNAM scheme was already well established by Jordan et al. (2011) to benefit both trait mapping and infusing diversity that was not accessible previously in the traditional plant breeding populations. In combination with genomewide sequencing approaches, these populations provide access to insights into the genetic architecture of important traits (Yang et al. 2014a, b). The major limitation in implementing these new advances routinely in the breeding programs is lack of data management skills and

database resources. In terms of applications of these tools in molecular breeding, the NGS-based genotyping platforms such as genotyping-by-sequencing (GbS) have the potential for background selection in traditional introgression breeding and genomic selection (GS) which is an emerging methodology in modern breeding programs. The main advantage and driving force for its implementation are availability of low-cost high-throughput genotyping platforms and access to high-throughput phenotyping facilities. These in turn feed the improved capacities of performance prediction of individual genotypes for quantitative traits. The genomic selection, to realize its full potential, will need to address specific issues related to the theory of GS such as design of training populations, predicting efficacy under an altered marker and population variable scenario, and an approach for integration of GS in ongoing breeding schemes (both in scale and dimension). Similarly, considering the different level of advancement of constituent technologies involved in GS, an appropriate resource investment strategy for every single breeding program needs to be developed for maximizing the returns in terms of genetic gain.

7 Future Prospects

Sorghum is an important failsafe crop providing food–feed–fodder–fuel to most of the resource-poor farming communities in drylands where it is a staple crop. The wide repertoire of germplasm, genetic, genomic, and breeding information/resources positions sorghum as one of the C_4-model crop species. Advancement in NGS, high-throughput phenotyping, and recent developments in advanced breeding populations will drive the new cycle of genetic gain in sorghum across agroecological zones. The sorghum research community has access to large genetic (germplasm with unique traits) and genomic (SSRs, SNPs, high-density genetic maps, genome sequence) resources, and many QTLs/candidate genes associated with agronomic traits are known in sorghum. These marker–trait associations need to be validated independently in breeding populations and suitable marker assays such as the KASPar SNP assay need to be generated. The integration of these new tools along with new-generation breeding populations will further help accumulate the required information to develop the framework for implementing genomic selection for sorghum improvement and thus historical division between breeding and genomics will become increasingly blurred.

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Genomic Approaches for Improving Grain Quality of Sorghum

10

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Abstract

Sorghum grain provides an important calorific source for millions of people living in developing countries and is a principal animal feed and source of gluten-free flour for the livestock and food processing industries of developed nations. A versatile grain, sorghum is also widely utilized in the production of alcoholic beverages in countries such as China and several countries in sub-Saharan Africa, where the liquor *baijiu* and beer are a major end-use, respectively. Renowned as a hardy crop, sorghum is relatively drought tolerant and can be grown on marginal lands and is adaptable to a wide range of environmental conditions, giving this species particular advantages over other cereals. Despite its inherent benefits, sorghum has not proven to be a major alternative to the other notable cereals such as wheat and maize, due to significant problems concerning the low amount of specific essential amino acids, for example, lysine, lower protein content, lower starch digestibility, and smaller grain size, which has implications for the traits mentioned above as well as acting as an impediment to efficient grain handling in cereal-processing industries. The challenges in enhancing sorghum grain quality are not insurmountable and great strides have already been achieved in a relatively short time via scientific breeding to enhance grain yield and provide abiotic and biotic stress resistance. As the sorghum market has matured, demand for higher quality grain, whether for alcohol production or animal and human consumption, is increasing. Although yield and disease resistance are still the primary focus of breeders, advances in genomics, online bioinformatic data repositories, high-throughput phenotypic screening such as near-infrared reflectance (NIR), and the increasing affordability of

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next-generation sequencing, have allowed breeders to incorporate improved grain quality parameters into their programs. This chapter elaborates recent advances in genomics that have provided researchers with the tools to solve several of the issues surrounding grain quality in sorghum as well as future directions for experimentation.

1 Introduction

Global demand for food is set to grow well into the twenty-first century where the human population has been projected to reach 9.6 billion by the year 2050 (Gerland et al. 2014). The focus on food security is not only to increase the food supply but also to improve food quality, especially in relation to nutritional value (Godfray et al. 2010). Sorghum [*Sorghum bicolor* (L.) Moench] being the fifth most important cereal crop in the world after wheat, rice, maize, and barley, is the dietary staple for more than 500 million people in 30 countries and is grown over 40 million hectares of land in up to 105 countries with an estimated production of around 60 million metric tons in 2012–2013 (http://www.fas.usda.gov/psdonline/psdHome.aspx, Rakshit et al. 2014).

Genetic studies and molecular breeding approaches require genomic resources such as molecular markers, genetic maps, and sequence information, which are readily available for sorghum because of its small diploid genome and role as a model crop species for C_4 plants (Ritter et al. 2008). From the extensive use of mapping and breeding populations through to the development of nested associated mapping (NAM; Mace et al. 2013a) and mutant (Xin et al. 2008) resources, the tools available for genetic trait dissection are improving constantly in sorghum (Luo et al. 2016).

The implication of genomic approaches to fill gaps in knowledge about plants has proven useful for both a basic understanding of plants and as a means to devise strategies to improve crop plant species. Here we present and discuss specific examples where the implementation of genomics tools has advanced knowledge dramatically, particularly in relation to grain quality. Although genomics-level approaches have only recently been applied to sorghum, where the first draft genome was published in 2009 (Paterson et al. 2009), extensively resequenced in 2013 (Mace et al. 2013b), and analyzed via a spate of high-density genotyping by sequencing (GBS) publications (Morris et al. 2013; Wang et al. 2014; Mindaye et al. 2015), it is anticipated that new datasets will continually be produced as our understanding of sorghum biology evolves.

2 Application of Sorghum Grain in Food Products

Cultivated sorghum in Africa and Asia is mostly used for human consumption providing millions of people with dietary starch, proteins, vitamins, and minerals. Equipped with many notable attributes including adaptation to drought, waterlogging, and saliferous soils, sorghum plays an important role in the food security of people in arid and semi-arid regions (Belton and Taylor 2004).

Sorghum is consumed as whole grain or as flour for a broad range of dishes and beverages. In parts of Africa, grain sorghum is ground into flour and processed into thin or thick porridges. Fermented sorghum bread called *kisra* (Sudan) or *injera* (Ethiopia) is the staple diet in the Horn of Africa where sorghum was first domesticated (Tinay et al. 1979; Belton and Taylor 2004). Similarly in India, sorghum grain is consumed in several forms such as unleavened bread called *roti*, boiled grain, and also thick and thin porridge (http://www.fao.org/). Other foods incorporating sorghum grain are tortillas (Latin America), *couscous* (Africa), traditional infant porridge called *nasha* (Sudan), and baked products (United States, Japan, Africa; Dicko et al.

2006). Furthermore, in some African countries, instant baby porridge that is made from pre-cooked sorghum flours with added vitamins and proteins is commercially available.

Sorghum is also a chief ingredient for the production of beer and alcoholic beverages in China and several African countries (Dicko et al. 2006; Taylor et al. 2006). The opaque beers of Southern Africa and malted dark beers found in West Africa are examples of alcoholic beverages produced in sub-Saharan Africa. In other developed countries such as the United States, parts of Southern America, and Australia, sorghum grain is mainly used for animal feed. The grain is fed milled or steam flaked to livestock as a major energy source (Godwin 2004). However, there is an increasing demand for the utilization of sorghum grain as human food in developed and developing countries, partly caused by the cereal's nongluten attributes that provide a cereal alternative to people suffering from gluten allergies (Pontieri et al. 2013). In fact, sorghum grain can be processed into gluten-free bread (with addition of 20–50 % sorghum flour to the wheat flour), cookies, cereals, pasta, and snacks.

The other increasing focus upon whole grain sorghum is its low glycemic index (GI) and human health attributes. Compared to cereals such as wheat or rice, all sorghum-based food products display significantly lower GI and glycemic load (GL) which may help in decreasing post-prandial blood glucose levels and aid in the prevention and control of diabetes (Prasad et al. 2015).

In addition to providing a source of starch and protein, sorghum grain is an important source of more than 20 micronutrients (BSTID-NRC 1996; Dicko et al. 2006). It is a richer source of several B vitamins (pantothenic acid, niacin, folate, and biotin) than maize (BSTID-NRC 1996), and provides higher levels of iron and zinc than rice (Dicko et al. 2006; Chan et al. 2007). Furthermore, recent studies suggest that future increases in iron and zinc should be achievable through breeding programs (Hariprassana et al. 2014; Badigannavar et al. 2016). For further details readers may refer to Chap. 4.

3 Nutritional Constraints of Sorghum Grain

Despite the potential of sorghum to be a leading crop to ensure food security for people in the least developed world and its potential as a raw material for many industrial applications, its grain still suffers from nutritional deficiencies, which make it inferior to the other major cereals. For instance, direct comparisons between maize and sorghum show that sorghum grain is remarkably similar in its nutritional profile in terms of structure and chemical composition; however, sorghum is known to have deficiencies in some essential amino acids such as lysine, tryptophan, threonine, and methionine (Wu et al. 2009). These amino acids are essential for human growth and development and particularly important for people in poorer regions where sources of nutritious food are limited. Added to this, digestibility of sorghum grain is lower than that of maize and other cereal species, especially when cooked (Duodu et al. 2003). Several studies have shown that proteins of wet cooked sorghum are significantly less digestible than the proteins of other similarly cooked cereals (Axtell et al. 1981; Maclean et al. 1981; Duodu et al. 2003). There are several factors contributing to poor protein and starch digestibility in sorghum including the extent of protein-starch interactions, type of starch, physical form of starch granules, degree of disulphide bond crosslinking of the sorghum storage proteins, as well as the large presence of tannins in certain sorghum cultivars (Rooney and Pflugfelder 1986; Duodu et al. 2003).

The most abundant component of the dry grain is starch. Starch is stored as crystalline or semicrystalline water-insoluble granules with an

internal lamellar structure packed within the endosperm, consisting of two types of glucan polymers, plus trace amounts of lipids and proteins (Buleon et al. 1997). The two principal components of starch in sorghum grains are amylose and amylopectin, which differ in degree of polymerization (DP) of glucan chains and frequency of branches. The length of glucan chains and the proportion of amylose and amylopectin molecules are the important factors that affect the size, structure, and function of starch (Charles et al. 2005).

Amylose consists of linear glucan polymer, composed of α-(1 → 4) linked glucose residues, with approximately one branch of α-(1 → 6) linkages for every 1000 glucose residues (Takeda et al. 1986, 1987). Amylose has a molecular weight of around 100 kDa and is only 20 to 30 % of total starch compared with amylopectin (Buleon et al. 1997). Amylopectin is a larger molecule with a molecular weight of around 104–106 kDa and has frequent branches, consisting of hundreds of short α-(1 → 4) glucan chains joined together by an α-(1 → 6) linkage (Buleon et al. 1997). Branch points are more frequent with approximately one per every 20 glucose residues in amylopectin (Takeda et al. 1987).

There are three parts of sorghum kernel: outer pericarp, embryo (germ), and storage tissue (endosperm; Rooney et al. 1981). Endosperm is made up of the outermost aleurone layer, peripheral layer (subaleurone), corneous, and floury areas (Rooney et al. 1981; Wong et al. 2009). The distribution of starch granules within aleurone cells is limited but the presence of autolytic enzymes such as amylases and protein inhibitors, as well as water-soluble vitamins, minerals, and spherical bodies containing protein and lipid are abundant.

Compared to maize, sorghum has a bigger fraction of peripheral endosperm and this section of sorghum grain is particularly dense, hard, and resistant to water penetration and digestion as well as to enzymatic degradation (Rooney and Pflugfelder 1986). The peripheral endosperm is located beneath the aleurone layer and the corneous endosperm is located beneath the peripheral endosperm. The largely packed starch granules contained within both of these endosperm layers are surrounded by numerous protein bodies that are embedded in a dense matrix of dried endosperm cells. The matrix, comprised mainly of protein and carbohydrates, is relatively resistant to water and hydrolytic enzymes. Compared with starch granules present in the peripheral and corneous endosperm, starch granules contained within the floury endosperm are the highest in numbers and are more accessible to enzymatic hydrolysis (Rooney et al. 1981; Wong et al. 2010).

Typically in sorghum grain, starch is composed of a mixture of 30 % amylose and 70 % amylopectin (Rooney and Pflugfelder 1986). However, phenotypes associated with endosperm starch lacking amylose have been recognized in sorghum (Karper 1933). In these varieties the endosperm resembles hard opaque wax and is thus referred to as "waxy" (Wong et al. 2010). This condition is due to a lack or inactive function of granule-bound starch synthase (GBSS), an enzyme that synthesizes amylose (Pedersen et al. 2007). Starch granules and the protein matrix that surround them are more digestible in waxy sorghum compared with the nonwaxy grains (Rooney and Pflugfelder 1986).

Protein typically makes up about 12 % of grain dry weight (Wong et al. 2010) although this varies among cultivars. The major class of protein is the seed storage proteins (prolamins), which are deposited as protein bodies approximately 1–2 μm in diameter. The prolamins of sorghum are named kafirins (Shull et al. 1992) and include alpha (α), beta (β), gamma (γ), and delta (δ) kafirin families. Of these, α-kafirin encompasses about 80 % of total kafirins, and is concentrated in the core of the protein body whereas β- and γ-kafirins are primarily found at the surface of the protein body (Shull et al. 1992; Oria et al. 2000). Cysteine-rich β- and γ-kafirins are linked together by disulphide bonds between themselves, as well as with α-kafirin forming oligomers of polypeptide. The formation of disulphide crosslinked protein oligomers and polymers increases with cooking. These enzymatically resistant protein polymers prevent

access to and restrict digestion of the more digestible and centrally located α-kafirin within the protein body (Oria et al. 2000; Duodu et al. 2003). Highly digestible protein is shown to have better nutritional value because it provides more amino acids for absorption after proteolysis than protein with lower digestibility (Blackburn 1981; Duodu et al. 2003).

4 Power of Omics Methodologies

The real power of omics-level methodologies to define gene–protein–trait relations becomes clear when it is coupled with an integrated approach targeted at the genomic, transcriptomic, and proteomic levels. Several pertinent examples involving sorghum exist in the literature such as the work of Cremer and others, who utilized the published gene set of sorghum alongside 2D protein electrophoresis and MALDI-TOF protein fingerprinting to determine the allelic variation at the kafirin loci and to determine the effects of this genetic diversity on protein expression (Cremer et al. 2014a). Their results highlighted reductions in alcohol-soluble protein in β-kafirin null lines as well as a range of redox active proteins affecting storage protein biochemistry. They concluded that redox states of endosperm proteins, of which kafirins are a subset, could affect quality traits in addition to the expression of proteins with direct implications for sorghum's value in food, feedstock, and biomaterial usages. Likewise, Campbell et al. (2015) linked the allergenic components of the *S. halepense* (Johnsongrass) pollen transcriptome to that of the actual Johnsongrass pollen proteome and identified immunologically relevant allergens that elicit hay fever. Environmental monitoring of allergenic components by measurement of sequence tags in air samples and the development of allergic rhinitis therapeutics is expected to arise from such work.

More recently, a comprehensive analysis linking sorghum grain genomics and transcriptomics explored the role of domestication and human selection upon sorghum grain starch synthesis and its implications for current breeding programs and the desire to improve sorghum grain quality and yield (Campbell et al. 2016). They determined that many genes within the primary starch synthesis pathway had a clear reduction in nucleotide diversity between the landraces and wild lines demonstrating that the ancestral effects of domestication are still clearly distinguishable. Furthermore, the allelic diversity present within a number of starch synthesis loci, including the identification of nonsynonymous single nucleotide polymorphisms (SNPs), providing researchers with an opportunity to conduct further starch structural studies and enhance our understanding of starch synthesis.

Quantitative trait locus (QTL) analysis has been an informative tool for breeding programs and functional genetics. A disadvantage of the approach is that the availability of markers, rates of recombination in the vicinity of a QTL, and the feasibility of handling the required population size, limit resolution of a QTL interval. Genotype by sequencing (GbS) marker systems such as diversity array technique sequencing (DArTseq), coupled with genomewide association studies (GWAS) is the next-generation sequencing (NGS) equivalent of QTL analysis for traits of interest. Numerous studies employing GbS and GWAS analysis to dissect various genetic traits now exist for sorghum (Morris et al. 2013; Hayes et al. 2015; Wang et al. 2014). Generating very high marker densities (tens of thousands of markers) with known physical locations within the genome, GbS technology is targeted at high-resolution mapping and detailed genetic dissection of traits. As in QTL analysis, GWAS begins with the identification of lines that possess contrasting phenotypes for the trait of interest. No prior set of markers is required to facilitate GWAS, as linkage of traits to genomic regions should harbor polymorphisms between the lines being compared that are useful as "markers". GWAS overcomes the main limitations of traditional QTL analysis which is that only allelic diversity that segregates between the parents of a particular F_2 cross or within the recombinant inbred line (RIL) population can be assayed and the amount of recombination that occurs during the creation of the RIL population places a limit on the mapping resolution (Korte and Farlow 2013).

5 Genomics of Starch Synthesis and Improvement

5.1 Improving Grain Quality: Engineering Increased Grain Size

As a crop, sorghum delivers lower yields than the other major cereals. This is primarily due to the smaller grain size of sorghum (100-seed weight 2.5–3.5 g). As indicated above, starch is the major component of sorghum grain, and hence it follows that if starch production is enhanced it will lead to increased grain size. The process of grain filling (which determines grain weight) in wheat and barley is typically sink-limited rather than source-limited except under extreme stress conditions, implying that there is potential to increase sink strength without the need to increase photosynthetic capacity (Serrago et al. 2013). Several candidate genes have been identified that have the potential to increase starch levels in grain through transgenic approaches. The recent development of an efficient transformation system for sorghum (Liu et al. 2014) makes transgenic approaches a promising strategy to increase seed weight and in turn grain yield in sorghum. For further detail readers may refer to Chap. 12.

A simplified version of the metabolic pathway for starch biosynthesis in cereal endosperm is shown in Fig. 1. The step with the greatest flux control in this pathway is the conversion of glucose-1-phosphate and ATP into ADP-glucose and pyrophosphate. The process is catalyzed by the enzyme ADP-glucose pyrophosphorylase (AGPase). AGPase is, therefore, an obvious candidate for overexpression in order to upregulate starch biosynthesis. In most plant tissues (both source and sink) AGPase is localized in plastids. However, in cereal endosperms there is an additional cytosolic form that produces approximately 85–95 % of the ADP-glucose that is used for starch synthesis in the endosperm (Denyer et al. 1996; Thorbjørnsen et al. 1996). This cytosolic form has been another target for studies aiming to upregulate starch biosynthesis in cereal seeds.

AGPase is a heterotetrameric enzyme made up of two large subunits and two small subunits (Tuncel and Okita 2013). In maize, the cytosolic forms of the large and small subunits are encoded by the genes *Shrunken2* and *Brittle2*, respectively. The sorghum genome contains close homologues of these genes, which are strongly expressed during grain filling (Campbell et al. 2016). Transgenic approaches have been used to overexpress the *shrunken2* gene in several cereals other than sorghum, including maize (e.g., Wang et al. 2007; Li et al. 2011; Hannah et al. 2012), wheat (e.g., Smidansky et al. 2002, 2007; Kang et al. 2013), and rice (e.g., Smidansky et al. 2003; Lee et al. 2010). Enhanced versions of the *shrunken2* gene harboring naturally occurring mutations that result in increased seed weight due to reduced inhibition of the enzyme by inorganic phosphate (Giroux et al. 1996) and increased thermostability (Greene and Hannah 1998) have been typically used in these studies. The general transgenic strategy with these candidate genes has been to express the transgenes under the control of an endosperm-specific promoter. Interestingly, the choice of endosperm-specific promoter has been important in determining the phenotype that is obtained. When seed-storage protein promoters were used (such as maize zein promoters or wheat and rice glutellin promoters), the resulting transgenic plants showed that seed sizes increased by 11–33 % (Wang et al. 2007; Lee et al. 2010; Li et al. 2011). However, when the *shrunken2* promoter was used, yield increases (of up to 64 %) were due to increased seed number rather than increased seed size (Smidansky et al. 2002, 2003, 2007; Hannah et al. 2012). Subsequent analysis in maize has shown that although the *shrunken2* promoter is predominantly expressed in endosperm, it is also expressed in floral meristems and in ovary wall tissue (Chrimes et al. 2005; Hannah et al. 2012). The upregulation of AGPase (and therefore at least transient accumulation of starch) in these "off-target" tissues presumably leads to enhanced floral initiation and/or reduced abortion of seeds that have already set, which in turn results in the observed increase in seed number. In most of the above studies, only a

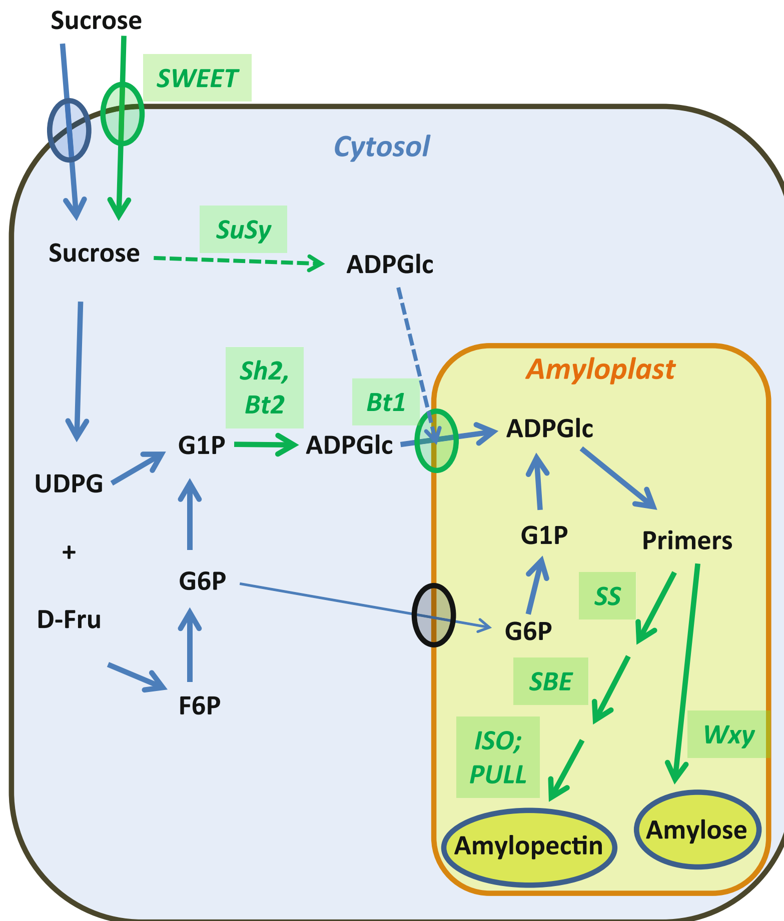

Fig. 1 An overview of the starch biosynthesis pathway in cereal endosperm. *Solid arrows* show the conventional pathway, while *dashed arrows* show an alternative pathway for ADPGlc synthesis mediated by SuSy. The cytosol is shown in *blue*, and the amyloplast is shown in *yellow*. Key enzymes mentioned in the text are shown in *green*: *SWEET*, member of the *SWEET* transporter family; *SuSy*, sucrose synthase; *Sh2*, Shrunken2 (large subunit of AGPase); *Bt2*, Brittle2 (small subunit of AGPase); *Bt1*, Brittle1 (amyloplastic ADPGlc transporter); *SS*, starch synthase; *SBE*, starch branching enzymes; *ISO*, isoamylase; *PULL*, pullulanase; *Wxy*, Waxy (granule-bound starch synthase). Key intermediates include: UDPG, uridine-diphosphate-glucose; D-Fru, D-fructose; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; G1P, glucose-1-phosphate; ADPGlc, Adenosine-diphosphate-glucose

single subunit of AGPase was overexpressed. Inasmuch as the enzyme consists of two subunits, it would be expected that overexpression of both subunits simultaneously would lead to greater yield increases than overexpression of just a single subunit, which was indeed observed in maize (Li et al. 2011).

As indicated above, the rate-limiting step for starch biosynthesis in endosperm is the production of ADP-glucose by AGPase. When AGPase is overexpressed in transgenic plants, however, there is evidence that the rate-limiting step switches to the subsequent step, which is the transport of ADP-glucose into the amyloplast (Fig. 1). This evidence comes from the fact that the increase in seed weight is less than the increase in ADP-glucose levels in these transgenic plants (Nagai et al. 2009). The amyloplast ADP-glucose transporter in cereal grain is encoded by the homologues of the maize *brittle1*

gene (Bowsher et al. 2007; Kirchberger et al. 2007), and sorghum contains a near identical homologue of this gene. It might be expected, therefore, that maximum yield increase would be obtained in transgenic plants that overexpress both AGPase subunits and the amyloplastic ADP-glucose transporter in endosperm tissues. This is yet to be achieved.

In most of the examples cited above, the analysis of AGPase-overexpressing transgenic plants was done under controlled glasshouse conditions. In two cases, however, transgenic plants were tested under field conditions. In the first, transgenic wheat plants expressing enhanced *shrunken2* under the control of the *shrunken2* promoter showed increased seed number only when grown under nonlimiting conditions (low density with irrigation; Meyer et al. 2007). This is contrary to the findings of Serrago et al. (2013) described above. A subsequent report investigating transgenic maize expressing the same transgene showed that significant increases in yield under field conditions were dependent on elevated temperatures (>33 °C) during the first four days after pollination (Hannah et al. 2012). It is not clear whether such elevated temperatures occurred in the trials conducted by Meyer et al. (2007). The requirement for elevated temperatures perhaps fits well with relatively high heat and drought tolerance of sorghum and its importance in the semi-arid tropics. More examples of field testing of cereals engineered to overexpress AGPase will be required in order to determine how agronomically useful this strategy may be.

Another two genes that are worth mentioning here as potential targets for overexpression in sorghum grain in order to increase grain size are *sucrose synthase* (*SuSy*) and the recently described *SWEET* sugar transporters from maize and rice (Eom et al. 2015). The interest in *SuSy* stems from an alternative (or additional) model for starch synthesis, in which *SuSy* (rather than AGPase) plays a key role in the production of ADP-glucose (see for review Bahaji et al. 2014). This model does not yet seem to have gained widespread support; however, it has recently been demonstrated that overexpression of *SuSy* in wheat endosperm leads to increased levels of ADP-glucose and starch (Li et al. 2013). *SWEET*s are a novel, recently described family of sugar transporters that includes both sucrose and hexose transporters (see, for review, Eom et al. 2015). Very recent work in maize and rice indicate that one member of this family is expressed in the basal endosperm transfer layer, and is involved in grain filling (Sosso et al. 2015). It follows, therefore, that upregulation of the sorghum homologue of these genes may lead to increased carbohydrate import into the grain and subsequent increases in starch production.

5.2 Improving Grain Quality: Engineering Starch with Health Benefits

As indicated above, sorghum grains typically contain about 30 % amylose and 70 % amylopectin (although these ratios differ among cultivars). This starch component provides a major source of calories in the human diet, but in recent years there is increasing evidence that the ratio of amylose to amylopectin has a big influence on digestibility within the human gastrointestinal tract. In particular, high proportions of amylose in grain lead to higher proportions of so-called “resistant starch” (which remains undigested in the small intestine and enters the large intestine in an intact form), which in turn leads to a reduced post-prandial glucose response and therefore a reduced GI (e.g., Granfeldt et al. 1995; Åkerberg et al. 1998; Behall and Hallfrisch 2002; Hallström et al. 2011). Increasing the amylose content of cereal grains (including sorghum) is, therefore, attracting interest particularly in developed nations where levels of obesity (and associated health issues) are reaching epidemic proportions.

As shown in Fig. 1, the synthesis of amylopectin is controlled by multiple isoforms of starch synthases and starch branching enzymes, and therefore these enzymes are obvious candidates for downregulation in order to alter the amylose:amylopectin ratio in transgenic plants. Indeed, natural *amylose extender (ae)* mutants of

maize, which have elevated amylose levels in grain, have been shown to lack activity of starch branching enzyme *SBEIIb*, which is the major isoform of this enzyme in maize (Hedman and Boyer 1982). A number of studies in cereals have demonstrated increased amylose following downregulation of starch branching enzymes. The first of these used RNAi constructs targeting the *SBEIIa* and *SBEIIb* genes in bread wheat (Regina et al. 2006). The construct targeting *SBEIIb* effectively downregulated *SBEIIb* expression but did not show any effect on starch composition. The construct targeting *SBEIIa*, however, resulted in the downregulation of both *SBEIIa* and *SBEIIb*, and resulted in amylose accumulation to >70 % in grains. Furthermore, the authors recorded positive effects on gastrointestinal health in rats that were fed with the high amylose grain. The *SBEIIa* gene was also effectively downregulated in durum wheat, resulting in accumulation of amylose up to 75 % (Sestili et al. 2010). In barley, RNA-mediated silencing of the *SBAIIa* or *SBEIIb* genes alone recorded little impact on amylose:amylopectin levels, but simultaneous downregulation of both by >80 % led to accumulation of >70 % amylose (Regina et al. 2010).

From the above studies it is evident that the starch branching enzyme isoforms that need to be targeted are species dependent. This predominantly reflects differences in the expression level of the isoforms. For instance, in maize grain there is approximately 50 times more *SBEIIb* than *SBEIIa* (Gao et al. 1997) and therefore, mutations of *SBEIIb* give rise to the *ae* phenotype. In contrast, wheat grain has approximately fourfold higher expression of *SBEIIa* compared to *SBEIIb* (Regina et al. 2005) and therefore, *SBEIIa* is a more effective target. In barley, there is approximately equal expression of these two genes (Sun et al. 1998) and both need to be downregulated in order to obtain the *ae* phenotype.

The *SBEI* gene has also been a target for downregulation in tandem with *SBEII* isoforms. For instance, transgenic rice with almost 65 % amylose was generated using a double antisense construct targeting both *SBEI* and *SBEIIb*, and these high amylose grains led to improved indices of animal health when fed to rats (Zhu et al. 2012). These feeding trials confirm the observations of Regina et al. (2006) described above. The most extreme result was obtained in barley engineered to simultaneously downregulate all three SBE isoforms (*SBEI*, *SBEIIa*, and *SBEIIb*), which resulted in grain that accumulated only amylose (Carciofi et al. 2012).

A common effect in the studies described above is a reduction in grain weight in transgenic lines with enhanced amylose levels resulting from downregulation of SBE. A recent study in maize suggests that this yield penalty can be overcome by simultaneously overexpressing AGPase subunits, sucrose synthase, and granule-bound starch synthase (to direct more carbon into the starch biosynthesis) and downregulating *SBE* isoforms (Jiang et al. 2013).

5.3 Starch Debranching Enzymes

The starch debranching enzymes include isozymes of isoamylase, and a single locus of pullulanase (known as limit dextrinase in wheat and barley). The functional role of the starch debranching enzyme, pullulanase, and its influence upon starch structural properties is not well understood. Knockout mutations for this gene in maize and rice have not revealed clear grain phenotypes (Dinges et al. 2003; Fujita et al. 2009), but a distinct relationship between a particular pullulanase allelic variant and increased sorghum grain digestibility has been demonstrated (Gilding et al. 2013). The common allele, known as *SbPUL-GD*, is found in approximately 90 % of cultivated sorghums. Another allele, *SbPUL-RA*, differs from the common allele at only two amino acid residues in the N-terminal end of the resultant protein. It has been established that in genotypes homozygous for *SbPUL-RA* there is a twofold increase in enzyme activity in both leaves and developing endosperm. This has been shown to be a result of enzyme activity and there is no difference in gene expression (Gilding et al. 2013; Campbell et al. 2016). No

clear connection between the high digestibility allele type (*SbPUL-RA*) and end use has been established; however, there is a well-defined partition in the gene tree between genotypes carrying this allele and the others carrying its less digestible counterpart (*SbPUL-GD*) as a result of balancing selection acting upon this gene during domestication and subsequent modern plant breeding (Gilding et al. 2013; Campbell et al. 2016).

6 Genomics of Seed Storage Proteins

The major sorghum seed storage proteins are the prolamin family of kafirins, which share a high level of homology with the maize zein family (Garratt et al. 1993; Watterson et al. 1993). The kafirins are stored in discrete protein bodies in the endosperm (Shewry and Halford 2002), and these bodies tend to form a very tight matrix around the starch granules (Hamaker and Bugusu 2003), particularly so in the outer peripheral and vitreous endosperm.

The most abundant of these prolamins are the α-kafirins, encoded by a multigene family (Xu and Messing 2008) and generally making up 70 % of the prolamin content (Hamaker et al. 1995). The remainder of the prolamins are the β- and γ-kafirins, which have moderate to high methionine and cysteine contents, with a high level of disulphide bonds, rendering them highly protease resistant. There is also a quite minor component, the δ-kafirins which comprise less than 1 % of total seed protein (Izquierdo and Godwin 2005). Within the protein bodies, the β- and γ-kafirins are predominantly on the periphery, where their high level of inter- and intramolecular disulphide bonds make overall protein digestibility lower than other cereals (Oria et al. 2000; Belton et al. 2006; Emmambux and Taylor 2009).

Genomics approaches to understand and improve the nutritional quality of sorghum grain have shown allelic variation for the kafirins. Laidlaw et al. (2010) showed considerable allelic diversity for the β- and γ-kafirins, but only two major allele classes for the δ-kafirins. Interestingly, this study revealed the presence of lines with a β-kafirin null allele, with a frameshift insertion leading to a premature stop codon, which is shown to be under balancing selection in a diverse range of sorghum germplasm (Frere et al. 2011). Proteomic and end-use analysis showed that the diverse kafirin alleles have a significant impact on grain digestibility (Cremer et al. 2014a), which also had an impact on ethanol conversion efficiency (Cremer et al. 2014b) and dough qualities (Shewaygra et al. 2012).

A point mutation in the signal peptide of an α-kafirin gene results in an aberrant periphery of the sorghum protein body, leading to enhanced digestability (Wu et al. 2013). Furthermore, this indirectly led to increased lysine content due to an increased proportion of non-kafirin proteins. Lysine is the most limiting amino acid in cereals, and sorghum has the lowest lysine content among the major cereal grains (Henley et al. 2010).

7 Genomics of Nonstarch Polysaccharides

Nonstarch polysaccharides (NSP) is the term given to polysaccharides other than starch that generally form the primary constituents of cell walls and are concentrated more abundantly in the outer rather than inner layers of grains. From a nutritional perspective, NSPs form one of the core components of dietary fiber, which has been strongly associated with enhanced health effects (Nugent 2005a, b). Dietary fiber is known to influence the extent and rate to which blood glucose increases after consumption of a carbohydrate food. A significant health benefit of dietary fiber is the ability to lower the glycemic response of the foods in which it is a major constituent. Foods rich in dietary fiber release glucose more slowly into the bloodstream which is relevant towards the prevention of disorders such as diabetes, obesity, and gastrointestinal cancer (Topping 2007; Babio et al. 2010). Additionally, a higher level of soluble

polysaccharides in a biofuel feedstock improves saccharification to provide more fermentable sugars for conversion into bioethanol (Lin and Tanaka 2006).

NSPs are structural polysaccharides as opposed to starch, which is chiefly a storage polysaccharide. The most common types of NSP are cellulose, arabinoxylan, and (1 → 3), (1 → 4)-β-D-glucan (β-glucan). Arabinoxylans are 1–4 linked β-D-xylopyranosyls, which can be substituted at the O-2 and/or O-3- atomic positions with α-L–arabinofuranose (Verbruggen et al. 1993), whereas the β-glucans are soluble NSPs of glucose chains linked via mixed β-(1,3)- and β-(1,4)-glycosidic linkages (Lazaridou and Biliaderis 2007). These structural molecules are central to the cell wall matrix of cereals, which when combined with lignins, cell wall proteins, and polyphenolic substances, results in a firm, inextensible cell wall.

The proportion of NSPs within cell walls of grain varies among species and between tissues within the grain itself. Wheat, barley, and oats contain upwards of 10 % w/w NSPs such as β-glucan and arabinoxylans (Collins et al. 2010), in contrast to sorghum grain which comprises only 3.4–7.3 % w/w (Bach Knudsen et al. 1988; Verbruggen et al. 1993). Specifically, the amounts of β-glucan found within cereal species (wholegrain flour) can range from 4 to 10 % (w/w) in barley and oats, through approximately 1 % (w/w) in maize and wheat, and as low as 0.1–0.4 % (w/w) in sorghum and rice (Collins et al. 2010; Niba and Hoffman 2003; Betts et al. 2015). Estimates of arabinoxylan amounts in total grain range from 4 to 9 % w/w in wheat, but drop to around 2–5 % w/w in rice and maize (Collins et al. 2010). The most recent estimates for arabinoxylan content in sorghum ranges from 1.5 to 3.6 % w/w (Betts et al. 2015).

High β-glucan and arabinoxylan levels have been demonstrated to increase the risk of disease in poultry due to the sticky feces produced when birds are fed grain diets (Bedford and Morgan 1996). Furthermore, β-glucans and arabinoxylan, particularly from barley, can likewise create problems for the brewing industry by increasing the haze produced in beer (Jadhav et al. 1998). The objective to manipulate NSPs as an important grain quality trait largely depends on the possible end use intended for the sorghum grain. In the context of manipulating NSPs towards the goal of creating sorghum as a useful functional food with human health benefits, lines recognized with high β-glucan, high soluble NSP, high protein, and low starch content would be desirable. Conversely, sorghum genotypes with reduced NSP levels, more digestible protein, and higher starch contents would be ideal for livestock feeds. The manipulation of NSPs with regard to the biofuel industry would see sorghum grain with higher levels of soluble polysaccharides, such as β-glucan, which would aid the process of saccharification, providing more fermentable sugars for conversion into bioethanol. And with respect to the brewing industry, lower amounts of viscous polysaccharides are preferred for beer making.

Betts et al. (2015) examined the influence of environment vis-à-vis genetic variation on β-glucan and arabinoxylan levels in grain sorghum by testing 10 genotypes across five environments. Genotype was the dominant source of variation for both β-glucan and arabinoxylan content (69–74 %), with environment responsible for only 5–14 %. These results also mirrored the G × E effects on sorghum grain popping ability (Rooney and Rooney 2013). All these studies suggest significant genetic influence on cell wall composition in sorghum grain. It also means that utilization of genotypic variation in breeding programs of wild genotypes and landraces from diverse geographic origins may make a positive contribution to altering NSP levels in sorghum. Towards this goal, deployment of rapid and inexpensive phenotyping technologies such as near-infrared reflectance (NIR) spectroscopy assume much significance (Blakeney and Flinn 2005). Another avenue for altering the levels of NSPs is through the utilization of transformation technology. In addition to the standard manipulation techniques such as overexpression or RNAi, new technology such as CRISPR/Cas9 (Mali et al. 2013), offers a unique opportunity.

The search for candidate genes involved in arabinoxylan synthesis identified genes in the glycosyl transferase (GT) families, including GT43, GT47, and particularly GT61 (Mitchell et al. 2007; Pellny et al. 2012). Although there have been no bioinformatics or genetic studies to date concerning possible candidate genes of sorghum involved in arabinoxylan synthesis, substantive evidence has been displayed in other cereals. Clear proof of function of candidate genes from the GT61 family of rice and wheat was shown when particular genes from the GT61 family led to the addition of α-(1,3) linked arabinofuranose to xylan in *Arabidopsis*, which otherwise lacks arabinosylation ability. This showed that these genes encode α-(1,3)-arabinosyltransferase activity (Anders et al. 2012). In a separate study, Chiniquy et al. (2012) demonstrated that a rice member of the *GT61* family, called *XAX1*, was responsible for adding the xylose residues in Xylp-(1 → 2)-α-Araf-(1 → 3) substitutions. Furthermore, suppression of wheat GT61 (TaXAT1) resulted in a 70–80 % decrease in the amount of α-(1,3) linked arabinofuranose in arabinoxylan of mature starchy endosperm (Anders et al. 2012), whereas suppression of GT43 (TaGT43_2) or GT47 (TAGT47_2) genes reduced the total amount of arabinoxylan in wheat endosperm walls by 50 % (Lovegrove et al. 2013). In both studies, these dramatic physical changes to the endosperm cell wall of these transgenic genotypes did not affect average grain weight or germination.

In contrast, the genes involved in the biosynthesis of β-glucans are now well characterized and are linked to the cellulose synthase-like (*Csl*) family of genes that includes members of the *CslF* and *CslH* clades of the cellulose synthase gene superfamily. In particular, the *CslF6* gene of barley when transformed into *A. thaliana* resulted in the biosynthesis of β-glucans in the cell walls of transgenic plants (Burton et al. 2006; Doblin et al. 2009), whereas in wheat downregulation of this gene resulted in a marked decrease of β-glucan in the endosperm (Nemeth et al. 2010). In fact, when the *CslF6* gene of barley was overexpressed by an endosperm-specific promoter, the resultant transgenics had an increase of more than 80 % of β-glucan levels in the grain (Burton et al. 2011). Furthermore, a mutant barley line with a lesion in the *CslF6* gene had no β-glucan content in its grain (Taketa et al. 2012). Transcript profiling of orthologues from the *Csl* family in sorghum across several tissues has revealed that *CslF6* transcripts predominate in these tissues and are likely responsible for β-glucan synthesis (Ermawar et al. 2015).

8 Future Directions

The science of molecular genetics and its application to plant breeding and biotechnology has finally achieved a level of technological sophistication and genomewide scope such that the analysis of genes and their respective functions can be affordably carried out by institutions without multimillion dollar budgets. Sorghum's small diploid genome and role as a model crop species for C_4 plants has meant that a plethora of genetic resources, online genomic databases, and advanced breeding populations (NAM, MAGIC) have become available to public research institutions and breeding programs worldwide. In conjunction with these bioinformatics resources, successful development and employment of sorghum transformation technology has become a reality and has allowed thorough characterization of genes and their respective functions. The future combination of genomic level sequencing encompassing diverse African germplasm with CRISPR/Cas9 technology that can specifically silence or manipulate numerous genes, means that the task of assigning function to the tens of thousands of uncharacterized genes within the sorghum genome is now conceivable and the goal of improving sorghum quality can be readily attained.

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11 Genetic Manipulation of Root System Architecture to Improve Drought Adaptation in Sorghum

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Abstract

Drought is one of the most important abiotic stresses and severely affects global agricultural production. Root system architecture (RSA) is the key determinant of water acquisition under moisture stress, and therefore has utility in breeding for drought tolerance in sorghum. Various components of RSA are known to influence drought tolerance in sorghum without any negative impact on yield. The growth angle of nodal roots is an important target trait for improving drought tolerance. Genetic variation for nodal root angle has been reported in sorghum, and this has been associated with grain yield under drought stress. Rapid advances in sorghum genomics have led to the identification of various quantitative trait loci (QTL) governing RSA, but the accuracy and preciseness in identification of QTL is the major hindrance in development of drought-tolerant cultivars through genetic manipulation of root traits. Hence, the complex genetic control of RSA and the lack of a high-throughput phenotyping platform have hampered integration of selection for RSA in breeding programs. These limitations can be overcome by designing a robust phenotyping platform

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that can maximize heritability and repeatability of RSA. Inclusion of the extensive phenotyping information with the recently developed genomic resources of sorghum will lead to mining of alleles that govern RSA and tailor a cultivar harboring genes for RSA that improve sorghum production under drought stress. This chapter provides an overview of the latest developments in RSA research in sorghum and gives direction to future breeding strategies to enhance the genetic gain for root traits.

1 Introduction

Drought is one of the most important abiotic stresses that can severely affect crop production by preventing plants from expressing their full yield potential (Mitra 2001). Moisture stress alone causes 70 % of agricultural yield loss across the globe (Jha et al. 2014). Worldwide, sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop after wheat, rice, maize, and barley. Sorghum is predominantly cultivated in dryland areas of the world due to its good adaptation to moisture-limited environments. World sorghum production has had a slight increase from 60–65 metric tons over the past decade (Mutava et al. 2012), in spite of reduction in area to the tune of 154,000 ha year^{-1} (Rakshit et al. 2014). However, predictions of increased frequency of drought as a result of accelerated climate change (IPCC 2012) may adversely affect its yield potential in the semi-arid tropics, imposing a threat to the food security in these regions. Therefore, genetic improvement of sorghum is necessary for drought adaptation in variable climatic conditions and this has been the major focus of sorghum breeding programs around the world.

Because of the unpredictable nature of the timing and intensity of occurrence of drought stress, breeding for drought tolerance has always been a challenging task for sorghum. Past efforts to improve drought tolerance in sorghum were largely focused on genetic and physiological aspects of aboveground plant parameters, namely stay-green (Rosenow et al. 1983; Tuinstra et al. 1996; Sanchez et al. 2002; Vadez et al. 2011; Jordan et al. 2012; Borrell et al. 2014), photosynthetic parameters (Tingting et al. 2010; Li et al. 2011), canopy temperature and leaf rolling (Blum et al. 1989; Mutava et al. 2012), and osmotic adjustment (Girma 1989; Girma and Krieg 1992; Patil and Ravikumar 2011). However, very few efforts have been made to improve the root system architecture (RSA), which is the key determinant of the ability of a plant to access water and nutrients to support shoot growth in moisture stress conditions. Studying the root system is complex, tedious, and expensive compared with aboveground components.

RSA refers to the spatial and temporal configuration of roots in the soil and is used to describe the shape and structure of root systems (de Dorlodot et al. 2007). In cereals, RSA is of fundamental importance to soil exploration and belowground resource acquisition, which ultimately determines the yield potential of cultivars in drought-prone environments. Simulation studies on maize suggested that manipulation of RSA and associated soil water capture are the main reasons for increasing yield trend of the US corn belt (Hammer et al. 2009). Likewise, studies on wheat demonstrated that selection for narrow root angle and higher number of seminal roots could significantly improve the water capture mechanism in dryland cropping regions (Asseng and Turner 2007; Manschadi et al. 2008; Lilley and Kirkegaard. 2011). Taking into account the ample genetic variability in root traits in different crops (e.g., rice, Kato et al. 2006; wheat, Manschadi et al. 2008; Nakhforoosh et al. 2015; sorghum, Singh et al. 2011), it can be expected that genetic manipulation of RSA is a feasible agronomic option to improve drought adaptation of sorghum. With the aid of high-throughput root

phenotyping platforms and advanced genotyping tools, genes related to root morphological characteristics and physiological functions can be identified or cloned, which will present an opportunity for further improvement of crop productivity in moisture-limited environments.

Sorghum, by virtue of its adaptation to moisture-limited environments, is known to possess a vertically oriented, uniformly distributed, and deep root system, but very little is known about the role of various RSA component traits in plant adaptation to harsh environments. Nodal roots are particularly important in sorghum as they have a major influence on development of RSA as the plant matures (Blum et al. 1977; Singh et al. 2012). However, genetic control and physiological aspects related to water and nutrient transport via RSA and the nodal root system are barely explored. One reason for the scarcity of data on sorghum root characteristics is the unavailability of a high-throughput phenotyping platform for robust root screening that can be applied on a large scale. Nonetheless, screening techniques for rooting depth, root angle, and distribution have been developed for other cereals (Oyanagi et al. 1993; Bengough et al. 2004; Hargreaves et al. 2009) and combined with the advancement in genotyping tools, this has led to the identification of a number of quantitative trait loci (QTLs) and candidate genes related to root morphological characteristics and physiological functions for those crops. This has opened an opportunity for further improvement of crop productivity in moisture-limited environments through root system modifications. None of these techniques has been applied or tested at the scale of a breeding program, in particular in sorghum. Research at University of Queensland, Australia on the morphology and genetics of sorghum root systems identified genotypic variation in nodal root angle that can inform breeding priorities in selection for RSA (Singh et al. 2010, 2012).

This chapter provides a review of RSA research in sorghum. First, we provide an overview of recent developments in root phenotyping platforms that can thoroughly investigate the structural and functional aspects of RSA. Second, we provide an overview of cereal root structure with particular reference to sorghum influencing drought tolerance and impact of moisture stress on RSA. Additionally, we provide a critical appraisal of existing genetic variation for root traits and their genetic control. Finally, the prospects of deploying root traits to improve drought tolerance through molecular breeding approaches are discussed.

2 High-Throughput Phenotyping Platforms for Screening Root Architecture

Recent availability of multiallelic resources such as backcross (BC)-nested association mapping (NAM) population (Jordan et al. 2011), which offer power to dissect complex quantitative traits along with advances in genomic resources including development of the consensus genetic map (Mace et al. 2009) and availability of resequencing data (Mace et al. 2013) in sorghum have the potential to provide a thorough insight on genetic control of RSA. Despite rapid advances in sorghum genomics, the scarcity of low-cost and high-throughput phenotyping platforms for screening RSA parameters remains a major obstacle to elucidate the underlying genetic control and to incorporate such traits in breeding programs through marker-assisted selection (MAS). A detailed list of various phenotyping methods utilized for studying the genetic and physiological basis of root traits in cereals is presented in Table 1. Currently, the most widely used methods for RSA phenotyping are those that are carried out in controlled conditions using hydroponics, gel chambers, soil, and other artificial substrates (Clark et al. 2011, 2013; Planchamp et al. 2013). Supported with novel 2D and 3D imaging platforms, these methods allow measurement of root traits with high resolution, precision, and accuracy (van Weele et al. 2003; Bengough et al. 2004; Basu et al. 2007; Clark et al. 2011). These methods have proven their utility in characterization of embryonic seminal roots in cereals (Hund et al. 2009; Planchamp et al. 2013; Richards et al.

2015). However, these methods are of little importance for sorghum as seminal roots do not constitute the major proportion of the mature root system and the postembryonic nodal roots dominate the RSA of mature plants. Furthermore, late appearance of nodal roots (sixth leaf stage, nearly 3 weeks after planting) and the larger size of the root and shoot system at this stage impose restrictions on utilizing these methods for phenotyping nodal roots mainly for genetic mapping studies. Despite their major importance, only few methods are available for phenotyping of nodal roots. The split pot system in wheat (Volkmar 1997) and paper pouch method in maize (Hochholdinger et al. 2004a) were developed to differentiate primary seminal roots from nodal roots. Rostamza et al. (2013) utilized the split pot system adopted from Volkmar et al. (1997) to investigate the effect of varying water supply on nodal roots of sorghum. However, these methods are not suitable for large-scale phenotyping of nodal roots. Singh et al. (2010) proposed a root chamber-based method to screen nodal root angle in sorghum precisely. The root chamber method is a greenhouse-based high-throughput phenotyping platform designed to study nodal roots in sorghum. The phenotyping platform includes specially built root observation chambers (60 cm × 40 cm × 3 mm) filled with soil. These chambers support the growth of sorghum plants up to the sixth leaf stage and the transparent Perspex sheets on both sides enable viewing and capturing images of nodal roots. This phenotypic platform has proven its utility in quantifying the genetic variation for nodal root angle across a diverse range of inbred lines and a biparental mapping population (Singh et al. 2011; Mace et al. 2012). Le Marié et al. (2014) proposed a paper based on nondestructive, high-throughput phenotypic method called "rhizoslides" in maize. The advantage of rhizoslides over previously developed paper-based methods is that they allow characterization of nodal roots in cereals by physically separating them from embryonic seminal roots through different layers of rhizoslide sandwich construction. Both root chambers and rhizoslides were designed primarily to phenotype nodal roots at the seedling stage, which makes them unsuitable to phenotype RSA at the adult plant stage. As an alternative, Traschel et al. (2011) proposed a field-based method "shovelomics", which visually scores post-embryonic root architectural traits at the flowering stage. In shovelomics, roots were excavated by standard shovels, which are capable of removing a soil cylinder of 40 cm diameter and 25 cm depth. After excavation roots were thoroughly washed in water containing a mild detergent to remove soil. The washed roots were visually scored for root angles using a 1 (shallow root angles, 10°) to 9 (steep root angles, 90°) scale. In addition to visual scoring, root angles were measured by placing washed roots on a phenotyping board fitted with a large protractor. Significant correlations between measured and visually scored trait values for growth angles of crown and brace roots confirmed accuracy and reliability of the method. The shovelomics approach has been utilized to quantify visually the excavated structure of the root crown in 218 recombinant inbred lines (RILs) of maize (Traschel et al. 2011). However, manual scoring of RSA traits is prone to error and imposes limitations on both the number of traits measured and number of genotypes screened. Recently, a high-throughput imaging system, which facilitates estimation of root traits based on digital images taken under field conditions was proposed to overcome the problems associated with manual scoring of the shovelomics approach (Bucksch et al. 2014).

Inaccurate phenotyping due to the lack of high-throughput root phenotyping platforms often leads to imprecise QTL identification. Therefore, immediate attention needs to be placed towards developing high-throughput phenotyping methods because it may be the only way to derive valid conclusions. However, each phenotyping method has its own limitations. For instance, extracting the intact roots and avoiding damage to finer components of RSA such as root tip and root hairs is the major problem of field-based screening methods. The currently used controlled environment

Table 1 List of various phenotyping methods used for characterization of RSA in cereals

Phenotyping method	Screened root traits	Stage for phenotyping	Crop	Reference
Clear pot method	Seminal root number (SRN), seminal root angle (SRA)	5 DAS (SRA) 11 DAS (SRN)	Wheat	Richard et al. (2015)
Gellan gum growth method	Median and maximum number of roots, average root radius, specific root length, total surface area, total root length, depth, volume, length distribution, maximum horizontal width, and width-to-depth ratio	14 DAS	Rice	Iyer-Pascuzzi et al. (2010)
Gel observation chamber method	Root length, elongation rate, longest root, deepest root, SRN, and SRA	9 DAS	Barley, wheat	Bengough et al. (2004), Manschandi et al. (2008)
Growth pouch method	Axile and lateral root growth, SRA, SRN	3–7 DAS (axile and lateral root growth 20 DAS (SRN and SRA)	Maize, wheat	Hund et al. (2009), Richard et al. (2015)
Hydroponics	Root thickness, dry root weight, root volume, RLD, primary root growth, total root growth	4 DAS	Rice, maize	Clark et al. (2013)
Rhizoslides	Embryonic and post-embryonic root traits	20 DAS	Maize	Le Marie et al. (2014)
Root box method	Number of roots, root length, root thickness, dry root weight	56 DAS	Rice	Kono et al. (1987), Price et al. (2002)
Root chamber method	Nodal root angle	21 DAS	Sorghum	Singh et al. (2010)
Root Trak method (X-ray computed tomography)	Maximum root system depth and maximum root system width	21 DAS (maize) 10 DAS (wheat)	Maize, wheat	Mairhofer et al. (2012)
Soil coring method	Rooting depth, root penetration rate, density, deeper rooting length, and root distribution	At maturity	Wheat	Wasson et al. (2014)
Shovelomics	Number of brace root whorls, brace root number, brace root angle, brace root branching, crown root number, crown root angle, crown root branching	At flowering	Maize	Trachsel et al. (2011)
Wax layer method/fabric cover method	Root penetration ability	24 DAS	Rice	Yu et al. (1995), Price et al. (2000)

phenotyping methods are also not an effective solution, because RSA is influenced by various soil parameters that cannot be reproduced in artificial growth systems. Mathematical simulation of RSA with models, which are better equipped to integrate the complex biological, physical, and chemical factors affecting roots in soil, can be utilized as an alternative approach for root phenotyping. Simulation packages, such as *SimRoot* have been specifically designed to study RSA and are capable of integrating soil heterogeneity and plasticity of root responses to soil factors into one mathematical framework (Lynch et al. 1997).

3 Root System Architecture in Cereals

3.1 RSA of Sorghum

Root system development of sorghum passes through the same sequence of events as that of the shoot. The hypocotyl or mesocotyl that emerges from the seed develops into leaves, stems, and reproductive organs and branches of the shoot. Likewise, for the root system, the radicle or primary root emerges first followed by the emergence of secondary roots, nodal roots, and lateral root branches. The structural and functional aspects of root and shoot are quite different, but the growth and development of these two systems are interdependent (Gregory 1983; Klepper et al. 1984).

In general, the RSA of cereals is complex and comprises several root types at various developmental phases. It is primarily composed of embryonic seminal and post-embryonic shoot-born nodal roots (Esau 1977; O' Toole and Bland 1987; Gregory 2006). In other cereals including maize, wheat, and rice, secondary seminal roots emerge about four days after germination, bearing first- and second-order lateral branches (Feldman 1994). Sorghum, however, is characterized by a sole primary seminal root (Singh et al. 2010) that originates as a radicle in the embryo and becomes visible only two or three days after germination. The post-embryonic shoot-born roots emerge from consecutive underground and aboveground nodes of the stem and are called nodal and brace roots, respectively (Hochholdinger et al. 2004a; Singh et al. 2010). Considerable variation has been reported in the timing of emergence of nodal roots in cereals. For example, the nodal root in maize emerges approximately 7 days after germination (second leaf stage), whereas in sorghum it appears approximately 21 days after germination (fifth to sixth leaf stage; Feldman 1994; Singh et al. 2010). Successive flushes of nodal roots emerge from successive stem internodes at a rate that is similar to leaves, such that the total number of nodal roots increases as the shoot grows (Klepper et al. 1984). By contrast, brace roots emerge much later, approximately 7 weeks after germination (Hochholdinger et al. 2004b).

The root system of sorghum grows around 2–3 cm per day (Dardanelli et al. 1997; Whish et al. 2005; Singh et al. 2010) and its maximum size is reached around anthesis (Gregory 2006). However, some roots survive for only a few days and remain short, whereas others may continue to grow for weeks and grow much longer. Contradictory reports on the functional life of various root types create complications in attempting to understand the development of the root system. For instance, some researchers reported that seminal roots can persist and remain functional throughout the life cycle of the plant (Kiesselbach 1949; Kausch 1967; Kozinka 1977; McCully and Canny 1988; Gregory 2006), whereas others have observed that the primary and other seminal roots die after the development of nodal roots (Lawson and Hanway 1977; O' Toole and Bland 1987; Feldman 1994). In sorghum, the seminal root system generally constitutes only 1–14 % of the mass of the entire root system (Singh 2010). Routley et al. (2003) reported that sorghum nodal roots may grow to depths of 2 m by the initiation of flowering, and can efficiently extract water to a distance of 1.6 m from the plant. Generally, growth of nodal roots ceases after flowering. However, in some of the stay-green sorghum genotypes active growth of nodal roots has been reported up to the grain-filling phase (Robertson et al. 1993).

Anatomical organization of roots has not been well studied in sorghum. It has been observed that sorghum genotypes with large xylem vessel diameter in both seminal and nodal roots were better adapted to drought-prone environments (Amelework et al. 2015). Hochholdinger et al. (2004b) provide a comparative overview of root anatomy of cereals and *Arabidopsis*. Generally, the cereal roots comprise 8–15 layers of root cortical cells and one endodermal cell layer, whereas the *Arabidopsis* roots comprise a single endodermal and cortical layer. In cereals, the quiescent center (QC), a central region of the root tip, consists of 800–2000 cells whereas the *Arabidopsis* QC contains only four cells (Jiang et al. 2003). Lateral roots in cereals originate

from pericycle and endodermis cells, whereas in *Arabidopsis* lateral roots originate only from pericycle cells (Fahn 1990; Beeckman et al. 2001). Vascular cambium, which replaces the dead or old xylem vessels with new ones, is absent in cereal roots, yet is an integral part of dicotyledonous roots. Vascular segmentation offers flexibility over the lack of cambia by forming "safety zones" in cereal roots, which protect them from cavitation and embolism at the root–shoot junction during moisture or freezing stress (Luxova 1986). Functional xylem anatomy reveals that the development of vascular segmentation in the root–shoot junction varies among cereal species. For example, cereal species, namely, rye, wheat, and barley, which develop several seminal roots, exhibit a high degree of vascular segmentation that results in formation of safety zones at the root–shoot junction. On the other hand, sorghum, which typically develops a single primary seminal root, contains unsafe vessels and exhibits a smaller degree of vascular segmentation (Luxova 1989; Aloni and Griffith 1991). Appearance of nodal roots in later stages of plant development provides some flexibility over the lack of vascular cambia in sorghum.

3.2 Genetic Variation for RSA in Sorghum

To a large extent, success of any crop improvement program depends on existing genetic variability in crop germplasm. Keeping this in view, studies were conducted to investigate the extent of genetic variation for root traits in sorghum. For instance, Damodar et al. (1978) observed significant genetic variation for vertical distribution of roots for 11 sorghum genotypes evaluated in moisture-limited environments. Wright et al. (1983) reported greater root length density for drought-tolerant sorghum cultivar E-57 especially below 80 cm, compared with a drought-susceptible sorghum cultivar, TX-671. As a result, the tolerant genotype extracted more water from below 80 cm after the booting stage. Similarly, Irwin et al. (1985) identified two distinct phenotypic classes for root angle in a set of 11 hybrids in sorghum, which included a horizontally oriented and a more vertically oriented nodal root system. However, their study did not relate the structural difference to function. Observation of distinct classes for root orientation among genotypes provides ample scope for its improvement through breeding. Likewise, Singh et al. (2011) observed a wide range of nodal root angle (14°–50°) in a set of 44 inbred lines and 30 hybrids. In addition to nodal root angle, they observed significant genetic variation for root length, root diameter, and root dry weight among the inbreds and hybrids. Interestingly, they observed a weak association between nodal root angle and plant size (root weight, shoot weight, and leaf area). The weak association indicates that nodal root angle and plant size are governed by different mechanisms for drought adaptation to water-limited environments. Mace et al. (2012) also observed a wide range of genetic variation for nodal root angle (14.6°–32.3°) in a set of 141 recombinant inbred lines of sorghum derived from a cross between inbred lines B923296 (narrow root angle, 18.3°) and SC170-6-8 (wide root angle, 32.3°).

In any crop species, landraces offer a great genetic potential for both biotic and abiotic stress tolerance due to their adaptation to wide agroecological niches and they are not subjected to selection over a long period of time. Sorghum landraces by virtue of their wide adaptation to moisture- and nutrient-limited environments offer great scope for genetic manipulation of RSA. Keeping this in view, Ali et al. (2009) investigated the genotypic variation for root traits in local landraces of Pakistani origin and observed considerable genetic variation for dry root weight, which is an important determinant of drought tolerance in sorghum.

Although a vast genetic variation for many drought-adaptive traits has been reported in sorghum, surprisingly very limited work has been done on the RSA. The above-mentioned reports have presented sufficient evidence of genotypic variation for root traits; however, these studies have focused on a limited set of genotypes with a narrow genetic base. Thus an exhaustive search

of potential donors to root traits is the foremost step in genetic manipulation of RSA to enhance drought adaptation of sorghum. Given the context, a list of the genotypes identified as a potential source for root traits contributing to drought tolerance in sorghum is presented in Table 2. These sources can be utilized as potent sources for introgressing the quantitative trait loci governing superior root traits to drought-susceptible genotypes.

3.3 Impact of Moisture Stress on RSA of Sorghum

Soil water content strongly influences root morphology, root anatomy, and the overall pattern by which different components of RSA contribute to water transport (Salih et al. 1999). Various authors have studied the impact of moisture stress on the sorghum root system. Nivedita et al. (1992) reported that a gravimetric moisture content of less than 19 % and bulk density of 1.65 g cm^{-3} were detrimental to germination as well as seedling emergence in sorghum and pearl millet under moisture stress. Passioura (1982) reported that extreme moisture stress imposes a negative effect on RSA by impeding growth and development of nodal roots, which often results in complete crop failure. A contrasting distribution profile of nodal roots under varying soil water regimes has been observed in sorghum. For instance, watering of the top soil around the plant crown can lead to the formation of new nodal roots, whereas the extension rate of existing nodal roots is inhibited (1984). This type of growth pattern ultimately results in a horizontally distributed and shallow RSA. In contrast, a marked reduction in the number of new nodal roots and an increase in the extension rate of existing roots were observed when growing conditions changed from wetter to drier (Jordan et al. 1979), resulting in a more vertically oriented RSA with more uniform root distribution at deep soil layers. Likewise, Pardales and Kono (1990) observed that seminal and nodal roots responded differently to rewatering of stressed sorghum plants, with a significant increase in both the number and length of nodal roots, but no response of seminal roots to rewatering. Rostamaza et al. (2013) performed a comparative analysis of the response of sorghum and pearl

Table 2 Sources of superior root traits contributing drought tolerance in sorghum

RSA component	Source	Reference
Narrow nodal root angle	R993396, B923296, SC56-14E, BTx642, R931945-2-2, ATx642 × RQL36	Singh et al. (2011)
Wide nodal root angle	SC999, SC170-6-8, BTx623, ISI2611C, ATx623 × RTx7000	Singh et al. (2011)
Vertically oriented RSA	Aispuri	Damodar et al. (1978)
Extensive lateral root development	IS 9673, IS 9183, B 36-1, and IS 9357	Bhan et al. (1973)
Root length density	FJS-1, FJS-11, FJSS-17, SSM1611, IS16101, SSM249	Ali et al. (2009), Chopart et al. (2008)
Root weight	AS2752, AS5057, AS4289, MS7819, IS5379, AS8038, AS6616, K3, MS7837, ISI2611C, B923296	Vinodhana and Ganesamurthy (2010), Singh et al. (2011)
Root thickness	QL 36, SPV 570, SC636-6,	Singh et al. (2011), Rajkumar et al. (2013)
Higher number of brace roots	Sansui	Li et al. (2014)
Higher number of primary and secondary roots	IS 1183, IS 1137	Bhan et al. (1973)

millet nodal roots to varying moisture regimes. The investigation revealed that differences between two species were associated with root branching and nodal root length which influenced the water uptake pattern. Faster root branching rate and increased nodal root length were the key factors for more plastic response of pearl millet than sorghum to moisture stress.

Root elongation rate (RER) is one component of RSA that is strongly affected by a variety of soil factors: water and nutrient availability (Dunbabin et al. 2000), soil temperature (Diggle 1988; Pages and Jordan 1989), and soil bulk density (Clausnitzer and Hopmans 1994). In general, low soil water content inhibits RER (Salim et al. 1965) and leads to thinner roots (Sharp et al. 1988) and poor lateral branching (Stasovski and Peterson 1991). Changes in growth angle of roots have also been observed with varying moisture regime in cereals (Oyanagi et al. 1993). Soil water content along with several other factors therefore can play an important role in modifying root architecture and functioning for plant survival and growth in given environments.

3.4 Key Components of RSA Influencing Drought Tolerance in Sorghum

Various components of RSA in terms of their number, length, diameter, weight, volume, density, surface area, and elongation rate determine the capacity of sorghum genotypes to survive and grow in drought-prone environments. Sorghum genotypes adapted to moisture-limited environments are often characterized by a deep and vigorous root system (Blum et al. 1997). Bibi et al. (2012) assessed the genetic potential of different sorghum accessions to drought tolerance at the seedling stage and reported that root length was the highest contributor towards drought tolerance. Many studies have identified dry root weight and root length density (RLD) as reliable and easiest root components to determine the drought tolerance in sorghum (Nour et al. 1978; Matsuura et al. 1996; Ali et al. 2009). A study by Robertson et al. (1993) in sorghum indicated a low RLD threshold of 0.2 cm cm^{-3} above which any increase in RLD would not increase water extraction. However, Cherif-Ari et al. (1990) reported a RLD threshold from 0.29 and 0.86 cm cm^{-3} for sorghum genotypes subjected to moisture stress. Thresholds for sorghum are generally below those found for maize, for which values of around 0.5–0.6 cm cm^{-3} (van Oosterom et al. 2016) and 1.70–2.56 cm cm^{-3} (Aina and Fapohunda 1986) have been reported. Blum and Arkin (1984) observed higher total cumulative root length and RLD for the late as compared to early maturing sorghum genotype under moisture stress. RLD beyond the threshold can indicate excessive dry mass allocation to roots at the expense of reproductive organs, which can negatively affect drought adaptation (van Oosterom et al. 2016).

Apart from these components, spatial distribution of roots and rooting depth are the critical factors for determining the drought-tolerance potential and survival of plants in drought-prone environments. The importance of spatial distribution of roots and rooting depth for crop productivity arises from the fact that soil resources are haphazardly distributed in time and space and are subjected to localized depletion in stress environments (Robinson 1994). This is especially important in crops such as sorghum, as they are frequently grown in moisture-limited environments. Root growth angle is an important determinant of RSA that strongly influences the spatial distribution and rooting depth in sorghum. In fact, the root angle spread at an early growth stage can serve as a useful predictor of the distribution and root biomass at the adult stage (Singh 2010) and may serve as a good reference point for comparison to other environmental conditions. Studies have demonstrated that the vertical distribution pattern of roots as a consequence of narrow root angle enhances the rooting depth, which is important if water availability in the upper soil layers becomes insufficient in terminal drought-stress environments (Manschandi et al. 2008; Hammer et al. 2009; Uga et al. 2011). On the other hand, wide root angle, which results in more horizontally distributed and shallow

RSA, is more relevant in skip-row-management systems in water-limited environments (McLean et al. 2003; Whish et al. 2005).

In sorghum, Singh et al. (2012) carried out a thorough investigation to study the effect of nodal root angle on the water extraction pattern of mature sorghum plants. Four inbred lines with contrasting nodal root angle, SC170-6-8, SC636-6, B923296, and R931945-2-2, at the seedling stage were grown up to flowering in large rhizotrons. The root systems of B923296 (narrow angle) and SC170-6-8 (wide angle) showed clear differences in vertical and horizontal distribution of roots at flowering. B923296 had more roots visible against the glass surface of the rhizotrons than SC170-6-8 at 100 cm depth immediately below the plant, whereas SC170-6-8 had more roots visible than B923296 at 80 cm depth, 120 cm away from the plant. These findings suggest that nodal root angle at the seedling stage can affect the spatial distribution of roots of mature plants. Genotypes with narrow root angle potentially increase root distribution at depth, which is important if water availability in the upper soil layers becomes insufficient to enable the crop to complete its life cycle. On the other hand, a genotype with wider root angle but shallower root system may be able to exploit small events of in-season rainfall from the upper soil layer.

The role of xylem vessel diameter has been clearly demonstrated in influencing drought tolerance in cereals. For instance, xylem vessel diameter has been used as an effective selection criterion in spring wheat breeding programs in Australia (Richards and Passioura 1989) and rice breeding programs in Asia (Yambao et al. 1992; Sibounheuang et al. 2006; Abd Allah et al. 2010), where the goal is to improve water acquisition from drying soils. In sorghum, Bawazir and Idle (1989) reported significant genetic variation for xylem vessel conductivity in seminal and nodal roots of nine genotypes in response to moisture stress. Genotypic differences in drought tolerance between sorghum cultivars "Tabat" (susceptible) and "Gadambalia" (tolerant) have been associated with anatomical components of RSA (Salih et al. 1999). As compared to drought-susceptible cultivar Tabat, the RSA of drought-tolerant cultivar Gadambalia was characterized by its ability to produce a smaller number of late metaxylem vessels and presence of sclerenchyma sheath around the vascular system, which reduced the axial water flow during moisture stress. Anatomical traits, root cortical aerenchyma (RCA), cortical cell file number (CCFN), and cortical cell size (CCS), were reported to reduce the metabolic cost of root growth in maize under moisture and nutrient-limited environments (Lynch 2015). Zhu et al. (2010) compared maize recombinant inbred lines (RILs) differing for RCA formation under moisture stress in the field and soil mesocosms in greenhouse. In field conditions, lines with high RCA had high RLD and produced 30 % more shoot biomass at flowering compared with the lines with low RCA. On average, high RCA lines yielded eight times more than low RCA lines. In mesocosms, high RCA lines were characterized by less seminal root respiration, deeper rooting, and greater shoot biomass compared with low RCA lines. These findings suggested that RCA deserves consideration as an important component of RSA to improve drought tolerance in cereal breeding programs. Therefore, there is an urgent need to explore the genetic basis and functional aspects of root anatomical traits in sorghum. Identification of genomic regions governing root anatomical traits would greatly facilitate their use in sorghum breeding programs.

4 Genetic Control of RSA in Sorghum

4.1 Gene Action and Heritability

Prior information on genetic control, prevailing gene action, and estimates of heritability is mandatory for designing a suitable breeding strategy for genetic improvement of any trait. However, information on gene action and heritability of sorghum root traits is very limited. Jordan et al. (1979) showed that root characters, root length, mass, and volume, were

polygenically controlled. Vinodhana and Ganesamurthy (2010) reported higher estimates of phenotypic and genotypic coefficient of variation coupled with high broad sense heritability and expected genetic gain for root volume in sorghum. Likewise, high heritability estimates and genetic advance for other traits, namely, root length, number of roots per plant, root fresh weight, root dry weight, and root-to-shoot ratio in sorghum have been reported (Thudi 2004; Ali et al. 2009; Rajkumar et al. 2013). Such high estimates for root traits in sorghum indicate that they are more likely to be controlled by additive gene action and selection will be effective for these traits to improve drought tolerance in sorghum breeding programs. Singh et al. (2011) assessed the inheritance pattern of nodal root angle and dry root weight in a set of 44 inbred lines and 30 hybrids. It was observed that both traits were polygenically controlled and exhibited moderate heritability. Although the heritability of nodal root angle was moderate, its genetic architecture was not simple, as illustrated by the significant specific combining ability (SCA) effects and the high contribution of male and female interactions to the heritability.

Drought tolerance in plants is often associated with the capacity of the roots to absorb available nutrients under moisture-limited environments (Amelework et al. 2015). For instance, Shangguan et al. (2005) reported that hydraulic conductivity of seminal and nodal roots in sorghum was strongly influenced by phosphorus availability under water deficiency. Keeping this in view, efforts were carried out to find the gene action underlying root length, root dry weight, and root/shoot dry weight under low phosphorus availability using the F_2 segregating generation derived from the cross between "B69" and "Numbu" in sorghum (Trikoesoemaningtyas et al. 2015). The investigation elucidated that additive gene action is involved in genetic control of root length, root dry weight, and root/shoot dry weight. In addition to this, high heritability and moderate genotypic coefficient of variation (GCV) indicated that root length and root dry weight can be used as selection criteria in low-P condition at the seedling stage.

In general, high to moderate heritability estimates were reported for the majority of root traits in sorghum. However, heritability estimates were thus far mainly calculated based on replications within homogeneous experimental conditions under a controlled environment (laboratory/greenhouse). A decreasing trend in heritabilities under low nitrogen condition have been observed for root traits such as root angle and branching density in maize due to the pronounced effect of soil heterogeneity (Cai et al. 2012; Colombi et al. 2015). Therefore, it remains to be elucidated whether heritabilities of root traits stay high in sorghum, when compared across years and soil environments.

4.2 Quantitative Trait Loci (QTLs) Governing RSA

A number of QTLs governing various root traits in cereals have been identified, which can be introgressed into drought-susceptible cultivars through marker-assisted selection (MAS). Among the cereal species, rice has been thoroughly investigated for QTLs controlling RSA and some of them explained up to 50 % of phenotypic variability for root traits (Price et al. 1997, 2000, 2002; Uga et al. 2011). Although various QTLs governing yield and yield components, stay-green, flowering, and maturity under moisture stress have been mapped on 10 linkage groups of sorghum (Sanchez et al. 2002; Borrell et al. 2006), information on QTLs governing RSA is still in its infancy in sorghum. A detailed description of various QTLs governing sorghum RSA is presented in Table 3. Mace et al. (2012) for the first time carried out a comprehensive genetic mapping study to dissect the genetic control of RSA in sorghum. They identified four major QTLs governing nodal root angle and three QTLs controlling root dry weight (Table 3) in a recombinant inbred line (RIL) population derived from a cross between two inbred sorghum lines with contrasting root angle (B923296/SC170-6-8). Interestingly, BLAST analysis revealed that three of the four nodal root angle QTLs were homologous to

Table 3 Details of different QTLs identified for various root traits in sorghum

Root trait	Mapping population	Markers	Identified QTLs	Linkage group	Phenotypic variation (%)	Reference
Root angle	B923296 × SC170-6-8	377 DArT markers	*qRA1_5*, *qRA2_5*, q*RA1_8*, *qRA1_10*	LG 5, 8 and 10	58.16	Mace et al. (2012)
Dry root weight	B923296 × SC170-6-8	–	*qRDW1_2*, *qRDW1_5*, *qRDW1_8*	LG 2, 5 and 8	32.08	Mace et al. (2012)
	E36-1 × SPV570	938 markers (270 nongenic nuclear SSRs, 530 EST-SSRs and 138 SNPs)	*qRD4*	LG 4	9.21	Rajkumar et al. (2013)
Fresh root weight	E36-1 × SPV570	–	*qRF4*	LG 4	9.21	Rajkumar et al. (2013)
Root length	E36-1 × SPV570	–	*qRL4*	LG 4	8.33	Rajkumar et al. (2013)
Root volume	E36-1 × SPV570	–	*qRV1*, *qRV4*	LG 1 and 4	27.05	Rajkumar et al. (2013)
Number of roots/plant	E36-1 × SPV570	–	*qRN1*	LG 1	17.87	Rajkumar et al. (2013)
Root/shoot ratio	E36-1 × SPV570	–	*qRS10*, *qRS10.1*	LG 10	16.03	Rajkumar et al. (2013)
Number of brace roots	Sansui × Jiliang 2	326 SSR markers	*qRT6*, *qRT7*	LG 6 and 7	59.5	Li et al. (2014)

previously identified root angle QTLs in rice and maize. The locations of the QTLs identified in the investigation were also projected onto a sorghum consensus map (Mace et al. 2009). Importantly, all four nodal root angle QTLs identified in this study were colocated with previously identified QTLs for stay-green. The colocation of the QTLs indicated the putative genetic association between nodal root angle and the stay-green drought response in sorghum. Furthermore, a putative association between three QTLs governing nodal root angle and grain yield was identified through single marker analysis conducted on yield data recorded in a subset of the mapping population grown in hybrid combination with three different tester lines. Similarly, Rajkumar et al. (2013) reported colocalization of QTL for root volume with QTL for root fresh weight and root dry weight on chromosome number 4 of sorghum. The colocalization of QTLs for root traits in sorghum can assist in their introgression in drought-susceptible cultivars through MAS utilizing the same linked markers. Further association analysis revealed that root-related QTLs identified were quite distinct from the QTLs governing yield component traits identified in the same population. However, a nonsignificant but positive association was observed between root traits and seed yield/plant. It is thus possible to combine grain yield and desirable root traits to enhance productivity under moisture stress.

Root architecture is the key factor in determining the phosphorus uptake of the cereals in harsh environmental conditions (Doumbia et al. 1993, 1998). In rice, a major QTL, *phosphorus uptake 1* (*Pup1*) was mapped on the long arm of chromosome 12 (Heuer et al. 2009). High-resolution mapping of *Pup1* locus identified a gene designated *phosphorus-starvation tolerance 1* (*OsPSTOL1*), which is known to increase P uptake through enhancement of early root growth and development under low-P conditions (Gamuyao et al. 2012). Hydraulic conductivity of seminal and nodal roots in sorghum is known to be strongly influenced by phosphorus availability under water deficiency (Shangguan et al. 2005). Hufnagel et al. (2014) investigated the role of *SbPSTOL1,* an homologous gene of the rice *OsPSTOL1* in sorghum on plant performance under low-P conditions. Two association mapping panels—sorghum association panel subset (SAPst) and West African association panel (WAP)—were rigorously phenotyped for P uptake and root morphology in hydroponics, and grain yield under low-P conditions in Brazil and Mali. The investigation elucidated *SbPSTOL1* alleles involved in reducing root diameter under low-P condition in hydroponics and increased root surface area in low-P soil. Furthermore, the role of *SbPSTOL1* was validated through linkage mapping in a large RIL population derived from the sorghum parents with contrasting root morphology. Interestingly, *SbPSTOL1* alleles were colocalized with QTL governing root length, root diameter, and root surface area under low-P conditions in sorghum.

4.3 Marker-Assisted Selection (MAS) for RSA

Genetic studies confirmed that the RSA of sorghum is a complex character, which is controlled not only by major-effect QTLs but also by plenty of minor QTLs exerting smaller effects on the root phenotype. Additionally, the presence of genotype × environment interactions also hampers the progress of RSA improvement using a conventional breeding approach. Therefore, several molecular breeding schemes have been developed recently that have enabled the detailed dissection of the complex root traits. Among various molecular breeding schemes, thus far marker-assisted backcrossing (MABC) in rice (Steele et al. 2006) and advanced backcross QTL (AB-QTL) analysis in barley (Naz et al. 2014) are the methods utilized for incorporation of desirable root traits in different genetic background in cereals. The best evidence of successful utilization of a molecular breeding scheme to pyramid various root traits to improve crop drought tolerance is the development of a highly drought-tolerant cultivar “Birsa Vikas Dhan 111,” which is developed by introgressing quantitative trait loci governing deeper rooting length through MABC in rice (Steel et al. 2013). To the best of our knowledge, to date there is no report on marker-assisted introgression of QTLs governing superior root characters to drought-susceptible genotypes in sorghum. The major reason for this is the lack of high-throughput phenotyping platforms for accurate and efficient screening of root traits in large breeding populations (Francia et al. 2005). In addition to this, imprecise QTL identification, inconsistency in validation of QTLs detected in controlled and field conditions, their unstable expression across the populations and environments, and unfavorable epistatic interaction have adversely affected the breeding programs entailing manipulation of RSA through MAS. For these reasons, there have been very few reports on the use of MAS for improvement of RSA in cereal breeding programs and there is no report on marker-assisted introgression of QTLs governing superior root characters to drought-susceptible genotypes in sorghum. A holistic molecular breeding approach combined with rigorous controlled and field-based phenotyping of root traits for genetic manipulation of RSA in sorghum has been proposed (Fig. 1) in this chapter. The approach is followed after the development of high-throughput phenotyping and genotyping methods. The best germplasm sources for an effective screening of root traits are multiparental breeding populations, core and mini-core collections, and diversity

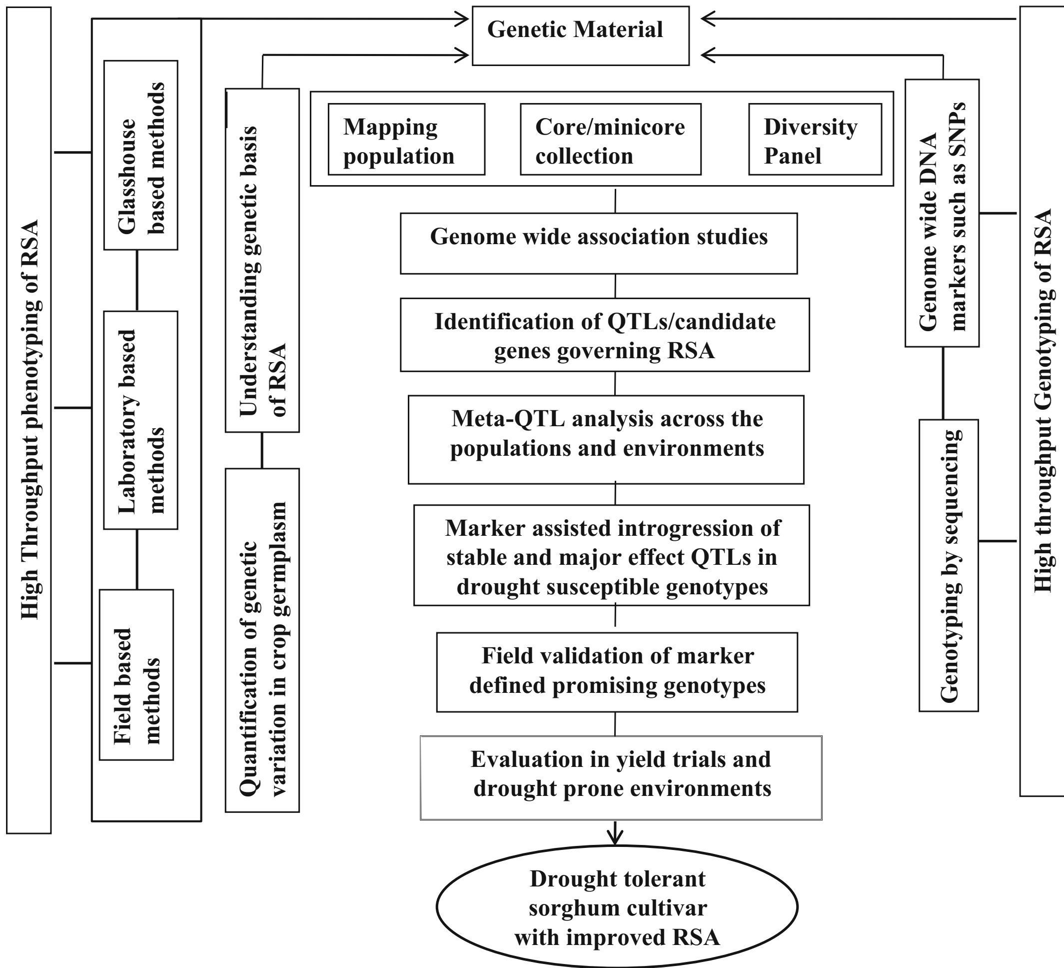

Fig. 1 Overview of molecular breeding approach to manipulate the root system architecture (RSA) in sorghum

panels. This approach combines QTL mapping and genomewide association studies (GWAS) with MAS. Last, this approach involves multilocation field testing of the marker-defined promising genotypes to assess their performance for the ultimate breeding target (grain yield).

5 Summary and Outlook

Genetic manipulation of RSA holds great promise to enhance productivity of sorghum under water deficit. The majority of the RSA components discussed in this chapter have shown genetic variation among crop genotypes, and thus could be integrated in breeding programs. However, quantification of genetic variation and genetic control of root traits is at its beginning stage. Therefore, an exhaustive characterization of diverse germplasm sources, namely core collections, diversity panels, multiparental breeding populations, and landraces, is required for identification of appropriate donors for superior root traits. In addition to the mainstream gene pool, wild species can serve as potential donors for the root traits due to their ability to colonize a wide range of moisture and soil regimes and to withstand harsh and nutrient-limited environments. Potential donors for root traits have been observed in wild species of barley (Grando and

Ceccarelli 1995), rice (Liu et al. 2004), and wheat (Reynold et al. 2007; Placido et al. 2013). However, wild sorghum species are still unexplored for root traits. Utility of RSA components such as nodal root angle, dry root weight, and RLD is fairly well established to influence drought tolerance in sorghum. In addition to this, traits such as nodal root angle at the early seedling stage have been suggested as proxy traits to determine drought tolerance of adult sorghum plants. However, a challenging obstacle to the deployment of these RSA components in sorghum breeding is the difficulty in evaluating root phenotypes of a large number of breeding lines or multiparental mapping populations. Therefore, efforts should be directed towards development of robust root screening platforms that are capable of (i) expressing high heritability for the measured component trait, (ii) minimizing the G $\times$ E interaction, (iii) screening the root trait at the early seedling stage to shorten the selection cycle and speed up genetic improvement, and (iv) finally, establishing the genetic correlation between the root trait phenotyped on the platform and ultimate breeding objective.

QTLs have been identified for traits related to root morphology, which has resulted in a great magnitude of knowledge and better understanding of the genetic control of RSA in sorghum. However, the root anatomy of sorghum is very poorly understood at present. Therefore, immediate attention needs to be paid towards identification of genomic regions governing root anatomical phenes, which will greatly facilitate their use in breeding programs. Meta–QTL analysis followed by cloning of QTLs, which are stable across populations and environments, will provide a driving force in molecular breeding for RSA because a cloned QTL can offer a reliable marker for MABC. However, the impact of a cloned QTL or candidate gene underlying the QTL region on plant productivity needs to be tested in a given environment. Furthermore, transformation of the knowledge acquired from genomics-oriented approaches into a drought-tolerant high-yielding cultivar with improved RSA is the most daunting challenge faced by breeders.

Efforts should also be directed towards understanding the physiological mechanisms that control functional aspects of RSA and its impact on crop performance in the field. Understanding the physiological mechanism and signaling behavior of roots in response to stress and subsequent physiological alterations in shoots will certainly assist plant breeding efforts towards RSA improvement in sorghum. Indeed, a multidisciplinary approach is required to integrate growing omics techniques with plant physiology, agronomy, and breeding to improve productivity of sorghum under drought through genetic manipulation of RSA.

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12 Genetic Transformation for Functional Genomics of Sorghum

Monika Dalal

Abstract

Plant transformation is an essential requirement for fundamental research in functional biology and for crop improvement. Sorghum is primarily a recalcitrant crop for tissue culture and transformation. It has taken three decades of painstaking optimization efforts to reach a transformation efficiency of 20 and 30 % through particle bombardment and *Agrobacterium*-mediated genetic transformation in sorghum, respectively. This chapter describes the different variables that were analyzed for the success of tissue culture and transformation in sorghum. These factors include type of explants, culture media, hormone combinations, methods of gene transfer, vectors, selection marker genes, and so on. Furthermore, efforts for deployment of this technique for sorghum improvement in the area of biotic and abiotic stress tolerance, and improvement of nutritional quality are discussed.

1 Introduction

For plant biologists, sorghum acts as a model system for C_4 plants. Its diploid, relatively small (750 Mb) and sequenced genome makes it suitable for comparative genomics and translational research among grasses. The remarkably diverse sorghum germplasm represents a large gene pool for fundamental research and agricultural exploitation. Primarily there are three ways—genetics, genomics, and genetic engineering—through which this vast resource can be exploited and understood. However, functional analysis of gene(s) underlying a qualitative or quantitative trait, or those identified through various omic techniques and to modify or introduce a gene, would require an efficient genetic transformation method.

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2 The Need for Sorghum Transformation

In the era of next-generation sequencing (NGS), almost every fortnight, a genome is decoded, sequenced, and added to the repertoire of genome sequence databases. The virtue of structural genomics lies in gene discovery, dissecting the pathways and networks of genes and development of markers. The ultimate aim is to understand the fundamental processes of plants and crop improvement. Therefore, post-2000, when the first plant genome was sequenced (The *Arabidopsis* Genome Initiative 2000), there was a surge of reports on functional analysis of genes and to find the function of almost all the 25,000 genes predicted for the *Arabidopsis* genome. Fifteen years after release of the *Arabidopsis* genome sequence, there are many genes with hypothetical functions. Still the unprecedented knowledge of genes and their functions has come from *Arabidopsis*. This success is attributed to the floral dip method of transformation (Clough and Bent 1998) which revolutionized the genetic transformation and gene discovery in *Arabidopsis*. Since a facile transformation was made available in this model plant system, a number of genes from other species have been validated for gene function in *Arabidopsis*.

This validation may hold true for genes that are highly similar or conserved during evolution. In the rice genome, the percentage of such genes that are homologous to *Arabidopsis* is 71 % out of 37,544 genes (The International Rice Genome Sequence Project 2005). In the sorghum genome about 24 % of the genes are grass-specific and 7 % are sorghum-specific (Paterson et al. 2009). Thus in spite of synteny and the presence of highly conserved genes, there are species-specific differences that are yet to be explored. The orthologous genes may acquire additional or distinct functions in addition to their predicted function during the course of evolution. This reiterates the need to analyze the function of the gene in its own biological context. Hence, a simple and efficient method of transformation is a must for analysis of gene function that may be specific to a particular plant species.

In addition, there are areas of crop improvement in sorghum such as insect resistance, modified lignin content and composition, and biofortification that would require molecular breeding as well as biotechnological interventions. The genes for crop improvements may come from other species that are not naturally crossable or these can also be edited or redesigned in their native background (Gaj et al. 2013; Jiang et al. 2013). This has led to renewed interest in developing an efficient method of plant transformation in sorghum. The following sections describe in detail the methods of regeneration, transformation, and transgenic development for improvement of various traits in sorghum.

3 Transient and Stable Transformation

Plant transformation can be of two types, transient and stable. Transient transformation is carried out for short-term study, as a rapid and high-throughput screening process, or for analyzing the subcellular/organelle localization of protein. In the transient method, the heterologous gene is expressed but the gene is not integrated into the genome. These studies can be done in leaf mesophyll cells using protoplasts (Yoo et al. 2007) or in intact plant (Ma et al. 2012), BY2 cell suspension cultures (Brandizzi et al. 2003), onion epidermal cells (Scott et al. 1999), and callus cultures. The most common methods for introducing the DNA into cells for transient expression include direct DNA delivery through particle bombardment, electroporation, *Agrobacterium* infiltration, and through viral vectors.

In stable transformation, there is integration of the heterologous gene into the host cell genome. This implies that the integrated gene will get replicated as part of the genome and transferred to descendants of the transformed cells (stable cell lines) and through gametes pass to the next generation. The ultimate aim in stable transformation is to generate plants with a uniformly modified genome throughout its plant parts. This facilitates the analysis of gene function and

introgression of gene(s) in a crop plant that is heritable.

The success of a genetic transformation or stable transformation depends on several criteria such as (i) a suitable explant, a target tissue for in vitro culture that has competence to regenerate; (ii) a suitable medium that facilitates efficient regeneration, (iii) method of DNA delivery; (iv) vectors and strains (in the case of *Agrobacterium* mediated transformation); and (v) selection of marker to select true transformants efficiently.

4 Sorghum Tissue Culture and Regeneration

Regeneration in tissue culture is a prerequisite for any transformation method. The regeneration relies on totipotency. In plants, many somatic cells, including fully differentiated types have the capacity to regenerate into whole plants. This phenomenon of totipotency depends on competence of the tissue used, that is, its ability to get induced to follow a developmental path or process. Plant cells can regenerate through somatic embryogenesis or organogenesis. In the case of monocots in general and sorghum in particular, somatic embryogenesis has been a most commonly observed phenomenon for regeneration. However, shoot organogenesis has also been reported (Harshavardhan et al. 2002; Nirwan and Kothari 2004).

4.1 Explant

Many sorghum explants were explored for regeneration capability. These include leaf segments (Pola and Saradamani 2006), mature embryo (MacKinnon et al. 1987; Nirwan and Kothari 2004), immature embryo (Casas et al. 1993; Howe et al. 2006; Gurel et al. 2012; Liu and Godwin 2012), immature inflorescence (Cai and Butler 1990; Kaeppler and Pedersen 1996; Casas et al. 1997; Jogeswar et al. 2007), anthers (Kumaravadivel and Rangasamy 1994; Can et al. 1998), and shoot meristem (Seetharama et al. 2000; Harshavardhan et al. 2002; Maheswari et al. 2006; Sai Kishore et al. 2006). Regeneration using mesophyll protoplast (Sairam et al. 1999) and root transverse thin cell layers (tTCLs) has also been reported (Baskaran et al. 2006). Among these explants, immature inflorescence and immature embryo have been found to be most embryogenic in nature. Jogeswar et al. (2007) reported a high frequency of somatic embryogenesis from immature inflorescence (after 50–60 days of sowing) in three different genotypes of sorghum, SPV 462, SPV 839, and M 35-1. A comparison between two explants, that is, immature embryos and inflorescence in five *Sorghum sudanense* and three *S. bicolor* genotypes revealed that immature inflorescence had more regeneration potential (46.48 %) than immature embryos (22.35 %) (Gupta et al. 2006). The higher regeneration potential was attributed to a relatively higher proportion of meristematic tissues in the form of floral meristems, rachis, and rachillae in immature inflorescence as compared to only scutellum, the source of meristematic cells in the immature embryo (Gupta et al. 2006). Nevertheless, immature embryos 12–15 days post-anthesis (1–1.5 mm in size) have been found to be more responsive and successful as far as genetic transformation of sorghum is concerned (Gurel et al. 2012; Liu and Godwin 2012). The maintenance and continuous supply of the specific developmental stage of the immature embryo poses a problem. The specific developmental stage provides a narrow window for work: slight deviation can increase variability and decrease the reproducibility. It has been reported that even the source of the embryos had a very significant impact on transformation efficiency. Field-grown embryos were found to have a higher transformation frequency than greenhouse-grown embryos (Zhao et al. 2000).

For genetic transformation, an explant that is easily available, meristematic in nature, and not too development stage specific (as in the case of the immature embryo) would be more ideal. Therefore, shoot meristem that can be conveniently obtained from germinating seeds might be a good option as an explant. Harshavardhan et al. (2002) analyzed the callus induction and

regeneration response from the shoot meristem culture of three sorghum genotypes, M 35-1, BTx623, and 296B. Depending on hormone combination in the medium, both direct organogenesis and somatic embryo formation were reported. A 5- to 7-day-old shoot meristem was found to be ideal (>80 %) for induction of multiple shoot bud initials. The regeneration frequencies of the three genotypes were in the range of 85–92 % (Harshavardhan et al. 2002). Multiple shoot induction from shoot meristem was also reported by Sai Kishore et al. (2006). The advantage of using shoot meristem from germinating seeds is that explant is available throughout the year; the chances of somaclonal variation can also be reduced by inducing direct organogenesis and the availability of multiple shoots in turn can take care of poor transformation efficiency of sorghum genotypes. Moreover, it was found that when shoot meristem is used as the explant, the different genotypes show relatively similar frequencies of regeneration. Therefore, it was proposed as a method with the least or no genotypic effect (Zhong et al. 1998; Harshvardhan et al. 2002).

4.2 Culture Medium

Media composition including the phytohormones and additives plays an important role in morphogenesis, developmental path (organogenesis or somatic embryogenesis), and regeneration time of explants. There are three phases in tissue culture: callus induction, regeneration, and rooting. Embryogenic callus cultures in sorghum can be of two morphotypes, compact and friable calli. These two morphotypes differ in morphology, texture, growth rate, and extent of embryo differentiation (Elkonin et al. 1995; Kaeppler and Pedersen 1997). Friable embryogenic callus has a higher growth rate, uniformity, and more easily gives rise to embryogenic cell suspensions than compact embryogenic callus (Elkonin et al. 1995). Addition of high concentrations of proline (3 g l^{-1}) and asparagine (2 g l^{-1}) to N6 medium led to friable embryogenic callus formation (Elkonin et al. 1995). The concentration of nitrate (NO_3^-) and its ratio with ammonium (NH_4^+) were shown as the main factors governing the morphotype of sorghum embryogenic callus (Elkonin and Pakhomova 2000). It was proposed that when the ratio of NO_3^-/NH_4^+ is 4:1, which is similar to that of N6 medium, friable embryonic calli are formed whereas at the higher level of NH_4^+ (20.6 mM) with relatively low NO_3^- concentrations, which is characteristic of the MS (Murashige and Skoog 1962) medium, a compact embryonic calli is formed (Elkonin and Pakhomova 2000).

4.3 Hormones and Hormone Combinations

Regeneration in tissue culture is highly dependent on the type of hormone and hormone combinations. Among auxins, 2,4-D (2,4-Dichlorophen oxyacetic) has been more regularly exploited for callus induction and BAP (6-Benzylaminopurine) and TDZ (Phenyl-N′-(1,2,3-thiadiazol-5-yl) urea) have been found to be more regenerative among cytokinins. TDZ is a substituted phenyl urea (N-phenyl-1,2,3-thidiazol-5-ylurea) and exhibits strong cytokinin-like activity similar to that of N6 substituted adenine derivatives (Mok et al. 1982). TDZ (5.0 μM) in combination with BAP (17.72 μM) and NAA (1.074 μM) gave rise to multiple bud induction, whereas 17.72 BAP and 2.69 μM NAA led to direct somatic embryogenesis in shoot meristem explants (Harshvardhan et al. 2002). In the case of immature inflorescence, and immature and mature embryo, 2,4-D in the range of 1.5–2.5 mg l^{-1} has been most successful for embryogenic callus induction (Casas et al. 1993, 1997; Nirwan and Kothari 2004; Nguyen et al. 2007; Liu and Godwin 2012). The frequency of embryogenesis was highest with 2,4-D followed by NAA, dicamba, picloram, and 2,4,5-T. A high frequency of globular embryos was induced from immature inflorescence grown on MS medium supplemented with 2.0 mg l^{-1} of 2,4-D and 0.5 mg l^{-1} of kinetin (Jogeswar et al. 2007).

Initially, NAA was used for sorghum root induction (Maralappanavar et al. 2000; Nirwan and Kothari 2003, 2004). A combination of three auxins (NAA, IBA, and IAA; Gao et al. 2005b; Gurel et al. 2009) or three auxins with copper sulphate was found to be good for in vitro sorghum root proliferation and resulted in 100 % survival of plantlets in glasshouse (Liu et al. 2013).

4.4 Additives

One of the major problems especially for embryos as explants is the release of phenolics and black pigmentation that negatively affects the growth and differentiation of tissues. This problem has been addressed either by including additives and antioxidants such as ascorbic acids or by physical treatment of the explants. Addition of 1 % PVPP (polyvinylpolypyrrolidone) and shortening of the subculture period (1-week interval) has been proved to be effective in reducing the phenolics and pigmentation (Zhao et al. 2000; Gao et al. 2005a, b).

The production of black pigments could also be reduced by addition of activated charcoal (AC; 1–5 g l^{-1}) in the callus induction medium. Nguyen et al. (2007) reported that addition of activated charcoal increased the survival of the immature embryo explants from 29 % (without AC) to 80 % (with AC); however, it inhibited the callus formation on surviving immature embryo explants (Nguyen et al. 2007). The inhibitory effect of AC on callus formation was attributed to adsorption of hormones from the medium.

Inclusion of additives such as casein hydrolysate, coconut water, or amino acids such as L-glutamine, proline, and asparagine in medium is a common practice in tissue culture. Casein hydrolysate is a mixture of amino acids, and coconut water has several nutritive factors and is a source of phytohormones. Amino acids are used as a source of organic nitrogen especially for somatic embryogenesis. The addition of proline and asparagine at high concentration (2–3 g l^{-1}) induces friable calli and reduces pigmentation and phenolics production (Elkonin et al. 1995; Carvalho et al. 2004). In contrast, Nguyen et al. (2007) found that addition of proline (2 g l^{-1}) increased the production of black pigment in their experiment. There can be several reasons for such variable results. Elkonin et al. (1995) had proposed that the presence of both proline (3 g l^{-1}) and asparagine (2 g l^{-1}) was required for reducing the pigmentation and the positive effect was reported with N6 medium (Elkonin et al. 1995). Moreover, the pigmentation can also be callus morphology dependent with compact embryonic calli more prone to pigmentation (Elkonin et al. 1995) or genotype dependent (Kaeppler and Pederson 1997).

Variable effects of coconut water in the medium were also reported. Addition of coconut water reduced the pigment production and improved callus induction in sorghum genotype P898012 (Carvalho et al. 2004), although there was no positive effect on other genotypes (Kaeppler and Pederson 1996; Nguyen et al. 2007). It is difficult to generalize the effect of complex and natural additives like coconut water. The variability in the composition of coconut water itself can be a major issue, as based on the age of the coconut the composition of the coconut water may differ.

4.5 Physical Treatments

Physical treatment of immature seeds or embryos has been shown to have a positive effect on explant survival and callus formation. A pre-treatment of immature seeds (before dissecting the immature embryo as explants) at 4 °C for 1–3 days resulted in decreased black pigmentation and increased survival of embryos. The pre-treatment for 1 day was found to be optimum, as treatment for more than one day resulted in germination of embryo which reduced the callusing ability (Nguyen et al. 2007). However, no such positive effect of cold treatment was observed on survival or callus induction from P898012 and Tx430. In fact, the cold treatment of spikes for 5 days decreased the frequency of immature embryo survival, callus production, and the expression of GFP (Gurel et al. 2009).

4.6 Genotype Selection

Genotype selection plays an important role in tissue culture as well as transformation. Frequently a genotype that is most responsive to regeneration may not be as efficient for transformation. Therefore, identification of a genotype suitable for both regeneration and transformation is a must. Since the 1990s, several sorghum genotypes have been screened for their response to either regeneration or transformation or both. Among these Tx430 and P898012 have been the most promising genotypes for transformation (Jeoung et al. 2002; Carvalho et al. 2004; Sato et al. 2004; Liu and Godwin 2012; Wu et al. 2014). The accession no. 214856 of the Ethiopian Plant Genetic Resources Center (Addis Ababa) was also reported to be efficient in callus induction and regeneration and was used for particle bombardment (Tadesse et al. 2003). In the Indian context, regeneration in genotypes such as 296B, SSV 84, and M 35-1 has been successful but the regeneration frequency is very low, between 14–18 % (Seetharama et al. 2000). Gupta et al. (2006) screened five *S. sudanense* and three *S. bicolor* genotypes using immature embryo and immature inflorescence. The regeneration frequency of *S. sudanense* was higher (38.91 %) than *S. bicolor* (26.93 %) and it was proposed that the genotypic effects on regeneration can be minimized by immature inflorescence culture. It was also observed that the extent of variability was more in plant regeneration traits than in callus induction traits (Gupta et al. 2006).

5 Transformation in Sorghum

Agrobacterium tumefaciens, a soil bacterium, has revolutionized the genetic transformation process in plants. Since the first report in the 1980s (Bevan et al. 1983; Fraley et al. 1983; Herrera-Estrella et al. 1983), *Agrobacterium* has been routinely used for transformation of many plant species. The genus *Agrobacterium* has a broad host range including many dicots, monocots, and gymnosperms (Gelvin 2003), but initially only dicot plants were considered as the host for *Agrobacterium* and hence monocot plant transformation by *Agrobacterium* progressed at a slow pace. Consequently, alternate methods of transformation were developed such as direct DNA transfer into protoplast and the biolistic method of gene transfer. Moreover, the recalcitrant nature of monocots for regeneration hampered the progress of genetic transformation.

5.1 Transformation by Direct DNA Transfer

In sorghum, the uptake of plasmid DNA by electroporation of sorghum protoplasts and transient expression of chloramphenicol acetyltransferase (*CAT*) gene was analyzed in 1986 (Ou-Lee et al. 1986). However, the first report of stable integration was published by Battraw and Hall (1991) where direct DNA transfer was carried out by electroporation of sorghum protoplasts and successful integration of *Npt*II or *Npt*II-*GUS* genes in the genome of resistant calli was shown. In the same year, the biolistic technique for sorghum transformation was first exercised on suspension cell cultures of sorghum by transforming the kanamycin or hygromycin resistance gene along with the *GUS* reporter gene by Hagio et al. (1991). Subsequently, the first successful transgenic sorghum was developed using particle bombardment of an immature embryo in 1993 (Casas et al. 1993). With this method the initial transformation efficiency of 0.286 % was reported. Later immature inflorescence was also used for sorghum transformation by particle bombardment (Casas et al. 1997). There was almost no significant improvement in transformation efficiency until 2010 as evident from the reported efficiency of 0.545 % in 1998 (Zhu et al. 1998a, b) to 0.77 % in 2010 (Grootbroom et al. 2010). However, efforts were made to optimize the conditions for particle bombardment with respect to choice of explants, promoters, and selection marker. A helium pressure of 1100 or 1300 psi with a target distance of 6 cm gave the highest transient *GUS* expression for immature embryo and shoot tips (Tadesse et al. 2003). The efficient expression of a gene is also governed by the

strength of the promoter. The 35S promoter from cauliflower mosaic virus (*CaMV35S*) has been the most extensively used promoter for constitutive overexpression of genes. However, it was found that *CaMV35S* promoter was more suited for dicot species than for monocot species. Therefore, to identify the strong constitutive promoter for expression of heterologous genes in sorghum, a comparative transient *GUS* expression study with constitutive promoters, namely *CaMV35S* (dual Cauliflower mosaic virus 35S promoter), *Ubi* (maize *ubiquitin 1*), *Act1* (rice *actin 1*), and *Adh1* (rice *alcohol dehydrogenase 1*) was carried out (Hill-Ambroz and Weeks 2001). The expression of *GUS* was very low in the bombarded embryonic tissue and did not reveal any significant difference in the strength of these promoters. Later, Jeoung et al. (2002) carried out transient assays using *CaMV35s, Ubi*, and *HBT* promoters using both *GUS* and *GFP* as reporter genes. *HBT* is a chimeric promoter with the 35S enhancer fragment fused to the basal promoter of the C_4-*pyruvate orthophosphate dikinase gene* (*C4PPDK*). The *GFP* expression by *Ubi* was highest, followed by *CaMV35S*, and *HBT* promoter (Ubi1 > CaMV35S > HBT). The four promoters *CaMV35s, Ubi*, *Act1*, and *Adh1* analyzed by Hill-Ambroz and Weeks (2001) were compared for transient *GUS* expression in immature embryos and shoot tips using particle bombardment by Tadesse et al. (2003). Based on the number and size of the blue foci (*GUS* expression) the strength of the promoters was ranked as *ubi*1 > *act*1 D > *adh*1 > CaMV35S.

The constitutive expression of proteins may not be required and may be undesirable as many a time it results in pleiotropic effects in plants. Moreover, for spatiotemporal-specific genetic modifications it would be essential to have specific promoters. Therefore, to engineer sorghum grain for quality or molecular farming, Ahmad et al. (2012) characterized the promoter of the seed storage α-kafirin gene from sorghum. The α-kafirin promoter (α-kaf) contained endosperm specificity-determining motifs, prolamin-box, the O2-box 1, CATC, and TATA boxes required for α-kafirin gene expression in sorghum seeds. Two constructs having the *GFP* reporter gene driven by α-kafirin promoter and constitutive (*Ubiquitin*) promoter were bombarded into various sorghum and sweet corn explants. All the explants bombarded with *Ubi-GFP* showed *GFP* expression, whereas with the *α-kaf-GFP* construct, *GFP* expression was detected only in seeds. This showed that *α-kaf* promoter is seed specific and hence can be exploited for seed-specific genetic modifications.

As for the selection marker, initially the *Npt*II selection marker was used (Battraw and Hall 1991; Hagio et al. 1991) but later almost all the reports of the biolistic method of sorghum transformation used *Bar* as the selection marker gene (Casas et al. 1993, 1997; Zhu et al. 1998a, b). The poor transformation efficiency reported in these cases may be due to the use of *Bar* as the selection marker. It was shown that regeneration from herbicide-resistant calli gets inhibited due to the release of phenolic substances during herbicide-based (phosphinothricin/bialaphos) selection (Casas et al. 1997; Tadesse et al. 2003), which results in a lower percentage of regenerated plants and hence overall a lower transformation efficiency.

A major leap of 20.7 % transformation efficiency was achieved by Liu and Godwin (2012). The increase in efficiency was attributed to improvement in various aspects of sorghum tissue culture, transformation, and selection strategy which are as follows.

- Basal medium: MS medium containing 4.44 g/L MS powder with Gamborg vitamins, 30 g l^{-1} sucrose and 8 g l^{-1} agar
- Genotype: Tx430, a highly transformation efficient genotype
- Explants: Immature embryos
- Callus induction medium (CIM): MS medium supplemented with 1 g l^{-1} L-proline, 1 g l^{-1} L-asparagine, 1 g l^{-1} potassium dihydrogenphosphate (KH_2PO_4), 0.16 mg l^{-1} $CuSO_4$, and 1 mg l^{-1} 2,4-D
- Osmotic medium: MS medium supplemented with 0.2 M D-sorbitol and 0.2 M D-mannitol
- Regeneration medium: MS medium supplemented with 1 mg l^{-1} BAP, 1 mg l^{-1} IAA, and 0.16 mg l^{-1} $CuSO_4$

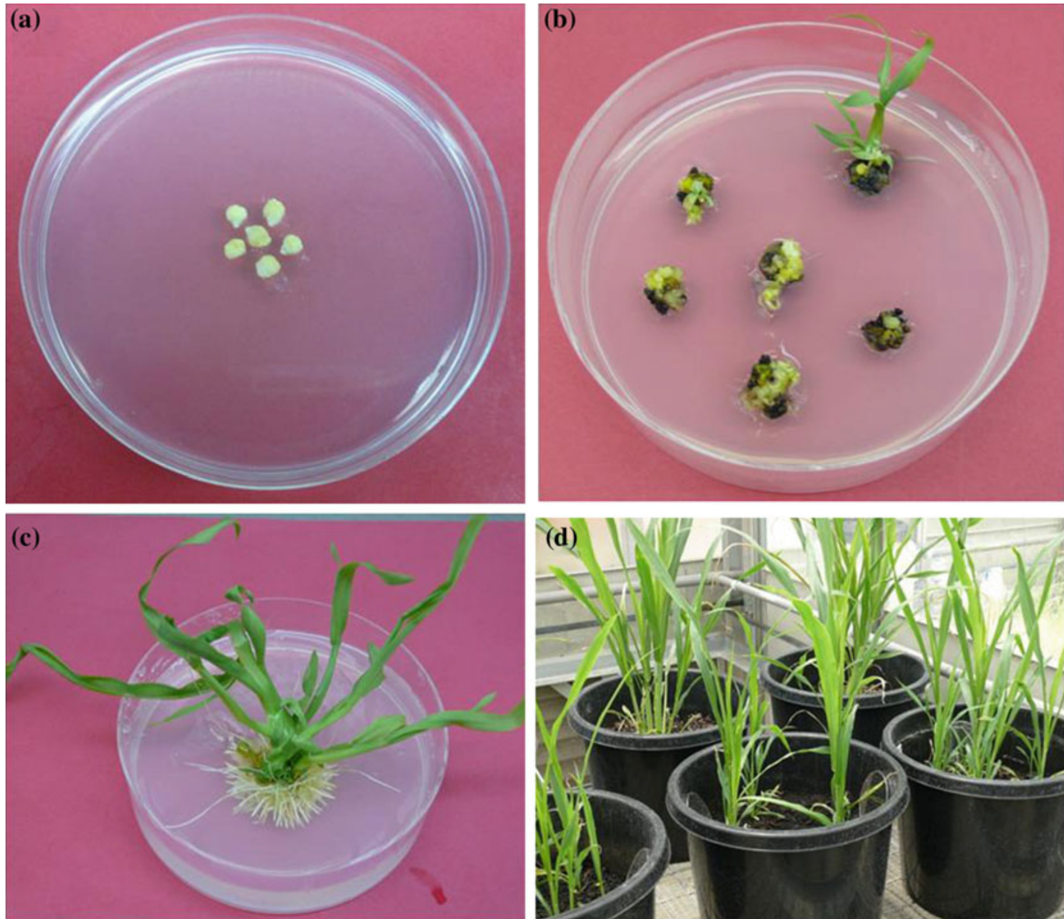

Fig. 1 Development of transgenic sorghum plants by microprojectile method. **a** immature embryo-derived calli used for transformation. **b** Shoot regeneration on selection media after 6 weeks. **c** Root proliferation in putative transgenic plantlet after 4 weeks in rooting medium. **d** putative transgenic plants in glasshouse (Figure reproduced with permission from authors Liu and Godwin 2012)

- Promoter, reporter, and selection marker gene: Ubiquitin promoter, GFP as reporter gene, *Npt*II as selection marker gene, and geneticin as selective agent

Based on the above modifications, more than 95 % of immature embryos formed callus, ~70 % showed regeneration, and of these more than 90 % of transformants were fertile and displayed normal morphology (Fig. 1; Liu and Godwin 2012).

A method of direct DNA transfer in sorghum was proposed by Wang et al. (2007). The method involved transformation of plasmid into the pollen through ultrasonication followed by pollination of stigmas of the male sterile line. The stable integration of the gene was analyzed by southern blot thereby confirming the success of the transformation. This is a simple method, however, it suffers from problems common with direct DNA transfer methods such as multicopy insertion, damage of target tissue (pollen) after sonication, and low transformation efficiency.

5.2 *Agrobacterium*-Mediated Transformation

Among the transformation methods available, *Agrobacterium* remains the first choice for

genetic transformation. This is because direct delivery through particle bombardment results in multicopy insertion and silencing. Moreover, the high cost of instruments and operation (consumables) becomes a hurdle in particle bombardment. *Agrobacterium*-mediated transformation, on other hand, is a low-cost method with low copy and stable insertions. However, genotype specificity and competence for transformation remains a limitation.

The first successful *Agrobacterium*-mediated production of stably transformed sorghum plants was published by Zhao et al. (2000) with 2.12 % transformation efficiency. The efficiency was further improved incrementally from 3.30 % (Gao et al. 2005b) to 8.3 % (Gurel et al. 2009). To increase the transformation efficiency, several factors including explant competence for transformation, pre-treatment of explants, *Agrobacterium* strains, promoter selection, and reporter genes were considered. Also when comparing *GUS* and *GFP* gene as screenable or reporter genes, *GUS* was found to be inconsistent in transformed callus or transgenic tissues (Carvalho et al. 2004). A reduction in *GUS* activity was reported in most of the transgenic plant tissues analyzed, which was attributed to the phenolics released by the sorghum tissues. *GFP* was superior to the *GUS* gene as a reporter gene for both transient and stable transformation of sorghum (Jeoung et al. 2002). Among selectable markers, hygromycin phosphotransferase (*hph*) was efficient in discriminating the transformed and nontransformed calli and seedlings (Carvalho et al. 2004).

The use of drug resistance and herbicide resistance marker genes for transgenic development has issues of public acceptance and biosafety. There is concern for use of drug resistance marker genes as it is anticipated that the drug resistance gene may be transferred to infectious bacteria, rendering the antibiotics ineffective for human and animal health applications. The horizontal transfer of an herbicide resistance gene from sorghum to the weedy relative, Johnsongrass (*S. halepense* [L.] Pers.), can be a major concern. Therefore, to address this issue Gao et al. (2005b) successfully used *phosphomannose isomerase* (*PMI*) gene from *Escherisia coli* as the selectable marker and mannose as the selective agent for sorghum transformation. The *PMI* gene catalyzes the reversible isomerization of mannose-6-phosphate to fructose-6-phosphate which enables growth on mannose-containing media. It also reduces the chances of accumulation of the inhibitory or derivative agents (Joersbo et al. 1998). The concentration of mannose required for the efficient selection of sorghum was the same as that reported for maize, but twofold less than that used for rice and twofold more than that used for wheat selection (Gao et al. 2005b).

Among *Agrobacterium* strains EHA105, EHA101, LBA4404, and AGL1 (Jeoung et al. 2002; Carvalho et al. 2004; Gao et al. 2005b) in combination with supervirulent binary vectors containing the *virB*, *virC*, and *virG* genes (pTOK233; Carvalho et al. 2004), hypervirulent pTiBo542 (Jeoung et al. 2002), PHP149, PHP166, and PHP32269 (Wu et al. 2014) were used for sorghum transformation.

Various physical treatments of explants to increase the competence for *Agrobacterium* transformation were explored. An increase in the transformation efficiency to 5 % was reported by cold pre-treatment of immature seeds due to improved callus formation and survival (Nguyen et al. 2007), and a pre-treatment of immature embryos at 45 °C for 3 min and cooling to 25 °C before *Agrobacterium* infection improved the efficiency to 8.3 % in sorghum variety P898012 (Gurel et al. 2009). Recently, a significant increase (33 %) in transformation frequencies was reported in sorghum (Wu et al. 2014). Such a significant increase in transformation efficiency was based on already available leads and some new modifications. They used immature embryos of sorghum variety TX430 as the target tissue which is known for high transformation efficiency. The seven-step procedure described by Wu et al. (2014) is as follows.

1. *Agrobacterium* infection: Infect freshly isolated embryos with *Agrobacterium* (either AGL1 or LBA4404) suspension (OD = 0.7 at 550 nm) in PHI-I (medium composition

"treatment c"; Zhao et al. 2000) medium for 5 min.

2. Cocultivation: Culture the infected embryos on PHI-T medium for 4–7 d at 25 °C in the dark.
3. Resting: Transfer the infected embryos to DBC3 medium (Cho et al. 1998) plus 100 mg/l carbenicillin (for LBA4404) or 100 mg l^{-1} cefotaxime and 150 mg l^{-1} timentin (for AGL1) for 7–10 d at 28 °C in the dark for resting.
4. Selection: Start selection on 14th day post-infection on DBC3 plus appropriate selection for Agrobacterium strain. For imposing selection:
    (a) Add 12.5 g l^{-1} mannose and reduce the maltose concentration to 5 g l^{-1} if using PMI as the selection marker, or add 3 mg l^{-1} bialaphos if moPAT is used as the selection marker.
    (b) Subculture on fresh medium every 2–3 wk for a total duration of approximately 2.5–3 mo.
5. Shoot development: Culture on PHI-XM medium for 2–3 wk in the dark, and then for one wk under light.
6. Root development: Culture on PHI-Z medium for 2–3 wk in the light.
7. Transplant the regenerated plantlets into soil for growth to maturity and seed production.

There were two major determinants for the increase in transformation efficiency, medium composition during cocultivation and regeneration, and choice of *Agrobacterium* strain (Wu et al. 2014). The medium DBC3 (Cho et al. 1998) which was primarily described for barley transformation, was used during resting and selection steps. Because use of DBC3 during cocultivation lowered the transformation frequencies, cocultivation was carried out on PHI-T medium (without copper and BAP). This modification was proposed as the most important component that significantly improved the sorghum transformation efficiency. The second factor was the use of *Agrobacterium* strain AGL1. Use of this strain resulted in about 30 % transformation efficiency, whereas LBA4404 gave an average transformation frequency of 9.7 % (Wu et al. 2014). The authors used two different selectable markers, *phosphomannose isomerase* (*PMI*) and codon modified *phosphinothricin acetyltransferase* (*moPAT*). The high-throughput transformation revealed that there was no significant effect of selection markers, *moPAT* and *PMI*, on sorghum transformation efficiency (Wu et al. 2014).

6 Sorghum Improvement Through Genetic Transformation

6.1 Insect-Pest Resistance

There are at least 150 insect species reported as pests of sorghum worldwide (Guo et al. 2011). Among these, spotted stem borer (*Chilo partellus*), shoot-fly (*Atherigona soccata*), and aphids are the major challenges for sorghum productivity. Stem borer attacks the young crop and feeds in the whorl and damages the shoot apical meristem leading to deadheart. Deadhearts can terminate plant growth and development or result in excessive tillers that are barren. Stem tunneling can result in lodging. Girijashankar et al. (2005) developed transgenic sorghum plants resistant to spotted stem borer by expressing a synthetic *cry1Ac* gene from *Bacillus thuringiensis* (*Bt*). The gene was driven by a wound-inducible promoter from the maize protease inhibitor gene (*mpiC1*). The transgenic plants expressed the Cry protein at low levels (1 and 8 ng Bt protein per gram of fresh leaf tissue) after 12 h of mechanical wounding. The leaf disc insect bioassays of the transgenic plant with the neonate larvae of the spotted stem borer led to 60 % reduction in leaf damage, 40 % larval mortality, and the surviving larvae showed 36 % reduction in weight over those fed on control plants. However, the shoot bioassays on two lines that showed high resistance in leaf disc assay recorded insect mortality of <25 %. There was no significant difference in the weight of the surviving larvae reared on transgenic lines from

those reared on nontransgenic control plants and that was evident by actively tunneling inside the transgenic shoots by the surviving larvae. This suggests that a threshold level of insecticidal protein expression in the transgenic plant is required to achieve a higher level of resistance against insect pest. Recently, 14 independent transgenic lines carrying two different classes of *B. thuringiensis* toxin genes, *cry1Aa* and *cry1B*, were developed in two elite parental lines, CS3541 and 296B (Visarada et al. 2014). The transgenic plants expressed Bt protein in the range of 35–500 ng g^{-1} fresh leaf tissue. In leaf bioassays, the transgenic plants showed 55–78 % less leaf damage as compared to nontransgenic controls. In whole plant assays, transgenic sorghum expressing the Bt gene showed significantly less leaf damage and reduced stem tunneling (2.4–31.5 %) compared to that of control (50–60 %).

6.2 Disease Resistance

Chitinase and *chitosanases* are the most commonly used candidate genes for engineering fungal resistance in plants. Chitinases endolytically hydrolyze the β-1, 4-linkages of chitin, a major component of fungal cell walls, whereas chitosanases hydrolyze the β-1,4-linkages between N-acetyl-D-glucosamine and D-glucosamine residues in a partially acetylated chitin polymer. Initial efforts for improving fungal resistance in sorghum were made by introducing the rice chitinase gene *G11* in sorghum (Zhu et al. 1998a, b). Later Krishnaveni et al. (2001) reported that constitutive expression of a gene encoding a class I rice chitinase in sorghum conferred increased resistance to the stalk rot caused by the *Fusarium thapsinu* fungus. To impart resistance to anthracnose, a fungal disease caused by *Colletotrichum sublineolum*, transgenic sorghum plants harboring both *chitinase* (*harchit*) and *chitosanase* (*harcho*) from *Trichoderma harzianum* were developed in Kenyan genotype KAT 412 (Kosambo-Ayoo et al. 2011). The infection assays on excised leaf segments and in one-week-old seedlings revealed that transgenic line KOSA-1 was significantly more tolerant to anthracnose than the parent wild-type, KAT 412.

6.3 Drought and Salt Tolerance

To engineer sorghum for drought and salt tolerance sorghum plants expressing the bacterial *mannitol-1-phosphate dehydrogenase* (*mtlD*) gene were developed in genotype SPV 462 (Maheswari et al. 2010). The transgenic leaf segment showed better leaf water retention under PEG 8000 and showed higher germination rate under 200 mM NaCl stress as compared to that of nontransgenic plants. The transgenic seedlings showed higher root and shoot lengths after 15 days of NaCl stress and significant stress recovery in both root and shoot length.

Taking the lead from the enhanced abiotic stress tolerance of rice transgenics expressing *OsCDPK-7* (*calcium-dependent protein kinases*; Saijo et al. 2000), sorghum transgenic plants constitutively expressing *OsCDPK*-7 were developed (Mall et al. 2011). CDPKs sense modulations in cellular calcium levels that occur due to exposure of plants to different environmental stresses. The transgenic sorghum plants did not show any improvement in cold or salt stress over nontransgenic control. Moreover, the transgenic plants bore a lesion mimic phenotype and showed upregulation of a number of pathogen-related proteins. Inasmuch as CDPKs are involved in a number of cellular signaling pathways (Schulz et al. 2013), their constitutive overexpression can lead to pleiotropic effects in plants. The species-specific background may have a role as success of *OsCDPK* transgenics in rice could not be re-created with sorghum.

6.4 Nutritional Quality

The major concern with sorghum grain is its lower nutritive quality due to its low content of essential amino acids such as lysine, and lower wet-cooked digestibility of its protein as compared to other cereals (Grootboom et al. 2014).

This results in sorghum having a very low protein digestibility corrected amino acid score (PDCAAS). By improving the nutritive quality of the sorghum grain, a major issue of malnutrition in developing countries can be addressed. To increase the lysine content in grain, a high lysine gene, *HTl2*, encoding α-hordothionin protein from *Hordeum vulgare* that contains 27 % lysine residues, was expressed in sorghum under the control of 27 KD maize γ-zein promoter. To develop marker-free transgenic plants a cotransformation vector strategy with a superbinary vector containing two unlinked T-DNA cassettes, one carrying the lysine-rich *HTl2* gene and another with the herbicide-resistant *Bar* gene as selectable marker was used (Zhao et al. 2003). Three of the transgenic lines expressed high lysine content and one of the event (P898012-3) showed segregation of the *Bar* and *HTl2* genes. A 40–60 % increase in lysine was reported in the hemizygous seeds of this event (Zhao et al. 2003).

Another approach to increase lysine content in grain was based on the study in maize where it was found that altered expression of plant *lysyl tRNA synthetase* may promote tRNA misacylation and translational recoding of lysine thereby significantly enriching the lysine content of grain (Wu et al. 2007). Using a cotransformation strategy, two plasmids, *pZY101-TC2* (altered $tRNA^{lys}$) and *pZY101-SKRS* (sorghum lysyl tRNA synthetase), containing *Bar* and target gene(s) in separate T-DNA regions were used for generating marker-free transgenic sorghum plants (Lu et al. 2009). The analysis of selected T1 progeny revealed independent segregation of these genes and generation of marker-free sorghum transgenic plants. However, no analysis of lysine content in the transgenic grains was reported in the mentioned study.

The poor digestibility of sorghum grain protein has been attributed to the nature and arrangement of the kafirin storage proteins. The α-, β-, and γ-kafirins constitute about 70–80 % of total endosperm protein. Among these, α-kafirins comprise about 80 % of total kafirin endosperm proteins. The α-kafirins are located in the center and are surrounded by β- and γ-kafirins at the periphery of the protein body. The arrangement and the increased crosslinking through disulphide bonds of β- and γ-kafirins block access to the more digestible α-kafirin core (Oria et al. 2000), thereby negatively affecting the digestibility of the cooked and uncooked sorghum (Wong et al. 2009). Therefore, it was postulated that reduction in γ-kafirins might increase the digestibility. In addition to poor protein quality and undigestibility, the viscoelasticity or the functionality of sorghum dough is also a major problem for end-use food and feed purposes. Therefore, to target both digestibility and functionality, Kumar et al. (2012) generated three types of transgenic lines. Two independent sets of transgenic lines with reduced γ-kafirin, or 29-kDa α-kafirin storage protein to increase the digestibility, and a third set of transgenic lines with expression of wheat Dy10/Dx5, a hybrid high-molecular-weight glutenin protein, to increase the viscoelasticity of the dough. The downregulation of γ-kafirin alone did not affect the protein digestibility of cooked flour samples. However, reduction in accumulation of 29-kDa α-kafirin resulted in distortion of protein bodies and enhanced protein digestibility in both raw and cooked samples. In another study, cosuppression of kafirin subclasses α-, γ-, and δ-kafirin increased the cooked protein digestibility by 30 %, improved the amino acid score by 0.4, and protein digestibility corrected amino acid score by 0.5 compared to the non-transgenic (Da Silva et al. 2011). But the study showed that suppression of fewer subclasses (i.e., γ1 and δ2) did not improve the cooked protein digestibility significantly. Conversely, Grootboom et al. (2014) reported that cosuppression of only two genes, γ-kafirin-1 (25 kDa) and γ-kafirin-2 (50 kDa), significantly increased sorghum in vitro digestibility of transgenic lines (39.2 %) as compared to control (28.5 %). The cosuppression of α-kafirin A1 (25 kDa), in addition to the two genes in the study increases the digestibility further (53.7 %) as compared to control. In both the above studies grains of the transgenic lines had floury endosperm.

Tannin present in the pigmented testa of some sorghum cultivars is one of the factors that

reduces protein digestibility. However, presence of tannin is also associated with grain mold resistance in sorghum and human health benefits due to antioxidant activity. Therefore, an optimum level that meets the requirement of plants as well as human health would be ideal. Wu et al. (2012) reported cloning of the *Tannin1* (*Tan1*) gene by fine mapping through metaquantitative trait locus (QTL) analysis. The study identified two nonfunctional alleles of *Tan1* (*tan1-a* and *tan1-b*) with 1 and 10 bp deletion in exons of the *WD40* gene resulting in a nontannin phenotype. Based on end use, this gene can be exploited through molecular breeding and transgenic approach for custom designing of sorghum genotypes.

Efforts were also made to develop biofortified sorghum lines with increased provitamin A content. The transgenic sorghum contained about twofold to ninefold higher β-carotene content based on a dry weight basis (DW). A carotenoid bioaccessibility test revealed that transgenic sorghum event Homo188-A contained the highest (fourfold to eightfold higher) bioaccessible β-carotene content, as compared to nontransgenic sorghum (Lipkie et al. 2013). Detailed genomic approaches of quality improvement are elaborated in Chap. 10.

7 Conclusion and Future Prospective

Improvement of sorghum for food and nutritional security of millions of people from Asia and Africa is a necessity. The improvement can be brought about by a combination of conventional and molecular breeding, and biotechnological approaches. Sorghum, which was known for its recalcitrant nature for regeneration and transformation, is slowly coming off its tag. The regeneration process has been optimized and the transformation efficiency up to 20 and 30 % has been realized by the biolistic method and *Agrobacterium*-mediated genetic transformation of sorghum, respectively. Significant progress has been made in increasing the protein quality, digestibility, and biofortification of sorghum. However, traits such as insect pest resistance still remain a challenge due to lack of resistance sources in the germplasm. Although transgenic sorghum harboring Bt genes has been developed, yet the level of resistance may not be substantial in actual field conditions. Therefore, expression levels need to be increased. There is a need to identify tissue-specific strong promoters to improve the tissue-specific expression levels as constitutive expression may not be desired in all cases. The success in generating marker-free sorghum transgenic has been encouraging. A proof of concept for genome editing in sorghum (Jiang et al. 2013) has opened new avenues for basic as well as applied research in sorghum. Sorghum is mostly self-pollinated; however, 0.6–6 % cross-pollination does occur depending on wind, flowering time, species, and so on. There have been studies where gene flow from cultivated to wild and vice versa have been reported (Schmidt and Bothma 2006; Tesso et al. 2008). Africa is the place of origin and diversity of sorghum. Therefore, permission, acceptance, and sustaining these engineered sorghums in Africa at present remains debatable. Nevertheless success in sorghum transformation would be immensely useful in gene discovery and crop improvement. Progress in sorghum transformation has opened up avenues for functional analysis of identified genes through overexpression, genome editing and knock-out studies.

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Cloning of Economically Significant Sorghum Mutant Genes

13

Sandeep R. Marla

Abstract

Mutations, occurring either naturally or induced, are permanent alterations of the nucleotide sequence in organisms. These alterations occurring throughout the evolution of a species are responsible for creating immense genetic diversity. Such natural variation generated by mutations has been selected and extensively utilized in crop improvement in several agronomically important crops. In sorghum, mutations producing key agronomic traits such as nonshattering, dwarfing, photoperiod insensitivity, improved protein digestibility, and brown midrib phenotype resulted in establishment of sorghum as the fifth major cereal crop of the world. In addition to successful utilization of these mutations in breeding, understanding the mechanistic basis underlying these traits is equally important to assist in trait advancement. Hence, mutations underlying important agronomic traits were identified using approaches such as positional or map-based cloning, candidate-gene approach, and whole genome sequencing. This chapter provides an overview of key sorghum mutations that resulted in evolution of sorghum as a major source of food, forage, and bioenergy. Additionally, cloning strategies used to identify the underlying mutations and mechanistic basis of the phenotype of interest are discussed. Identification of mutations underlying agriculturally important traits can assist in developing molecular markers to enable precise introgression of selected traits into elite inbreds used in sorghum improvement programs.

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1 Introduction

Sorghum (*Sorghum bicolor*) is an important cereal grain crop that originated ~10,000 years ago in the subtropical regions of Africa. From its center of origin in Africa, sorghum was distributed to China, India, the Middle East, and the

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United States. Movement of sorghum across a diverse range of climates, from the tropics to the temperate regions of the world, resulted in the development of immense genetic diversity observed in sorghum collections. In plants, genetic variation is created naturally due to transposons, insertion or deletion mutations, or unequal crossing over between alleles. Additionally, new genetic variants or mutants can be generated using gamma-ray irradiation and chemical mutagens such as ethyl methanesulfonate (EMS), diethyl sulfate (DES), nitrosoguanidine (NTG), and N-methyl-N-nitrosourea (MNU), or by taking advantage of transgenic and modern gene-editing approaches such as zinc-finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and the most recent clustered regularly interspaced short palindromic repeats (CRISPR). Currently, sorghum germplasm diversity collections across the world are known to possess ~100,000 accessions (see Chap. 4). However, only a small fraction of genetic diversity present is being utilized in sorghum improvement mainly due to lack of proper understanding of germplasm and the genetic mechanisms underlying different agronomically important traits.

Traditional sorghum plants were typically tall in height (2–3 m), late flowering as plants required short days for transition from the vegetative to reproductive stage of development, contained tannins in seeds, and shattered seeds at maturity before sorghum domestication. All these characteristic sorghum traits are believed to have played a key role in their adaptability to different climates around the world. However, these phenotypic traits are not ideal for large-scale sorghum production as they adversely affect seed harvesting, delay maturity, and reduce grain yield and protein digestibility; therefore, the yield and nutritional value are not stable. Moreover, presence of tannins reduces protein digestibility and the nutritive value of sorghum. Therefore, sorghum mutants containing agronomically beneficial traits such as nonshattering grains, reduced plant height, photoperiod insensitivity, nontannin seeds, and acyanogenic accessions were artificially selected from diverse sorghum accessions and successfully utilized in sorghum breeding. Cloning of a few key genes of agronomic importance and mechanisms underlying such traits are presented in this chapter.

2 Nonshattering Mutations Facilitated Sorghum Domestication

Shattering of seeds from panicles at maturity is observed in wild grasses that facilitate natural dissemination of seeds. Although being beneficial for plants, seed shattering is agronomically very undesirable as it results in shedding of seeds from panicles, making harvesting very laborious and causing significant yield losses (Li et al. 2006). To minimize the negative effects of seed shattering, ancient farmers selected sorghum plants that retained seeds on the panicles till harvesting. This artificial selection is believed to be one of the key events leading to the domestication of sorghums approximately 8000 years ago. Despite identifying and propagating nonshattering sorghums, it is most unlikely that the ancient farmers knew mutations were responsible for seed retention at maturity and the impact this single selection event would have on sorghum production. Similar to sorghum, the nonshattering trait possibly underwent a strong selection in other grass species as well such as rice, wheat, maize, and barley during their domestication (Lin et al. 2012).

To unravel the genetic and biochemical mechanisms that led to nonshattering seeds in sorghum, an important shattering gene, the *Shattering1* (*Sh1*, Sobic.001G152901), was isolated and characterized by Lin et al. (2012). Genetic crosses between seed-shattering-type wild species, *Sorghum virgatum* (SV) and Tx430, a nonshattering domesticated inbred, revealed seed shattering at maturity to be governed by a single dominant gene. Map-based cloning demonstrated that *Sh1* codes for the YABBY transcription factor involved in formation of the lignin abscission layer between the seed hull and pedicel in plants containing the wild-type *Sh1* allele. Genomic sequence

comparisons of *Sh1* from shattering *S. virgatum* with nonshattering sorghum accessions showed that nonshattering accessions contained three different haplotypes or allelic variants of *sh1*, suggesting three independent domestication events, resulting in the domestication of sorghum. Of the three alleles of *sh1* resulting from mutations, the first allele contained a single nucleotide polymorphism (SNP) at the intron–exon junction producing a splice-variant; the second allele has a 2.2-kb deletion in the genic region; and the third has alterations in the promoter region as observed in Tx430. These mutated forms of *sh1* produced a truncated protein in the first two cases and reduced expression in Tx430, thus preventing the formation of an abscission layer that resulted in the loss of the shattering phenotype in sorghums (Lin et al. 2012). Without these mutations, sorghum most likely would not have gained such prominence in present-day agriculture and possibly still remained as a wild grass in nature.

In addition to *Sh1*, a second gene involved in shattering was also identified in *S. propinquum* by Tang et al. (2013). Positional mapping using F_2 progeny generated by crossing nonshattering *S. bicolor* (*Sb*) with *S. propinquum* (*Sp*) mapped the new shattering loci to be 300-kb apart from the *YABBY* gene present on chromosome 1. Flanking DNA sequences from this interval were used as markers for hybridizing *Sp* bacterial artificial chromosome (BAC) libraries. Combining genomic alignments of *Sb* and *Sp WRKY* with association studies using 11 shattering and 13 nonshattering accessions, and semiquantitative RT-PCR of two putative candidate genes revealed a transcription factor, *WRKY* (Sobic.001G148000), to be the gene attributing to seed shattering. *Agrobacterium tumefaciens*-mediated transformation of nonshattering Tx430 inbred containing the *Sb* allele with the *SpWRKY* allele re-created the seed-shattering phenotype, confirming the role of WRKY transcription factor in seed dispersal (Tang et al. 2013).

Sb and *Sp WRKY* alleles differed as the *Sb* start codon ATG mutated to ATT in *SpWRKY*, causing a methionine (M) to isoleucine (I) substitution and creating a different translation start site. As a result, the *Sp*WRKY protein contained an additional 44 amino acids compared to *Sb*WRKY. Expression of *SpWRKY* decreased during flowering and seed maturity, whereas *SbWRKY* remained uniform at all stages of development, suggesting that the wild-type *Sb* allele was a negative regulator of shattering by modulating lignin synthesis. Only *SpWRKY* formed a lignin abscission layer at the seed–stalk interface causing dehiscence of seeds. Cloning of this new shattering gene showed that it resulted from a gain-of-function mutation and has an epistatic effect on the *Sh1* gene encoding YABBY transcription factor. Map-based cloning of *WRKY* and *Sh1* shattering genes divulged both of them to contain DNA-binding domains implicated in genes controlling cell wall biosynthesis. Cloning of *WRKY* also revealed the origin of this shattering gene as occurring recently after the divergence of *S. propinquum* from *S. bicolor*, hence this *WRKY* shattering gene did not play a role in the domestication of sorghums.

3 Altering Plant Architecture by Deploying Dwarfing Genes

Agriculturally important grasses such as rice, wheat, barley, and sorghum naturally grew tall in stature. Due to their tall architecture, these grasses were profoundly susceptible to lodging at crop maturity. As crop lodging is agronomically undesirable, reducing plant height by utilizing dwarfing mutations effectively decreased lodging at maturity. Hence, dwarfing mutations in rice (*semi-dwarf1I, sd1*), wheat (*Reduced height-1*, *Rht-1*), maize (*brachytic*, *br*), and sorghum (*dwarf*, *dw*) received increased attention. Additionally, plants with reduced height responded effectively to application of fertilizers and marked a drastic increase in rice and wheat yields during the 1960s in developing countries (Khush 2001). This phenomenal increase in food production resulted from combining dwarfing genes and genetically improved varieties and is popularly known as the “green revolution,” which provided global food security over the past 50 years.

In plants, height is controlled by phytohormones gibberellins (GA), brassinosteroids (BR), and auxins, by altering the distance between internodes. Identification of dwarfing genes in rice and wheat generated curiosity in other grasses, especially maize and sorghum. Tropical sorghums were extremely tall and had weak stalks and narrow leaves. To regulate plant height in sorghum, Quinby and Karper (1961) identified four loci contributing to plant height, popularly known as *dw1–dw4*. The most prevalent alleles at these loci were the wild-type (WT) alleles that are dominant in nature and attributed to internode elongation leading to increased plant height, whereas the dwarf mutants with short internodes were produced by recessive alleles resulting from natural mutations in the wild-type genes. These four independent recessive genes were utilized extensively in various combinations in sorghum breeding to modify plant architecture.

Of the four identified dwarf genes, *dw3* is the most extensively used in sorghum breeding. Sorghum plants containing the *dw3* gene are characterized by production of dwarf plants with compact lower stalk internodes and increased girth of the internodes. The *dw3* mutation in sorghum reduced lower stalk internode length by 40–50 % of their normal counterparts (Multani et al. 2003). In addition to reducing stalk length, *dw3* causes a substantial increase in cell number in the hypodermal region and modification of the vascular system. Detailed genetic analyses revealed that *dw3* is located on chromosome 7 and leads to dwarfing by the action of a single gene. Cloning of the dwarfing gene *dw3* (Sobic.007G163800), an orthologue of maize *br2*, revealed that this gene codes for a transmembrane protein of the adenosine triphosphate-binding cassette (ABC) transporter superfamily involved in auxin efflux (Multani et al. 2003). A duplication of 882-bp in the fifth exon resulted in production of a nonfunctional protein. By doing so, it possibly nullifies the ability to transport hormone auxin required for internode elongation.

Since its discovery, the *dw3* dwarfing gene has been successfully used in sorghum breeding from the 1950s. However, a constraint associated with this gene is its ability spontaneously to produce revertants, tall plants, at a frequency of 0.1–0.3 % depending on the genetic background. Detailed genetic analysis of the *dw3* revertants revealed the occurrence of unequal crossing over during meiosis at the 882-bp duplication site in exon 5 (Multani et al. 2003). Because of unequal crossing over, the 882-bp duplication event was eliminated in some plants and these plants reverted back to tall-type resembling the tall wild-type plants. One possible genetic mechanism to solve the problem of revertants is to utilize a stable source of *dw3* by identifying new mutations that will not undergo unequal crossing over (Multani et al. 2003). In sorghum inbred Tx2737, characterization of *dw3* showed that this gene does not possess the infamous 882-bp duplication event but contains a 6-bp deletion in the fifth exon (Barrero Farfan et al. 2012). This new *dw3* allele named *dw3–sd2* demonstrated the potential to solve the problem of reverting the phenotype of *dw3* and is currently being incorporated into the sorghum elite germplasm using marker-assisted breeding (Barrero Farfan et al. 2012).

In addition to *dw3*, another dwarf mutant *dw1* was isolated and characterized. Cloning of *dw1* (Sobic.009G229800) revealed that it codes for a putative membrane protein of unknown function (Hilley et al. 2016). An SNP in *dw1* produced a premature stop codon, thus eliminating the activity of *dw1* completely and resulting in a dwarfing phenotype. Characterization of *dw1* revealed it to be independent of the other dwarfing gene *dw3* and reduced the length of internodes uniformly throughout the plant. However, the mechanistic basis of how *dw1* regulates dwarfing in sorghum is unclear. Although other dwarf mutations *dw2* and *dw4* were also used extensively in sorghum breeding, the genetic basis of the underlying mutations and the mechanistic basis of dwarfing underlying these mutations await to be characterized.

In addition to these dwarfing genes, a gamma-ray irradiation screen produced additional dwarfing mutants designated *bending dwarfs* (*bdw1–4*; Ordonio et al. 2014). Characterization of these novel *bdw* mutants revealed

aberrations in GA and these mutants contained culm bending additional to reduced plant height. The *bdw1–4* genes encodes *SbCPS1* (ent-copalyl diphosphate synthase, Sobic.001G248600), *SbKS1* (ent-kaurene synthase, Sobic.006G211500), *SbKO1* (ent-kaurene oxidase, Sobic.010G172700), and *SbKAO1* (ent- kaurenoic acid oxidase, Sobic.010G007700), respectively (Ordonio et al. 2014). Characterization of *bdw1–4* revealed that they code for key enzymes involved in GA biosynthesis, a growth hormone responsible for increasing plant height. However, GA-related mutations are not utilized in sorghum breeding currently due to their culm bending phenotype.

4 Photoperiod-Insensitive Sorghums Modulate Flowering Time

Sorghum is a short-day tropical plant that requires less than 12 h of daylength (photoperiod) for transition from vegetative to reproductive stage of plant development. Induction of flowering is a complex process as it is regulated by environmental cues, especially photoperiod, to signal this switch from the vegetative phase. When photoperiod-sensitive sorghums are grown in temperate regions with long days, they tend to flower very late or don't flower at all. Late-flowering sorghums were extensively used in forage, sweet, and bioenergy purposes as delayed flowering increases biomass due to prolonged vegetative growth. However, delayed flowering is counterproductive for grain production in regions prevalent with post-flowering drought or erratic late-season weather.

To alleviate the problem of delayed flowering in temperate regions for sorghum grain production, photoperiod-insensitive mutants that flowered early (~60–80 days) under long days were selected and broadly utilized. In sorghum, six flowering-time or maturity loci, designated *Ma1–Ma6*, have been reported (Quinby and Karper 1961; Childs et al. 1997). The wild-type alleles at each locus are dominant and contribute to delay flowering under long days. Among the different flowering-time loci, *Ma1* and *Ma6* significantly increased flowering time under long days. To understand the mechanistic basis of delayed flowering in sorghum, three photoperiod-sensitive genes, *Ma1*, *Ma3*, and *Ma6*, were isolated and characterized.

The *Ma3* flowering-time gene was cloned based on physiological similarities between the sorghum mutant $ma3^R$ and *Arabidopsis PHYB* (*Phytochrome B*), which displayed reduced sensitivity to inhibitory photoperiods (Childs et al. 1997). Childs et al. (1997) screened an F_2 population, generated by crossing sorghum near-isogenic lines (NILs) of 100M (*Ma3*) with NIL 58M ($ma3^R$), with 21 polymorphic random amplified polymorphic DNA (RAPD) markers. The F_2 plants were self-pollinated to produce F_3 families. Forty F_3 families generated were evaluated for markers cosegregating with *Ma3*. The *Ma3*-linked RAPD markers were screened on 137 recombinant inbred lines (RILs) obtained by crossing BTx623 and IS 3620C, which mapped *Ma3* to the sorghum linkage group A. Sequencing of *PHYB* (Sobic.001G394400) present in this linkage group showed that the mutated $ma3^R$ allele contained a single nucleotide deletion 30 bp upstream of the termination codon, thereby producing a truncated protein which results in photoperiod-insensitive sorghum (Childs et al. 1997). Cloning of *Ma3* marks the first flowering-time or photoperiod-sensitive gene to be cloned in sorghum.

To understand the mechanisms regulating the *Ma1* flowering-time locus in sorghum, Murphy et al. (2011) carried out positional mapping of *Ma1* using three different mapping populations. The first population was obtained by crossing two photoperiod-insensitive inbreds ATx623 (*ma1Ma5*) and R.07007 (*Ma1ma5*). The resulting F_1 progeny were photoperiod-sensitive, flowered when subjected to short days, but did not show transition to flowering under long days. The F_1 hybrid obtained was then backcrossed with ATx623 to generate BC_1F_1 plants that were used for genetic mapping of *Ma1* to an interval of 700 kb on chromosome 6 which contained 34 genes. The second mapping population generated by crossing late-flowering 100M with photoperiod-insensitive BTx406, and screening the F_2

population narrowed the genetic interval to 240-kb region. The third mapping population was obtained by crossing 100M with photoperiod-insensitive cultivar Blackhull Kafir (*ma1*). Screening the resulting F_2 population, the mapping interval of the *Ma1* locus was further reduced to an 86-kb region that contained only one gene PSEUDORES PONSE REGULATOR 37 (*PRR37*; Sobic.006G 057900).

To affirm *PRR37* was the gene leading delayed flowering, *Ma1* cDNA was sequenced from different photoperiod-insensitive sorghum cultivars (Murphy et al. 2011). The BTx406 *ma1* (*Sbprr37-1*) allele contained one nucleotide deletion upstream of the pseudoreceiver domain producing a truncated protein. Blackhull Kafir *ma1* (*Sbprr37-2*) harbored three amino acid substitutions, two substitutions in the low conserved peptide sequence, and Lys162Asn in the highly conserved pseudoreceiver domain, suggesting this lysine to be responsible for altering protein function. The third *ma1* allele (*Sbprr37-3*) from ATx623 possessed Lys162Asn as in Blackhull Kafir; additionally it contained a premature stop codon at Q^{270}. Three independent alleles of photoperiod-insensitive *ma1* resulted in their loss of function, thus conferring photoperiod-insensitivity in sorghum. In addition to *Ma1* and *Ma3*, the third delayed flowering gene *Ma6* was identified by Murphy et al. (2014) in the BC_1F_1 population derived from a cross of Tx623 (*ma1Ma5ma6*) with R.07007 (*Ma1-ma5Ma6*) and backcrossing with male sterile ATx623. Cloning of *Ma1* enabled generating molecular markers for identifying *Ma1* plants and accounting for phenotypic variation caused by *Ma1*. Quantitative trait locus (QTL) analysis positioned *Ma6* on chromosome 6 at an interval of 276 kb containing 14 genes. This genomic interval contains an orthologue of previously identified rice flowering-time gene, *grain number, plant height, and heading date* (*Ghd7*; Sobic.006G004400). This gene encodes "CONSTANS, CO-like, and TOC1 (CCT)-domain" protein involved in repression of a known floral activator *SbEHD1* and other flowering genes. Comparison of the *Ghd7* sequence between *Ma6* and mutated *ma6* from ATx623 indicated that *ma6* contained a five-nucleotide insertion in the first exon, creating a frameshift in the coding sequence and resulting in a premature stop codon.

Cloning of three major sorghum flowering-time genes, *Ma3* (*PHYB*), *Ma1* (*PRR37*), and *Ma6* (*Ghd7*), facilitated the understanding of mechanisms controlling flowering time. Under long days, *PRR37* and *Ghd7* expression increased in the morning and evening at ~3 and ~15 h, respectively, from the start of the light cycle (Murphy et al. 2011, 2014). In contrast, under short days the *PRR37* and *Ghd7* signal was detected only in the morning and was completely absent during the evening. Expression of mutated *ma1* and *ma6* from photoperiod-insensitive sorghums was similar to that of *PRR37* and *Ghd7* under short days. These findings indicate photoperiod- and circadian-clock-dependent expression of *PRR37* and *Ghd7,* and the absence of *PRR37* and *SbGhd7* expression in the evening leads to initiation of flowering. Under long days, *PRR37* and *Ghd7* repressed *SbEHD1*, a grass-specific floral activator (Murphy et al. 2011, 2014). Additionally, *PRR37* repressed the activity of another important floral activator *SbCO*. Through *EHD1* and *CO*, *PRR1* and *Ghd7* repressed expression of florigens *SbCN8* and *SbCN12* and delayed flowering under long days; *PRR37* and *SbGhd7* are central repressors of genes encoding flowering initiation in response to photoperiod in sorghum (Murphy et al. 2011; Yang et al. 2014a, b). Characterization of *PRR37* and *Ghd7* demonstrated an additive effect on delayed flowering by increasing the days to flowering to ~175 d in *Ma1Ma6* cultivars, in contrast to ~54 or ~74 d in *Ma1*- or *Ma6*-containing cultivars, respectively (Murphy et al. 2014).

Although *Ma3* was the first cloned photoperiod-sensitive gene, the mechanistic basis underlying this flowering-time gene was not known until the cloning of *Ma1* and *Ma6*. Genetic and expression analysis of the $F_{2/3}$ populations generated using 100M (*Ma1Ma2 Ma3Ma4Ma5ma6*) and 58M (*Ma1Ma2-ma3RMa4Ma5ma6*) demonstrated *Ma3* to be epistatic to *Ma1* and *Ma6*, and *Ma3* is required

for photoperiod-regulated expression of these flowering repressors in sorghum (Yang et al. 2014b). Cloning of flowering-time genes in sorghum can aid in marker-assisted selection of sorghum germplasm improvement for bioenergy and grain production.

5 Improving Sorghum Protein Digestibility with Nontannin and Highly Digestible High-Lysine (*hdhl*) Mutants

Sorghum grain contains 7–16 % total proteins of the grain dry weight (Benmoussa et al. 2015). Tannins or proanthocyanidins (PAs) are oligomers and polymers of flavan-3-ols compounds in the pigmented testa layer present underneath the pericarp of sorghum grains (Wu et al. 2012). Tannin-containing sorghums are prevalent in areas with severe insect pressure and bacterial and fungal diseases, thus correlating tannin production with protection against biotic stressors. Additionally, tannins benefit human health because of their anticancer activity and antioxidant and radical scavenging functions (Kaufman et al. 2013). However, tannins impart a bitter flavor and reduce protein digestibility in humans and animals. Therefore, nontannin sorghum mutants present in sorghum accessions were preferentially selected and extensively used in sorghum breeding.

To identify the gene involved in tannin production, Wu et al. (2012) evaluated tannin production in a RIL population generated by crossing a nontannin inbred Tx430 with tannin-producing inbred ShanQui Red. Additionally, two RIL populations of Tx430/SC1345 (212 RILs) and Tx430/SC1103 (192 RILs) were screened for underlying genetic mechanisms of tannin production. Composite interval mapping and meta-QTL mapping showed two major QTLs, on chromosome 2 (*Tan2*) and chromosome 4 (*Tan1*), contributing to 54 % of the total tannins. Synthesis of tannins is dictated by the presence of dominant functional alleles at both these loci and recessive homozygous loss-of-function mutations at either loci resulted in nontannin sorghums. Isolation of the *Tan1* gene (Sobic.004G280800.1 or Sb04g031730.1) from Tx430 revealed that it contain a deletion of G at position 580, causing a frame shift in the second WD40 repeat domain that makes a truncated protein lacking 55 amino acids at the C-terminal region. This nontannin-producing allele was designated *tan1-a*. In addition to *tan1-a*, a new nontannin allele, namely *tan1-b* was detected in BTx623 inbred and it possessed a 10-bp insertion resulting in a truncated protein. These nontannin alleles validate that the *Tan1* gene is responsible for tannin production in sorghum grain. Molecular markers generated from the *Tan1* allele can be used to breed selectively for increased tannin content and eliminate agronomically undesirable traits closely linked to sorghum tannin genes (Wu et al. 2012).

In addition to tannins, predominant sorghum seed proteins known as kafirins reduce protein digestibility. Given that kafirins constitute ~70 % of the total seed proteins, malnutrition occurs when sorghum is consumed as the primary source of protein. Kafirins, based on molecular weight and sequence, are categorized into four classes, α, β, γ, and δ. They are hoarded in the endoplasmic reticulum (ER) in spherical protein bodies (PBs) (Wu et al. 2013). In the PBs, β- and γ-kafirins are present in the peripheral PB region, whereas α- and δ-kafirins are located in the inner regions. As α-kafirins are the most abundant and the last digested proteins among different kafirins, they are believed to be responsible for low protein digestibility in sorghum.

Identification of a P721Q, a high digestibile high-lysine (*hdhl*) mutant exhibiting high protein digestibility in a DES-derived mutant population was indicated as a promising solution for mitigating the problem of sorghum protein digestibility. Wu et al. (2013) crossed a P721Q-*hdhl1* mutant with Tx430 to generate a segregating F_2 population and identify the causal mutation. Genetic studies with the obtained F_1 and F_2 progeny demonstrated the P721Q-*hdhl1* mutation to be dominant and maintained by a single locus. The *hdhl1* mutant was cloned using

the candidate-gene approach based on the maize *floury2* mutant with higher protein digestibility. Amplification of the *α-kafirin* gene (Sobic.005G186300) from genomic DNA and cDNA revealed a single nucleotide exchange causing Ala21Thr substitution in the peptide sequence. This amino acid Alanine is highly conserved and is required for cleaving the *hdhl1* signal peptide. Mutation at this site results in production of irregular PBs, and improves protein digestibility. Therefore, *hdhl1* and nontannin sorghum mutants can be used extensively as a major source of protein diet to eradicate malnutrition in the sub-Saharan Africa and Southeast Asian populations.

6 Brown Midrib Mutations Enhance Bioenergy Efficiency and Forage Digestibility

Lignin, cellulose, and hemicellulose are the major constituents of plant secondary cell walls. Lignin is largely composed of three subunits, namely, p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) monolignols (Wang et al. 2015). Lignin provides mechanical strength to plant cells; however, lignin reduces forage digestibility by ruminant livestock and saccharification efficiency of biomass when used for energy production. Therefore, sorghum mutants containing lower lignin content in secondary cell walls, characterized by their reddish-brown midrib and brown stems, were identified and broadly utilized to minimize the negative effects of lignin. In sorghum, 38 brown midrib (BMR) mutants were generated by chemical mutagenesis using DES and EMS (Gorthy et al. 2013). Based on an allelism test, sorghum brown midrib mutants were assigned to four independent groups designated *bmr2*, *bmr6*, *bmr12*, and *bmr19* (Saballos et al. 2008). Additionally, six new brown midrib loci were identified based on novel mutants identified in different EMS screens (Sattler et al. 2014).

In contrast to positional mapping as explained for *Sh1* and *Ma1* cloning, sorghum brown midrib mutant *bmr26*, an allele of *bmr12* and *bmr18* mutants, was cloned using the candidate-gene-based approach combined with chemical analysis of cell wall composition (Bout and Vermerris 2003). A spontaneous mutation in the sorghum inbred P898012 produced *bmr26*, the first BMR mutant cloned in sorghum. Near-isogenic lines (NILs) for the BMR mutants identified from chemical mutagenesis screens were derived using wild-type siblings from the same head rows where the mutant was identified. Cell wall composition of *bmr26* and its wild-type NIL was measured using pyrolysis-gas chromatography mass spectrometry (Py-GC-MS). The *bmr26* contained reduced levels of syringyl residues and less esterified p-coumaric acid. Similar to *bmr26*, syringyl residues were lower in *bmr12*, *bmr18*, and maize *bm3*. The maize *bm3* contained mutations in the caffeic acid O-methyltransferase (COMT) gene catalyzing the penultimate step in monolignol biosynthesis (Vignols et al. 1995). As mutations in maize COMT produced the BMR phenotype, COMT seemed to be the most likely candidate-gene-producing sorghum midrib phenotype. Sequencing of the COMT gene (Sobic. 007G047300) from *bmr12*, *bmr18*, and *bmr26* showed single nucleotide substitutions in all three alleles resulting in a premature termination codon, leading to the *bmr* phenotype.

Similar to *bmr12*, a *bmr6* mutant was identified using the candidate-gene approach by Sattler et al. (2014). Gas chromatography-mass spectrometry (GC-MS) analysis of stalks of *bmr6* mutants indicated a significant decrease in H-, G-, and S-lignin subunits by 4.8-, 7.3-, and 17.7-fold, respectively (Sattler et al. 2014). An increase of minor lignin subunits, S-indene and G-indene by 9.5- and 8.3-fold, respectively, was observed in *bmr6* mutants, indicating loss of function of the *cinnamyl alcohol dehydrogenase* (*CAD*) gene responsible for the *bmr6* phenotype. Characterization of *CAD* indicated its role in catalyzing the last step of monolignol biosynthesis (Saballos et al. 2009). Availability of the sorghum reference genome from BTx623 made it possible to search for *Arabidopsis AtCAD4* and *AtCAD5* orthologues in sorghum.

Two putative *CAD* genes were identified in sorghum, but only one *CAD* gene (Sobic.004G071000) contained a nucleotide substitution that created an early termination codon, resulting in a null allele of *bmr6* and producing brown midrib phenotype (Sattler et al. 2009). In addition to the DES-derived *bmr6* reference allele, Scully et al. (2016) identified six independent alleles of *bmr6* in the BTx623 TILLING population (Xin et al. 2008).

In addition to *bmr12* and *bmr6*, the *bmr2* gene was identified using the map-based cloning approach. Genetic mapping was conducted by Saballos et al. (2012) on an F_2 population obtained by crossing AMP11 containing a *bmr2*-ref allele with Theis using 15 polymorphic SSR markers. The study positioned *bmr2* on chromosome 4. Fine-mapping of *bmr2* was conducted using newly generated molecular markers on 70 and 20 homozygous F_2 and F_3 plants, respectively, which narrowed the region to a 262-kb interval encompassing 22 genes. Among these is Sb04g005210, an *Arabidopsis* orthologue of 4-coumarate:CoA ligase *4CL1*, *4CL2*, and *4CL3* genes involved in lignin biosynthesis, therefore, making it the most likely candidate gene conferring the BMR phenotype. Comparing *4CL* sequences of *bmr2*-ref and a second *bmr2* allele generated using EMS with the wild-type revealed Gly111Asp and Gly262Arg substitution, respectively (Saballos et al. 2012). This supports *4CL* (Sobic.004G062500) as the gene conferring the BMR phenotype of *bmr2*. The missense mutations seem likely to effect the protein folding and result in aberrations in an early step in monolignol biosynthesis.

Cloning of three sorghum *bmr* mutant genes/alleles and identification of several new alleles can aid in understanding the mechanistic basis of lower lignin content in sorghum. Identification of several *bmr* genes can enable precise gene stacking to develop sorghums with high fodder digestibility and increased biofuel efficiency. Given the ability to survive and flourish under poor nutrient conditions, severe heat and drought, and the presence of several *bmr* mutants, sorghum is emerging as a major alternative to commercial crops that cannot withstand low water and high temperatures.

7 Acyanogenic Accession Curtails Seedling Toxicity

Cyanogenic glucosides are a group of allelochemicals produced in more than 3000 plant species to protect against herbivores and insects. These chemicals pose a serious risk for human and animal health because of their ability to release toxic hydrogen cyanide (HCN) upon tissue damage. Sorghum seedlings contain dhurrin, tyrosine-derived cyanogenic glucoside, produced in highest concentrations at early stages of seedling germination and young leaves, and decreasing as plant age advances. Dhurrin levels tend to increase under severe drought and post-application of nitrogen fertilizer, thus rendering sorghum unsuitable for animal consumption (Busk and Møller 2002). Hence, nondhurrin-producing mutants of sorghums are very vital for sorghum forage production.

In sorghum, dhurrin biosynthesis involves three key genes: *CYP79A1*, *CYP71E1*, and UDP-glucosyltransferase (UGT85B1; Hansen et al. 2003). EMS mutagenesis was conducted to generate dhurrin-free mutants, as naturally existing nondhurrin mutants were not identified in wild or cultivated sorghum. Mutagenesis was performed using seed treatment of BTx623 inbred with 0.1–0.4 % EMS, and M_1 plants were self-pollinated to generate M_2 plants (Xin et al. 2008). TILLING, a reverse genetics approach, of 264 M_2 plants using CEL1 endonuclease resulted in 10 mutants with G/C–A/T substitutions in *CYP79A1* (Sobic.001G012300), a rate-limiting enzyme catalyzing the first step of dhurrin synthesis (Blomstedt et al. 2012). These nucleotide substitutions resulted in two silent mutations, one premature stop codon, and seven different single amino acid substitutions in *CYP79A1*. Dhurrin content of different *CYP79A1* alleles was measured using Feigl–Anger (FA) paper, a high-throughput colorimetric technique (Feigl and Anger 1966). Among different *CYP79A1* alleles generated, P414L substitution resulted in a completely acyanogenic mutant, designated *tcd1* (*totally cyanide deficient1*; Blomstedt et al. 2012). Additionally, TILLING produced an acyanogenic *tcd2* mutant containing nucleotide

C-T transition in *UGT85B1*, resulting in a Q149* premature stop codon. In contrast to *tcd1*, *tcd2* plants were stunted with less root growth and very low seed production (Blomstedt et al. 2016).

In addition to acyanogenic *tcd1* and *tcd2* mutants, a novel EMS-derived acyanogenic mutant was identified using FA paper. To identify the gene underlying the new acyanogenic mutant, the genome of a single acyanogenic mutant was short read sequenced using an Illumina HiScanSQ and aligned to the sorghum BTx623 reference genome (Krothapalli et al. 2013). Next-generation sequencing (NGS) yielded 20 million reads providing ~17× coverage of the mutant genome. Comparing the BTx623 reference genome with the acyanogenic mutant revealed that it contained 577 SNPs in the coding region of this mutant genome. Simultaneously, six known genes involved in dhurrin production (biosynthesis: *CYP79A1*, *CYP71E1*, and *UGT85B1*; catabolism: *dhurrinase1*, *dhurrinase2*, and alpha-hydroxynitrile lyase) were sequenced to identify the mutation. Only the *dhurrinase2* (Sobic.008G080400) gene contained a G-to-A SNP transition resulting in a premature stop codon and a nonfunctional enzyme (Krothapalli et al. 2013). This new mutant allele was designated *dhr2-1*. Cosegregation analysis was performed on an F_2 population generated by crossing BTx623 with *dhr2-1*, 25 homozygous *dhr2-1* mutants were identified using KASPar assay, and FA assays revealed the absence of HCN under tissue damage, confirming mutations in *dhr2-1* to be responsible for the acyanogenic phenotype.

8 Herbicide Tolerance

Weeds are one of the major limiting factors in sorghum production around the world; they cause ~50 % yield losses if not managed. Manual weeding is commonly practiced in developing countries for weed management. As manual removal of weeds is laborious, sorghum producers currently rely on pre-emergent herbicides that provide broad-spectrum control. Pre-emergence herbicides often fail under low rainfall or severe rainfall after herbicide application, thus creating complications in weed management. One alternative is using transgenic approaches to generate herbicide-resistant sorghum as observed in maize, soybean, and cotton. However, sorghum lacks this luxury because of its relatively small acreage. Therefore, naturally occurring sorghum mutants that are herbicide-tolerant are the only promising post-emergence weed management strategy in sorghum production.

In sorghum, few accessions of Johnsongrass (*Sorghum halepense*) and some biotypes Bol-15, Bol-45, and Bol-71 of sudangrass (*Sorghum X drummondii*) manifested resistance to ACCase (acetyl-coenzyme A (CoA) carboxylase) inhibitors, herbicides known to control weeds effectively. Genetic studies involving herbicide-resistant wild sorghum accessions crossed with Tx623 inbred revealed resistance to ACCase inhibitors to be conferred by a single gene with partial dominance (Kershner et al. 2012). The mode of operation of ACCase inhibitors involves binding to the carboxyl-transferase (CT) domain of the *ACCase* gene to cause metabolite leakage and rapid cell death (Délye 2005). Previous research with grass weed black-grass (*Alopecurus myosuroides*) showed that mutations in the CT domain of the chloroplastic *ACCase* resulted in herbicide tolerance to two ACCase inhibitors, aryloxyphenoxypropionates (APPs) and cyclohexanediones (CHD; Délye et al. 2005). These findings indicated that a similar mechanism might be in play for sorghum herbicide resistance. Cloning of the sorghum *ACCase* (Sobic.006G030100) gene from herbicide-resistant sorghum accessions revealed a single nucleotide substitution that resulted in Trp-2027-Cys conversion in the CT domain (Kershner et al. 2012), thus preventing binding of ACCase inhibitors to confer resistance to these herbicides in sorghum. Cloning of the ACCase gene enables precise marker-assisted introgression of this gene from wild sorghums into elite sorghum inbreds, and can be used effectively to manage weeds in sorghum production.

9 Conclusions

Mutations have been extensively used in sorghum improvement programs around the world. A classic example of such a breeding effort is the sorghum conversion program of the United States of America during the 1960s wherein dwarfing (*dw1–dw4*) and photoperiod-insensitive (*ma1–ma4*) mutations were incorporated into exotic germplasm by a series of backcrosses using conventional breeding. As a result, most of the sorghum cultivated throughout the United States contains dwarfing and photoperiod-insensitive genes, except the sorghums used for bioenergy. Although mutations have been used in breeding, cloning and understanding the genetic and molecular nature of mutations are needed for designing better strategies for trait enhancement. A classic example is the dwarfing gene *dw3* utilized extensively in sorghum breeding, although revertants have always been a problem in using this dwarfing gene. Cloning of *dw3* revealed that it contains an 882-bp duplication in exon5 that was making plants dwarf (Multani et al. 2003). Given that *dw3* was cloned and the nature of the mutation identified as a duplication event, unequal crossing over was predicted to be responsible for revertants. This knowledge helped in identifying new sources of mutations in *dw3*, a 6-bp deletion and absence of 882-bp duplication were identified in Tx2737 and no revertants were observed, thus mitigating the problem of revertants in sorghum fields.

Additionally, identifying the genetic basis of mutants enables designing molecular markers to enable precise breeding and avoid linkage drag or eliminate the possibility of losing beneficial alleles that would have otherwise been eliminated completely from the germplasm. Positional cloning of rice *dro1* emphasizes the need for precise molecular markers in mutation breeding. In rice, deep-rooting gene *dro1*, associated with root architecture and drought tolerance, was tightly linked with tall stature (Uga et al. 2013). In the process of incorporating dwarfing genes into rice, *dro1* got completely eliminated from the International Rice Research Institute (IRRI) germplasm as dwarfing genes were incorporated based on phenotypic selection through conventional breeding. Loss of beneficial traits can be reduced or eliminated completely with marker-assisted breeding. Similar to rice, genes that are agriculturally important may have been lost during the sorghum conversion program, thereby underscoring the importance of marker-assisted selection in mutation breeding. Developing molecular markers for desirable traits can accelerate sorghum breeding; simultaneously non-benefitical alleles impact grain yield and quality can be expunged from the sorghum germplasm.

Sorghum being an orphan crop, complete potential of this drought- and heat-tolerant crop of tropical origin has not been achieved as observed in the case of agronomically important crops such as maize and rice. Despite the availability of enormous genetic diversity, only 5 % of the genetic variation is being used in sorghum breeding. Some of the most likely reasons include photoperiod-sensitivity, absence of a reference genome till 2009, dearth of sorghum transformation technologies, very limited funding for sorghum research, and little knowledge of the genes underlying different traits of interest. However, recent advances in the cloning of sorghum mutants such as *Sh1*, *Tan1*, *br*, *bmr, ma*, and *dhr2-1*, and the availability of two independent TILLING populations generated by Dr. Mitch Tuinstra at Purdue University (West Lafayette, IN) and by Dr. Zhanguo Xin at USDA-ARS (Lubbock, TX), marked a new era in the field of sorghum genetic improvement. Additionally, sorghum cultivation has gained immense popularity over the past decade due to its profound ability to withstand severe heat and drought environments and grow in soils with poor nutrients. Given the new resources available and ability of sorghum to survive harsh abiotic stresses, sorghum seems most likely the crop to entrust for achieving global food security. Hence, identification of new mutants for biotic and abiotic stresses combined with marker-assisted breeding is required to alleviate world hunger. By identifying new mutations underlying agronomically important traits can assist in breeding hardier crops to increase crop productivity to

meet global food requirements, and much-needed sources of clean fuel in a sustainable manner.

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TILLING and EcoTILLING for Discovery of Induced and Natural Variations in Sorghum Genome

14

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Abstract

TILLING is a reverse-genetic method to uncover the induced variation from the mutagenized population. Initially induced variation was detected by melting and reannealing the PCR products, cleavage of heteroduplex using CEL I endonucleases, and the resulting products separated and visualized on sequencing gels or capillaries. Subsequent sequence analysis in heteroduplex regions of individual plant DNA identified the mutation. In the past 6 years, mutation discovery through sequencing is becoming popular. Sequencing-based TILLING is less labor intensive with high specificity and confidence of mutation discovery. TILLING by sequencing has been reported in many crop plants including rice and wheat and is yet to be applied in sorghum. A conventional TILLING project has been reported in sorghum for agronomically important traits and also for the development of acyanogenic or low cyanogenic mutants in sorghum. EcoTILLING is another reverse-genetic approach that utilizes the same principles in the natural population instead of the mutagenized population. In this chapter, we discuss TILLING and EcoTILLING in sorghum and

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also TILLING by sequencing for the improvement of sorghum through the reverse-genetic approach.

1 Introduction

Genetic variability is the prerequisite for crop improvement, which forms the basis for selection and recombination breeding that aims at isolation of superior genotypes. Most genetic variability available today in plant collections is the result of past evolution involving spontaneous mutations, recombination, and exposure to the forces of natural selection. There are indications that a major part of domestication of crop plants occurred in discrete steps mediated by single gene mutations (Gottlieb 1984). In many cases, a cultivated species and its primitive ancestors are often reported to be differing in only a few major genes.

Because of the increased recognition of its importance, evaluation and characterization of sorghum germplasm have received the attention of plant breeders. Utilization of exotic and genetically diverse germplasm is needed to develop stable and high-yielding cultivars with a broad genetic base. This can be achieved through isolation of induced or natural allelic variation for any desired traits for which two genetic approaches are popular; one approach directly screens for the phenotype of a large number of germplasm accessions (forward genetics). This approach requires a high-throughput and rapid protocol that can identify variants for the trait under study, followed by a more precise but inevitably slower molecular characterization (Brady and Provart 2007). The main advantage of this approach is that it makes no assumption about the primary cause. Consequently, genes influencing the trait have to be identified. In the second approach, if genes influencing the trait (candidate genes) have been identified, one can screen for alteration in the gene under consideration and then characterize those lines carrying gene-level variations for the trait of interest (reverse genetics) (Brady and Provart 2007).

The first approach is not effective to screen a large number of natural or even artificial mutations. The major limitation of forward genetics is that phenotyping of a large collection is difficult and time-consuming, too insensitive for small targets and rare mutations which are usually missed, and that it demands huge land and financial resources. In contrast, many effective reverse-genetic approaches have come into existence recently but most of the approaches tend to be organism-specific. The routine reverse-genetic approaches used in microbes such as homologous recombination prove to be less efficient when applied to plants (Tissier and Bourgeois 2001; Henikoff and Comai 2003). Random insertion mutagenesis using either T-DNA or transposons has been successfully used in *Arabidopsis thaliana* to assemble mutant libraries, which cover the vast majority of its genes (http://signal.salk.edu). Silencing genes of interest using RNA-interference (RNAi) transgenesis has also become a popular reverse-genetic tool in a few plant species (Mansoor et al. 2006). Many of the currently used reverse-genetic approaches (RNAi, gene knockout, site-directed mutagenesis, transposon tagging) rely on the creation of transgenic material, the development of which is seldom feasible for many plant species due to their recalcitrance for in vitro transformation procedures and also application of these methods involves various ethical and policy issues (Barkley and Wang 2008).

Recently, a new reverse-genetic method called targeting induced local lesions in genomes (TILLING) has been developed to take advantage of new DNA sequence information and to investigate the functions of specific genes (McCallum et al. 2000; Comai and Henikoff 2006). TILLING also shows promise as a non-transgenic tool to improve domesticated crops by

introducing and identifying novel genetic variation in genes that affect key traits. TILLING was first developed in *Arabidopsis* (McCallum et al. 2000) and has been successfully applied to identify knockout mutations and provide allelic mutations in target genes from animals and plants (Barkley and Wang 2008). At present the application of TILLING in many plant species is well established. The technique has proved to be valuable in characterizing the function of many target genes in *Arabidopsis* (Greene et al. 2003).

EcoTILLING is a method that uses TILLING techniques to look for natural mutations in individuals, usually for population genetics analysis which was first reported in *Arabidopsis* (Comai et al. 2004) and subsequently used to detect natural allelic variation and the putative gene functions of agriculturally important crops (Gilchrist and Haughn 2005). This approach allows one rapidly to screen through many samples (germplasm) with a gene of interest to identify naturally occurring single nucleotide polymorphisms (SNPs) and/or small insertion-deletion (indel) variations.

The powers of TILLING and EcoTILLING in discovering new traits have been clearly demonstrated by various studies, namely, acyanogenic mutant in sorghum (Blomstedt et al. 2012), high Brix tomato (Bauchet 2002), reduced seed erucic acid content in rapeseed (Wang et al. 2008), and reduced allergen in peanut (Knoll et al. 2009). TILLING approaches have also been successfully employed in isolation of mutants in the starch biosynthetic pathway (Stemple 2004; Slade 2012). Reports of waxy mutant in wheat (Stemple 2004) and high amylose mutant in wheat and maize (Slade 2012) are good examples of effectiveness of reverse-genetic techniques in trait discovery.

The TILLING process involves PCR amplification of pooled DNA (mutant and wild-type) in the presence of fluorescently labeled primers. Mismatched heteroduplexes are generated between wild-type and mutant DNA by melting and reannealing the PCR products (Graham et al. 2005). Heteroduplexes are incubated with the endonuclease CEL I that cleaves mismatched heteroduplex sites, and the resulting products are separated and visualized on sequencing gels or capillaries. Subsequent sequence analysis in heteroduplex regions of individual plant DNAs identifies the mutation. Conventionally, this is carried out in a Li-COR Sequencer. However, Li-COR-based heteroduplex analysis in detecting mutations in a high-throughput fashion involves high cost and is a cumbersome process (Tsai et al. 2011). Hence, to overcome the limitations, TILLING by Li-COR gels has been recently replaced by high-throughput TILLING via rapid-sequencing methods (Tsai et al. 2011).

TILLING and EcoTILLING platforms have been established for a variety of plants including important cereal crops such as maize (Till et al. 2003), wheat (Slade and Knauf 2005), and rice (Till et al. 2007). This chapter presents the current status and promising prospects of TILLING and EcoTILLING with special reference to their applications in sorghum.

2 Status of Functional Genomics in Sorghum

For any breeding program, identification of genes and their function is a prerequisite. In spite of many recent advances in omics studies, this still remains a major challenge in crop plants including sorghum. The available reverse-genetic approaches, T-DNA and transposan tagging, are still not very successful in sorghum as the gene transformation protocols are still inefficient and in addition there are no viable transposan-tagged populations reported thus far with identified active transposable elements (Chopra et al. 1999; Carvalho et al. 2005). Another reverse-genetic approach, the RNAi- mediated gene knockout/knockdown tool, has been employed in many crop plants but is yet to be applied in sorghum.

As with many other cereals, sorghum is also recalcitrant to tissue culture and consequent genetic transformation. Transformation efficiency in sorghum is very low and highly genotype-specific. Apart from these factors, horizontal dispersal of transgenes to the numerous wild and related species of sorghum is also a serious concern especially in the context of this crop being used as a food and

animal feed. Ethical issues and public perception on genetically modified (GM) crops further complicates acceptability of GM sorghum (Kresovich et al. 1987; Zhu et al. 1998). Sorghum being less important as a cereal crop, GM research is of lesser priority as compared to principal cereals such as rice, wheat, maize, and barley.

Very few researchers have reported successful sorghum transformation-based genetic improvement in comparison with other cereal crops (Masteller and Holden 1970; Cai et al. 1987; Ma and Liang 1987; Cai and Butler 1990; Casas et al. 1993; Kaeppler and Pederson 1997; Zhu et al. 1998; Zhao et al. 2000, 2003; Able et al. 2001; Emani et al. 2002; Tadesse et al. 2003; Gao et al. 2005). This clearly indicates the technical barrier in the use of genetic transformation as a method of crop improvement in sorghum which is discussed in detail in Chap. 12
.

With the advent of high-throughput sequencing using next-generation sequencing platforms, sequencing has become faster and cheaper. The relatively small genome size of sorghum (~736 Mb), existence of high genetic diversity, and the diploid nature of its genome and minimal level of gene duplication facilitate functional assignment in sorghum more effectively than many related cereals. These qualities further make sorghum a model system for C_4 crops including maize, pearl millet, and sugarcane. The sorghum draft sequence (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Sbicolor_er) of cultivar BTx623 serves as an important resource for the identification and isolation of novel and superior alleles of agronomically important genes from sorghum gene pools to deploy them suitably towards development of improved cultivars.

3 TILLING for Agronomic Traits

The first TILLING resource for sorghum was created by Xin et al. (2008) through ethylmethane sulfonate (EMS) mutagenesis of the BTx623 inbred line. They generated 1600 mutant lines with numerous phenotypes with altered agronomic and morphological traits in the M_2 and M_3 population. From 1600 mutants, a set of 768 mutant lines was used in an eightfold pooling strategy in 12 × 8 well formats for TILLING. There were a total of five mutations with a mutation density of 1/526 kb in four candidate genes: 1-aminocyclopropane-1-carboxylate oxidase (*ACO1*), caffeic acid O-methyltransferase (*COMT*), myoinositol kinase 1 (*MIK1*), and phytochrome A (*PHYA*). In this TILLING program two promising mutant lines P5A3 and P7H6 were identified in the target gene *COMT* with missense mutations at different positions of exon 2 that are associated with *bm* or *bmr* mutations in maize and sorghum, respectively. These mutants are characterized by a brown midrib in the leaves with reduced lignin content and increased digestibility. The sorghum mutant populations developed in the study have already been distributed to a number of sorghum researchers, and are serving as a valuable resource to isolate mutants for many other traits.

A combined biochemical screening and a TILLING approach was used by Blomstedt et al. (2012) to screen acyanogenic or low HCNp lines as well as mutant lines that accumulate high HCNp in mature stages. They generated 4200 M_2 plants and subsequently screened the mutants for their inability to produce HCN through Feigl–Anger assay (Blomstedt et al. 2012). Among 4200 M_2 screened, 264 were found to be putative mutants. Isolated putative mutants were screened for mutations in the key candidate genes in dhurrin biosynthesis, two cytochrome P450 s (*CYP79A1* and *CYP71E1*) and one UDP-glucosyltransferase (*UGT85B1*). The mutant line P414L with mutation in *CYP79A1* was found to be acyanogenic and the mutation in this line was in homozygous condition. The mutant showed a normal phenotype for all morphological features except for its slow growth at the early emergence of seedlings. These TILLING resources are available in the public domain for further trait-based crop improvement through the TILLING program.

4 EcoTILLING in Sorghum

EcoTILLING is a molecular technique developed with an objective to uncover natural genetic variation as opposed to induced mutations. EcoTILLING can aid in the discovery of natural variants and their putative gene function (Gilchrist and Haughn 2005). This approach allows one rapidly to screen through many samples with a gene of interest to identify naturally occurring SNPs and/or small indels.

A large magnitude of genetic variability is available in sorghum in which many of them were classified, characterized, and evaluated even though there are major gaps specifically for multigenic traits (Rosenow and Dahlberg 2000). ICRISAT maintains 36,774 accessions from 90 different countries which represent about 80 % of the variability present in sorghum (Eberhart et al. 1997). Most of the collections originated from developing countries in the semi-arid tropics. For example, 60 % of the germplasm with ICRISAT originated from India, Ethiopia, Sudan, Cameroon, Swaziland, and Yemen, the largest collection being from India. Upadhyaya et al. (2009) developed a sorghum mini-core collection consisting of 242 accessions that is available for further characterization through TILLING and EcoTILLING.

The USDA together with the Texas Agricultural Experimental Station maintains 42,221 germplasm accessions of sorghum as a part of the National Plant Germplasm System (NPGS; Dahlberg and Spinks 1995). A core collection of >200 accessions from the base collection of 42,221 have been developed at the USDA in Mayagüez, Puerto Rico. These collections represent genes for various agronomically important traits including maturity, drought resistance, plant height, pericarp color, and green bug, aphid, and downy mildew resistance (Dahlberg and Spinks 1995; Sharma et al. 2009).

The extensive examination of genetic diversity in sorghum germplasms and core collections based on origin, race, photoperiod sensitivity, seed quality, agronomic traits, and molecular markers has been established in sorghum. However, this variation has been poorly exploited in terms of crop improvement (Anas and Yoshida 2004; Abu Assar et al. 2005; Deu et al. 2006; Kayode et al. 2006).

An ecoallelic variation in 971 sorghum accessions was studied using genotyping by sequencing (GBS) by Morris et al. (2013). They identified 265,487 SNPs in sorghum germplasm. This genome-wide SNP variation in sorghum provides a basis for crop improvement. TILLING and/or EcoTILLING techniques will be a viable option for the exploitation of sorghum variability in terms of crop improvement which is successfully demonstrated for the isolation of acyanogenic sorghum.

5 Other Mutant Resources in Sorghum

Xin et al. (2009) developed 1600 M_3 annotated pedigreed mutagenized populations using EMS for sorghum genotype BTx623 and their preliminary characterization was carried out for various traits. They also characterized individual M_3 rows for multiple mutant phenotypes. These resources can also be utilized for further exploitation through reverse genetics. Subsequently, they developed 6144 M_4 annotated individually pedigreed mutated sorghum (AIMS) through the single-seed descent method. These mutant lines contain biologically important and agronomically viable mutants, such as brown midrib (*bmr*) mutants for improved biomass digestibility and ethanol production and erect leaf (*erl*) mutants for improved capture of canopy temperature and biomass yield. A set of useful mutations harboring potential mutations for dwarfness, early maturity, high lysine, and high protein digestibility have also been identified earlier.

6 TILLING and EcoTILLING by Sequencing

The discovery of mutations in agriculturally important crop plants is critical which is also important for further crop improvement programs. The conventional TILLING procedure

consists of mutagenesis, DNA isolation and pooling, and mutation discovery by any one of the methods, namely melting and reannealing the PCR products, cleavage of heteroduplex using CEL I endonucleases, and the resulting products separated and visualized on sequencing gels or capillaries. Subsequent sequence analysis in heteroduplex regions of individual plant DNA identifies the mutation. Recent development in high-throughput sequencing replaces all the labor-intensive and less efficient mutation discovery methods in the conventional TILLING program which is otherwise called TILLING by sequencing.

6.1 Methods and Protocols

Here, we present a protocol for TILLING or EcoTILLING by sequencing protocol that has been successfully employed in our laboratory for isolation of mutations in starch biosynthetic enzymes for altered starch composition in rice and flowering control genes for optimal partitioning of the vegetative and reproductive phases, for increasing number of branches, and synchronized maturity of mungbean for higher yield (publications under preparation). The general workflow of TILLING or EcoTILLING by sequencing is presented in Fig. 1.

6.1.1 Creation of Mutant/Germplasm Panels for TILLING/EcoTILLING

For TILLING and EcoTILLING, a population of mutants/germplasm panel is required. The numbers of mutants/germplasm accessions are fixed as per the wet lab capacity to fit in high-throughput format generally in multiples of 96 samples to fit in the microplates. The generation of mutants and formation of germplasm panels is similar to that of any classical TILLING/EcoTILLING work flow.

6.1.2 DNA Isolation and Quantification

The genomic DNA is commonly extracted using a DNeasy 96 Plant kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Assessment of DNA concentration is very important for normalization as well as optimization of PCR reaction. The DNA concentrations are measured with Tecan Nano Quant Infinite m200 pro or checked by using 1.5 % agarose gel electrophoresis by comparing with uncut λ DNA. Measured DNA is subsequently normalized to 5 ng/μl. The normalized DNA is used for further downstream applications.

6.1.3 Pooling and Super Pooling

The extracted genomic DNA from 768 individual samples is transferred to eight numbers of 2 ml deep-well plate in 12 × 8 format (row × column). From the 8 numbers of 1x DNA (96-well) plates, one 8x pooled plate is prepared from the 768 germplasm lines. The 8x pooled plate is further pooled across the columns (12) and rows (8) to prepare a superpool (pool of pool) plate. DNA from the 20 wells of the superpool plate is used for amplification of target genes.

6.1.4 Selection of Candidate Genes for TILLING or EcoTILLING

The candidate genes for TILLING/EcoTILLING are generally selected based on the specific trait to be improved. Key candidate genes whose role in governing a particular trait is well established or genes controlling rate-limiting enzymes of the biosynthetic pathways are usually chosen for the TILLING and EcoTILLING by sequencing program.

6.1.5 Construction of Gene Model for Key Candidate Genes

The gene models are built based on blasting protein and EST sequences of target genes with the genomic sequences using different bioinformatics pipelines such as CODDLE (codons optimized to discover deleterious lesions; http://www.proweb.org/input/) or Genewise (http://cbs.ym.edu.tw/services/genewise/).

6.1.6 Designing Primers to Capture TILLING/EcoTILLING Fragments of Target Key Genes

The TILLING or EcoTILLING fragments of candidate genes with maximum mutation

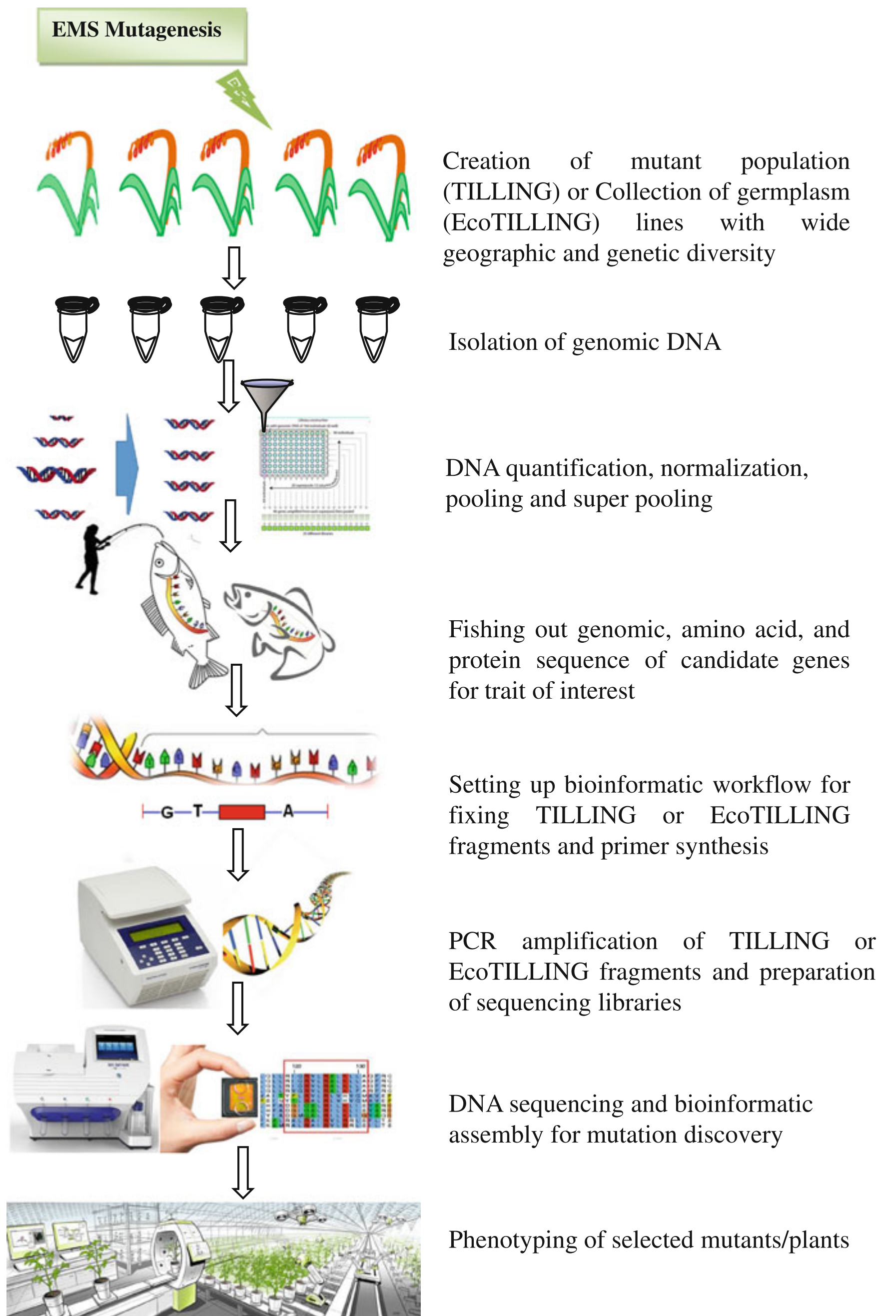

Fig. 1 The general workflow of TILLING or EcoTILLING by sequencing

probability for PCR amplification were usually fixed using the CODDLE (http://bioinfo.ut.ee/primer3/pipeline). Primers for TILLING or EcoTILLING fragments for target genes are designed with PRIMER 3 software embedded with CODDLE. For high robustness in PCR amplification, the primers were developed to meet the following criteria: (i) the amplified fragment must be genome-specific; and (ii) 3′ end complementarity and self-complementarity value of the primers should be below 3 and 8, respectively, to reduce the mispriming.

6.1.7 PCR for Amplification of TILLING or EcoTILLING Fragments

The long-range PCR is carried out in a reaction volume of 50 µl containing 10 µl of 5x longAMP Taq Reaction buffer (New England Biolabs), 1.5 µl of dNTPs (10 mM), 2 µl of each forward and reverse primer (10 µM), 2 µl of DMSO, 5 µl of pooled DNA (50 ng/µl), 2 µl of LongAmp® Taq polymerase (5 unit), and 25.5 µl sterile water. The following thermal cycling condition is followed: 94 °C for 2 min/30 cycles of 94 °C for 1 min, 60 °C for 50 s, 65 °C for 1 min/kb, with a final extension of 65 °C for 10 min. Amplified PCR products are analyzed in 1 % agarose gel for successful amplification.

6.1.8 Equimolar Pooling of PCR Products

The concentration of all the PCR products is measured individually by using Qubit (Applied Biosystems, Inc.) as per the manufacturer's protocol. To obtain final equimolar pooling of PCR products for all the amplified superpools for all the TILLING or EcoTILLLING fragments, the concentration of the individual amplified superpools is normalized to 20 ng/µl. For equimolar pooling across the gene fragments, calculations are made in consideration of the fact that larger amplicons need a greater number of copies than smaller fragments. The final PCR pool from an individual amplified superpool is prepared with the objective of having the final concentration of 2.0 µg of long amplicons for sequencing.

6.2 Next-Generation Sequencing of Pooled TILLING or EcoTILLING Libraries

6.2.1 Library Preparation

Library preparation is generally carried out using the Ion Xpress™ Fragment Library Kit, with 100 ng of DNA. Adapter ligation, size selection, nick repair, and amplification are performed as per manufacturer's instructions (Ion Xpress™ Fragment Library Kit—Part Number 4469142 Rev. B). Size selection is executed using the Lab Chip XT (Caliper Life Sciences, PerkinElmer) and the Lab Chip XT DNA 750 Assay Kit (Caliper Life Sciences), with collection between 175 bp and 220 bp. The Agilent 2100 Bioanalyzer (Agilent Technologies) and the related high-sensitivity DNA kit (Agilent Technologies) are used to determine quality and concentration of the libraries. Emulsion PCR and enrichment steps are carried out using the Ion Xpress™ Template Kit and associated protocol (Part Number 4469004 Rev. B).

6.2.2 Sequencing of TILLING or EcoTILLING Libraries

Individual libraries are barcoded and sequencing is carried out using any one of the next-generation sequencing platforms with various data output.

6.2.3 SNP Calling and Data Analysis

Filtering, trimming, and aligning of sequence TILLING or EcoTILLING fragments reference sequences with the specified coordinates, extracted form Genbank, are used with Torrent Suite 1.5. A variant caller is used for filtering the SNP from sequenced contigs. The parameters such as min-max distance, mismatch cost, length fraction, and similarity are selected in order to minimize reads and alignment ambiguities as well to detect rare SNPs. The minimum variant frequency and minimum coverage are set to 0.5 and 20, respectively, which gives variations on or above 0.5 %. These variations are considered SNPs.

6.2.4 Functional Validation of SNPs Through Sorting Tolerant from Intolerant (SIFT) Analysis

Discovered sequence variants are analyzed by the PARSESNP program (http://www.proweb.org/parsesnp/), which provides information on the location along with the details about amino acid changes and location of the amino acid changes and severity of mutations and provides information on the creation or loss of restriction sites caused by the induced polymorphisms. Addition, deletion, and substitution of each amino acid have the potential effect on protein function (Sim et al. 2012). SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html.) is a Web-based tool that predicts whether amino acid substitution affects protein function and structure based on sequence homology and the physical properties of amino acids (Ng and Henikoff 2003). The predicted SIFT score ranges from 0 to 1. The amino acid substitution is predicted as damaging if the score is <0.05, and tolerated if the score is >0.05.

6.2.5 Comseq Technique to Identify Mutation Through TILLING Approach

This approach combines the celebrated mathematical model of compressed sensing with next-generation sequencing as reported by Nida et al. (2016) in sorghum. This computational approach is used to identify de novo SNPs in a large panel of sorghum TILLING population. This approach is a cost-effective and highly robust tool for the identification of novel SNPs and subsequent utilization in crop improvement programs.

7 Conclusion

TILLING and EcoTILLING as reverse-genetic tools are high-throughput mutation detection systems that are very useful for mining non-transgenic allelic variations in crop genomes. These techniques have demonstrated high efficiency in the discovery of desirable mutants and germplasm resources. These tools are also extremely adoptable by plant breeders. The flexibility of the mutation and natural variant discovery system has allowed the technology to become widely applicable in many crop plants, including sorghum, rice, and wheat since its first development in *Arabidopsis* a decade ago. Subsequently, there is also growing interest in applying the technique in sorghum breeding programs that need trait-specific improvement. Unlike rice, wheat, and maize, a massive TILLING program is yet to be initiated in sorghum. The results from some preliminary studies have discovered promising mutants with acyanogenic or low HCN, brown midrib (*bmr*) with reduced lignin content and increased digestibility traits. This should encourage sorghum breeders to set up global TILLING and EcoTILLING programs in the near future.

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15 Sorghum-Associated Bacterial Communities—Genomics and Research Perspectives

Mandira Kochar and Pushplata Singh

Abstract

Sorghum is known to be intolerant to soil acidity and grows best in the pH range of 5.7–6.4. Consequently, its associations with metabolically versatile plant growth promoting bacterial strains in the rhizosphere are likely to be helpful as they will be able to support the growth of the host plant even under varying rhizospheric conditions by improving root growth. The mechanism of the phytohormones crosstalking with each other and integration of the signaling pathways have been deciphered to a large extent in plants, yet the interrelationship between the bacterial plant growth regulators has not yet been unraveled. In-depth understanding of sorghum genomics is necessary for improving sorghum composition and agronomic behavior. Along with this, innovative strategies and genomics and transcriptomics studies can be designed to understand the microbial communities associated with sorghum. These offer an environmentally sustainable approach to increase sorghum production and health.

1 Introduction

Improving productivity is the biggest challenge before agricultural scientists, and this is more challenging under the harsh conditions where crops such as sorghum are grown. Endogenous or genetically modified plant rhizosphere-associated bacteria can significantly enhance plant productivity (e.g., Berg 2009; Compant et al. 2005; Glick 2012; Schenk et al. 2012) as such strains have specific mechanisms to benefit plant hosts either directly or indirectly. These predominantly include rhizosphere bacteria that can utilize the nutrients secreted by the plants in the form of root exudates consisting of carbon and energy sources (Dobbelaere et al. 2003; Kochar et al. 2013). The rhizosphere environment is created by the release of carbon-rich root exudates, which act as nutrients for microbes making it a "hot-spot" for microbial growth (Morgan and White 2005; Hartmann et al. 2009). Endophytic environments correspond to

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microenvironments localized inside plant organs and tissues (Saito et al. 2007). In such environments, bacteria have been shown to have neutral, detrimental, or beneficial effects on plants (for reviews, see Berg 2009; Schenk et al. 2012). Successful colonization is determined by the bacterium's ability to maintain and grow on or around the root, thereby becoming rhizosphere competent. In order to successfully establish an association with the host plant, an active microbial population needs to recognize and utilize nutrients, signals, and toxins (Simons et al. 1996). In addition, studies have shown the importance of environmental factors in a rhizosphere bacteria–host plant relationship (Bashan 1998; Kochar et al. 2013; Bashan et al. 2013; Koul et al. 2015a).

2 Sorghum Rhizosphere: Allelopathic Root Exudates and Rhizospheric Bacteria

It is well established that microflora in the rhizosphere interact with the crop plants leading to synergistic reactions. Sorghum-associated bacteria have been isolated and identified in only a few studies (Budi et al. 1999a, b; Zinniel et al. 2002). Some of the identified bacteria such as *Klebsiella pneumoniae, Enterobacter cloacae,* and *Erwinia herbicola* exhibit plant growth promoting (PGP) traits such as nitrogen-fixation (Pedersen et al. 1978), whereas others, *Paenibacillus* sp. strain B2 and *Pseudomonas* spp., exhibit biocontrol capabilities (Budi et al. 1999a, b; Funnel-Harris et al. 2013). Interestingly, 13 definite and constant operational taxonomic units (OTUs) were observed in the rhizosphere and rhizoplane of sorghum grown in South Africa. These are *Acetobacter*, *Azospirillum*, *Bradyrhizobium*, and the cyanobacterial species, *Synechococcus* (Ramond et al. 2013). Furthermore, denaturing gradient gel electrophoresis (DGGE) analysis also identified the ubiquitous presence of cyanobacterial strains *Nostoc* sp., *Synechococcus* from the root, and *Pantoea* sp. from the shoot samples of sorghum. *Escherichia* sp. and *Vibrio* sp. were identified along with other uncultured bacteria in rhizosphere samples, and *Bacillus* sp. was the major bacterial taxa identified using DGGE in sorghum rhizoplane samples (Ramond et al. 2013).

Sorghum shows higher bacterial diversity in its rhizosphere and rhizoplane than endophytic diversity on the root, shoot, and stem (Ramond et al. 2013). This is due to different nutrient availabilities in the rhizosphere as a result of exudation of carbon- and nutrient-rich compounds by the sorghum roots. Exudates produced by different sorghum accessions vary in their chemical composition with sorgoleone as the primary constituent, and 5-ethoxysorgoleone and 2, 5-dimethoxysorgoleone being the other major components (Czarnota et al. 2003). Sorghum is allelopathic as it represses the growth of weeds and injures subsequently grown crops. This allelopathic potential is a result of the sorgoleone and a resorcinol analogue present in the root exudate (Netzly et al. 1988; Erickson et al. 2001; Czarnota et al. 2003; Dayan et al. 2003).

The sorghum rhizospheric and endophytic cyano/bacterial taxa (*Acetobacter* sp., *Azospirillum* sp., *Bradyrhizobium* sp., *Pantoea* sp., *Bacillus* sp., and *Synechococcus* sp.) include strains with known PGP traits, especially the diazotrophic nature and production of phytohormes (Lugtenberg et al. 1991; Bai et al. 2002; Kevin Vessey 2003; Loiret et al. 2004; Coelho et al. 2008; Terakado-Tonooka et al. 2008; Franche et al. 2009; Rout and Chrzanowski 2009; Saharan and Nehra 2011). These are ideal targets for genetic engineering for upregulation of PGP traits and improved root behavior (Malhotra and Srivastava 2006). The PGP diazotrophic bacteria provide additional N to plants (Kevin Vessey 2003; Berg 2009; Franche et al. 2009), contributing to the elevated N-uptake capacity of sorghum roots. The microbial *nifH* gene has been used as a reference gene to study rhizospheric and endophytic microbial diversity as well as in metagenomic studies to identify the diazotrophic communities present in sorghum and to confirm their role in providing bioavailable N to sorghum (Coelho et al. 2008, 2009a, b). Marked shifts in diazotrophic bacterial communities have been observed in rhizosphere versus nonrhizosphere soils, which is influenced by

cultivar type and amount of fertilizer used (low N vs. high N) as observed in the case of cultivar BRS 308 versus cultivar BRS 310 (Coelho et al. 2009a, b). Studies that show the effect of high or low N on diazotrophic microbial communities in natural soils are important and are a future research scope.

Various bacterial species that have been identified to be associated with sorghum are highlighted in Table 1. Interestingly, some studies also suggest microbial endosymbionts contribute significantly to *Sorghum halepense* invasions by augmenting the plant biomass, growth rate, and competitive effects, as well as herbivore defense (Rout 2011). This work shows that these invasive plant traits are microbially mediated and this novel invasion strategy is termed microbially enhanced competitive ability (MECA), wherein microbial associations significantly contribute to plant traits that are involved in or affected by invasion (Rout et al. 2013). Strong correlations between sorghum-associated bacterial community assemblages and geographical origin have been reported recently for rhizospheric samples (Ramond et al. 2013). Studies also point to the fact that soil structure and composition play a significant role in determining the sorghum-associated rhizospheric microbial communities rather than the sorghum plant itself (Hinsinger et al. 2009).

3 Nitrification Inhibition: Sorghum–Microbe Interaction

Nitrification, denitrification, ammonification, organic matter mineralization/immobilization, and nitrogen fixation are the key processes involved in N_2 cycling in soils. Nitrogen (N_2) present in the atmosphere is generally unusable by plants. To assimilate this as a nutrient, the nitrogenase enzyme present in nitrogen-fixing microbes catalyzes the conversion of N_2 to ammonia (NH_3). The transformation of organic nitrogen to the ammonia (NH_3) and ammonium (NH_4^+) forms or ammonification is followed by transformation of NH_3 to the nitrate form (NO_3^-) by nitrification (carried out by nitrifying bacteria, *Nitrosomonas* and *Nitrobacter*). A form of respiration, denitrification is carried out by microorganisms under low oxygen conditions wherein specific bacteria use NO_3^- to carry out their metabolic functions. Herein, NO_3^- is reduced to NO_2^- and N_2O (nitrous oxide)/N_2. N_2O is a potent greenhouse agent, an ozone-depleting catalyst, and presents a serious problem due to its long atmospheric lifetime of 114 years (Ravishankara et al. 2009).

Nitrification is one of the main causes of inefficient N use and N loss-associated environmental pollution as a result of leaching and denitrification. Nitrification–denitrification by bacteria has been established as an enzymatic process with nitric oxide (NO) being an obligatory intermediate (Molina-Favero et al. 2007) and is the principal agricultural source of N_2O emissions. The N_2O levels in the atmosphere have been increasing at an alarming rate and are expected to increase further unless remedial measures are taken to reduce these emissions (Galloway et al. 2008; Schlesinger 2009). In undisturbed ecosystems, ~10 % of the total N undergoes nitrification, whereas in modern agricultural systems ~95 % of total N streams through nitrification–denitrification (Subbarao et al. 2015). These modern systems are accountable for low-N recovery and use efficiency, have disrupted the N_2 cycling in nature, reduced soil organic matter, and altered soil properties along with its microbial activity (Dinnes et al. 2002). As a result high-nitrifying soil environments are created where NO_3^- accounts for ~95 % crop N uptake (Russell et al. 2009; Subbarao et al. 2013). Hence, the soil microbial biomass has been severely deteriorated in modern agricultural systems, leading to decoupling/discrepancies between N mineralization and plant N demand (Perveen et al. 2014).

In this scenario if nitrification is repressed, N recovery and its crop uptake can be improved, leading to reduced N_2O emissions (Subbarao et al. 2006a; Zakir et al. 2008). This can be achieved by the release of nitrification inhibitors (biological nitrification inhibition or BNI) from plant roots or the application of artificial inhibitors (allylthiourea, AT; nitrapyrin and

Table 1 Bacterial associations with sorghum

Ecological niche	Cultured/closest cultured match	Taxonomic group and known traits of closest match if available	References
Sorghum root	Various cyanobacterial 16S rRNA gene, including *Synechococcus* sp. clone R4CP3R1F09 [HQ018568.1]	Cyanobacteria	Ramond et al. (2013)
Sorghum shoot	Enterobacteriaceae, including *Pantoea agglomerans* strains CECRI-IOC29 IGCAR-17/07 and IGCAR-18/07; *Pantoea dispersa* strain BH103 and *Pantoea ananatis* strain JB1/KB-10511	γ-Proteobacteria	Ramond et al. (2013)
Sorghum rhizosphere	*Escherichia coli* strain G4M80 [GU646119.1]	γ-Proteobacteria	Ramond et al. (2013)
	Escherichia fergusonii strain BAN86 [JX415362.1]	γ-Proteobacteria	Ramond et al. (2013)
	γ-Proteobacteria, including *Vibrio sp. U32* [AY864627.1]	γ-Proteobacteria	Ramond et al. (2013)
	Paenibacillus sp.	Firmicutes-Bacillales	Coelho et al. (2008)
	Mesorhizobium loti AB367742.1	α-Rhizobiales	Coelho et al. (2009a, b)
	Azohydromonas australica AB188121.1	β-Burkholderiales	Coelho et al. (2009a, b)
	Klebsiella pneumoniae AY242355.1	γ-Enterobacteriales	Coelho et al. (2009a, b)
	Azohydromonas lata AB188122.1	β-Burkholderiales	Coelho et al. (2009a, b)
	Delftia tsuruhatensis AY544164.1	β-Burkholderiales	Coelho et al. (2009a, b)
	Bradyrhizobium sp. AB079620.1	α-Rhizobiales	Coelho et al. (2009a, b)
	Azospirillum brasilense	α-Rhodospirillales	Coelho et al. (2009a, b)
	Bacillus spp. KB122 [JQ623487], KB129 [JQ623488], KB133 [JQ623489], and KB142 [JQ623490]	Firmicutes-Bacillales	Grover et al. (2014)
	Pseudomonas sp., *P. chloraphis*	γ-Pseudomonadales; Phytopathogen biocontrol	Das et al. (2008)
Sorghum Rhizomes	*Xanthomonas melonis,* strain LMG8670 [Y10756]	γ-Xanthomonadales; plant pathogen	Rout and Chrzanowski (2009), Rout (2011)
	Agrobacterium Tumefaciens [M11223]	α-Rhizobiales; plant pathogen, organism fixes N	Rout and Chrzanowski (2009), Rout (2011)
	Sphingobium amiense strain YT [AB047364]	α-Sphingomonadales; N2-fixation in former,	Rout and Chrzanowski

(continued)

Table 1 (continued)

Ecological niche	Cultured/closest cultured match	Taxonomic group and known traits of closest match if available	References
		nonylphenol degradation in latter	(2009), Rout (2011)
	Pseudomonas jessenii, strain CIP105274 [AF068259]	γ-Pseudomonadales; produces Fe-siderophores, solubilizes PO4	Rout and Chrzanowski (2009), Rout (2011)
	Caulobacter vibroides strain 15252T [AJ227756]	α-Caulobacterales; Horizontal gene transfer reported with N_2-fixing organisms	Rout and Chrzanowski (2009), Rout (2011)
Sorghum rhizoplane	*Bacillus megaterium* strain JL35-9 [JN118434.1]	Firmicutes-Bacillales	Ramond et al. (2013)
	Various *Bacillus megaterium* strains including the pesticide degrading strain APDB9 [JX274543.1] and strain GMC5001-b [AB741472.1]	Firmicutes-Bacillales	Ramond et al. (2013)
	Bacillus megaterium strains AIMST 3.24.2 [HQ694028.1] and AIMST 1. Hb.20 [HQ670443.1]	Firmicutes-Bacillales	Ramond et al. (2013)

dicyandiamide, DCD; and 3, 4-dimethylpyrazole phosphate, DMPP) which can help to reduce the excessive nitrification (Slangen and Kerkhoff 1984; Smart and Bloom 2001).

By virtue of its role in determining N-cycling efficiency, nitrification is the most important process for retaining N in the ecosystem and curbing nitrification will minimize N-leakage and enable N-flow through NH_4^+ assimilation (Subbarao et al. 2013). Although plants and microbes have the ability to utilize NH_4^+ or NO_3^- as the N source, not much literature is available to identify the extent of their utilization in relation to the ecosystem (Boudsocq et al. 2009). Suppressing soil nitrification should thus not limit the availability of inorganic N for growth of the plants or microbial activity. To supplement this, H^+ are released by plant roots on NH_4^+ absorption and assimilation, which acidifies the rhizosphere, and improves the P pool available to the plants.

Among all important cereal crops cultivated (including wheat, barley, rice, and maize), only sorghum, which is adapted to low-N input environments, has been found to have significant BNI capacity (Subbarao et al. 2007). As an adaptation mechanism, BNI allows the plants to retain and use N efficiently in N-limiting ecosystems (Subbarao et al. 2015). Sorghum was found to release 20 allylthiourea units (ATU) g^{-1} root DW d^{-1} in normal conditions and this release of BNI compounds was shown to increase with growth stage and NH_4^+ supply (Subbarao et al. 2007; Zakir et al. 2008). Interestingly, plants grown with supplemented NH_4^+ released severalfold higher BNI compounds than NO_3^--grown plants (Zakir et al. 2008). Subbarao et al. (2015) hypothesized that the BNI release from sorghum roots may be a localized phenomenon confined to the part interacting with NH_4^+, not the bulk root, and their transport is driven by an ATPase for NH_4^+ uptake and assimilation.

Although some early reports hinted at the possibility of production of nitrification inhibitors from sorghum roots (Alsaadawi 1988), the first active constituent was identified in 2008 from the hydrophilic fraction as methyl 3-(4-hydroxyphenyl) propionate (MHPP; Zakir

et al. 2008). Recent work has identified two categories of BNI compounds: hydrophilic- and hydrophobic-BNIs. Addition of hydrophilic BNIs (10 $ATUg^{-1}$ soil) showed ~40 % inhibition of soil nitrification over a 30-day period. Sakuranetin (ED_{80} 0.6 μM; part of hydrophilic-BNI fraction) and sorgoleone (ED_{80} 13.0 μM; part of hydrophobic-BNIs fraction) were identified as active BNI components that inhibited *Nitrosomonas europaea* by blocking the ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) enzymes involved in NH_4^+ oxidation to NO_2^-. Sakuranetin has a stronger inhibitory activity (ED_{50} 0.2 μM) than MHPP (ED_{50} 100 μM; Subbarao et al. 2013). The other reason for *Nitrosomonas* inhibition could be that the disruption of the electron transfer pathway from BNI activity interferes with the metabolic functions and thus bioluminescence emission from the recombinant *N. europaea* (Subbarao et al. 2006a, b). Figure 1 depicts the model highlighting the role of BNI with respect to the nitrification and denitrification pathways and microbes that may be operating in the sorghum rhizosphere. Nitrification is carried out mainly by chemolithoautotrophic ammonia-oxidizing bacteria (AOB, *Nitrosomonas* spp.) and nitrite-oxidizing bacteria (*Nitrobacter*). However, this process may be more complex, involving autotrophic Archaea nitrifiers, heterotrophic nitrifiers, and anammox bacteria and plant interactions with these associated microbes.

The fungal, actinomycete, and ammonifier viable counts have been found to be reduced in Indian field soils of "very high" input (fertilizer and pesticide usage, e.g., chilies) in comparison to "lower input" production systems (e.g., black gram), causing a reduction in the fungi-to-bacteria ratio, and a drastic change in the eubacterial community and diversity profile over normal rates of fertilizer and pesticide usage thereby affecting soil health (Malhotra et al. 2015). This may very well

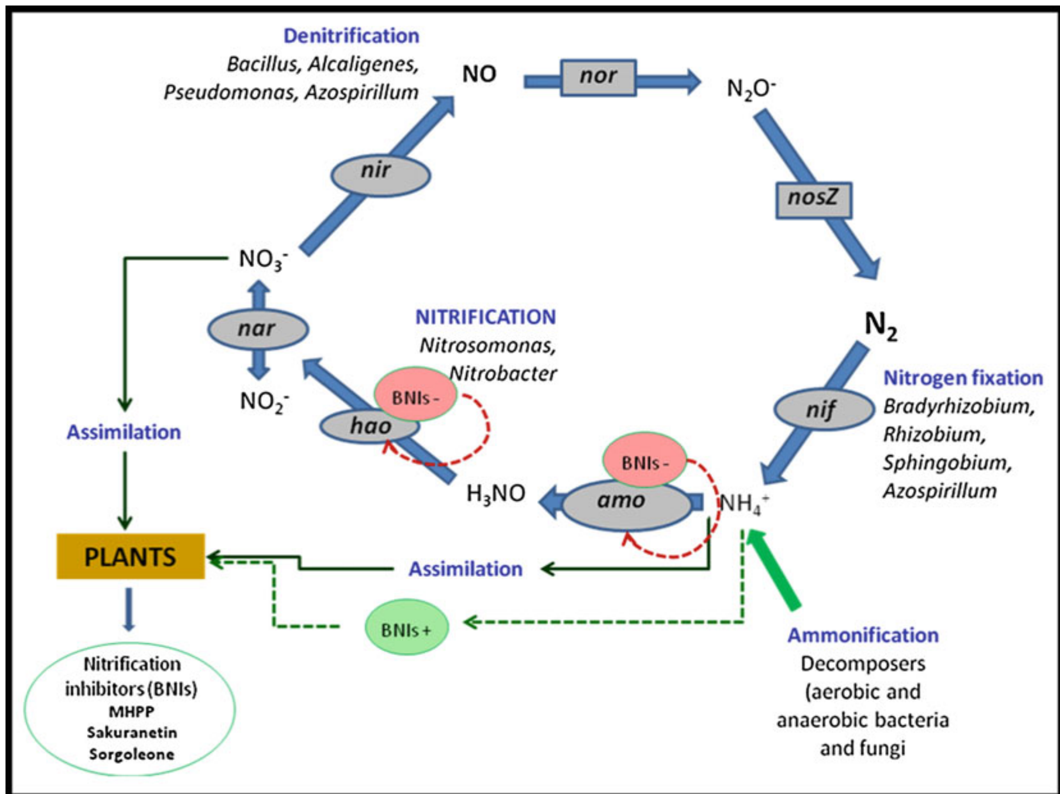

Fig. 1 Model highlightling the role of biological nitrification inhibition (BNI) with respect to the nitrogen cycle and nitric oxide metabolism by bacteria (Adapted and modified from Koul et al. 2015b.)

be the case with sorghum-associated microbial community dynamics due to its low input nature. Furthermore, it has also been shown that sorghum rhizospheric microbial communities are less variable than their endophytic counterparts and the main driver of these niche-specific assemblages is the geographical location and the total NO_3^- and NH_4^+ (nitrogen), carbon, and pH (Ramond et al. 2013). Endogenous NH_4^+ may be a regulator in adjusting phenylalanine ammonia lyase (PAL) activity or other related enzymes, which may be involved in other metabolic changes, including not only the production of BNI compounds but also signalling the membrane for BNI compound release (Zakir et al. 2008).

4 Mechanism of Sorghum–Microbe Interaction: Direct and Indirect

Microbes help to promote plant growth by different mechanisms and are classified according to their beneficial effects. For instance, biofertilizers include N_2-fixing bacteria, biomineralizers break down complex molecules to release simple usable nutrients, phytostimulators produce phytohormones and plant growth regulators that directly promote plant growth and bioprotectants, or biocontrol PGPRs protect plants from phytopathogens. Of these, the first three are categorized as direct effectors of PGP (includes the Azospirilla, Rhizobia, *Paenibacillus*, *Caulobacter*) and the latter are often referred to as indirect effectors of PGP (exemplified by the Pseudomonads). The readers are directed to Tables 1 and 2 for details of the microbes that affect plants. Although direct mechanisms of effector strains involve the utilization of microbial functions/products by plants, indirect mechanisms involve suppression of the deleterious effects of phytopathogens on crop yield.

PGPB-mediated direct enhancement of plant growth includes the fixation of nitrogen for the plant, and solubilization of inorganic and mineralization of organic phosphate complexes. In addition, the production of various phytohormones (including auxin-indole-3 acetic acid), vitamins, and gaseous molecules such as nitric oxide, are also implicated in direct improvement of root growth in terms of root hairs and lateral roots, and the synthesis of some enzymes (such as ACC deaminase) that modulate the level of the plant hormone ethylene also lead to improved plant growth (Dobbelaere et al. 1999a, b, 2003; Mandira Malhotra 2007; Kochar et al. 2013; Koul et al. 2015a, b).

Indirect effects are mediated by the ability to synthesize antifungal metabolites such as antibiotics, fungal cell wall-lyzing enzymes such as chitinases and glucanases, hydrogen cyanide, or the production of siderophores that chelate iron, thereby suppressing the growth of pathogens (Glick 1995; Bloemberg and Lugtenberg 2001; Das et al. 2008).

5 Crosstalk Between Various Pathways and Different Biofertilizers

The rhizosphere and its inhabiting microorganisms fulfill important ecological functions, for example, nutrient recycling, and are responsible for plant growth and health (Sørensen 1997). Additionally, this microenvironment is known as a “microbial hot-spot” where diverse interactions between organisms, beneficial as well as pathogenic, take place (Whipps 2001). A more robust root system will help the plant to anchor and acquire nutrients more effectively, leading to better growth. PGPB strains are known to employ one or more of the direct or indirect mechanisms in conjunction in the rhizosphere, and modulate the same in terms of prevailing environmental conditions.

Rout and Chrzanowski (2009) presented a conceptual framework of microbial processes occurring in the rhizosphere of the invasive *Sorghum halepense*. They explained the feedback loop based on N-cycling, the modification of resource availability that brings about the observed changes in the biogeochemical environment. The N_2 is fixed by N_2-fixing bacteria in the *S. halepense* rhizome, the majority NH_4^+ is then shuttled into the plant (with some rhizosphere loss), and the remainder is converted rapidly into NO_3^-. NH_4^+

Table 2 Different mechanisms employed by plant growth promoting bacteria to benefit sorghum

S. no.	Mechanisms	Bacterial genera	References
1	Root-associated nitrogen fixation	*Azospirillum, Cyanobacteria, Herbaspirillum, Klebsiella, Enterobacter cloacae, Erwinia herbicola*	Steenhoudt and Vanderleydern (2000), Rout (2011)
2	Production of plant hormones (e.g., auxin)	*Azospirillum, Serratia, Sinorhizobium*	Malhotra and Srivastava (2006), Malhotra and Srivatsava (2008), Golubev et al. (2011), Gujral et al. (2013)
3	Phosphate solubilization	*Serratia* sp., *Bacillus licheniformis, Pseudomonas* spp.	Gujral et al. (2013)
4	Production of signal molecules such as nitric oxide	*Azospirillum brasilense,*	Koul et al. (2015b)
5	Increased colonization by arbuscular mycorrhizal fungi	*Azospirillum, Pseudomonas*	Pacovsky (1988, 1989)
6	Aromatic hydrocarbon degrading, polycyclic aromatic hydrocarbons bioremediation	*Sinorhizobium*	Golubev et al. (2011)
7	Increased resistance to adverse conditions (drought, salinity, compost toxicity)	*Azospirillum, Pseudomonas, Arbuscular mycorrhizal fungi*	Yoneyama et al. (2007), Ali et al. (2009)
8	Biocontrol, antagonistic molecules such as cyclic peptides	*Pseudomonas chloraphis, Pseudomonas* sp.	Das et al. (2008), Begum et al. (2014)

and NO_2^- metabolism acidifies soil and promotes dissolution of hydroxyapatite, increasing available P, Ca^{2+}, and Fe^{2+} for plant uptake. These processes are promoted by the action of endophytic pseudomonads. These secondary microbial processes not only help condition soils but also favor the persistence of this successful invasive *Sorghum* species.

The environmental conditions hold the key as bacterial cell survival is of paramount importance before any PGP activity can be mined. Furthermore, a part of the root exudates released by the plants into the rhizosphere creates a conducive/favorable environment for diverse microbial growth thereby improving plant productivity. Microbes maintain a critical mass of cells so that they can excrete essential metabolites above a threshold concentration that is required by the associated host plant. NO, a small diatomic molecule, is gaining immense importance in the field of research that can be substantiated by an increase in the number of publications elucidating its role in mammals, plants, and most important, bacteria (Koul et al. 2015a). However, the exact working mechanism of NO is still widely debated due to its gaseous and highly reactive nature which causes experimental limitations. Also, inadequate information is available about various NO-responsive and influenced genes. Similarly, indole-3-acetic acid (IAA) has long been known as an essential phytohormone, but on IAA exposure, a number of bacterial genes are influenced. It is likely that IAA and NO produced by PGP bacteria may interact in the rhizosphere with other members of the rhizosphere microbiome and the presence of s54 transcription for genes involved in the metabolism of such plant growth regulators may open up a field of study under changing environments (nutrient and limiting environment conditions). The bacterial NO- and IAA-induced genes or proteins may play an important role in

signaling and sensing of other hormones by plants in the rhizosphere.

We recently developed a hypothetical model highlighting the crosstalk involving plant growth regulators, IAA (auxin) and NO (gasotransmitter) produced by the rhizosphere bacteria *Azospirillum brasilense* strain SM, based on their effect on *Sorghum* we observed in the lab (Koul et al. 2015a, b). This model emphasizes the importance of signaling involving IAA and NO and their likely common genetic regulation (Fig. 2). For better understanding of these interactions more information needs to be gathered about the genes and proteins involved in these processes and the points of interplay. Future efforts should be made to identify differentially expressed genes in response to these plant growth regulators and their relevance in improving plant growth as many of the PGP bacteria act as biofertilizers. Keeping in view the current available information, interplay between different plant growth regulators, for example, IAA and NO, cannot be ruled out in the rhizosphere bacteria.

Consequently, it is extremely important to study the extent of influence of microbial plant growth regulators IAA and NO so that their effect on plants can be judged for their appropriate action and application. It is also very important to consider the effect of the root exudate on PGPB gene expression. We have studied the effect of sorghum root exudate (SRE) on IAA and NO metabolism genes of the PGPB, *A. brasilense* SM and our unpublished results suggested that the indole-3-pyruvate decarboxylase (*ipdC*) gene expression did not change significantly in the presence of SRE. However, changes in expression of the nitrous oxide reductase (*nosZ*) and nitric oxide reductase (*norB*) were noted. In the case of *nosZ* expression, when strain SM was grown without tryptophan (IAA substrate) supplementation but in the presence of SREs no significant change in expression was noted. On the other hand, on tryptophan and SRE supplementation, a significant increase in *nosZ* was observed. In the case of *nor*B expression, a five- to sixfold increase was recorded when there was

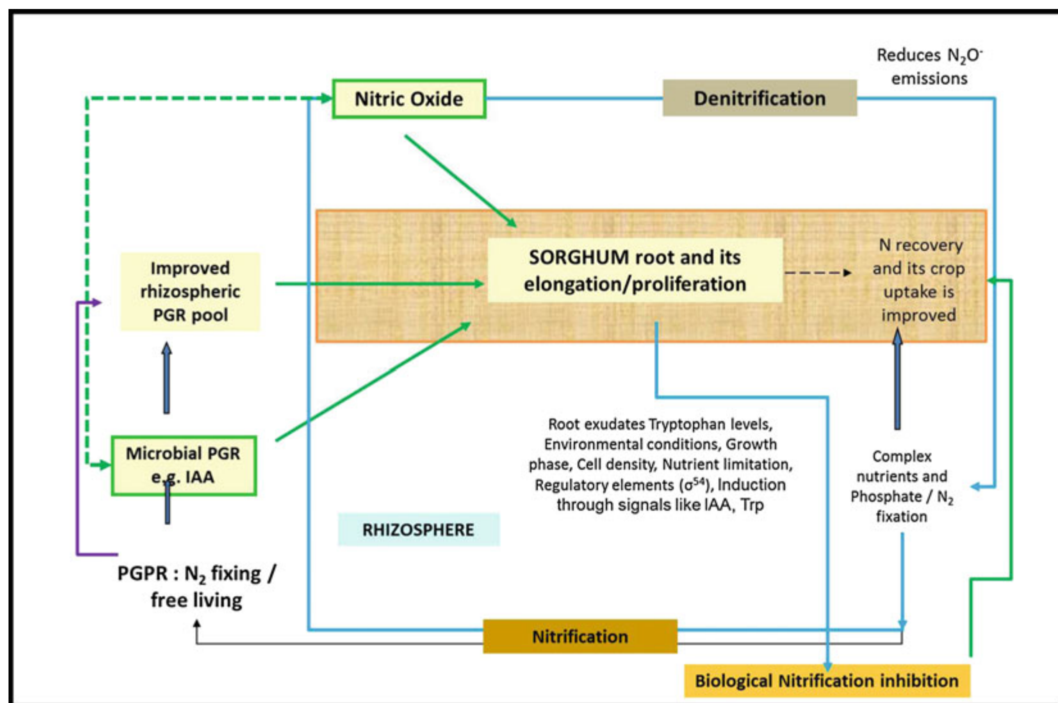

Fig. 2 Schematic representation of the crosstalk involving phytohormones/gasotransmitters produced by rhizosphere bacteria and their effect on the host plant (Modified from Koul et al. 2015a.)

no additional tryptophan supplement provided to the bacterial culture. When tryptophan supplementation was provided along with SREs the bacteria showed 15-fold higher expression. Because SRE contains BNI compounds such as sorgoleone, their effect on the genes involved in plant growth promotion needs to be looked into to determine the crosstalk between plant growth promotion and BNI.

6 Genomics and Transcriptomics of Sorghum Biofertilizers: Current and Future Perspectives

Studies carried out to understand structural dynamics of the microbial community in the rhizosphere reveal that it is influenced by different ecological factors that include plant productivity gradients (Horner-Devine et al. 2003; Schweitzer et al. 2008), herbivorous activity (Kuske et al. 2003), invasion by exotic plant species (Kourtev et al. 2002; Hawkes et al. 2005), and plant nutritional status (Carvalhais et al. 2013). However, recent studies indicate that the microbial community in the plant rhizosphere is primarily structured by the molecular signals relayed by plant root exudates (Fig. 3; Ramond et al. 2013) in addition to other abiotic and biotic factors. Root exudate initiates and regulates dialogues between the host and the microbial community (İnceoğlu et al. 2010). These molecular signals vary between different plant genotypes and thus are responsible for the development and activity of a diversified but specific microbial community in the plant root (Bailey et al. 2009).

Empirical data from several studies highlight the importance of the cultivar type (genotype) at both plant species (Perin et al. 2006) and variety (Wu et al. 2009; Coelho et al. 2009a, b) levels, when selecting nitrogen-fixing strains for use as inoculants for maize, sugarcane, and sorghum. A direct correlation between the cultivar type and bacterial community has been reported using culture-dependent methods for *Azospirillum* association with maize (García de Salomone and Dobereiner 1996) and rice (García de Salomone et al. 2012), and for *Sinorhizobium meliloti* association with alfalfa (Carelli 2000). Similar results were observed for sorghum cultivars and *Paenibacillus* using DGGE and sequencing methods for molecular identification (Coelho et al. 2007).

Conversely, the microbial community, including plant growth promoting species and biocontrol agents, release phytohormones and secondary metabolites that not only modify the root system architecture but also influence root functioning, plant growth, and response towards biotic and abiotic stresses (Vacheron et al. 2013). PGPB produce an array of hormones including auxin and cytokinin that influence the auxin-to-cytokinin ratio in plants. The balance between phytohormones is reported to be crucial in regulation of organogenesis and root architecture in plants (Aloni et al. 2006). The switch between low and high concentration of exogenously produced IAA regulates primary root elongation and development of lateral roots, decreased primary root length, and increased root hair formation, respectively (Vacheron et al. 2013). Furthermore, different plant genotypes produce different concentrations of tryptophan in their root exudates, which is an important determinant of IAA biosynthesis in plant-associated bacteria (Kamilova et al. 2006). Based on the presence of different intermediates, several biosynthetic pathways for IAA production have been identified in PGPB (Spaepen et al. 2007). Bacterial gene *ipdC*/*ppdC* that encodes the indole (or phenyl)- 3-pyruvate decarboxylase is a major regulator of the indole pyruvic acid pathway. *ipdC* mutants are often reported to have altered plant root morphology in comparison to wild-type strains (Brandl and Lindow 1998; Dobbelaere et al. 1999a, b; Malhotra and Srivastava 2006, 2008).

The conventional culture-dependent approach has identified various sorghum-associated plant growth promoting bacteria that beneficially influence nitrogen fixation (Budi et al. 1999a, b; Ramond et al. 2014). Culture-based studies along with molecular fingerprinting methods (DGGE and T-RFLP) in sorghum rhizospheres have shown the presence of *nifH* gene sequences from a diverse set of bacterial species. Such findings

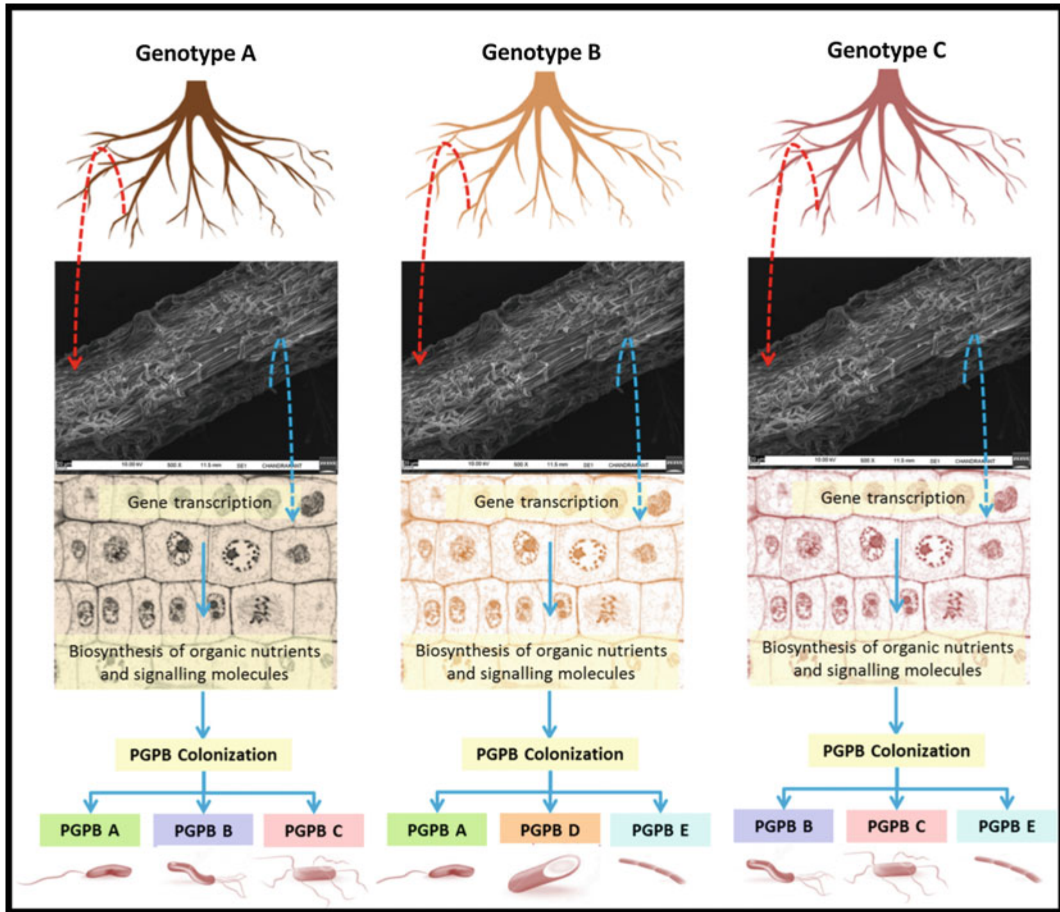

Fig. 3 Impact of plant genotype on rhizosphere communities and their functions and vice versa

emphasize that the source of bioavailable N_2 to *Sorghum* is a result of the various sorghum-associated diazotrophic microbial strains and is not limited to a single genome (Coelho et al. 2007; 2009a, b). Although mutagenesis-based studies in PGPB have identified some microbial genes/pathways responsible for modifying the physiology of *Sorghum* (Malhotra and Srivastava 2006, 2008; Koul et al. 2015b), the knowledge remains incomplete and not ready for translation for complete crop management.

A microbial community is described to exhibit emergent properties that none of its constituent microbes possess when independently evaluated (Konopka 2009). Furthermore, microbial community structure varies in different spatial and temporal scales depending upon host genotype and surrounding abiotic factors. The cumulative and differential effect of the microbial community on the host plant can only be mapped to a great extent through next-generation culture-independent strategies that utilize so-called "omic" techniques (metagenomics, metatranscriptomics, and metaproteomics). The crosstalk between the plant and microbial community can be elaborately deciphered by carrying out metagenomics, and whole genome transcriptomic analyses of the interacting partners under different physiological conditions and variable time points. An investigation of mechanisms involved in osmotic stress tolerance employing transcriptomic and microscopic strategies revealed a considerable change in the transcriptome of *Stenotrophomonas rhizophila*

DSM14405T in response to salt stress (Alavi et al. 2013). A transcriptome analysis of the root-colonizing bacterium *Bacillus amyloliquefaciens* FZB42 in response to maize root exudates revealed differential transcription of a total of 302 genes indicating their potential role in interactions of bacteria with plants (Fan et al. 2012). In depth analysis indicated that root exudates serve primarily as a source of carbon and energy for FZB42 and regulate a large set of differentially expressed genes. Another group of microbial genes that were significantly induced by plant root exudates encoded the nonribosomal synthesis of antimicrobial secondary metabolites (Fan et al. 2012).

With the knowledge gained through omics study, the rhizosphere can be aided and structured to benefit sorghum cultivars. In addition it is also possible to engineer a PGPB metabolically by introducing specific pathway genes, or refine the beneficial functions, or add new desirable functions, all aimed towards improving the health of the plants. Keeping their application potential in mind, and the fact that such genomics studies are lacking in biofertilizers associated with sorghum, future research should integrate comprehensive molecular/signaling and transcriptomic-/proteomic-based studies. The functional information from microbial populations of the sorghum rhizosphere through whole genome transcriptome microarray analysis is fast becoming a prerequisite to developing novel management strategies for optimum utilization and application of biofertilizers for sustainable agriculture.

7 Conclusions

The rhizosphere is a heterogeneous environmental niche not only in terms of its constituent microbial members but also the plentiful metabolic activities. The latter govern the physiological fitness of a strain and their interactions may prove both productive and counterproductive. Thus, analyzing any function in isolation may be important but not sufficient to understand the dynamics of this interaction. The complete picture needs to be looked at and all players in this interaction need to be identified. Although bacterial IAA has been identified as a signaling molecule in sorghum–microbe interactions that promotes root growth directly by stimulating plant cell elongation or cell division, it may also do so by influencing other bacterially produced plant growth regulators. However, one may be tempted to extrapolate certain plant signaling mechanisms to be operating in the case of plant–bacteria associations as well. No doubt a number of bacterial species have been reported in the literature that significantly benefit the growth of sorghum and can be applied at field levels in suitable formulations, yet a wide range of unanswered questions and the current limitations of existing biofertilizers leave a large scope for the use of genomics and transcriptomics studies. In addition to information on sorghum genotypes with high biomass and improved beneficial traits, a detailed understanding of the rhizosphere communities and microbes is required as these may be exploited to improve productivity and utilization of sorghum genotypes. Such strategies can help find solutions to challenges in the field application of biofertilizers with sorghum and many other important crop plants.

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