7 Sorghum

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7.1 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop, after wheat, rice, maize, and barley. A largely self-pollinated crop, it is grown on over 40 million hectares (USDA 2004) in both temperate and tropical regions. Sorghum is mainly grown as a rainfed crop by subsistence farmers in the semiarid tropical regions of Africa and Asia as well as by other farmers in the USA and Latin America. It is a suitable crop for drought and heat-stressed environments and can be grown from sea level to elevations in excess of 300 m, in high rainfall areas, in semiarid regions, and in different seasons.

7.1.1 Center of Origin

The origin of sorghum, an African grass, and its diversification into five major races and thousands of different genotypes began in the distant human past and is only partially known. However, the work of botanists, plant breeders, archaeologists, and geographers has uncovered the probable evolutionary pathway in the domestication of sorghum and the probable spatial dynamics of that evolution under cultural control. A great deal has been learned in the last few about the origins of the cereal and the people responsible for the domestication of sorghum races years. The Ethiopian region of Africa is the center of origin of sorghum (Mann et al. 1983) as it is rich in the number of snowdenian species and also contains several varieties of the durra type, which represents the highly evolved varieties among the cultivated races. From Ethiopia sorghum was taken to West Africa across the Sudan, from where it was first grown among the Mande people of the upper Niger. Also from Ethiopia sorghum was taken to East Africa, from where it was distributed among the Nilotic and Bantu people. From East Africa the sorghum spread to India during the first millennium and was taken from there to China in the early Christian era (Doggett 1976). Sorghum races in India are closely related to those in northeast Africa. From West Africa sorghum was distributed to the USA and other parts of the world through slave trade around the mid-19th century. Before 1900 full-scale cultivation of sorghum had started in the southern great plains of the USA.

7.1.2 Domestication

Sorghum has been carried to many new habitats in different environments to become a staple grain for millions of people. Sorghum has also been diversified into a sugar source, a construction material, a raw material for household implements, and a raw material for industry. The change from a harvested wild plant with much internal variability to an important resource for use and improvement is the result of management. Cultivated races of sorghum originated by disruptive selection and domestication in east central Africa from the wild snowdenian species, *Sorghum arundinaceum*. Human selection for cultivated characters (mainly nonshattering heads, large seeds and ears, easy threshability, and suitable height and maturity) and natural selection for wild type character resulted in divergence into polymorphic populations in the presence of considerable gene flow between the wild and cultivated types. These processes seem to have contributed to the evolution of durra, kafir, bicolor, cernum, and caudatum and other intermediate types. According to Doggett (1976), most of these types might have migrated to India and China around 4000 BC and 2000 BC, respectively.

Sorghum is adapted to a wide range of environmental conditions but is particularly adapted to drought. It has a number of morphological and physiological characteristics that contribute to its adap-

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tation to dry conditions, including an extensive root system, waxy bloom on the leaves that reduces water loss, and the ability to stop growth in periods of drought and resume it again when conditions become favorable. It is also tolerant to water logging and can be grown in high rainfall areas. It is, however, primarily a crop of hot, semiarid tropical environments with 400 to 600 mm rainfall that are too dry for maize. It is also widely grown in temperate regions and at altitudes of up to 2,300 m in the tropics.

7.1.3 Taxonomic Position

All commercial groups of sorghum such as grain sorghum, fodder sorghum, broomcorns, and sorgos are classified under a single botanical species *Sorghum bicolor* (L.) Moench. The genus *Sorghum* belongs to one of the 16 subtribes of the tribe Andropogoneae of the subfamily Panicoidae of the family Poaceae.

Classification of the Genus Sorghum

Among all the classification attempts, Snowden's (1936) is the most comprehensive and practicable to a certain extent.

Section	Eusorghum	
	Subsection	Arundinaceae
	Series	Spontanea and Sativa
	Subsection	Halepensia
Section	Para-sorghum	

Members of the subsection Arundinacea are diploids with $2n = 20$ chromosomes. The series Spontanea comprises wild species or races, and the series Sativa, the cultivated races. Using this basic structure, Snowden (1936) described 31 cultivated and 17 related wild species. These species are more appropriately considered as races of a single species.

Garber (1950) and Celarier (1959) divided the genus into six subgenera based on cytotaxonomic data: Eusorghum, which is the same as Snowden's section = Eusorghum, Chaetosorghum, Heterosorghum, Sorghastrum, Parasorghum, and Stiposorghum. Variation within these subgenera can best be described from the key outlined by Celarier (1959):

AA Nodes glabrous or minutely pubescent, first bloom of sessile spikelet many nerved (>10)

- A Sorghum: pedicellate spikelets staminate or neuter, awns small or wanting.
- B Pedicellate spikelets with glumes only, awns prominent.
  1. Heterosorghum: primary branch of panicle simple and not whorled, glumes of pedicellate spikelets subequal, lodicules ciliate
  2. Chaetosorghum: primary branch of panicle simple and not whorled, glumes of pedicellate spikelets unequal, lodicules glabrous

BB Nodes with distinct ring of hairs, first glume of sessile spikelet few nerved (<10)

1. Parasorghum: callus obtuse, awns <65 mm in length
2. Stiposorghum: callus pointed, awns >65 mm in length

Sun et al. (1994) used internal transcribed spacers of nuclear ribosomal DNA to evaluate the phylogenetic relationships within the genus *Sorghum*. They found that Chaetosorghum and Heterosorghum appear to be closely related to each other, and these two are more closely related to sorghum than to Parasorghum.

A simplified classification scheme of cultivated sorghums was proposed by Harlan and de Wet (1972) based on morphological characteristics that most present-day breeders have come to recognize and utilize. The International Plant Genetic Resources Institute (formerly IBPGR) Advisory Committee on sorghum and millet germplasm has recommended this classification to be used in describing sorghum germplasm. Their system of classification of cultivated races into five basic races and ten intermediate races and those of wild races into six spontaneous races is presented below:

1. Basic races:
   - Race 1 bicolor (B)
   - Race 2 guinea (G)
   - Race 3 caudatum (C)
   - Race 4 kafir (K)
   - Race 5 durra (D)
2. Intermediate races: (all combinations of basic races)
   - Race 6 guinea-bicolor (GB)
   - Race 7 caudatum-bicolor (CB)
   - Race 8 kafir-bicolor (KB)
   - Race 9 durra-bicolor (DB)
   - Race 10 guinea-caudatum (GC)
   - Race 11 guinea-kafir (GK)

Table 1. Characteristics of commercial grain sorghum types

Grain sorghum type	Brief morphological description	Geographical location
Durra	Hairy, rachises, flattened kernels and dry stalks	Mediterranean, Near East, Middle East
Shallu	Partly pubescent involute glumes, cone-shaped lax panicles, corneous kernels, dry and non-sweet stalks	India, tropical Africa
Guineense	Involute and nearly glabrous glumes and compact panicles	Central and Western Africa
Kafir	Awnless, compact cylindrical panicles and juicy non-sweet stalks	South Africa
Kaoliang	Stiff stalks, thick hard rind, stiff spreading and few panicle branches, and dry and no-sweet 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 non-sweet stalks	Sudan
Hegari	Rounded kernels, brown testa midcompact ellipsoid and branched panicles, and white kernels with a bluish-white appearance	Sudan

  - Race 12 guinea-durra (GD)
  - Race 13 kafir-caudatum (KC)
  - Race 14 durra-caudatum (DC)
  - Race 15 kafir-durra (KD)
3. Spontaneous races: *S. bicolor* ssp. *arundinaceum*
  - Race 1 arundinaceum
  - Race 2 aethiopicum
  - Race 3 virgatum
  - Race 4 verticilliflorum
  - Race 5 propinquum
  - Race 6 shattercane

Classification within the subgenera was further developed by de Wet (1978). The three species in the subgenera sorghum were recognized: Sorghum, two rhizomatous taxa, *S. halepense* and *S. propinquum*, and *S. bicolor*, representing all annual wild, weedy, and cultivated taxa. *S. bicolor* was broken down further into three subspecies: *S. bicolor* ssp. bicolor, *S. bicolor* ssp. *drummondii*, and *S. bicolor* ssp. *verticilliflorum* (formerly ssp. *arundinaceum*).

A commercial type of classification is used in the United States. Several commercial types occur and are given regional names. Extensive breeding has eroded the clear-cut differences among the various types. However, popular regional types such as durras, shallus, guineas, kafirs, kaoliangs, milos, feteritas, and hegaris are common in grain sorghum literature. These groups differ in their genetic characters as evidenced by the diversity resulting from intercrosses between the groups. Certain factors for disease reaction, insect resistance, heterosis, cytoplasmic male sterility, fertility restoration, and tillering tend to be associated with particular groups. Details of some of the more popular groups are given in Table 1.

7.1.4 Brief Morphology

Sorghum is a vigorous grass that varies between 0.5 and 5.0 m in height. It is usually an annual. It produces one or many tillers, which emerge initially from the base and later from stem nodes. The root system consists of fibrous adventitious roots that emerge from the lowest nodes of the stem, below and immediately above ground level. Roots are normally concentrated in the top 0.9 m of soil but may extend to twice that depth and can extend to 1.5 m in lateral spread. The stem is solid, usually erect. Its center can be dry or juicy, insipid or sweet to taste. The center of the stem can become pithy with spaces. Leaves vary in number from 7 to 24, depending on the cultivar. They are borne alternately in two ranks. Leaf sheaths vary in length from 15 to 35 cm and encircle the stem with their margins overlapping. The leaf sheath often has a waxy bloom. Leaves are from 30 to 135 cm long and 1.5 to 13 cm wide, with flat or wavy margins. Midribs are white or yellow in dry pithy cultivars or green in juicy cultivars. The flower is a panicle, usually erect,

but sometimes recurved to form a gooseneck. The panicle has a central rachis, with short or long primary, secondary, and sometimes tertiary branches, which bear groups of spikelets. The length and closeness of the panicle branches determine panicle shape, which varies from densely packed conical or oval to spreading and lax. Grain is usually partially covered by glumes. The seed is rounded and bluntly pointed, from 4 to 8 mm in diameter and varying in size, shape, and color with cultivar.

7.1.5 Cytogenetic Structure

Sorghum bicolor has a haploid chromosome number of 10, and it is classified as a diploid ($2n = 2x = 20$). Most species in the genus *Sorghum* are diploid with $2n = 20$, but several species, most notably *S. halepense*, are tetraploid ($2n = 4x = 40$). As the basic chromosome number in the Sorghastrae is five, it has often been hypothesized that sorghum may be of tetraploid origin. Meiotic chromosome pairing analysis did not provide any strong evidence of a tetraploid origin (Brown 1943; Endrizzi and Morgan 1955), but the large number of complementary gene loci seems to indicate a tetraploid origin. The application of fluorescent in situ hybridization (FISH) to sorghum chromosomes indicates that single-copy probes consistently identify two loci on separate chromosomes. This provides strong evidence that sorghum does in fact have tetraploid origins (Gomez et al. 1997).

Differences between chromosomes in subgenera of sorghum are detectable, but karyotypic analysis of sorghum chromosomes has been difficult due to similarities in chromosome size and structure (Huskins and Smith 1932; Doggett 1988). Karyotype analysis of several subgenera of the genus *Sorghum* indicates that chromosomes in the subgenus Eusorghum are distinctly different and smaller than chromosomes in the subgenera Parasorghum and Stiposorghum (Garber 1950; Celarier 1959; Gu et al. 1984). Gu et al. (1984) described the karyotype of *S. bicolor*, but only chromosome I (nucleolar organizing region) and chromosome IV (characteristic arm ratio) could be identified distinctly. Yu et al. (1991) were able to identify all ten chromosomes in *S. bicolor* using a combination of chromosome size, arm ratio, and C-banding patterns. C-banded karyotype for somatic metaphase chromosomes of sorghum (Combined Kafir 60) is presented in Fig. 1. Later, Kim et al. (2002) used fluorescence in situ hybridization (FISH) and integrated structural genomic resources, including large insert genomic clones in bacterial artificial (BAC) libraries, to identify ten chromosomes simultaneously. Recently, they (Kim et al. 2004) have determined linkage group identities and homologies for metaphase chromosomes of *Sorghum bicolor* ($2n = 20$) by FISH of landed BACs. They used relative lengths of chromosomes in FISH-karyotyped metaphase spreads of the elite inbred BT $\times$ 623 to estimate the molecular size of each chromosome and to establish a size based nomenclature for sorghum chromosomes (SBI-01 to SBI-10) and linkage groups (LG1 to LG10) (Table 2 and Fig. 2).

The genome size for *S. bicolor* and *S. halepense* has been reported to be 735 and 1,617 Mb, respectively (Laurie and Bennett 1985). Later Arumunganathan and Earle (1991) estimated the genome size of *S. bicolor* to be ca. 750 Mb while Peterson et al. (2002) reported 692 Mb.

7.1.6 Economic Importance

Sorghum is the fifth most important cereal crop in the world after wheat, rice, maize, and barley. It is cultivated annually on ca. 45 million ha, producing ca. 60 million MT of grain (USDA 2004) (Table 3). Sorghum grain is a major food in much of Africa, South Asia, and Central America and an important animal feed in the USA, Australia, and South America. In addition to these uses of the grain, sorghum crop residues and green plants also provide sources of animal feed, building materials, and fuel, particularly in dryland areas of the semiarid tropics (SAT). Grain sorghum is well known for its capacity to tolerate conditions of limited moisture and to produce during periods of extended drought, in circumstances that would impede production in most other grains. Sorghum leaves roll along the midrib when moisture-stressed, making the plant more drought resistant than other grain plants. Like corn, sorghum can be grown under a wide range of soil and climatic conditions. Unlike corn, however, sorghum's yield under different conditions is not so varied. Consequently, it is grown primarily in arid areas where corn would not make it without substantial irrigation.

Sorghum is an important part of the diets of many people in the world and is nutritionally rich (Table 4). It is made into unleavened breads, boiled porridge

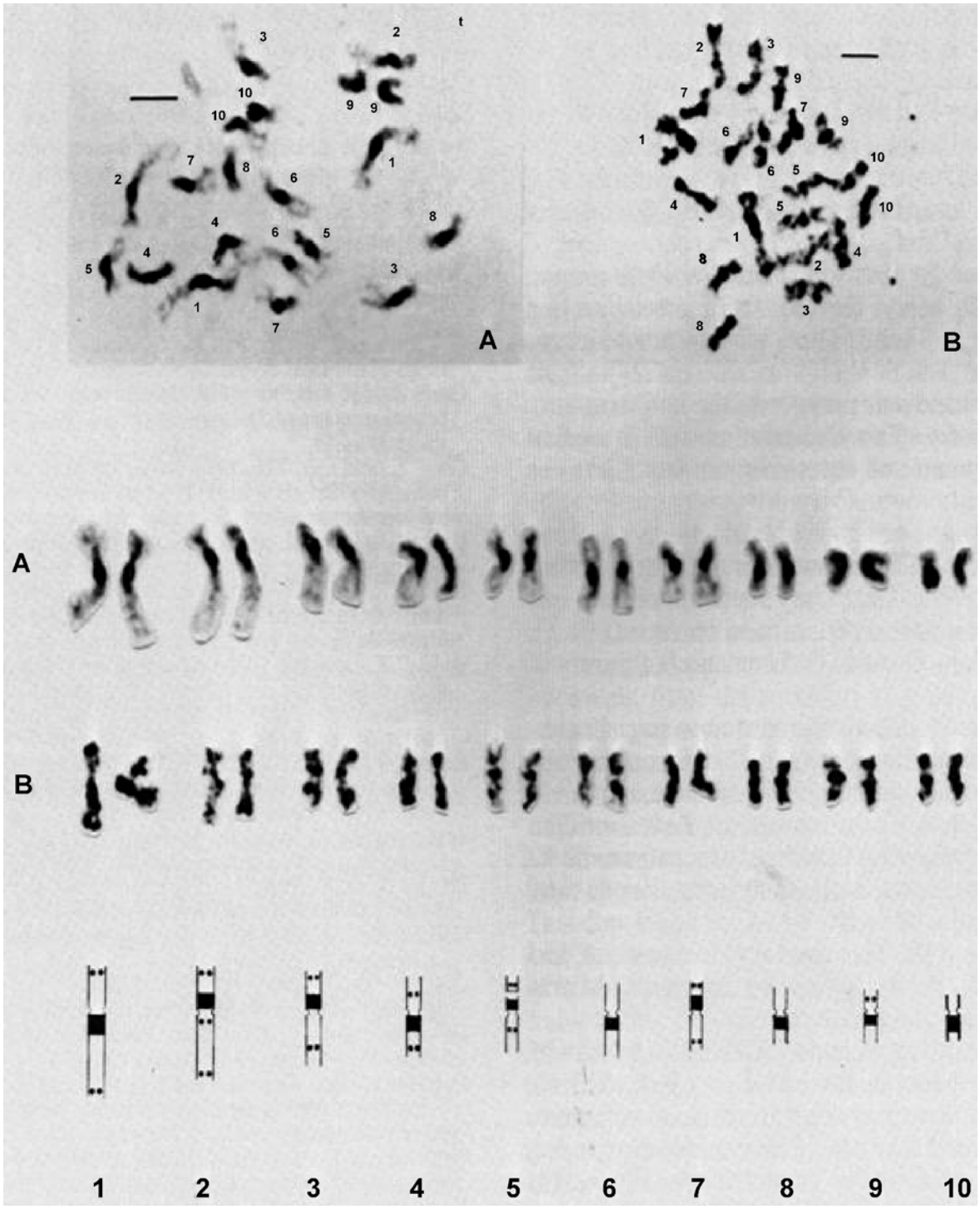


Fig. 1. C-band karyotype for somatic metaphase chromosomes of Combine Kafir 60, sorghum (Reprinted, with permission of Crop Science Society of America, from Yu et al. 1991)

Table 2. Relationship of FISH-based karyotype of sorghum and linkage groups comprising various linkage maps of sorghum genome. (Reprinted with permission of Genetics Society of America; from Kim et al. 2004)

Chromosome number[1]	SBI-01	SBI-02	SBI-03	SBI-04	SBI-05	SBI-06	SBI-07	SBI-08	SBI-09	SBI-10
Linkage group (LG)	LG-01	LG-02	LG-03	LG-04	LG-05	LG-06	LG-07	LG-08	LG-09	LG-10
LG in Menz et al. 2002[2]	A	B	C	D	J	I	E	H	F	G
LG in Pereira et al. 1994	C	F	G	D	J	B	A	I	E	H
LG in Bowers et al. 2003[3]	C	B	A	F	H	D	J	E	G	I
LG in Crasta et al. 1999	G, K	D	A	C	J	F	E	H	I	B
LG in Boivin et al. 1999[4]	C, K	F	G	D, L	J	B	A	I	E	H
LG in Whitkus et al. 1992	B, C	D	F, M	H	G	E	A	K, L	I	J
Fish Karyotype	See	Fig. 2								
Total length (μm)	5.11	3.87	3.85	3.5	3.44	3.15	3.13	3.07	2.98	2.94
Standard errot[6]	0.047	0.035	0.038	0.032	0.037	0.029	0.028	0.026	0.029	0.023
Relative length[7]	14.59	11.06	10.98	9.99	9.82	9.00	8.92	8.75	8.51	8.39
Estimated DNA content[8]	119.3	90.5	89.8	81.7	80.3	73.6	73.0	71.6	69.6	68.6
Arm ratio[9]	1.32	1.16	1.13	1.14	1.02	1.42	1.06	1.10	1.02	1.04

[1] Chromosomes were ordered and numbered according to their rank of the total length at metaphase (full contraction)

[2] Linkage group designations are identical to those described in Peng at al. (1999), Kong et al. (2000), Bhattramakki et al. (2000) and Haussmann et al (2002a)

[3] Linkage group designations are identical to those described in Chittenden et al. (1994) and Tao et al. (2000)

[4] Linkage group designations are identical to those described in Dufour et al. (1997)

[5] The chromosomes are displayed according to cytogenetic convention with the short arm at the top of the vertical chromosomes

[6] The sample size for measurement was 40

[7] Relative length = 100* (chromosome length/genome length)

[8] Estimated DNA content = Relative length × estimated genome size, i.e., 818 Mbp (Price et al. 2005)

[9] Arm ratio = length of arm/length of short arm

Table 3. Global area and production of Sorghum (USDA 2004)

Country	Area harvested (1,000 HA)	Production (1,000 MT)	Country	Area harvested (1,000 HA)	Production (1,000 MT)
Argentina	525	2,600	Lesotho	10	10
Australia	700	1,900	Mauritania	150	70
Benin	170	150	Mexico	1,800	6,300
Botswana	50	8	Morocco	25	15
Brazil	950	2,200	Mozambique	500	300
Burkina	1,450	1,300	Nicaragua	62	103
Burundi	55	65	Niger	1,500	650
Chile	0	0	Nigeria	6,800	8,050
China; Peoples Republic of	820	3,300	Norway	0	0
Colombia	60	170	Pakistan	400	230
Cote d'Ivoire	60	30	Paraguay	30	40
Dominican Republic	9	38	Peru	1	1
Ecuador	5	10	Philippines	0	0
Egypt	160	750	Romania	5	5
El Salvador	89	141	Rwanda	150	155
Eritrea	150	130	Saudi Arabia	180	200
Ethiopia	1,500	1,400	Senegal	210	160
EU-25	110	650	Somalia	225	150
Gambia; The	20	25	South Africa, Republic of	100	220
Ghana	300	320	Sudan	6,000	4,350
Guatemala	45	55	Swaziland	1	1
Guinea-Bissau	50	45	Taiwan	5	20
Haiti	115	90	Tanzania	750	580
Honduras	40	40	Thailand	160	280
India	9,900	8,500	Uganda	280	350
Iran	10	20	United States	2,799	11,050
Iraq	5	5	Uruguay	20	60
Israel	0	0	Venezuela	140	340
Japan	0	0	Yemen	320	260
Kenya	140	130	Zambia	40	25
Korea, Republic of	1	1	Zimbabwe	140	80

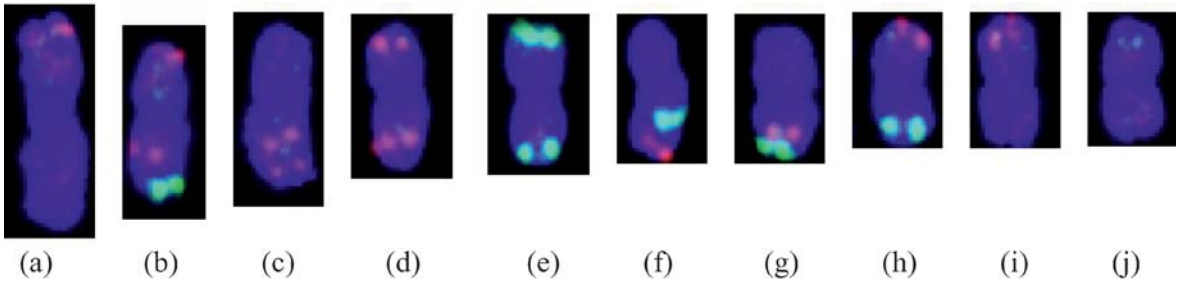


Fig. 2. FISH-based karyotype of sorghum. (**a**) LG-01. (**b**) LG-02. (**c**) LG-03. (**d**) LG-04. (**e**) LG-05. (**f**) LG-06. (**g**) LG-07. (**h**) LG-08. (**i**) LG-09. (**j**) LG-10. (Reprinted, with permission of Genetics Society of America, from Kim et al. 2004)

or gruel, malted beverages including beer, and specialty foods such as popped grain and syrup from sweet sorghum. In Africa, the straw of traditional tall sorghums is used to make palisades in villages or around a homestead. The plant bases are an important source of fuel for cooking, and the stems of wild varieties are used to make baskets or fish traps. Dye extracted from sorghum is used in West Africa to color leather red.

Some quantities of grain sorghums go into industrial uses. Sorghum starch is manufactured in the USA by a wet-milling process similar to that used for corn starch, then made into dextrose for use in foods. Starch from waxy sorghums is used in adhesives and for sizing paper and fabrics and is an ingredient in oil drilling "mud". The grain can be a source of butyl alcohol.

7.1.7 Breeding Objectives

Sorghum is grown in a wide range of physical conditions in locations ranging from the equator to over 50° N and 30° S. The crop is therefore subjected to a wide variety of temperature, day-length, and moisture regimes. Improved sorghum cultivars for a particular environment always involve breeding for adaptation to the specific climatic conditions found there. This is usually indicated by the appropriate crop duration for that environment and by acceptable and stable yield levels and appropriate grain qualities. The type of cultivar required for a target location also influences the objectives of the plant breeder. For example, the height of a pure-line variety for a specific environment and the heights of the parental lines of a hybrid for the same environment are likely to be different. In addition, improved cultivars for specific locations must

Table 4. Nutritional composition of sorghum*

Nutrient	Amount	Unit
Water	9.2	g
Energy	339.0	Kcal
Protein	11.3	g
Total lipid	3.3	g
Carbohydrate	74.6	g
Fiber, total dietary	n/a	g
Ash	1.57	g
Calcium	110.0	mg
Iron	3	mg
Magnesium	n/a	mg
Phosphorus	287.00	mg
Potassium	350	mg
Sodium	6	mg
Zinc	n/a	mg
Copper	n/a	mg
Manganese	n/a	mg
Selenium	n/a	mcg
Vitamin C	0	mg
Thiamin	0	mg
Riboflavin	0.26	mg
Niacin	3.53	mg
Pantothenic acid	n/a	mg
Vitamin B-6	n/a	mg
Folate	150.0	mcg
Vitamin B-12	0	mcg
Vitamin A	2,205	IU
Vitamin E	0.00	mg-ATE
Vitamin D	n/a	IU
Iodine	n/a	mcg

*Average values (per 100 g), taken from U.S. Department of Agriculture, Agricultural Research Service (USDA:ARS) 1998 USDA Nutrient Database, Release 12, Laboratory Home Page (http://www.nal.usda.gov/fnic/foodcomp)

possess resistance to the major constraints to production encountered and grain- and stover-quality factors appropriate for sorghum there. These constraints include biotic stresses such as diseases, insects, and parasitic weeds, and abiotic stresses, the requirements for which are usually quite different from one location to another. Resistance to these constraints is deliberately bred into cultivars by crossing resistant types with cultivars possessing other desirable traits and selecting plants with both resistance and desirable traits. Increased yields and improvement of quality are the main concerns of sorghum-breeding programs. On a global basis, sorghum breeding aims at specific objectives including high grain yields, higher fodder yields, disease resistance, insect resistance, drought tolerance, high temperature resistance, striga resistance, nutritional quality, cooking quality, and good stalk quality. In addition, development of suitable varieties to fit into various cropping patterns (intercropping and sequence cropping) in developing countries is another objective.

7.1.8 Classical Breeding Achievements

Kharif Sorghum

With the release of CSH I, the first commercial hybrid in 1964, sorghum became the second crop after maize in developing high-yielding hybrids using a cytoplasmic-genic male sterility system. Since CSH I, a total of 18 more hybrids have been released. The hybrids played a major role in raising productivity and production, particularly in the case of kharif sorghum. Yield potential shown by the hybrids CSH 5 to CSH 18 requires special mention. CSH 5 and CSH 6 had a yield potential of 34 q/ha, while CSH 9 produces 40 q/ha in. This further increase to 42 to 45 q/ha in CSH 16–CSH 18 recently.

Besides hybrids, 15 high-yielding varieties (CSV 1 to CSV 15) have also been released with medium maturity (Table 5). Higher preference was shown for dual-purpose varieties such as CSV 10, CSV 13, SPV 462, and CSV 15. A major advantage of varieties over hybrids is their relatively better grain quality and multiple resistance or tolerance against major pests and diseases. The recently released variety CSV 15 has established higher grain and fodder yield potential than hybrids CSH 5 and CSH 6 released two decades ago.

Rabi Sorghum

Improvement of rabi sorghum did not receive as much emphasis and effort as the kharif sorghum until the 1990s. However, some of the hybrids and varieties listed in Table 5 are specifically developed and recommended for rabi season where the fodder yield is more important than that in kharif sorghum. Therefore, rabi grain productivity must be accompanied by normal or better fodder productivity. From this point of view, gradual success was achieved from the first rabi hybrid CSH 7R to the latest hybrids CSH 15R and 18R.

7.1.9 Limitations of Classical Endeavors and Utility of Molecular Mapping

Plant-breeding efforts over the past six decades have contributed tremendously to the genetic improvement of cereals in terms of yield and quality. However, traditional approaches to crop improvement have several limitations, and increase in yield and productivity cannot be sustained indefinitely (Vasil 1994). Most sorghum-breeding programs have focused on agronomic performance to insure food security; however, grain quality is also an essential requirement for the development of improved cultivars. Sorghum proteins are not of superior quality. Limited lysine and the excess of leucine, which affects the leucine–isoleucine balance, are the primary limiting factors of sorghum protein quality. The hopes raised by those of the Ethiopian high-lysine sorghums that are late, photosensitive, and possess shriveled seeds, as well as those of P7212, an opaque mutant and N94 with shriveled seeds, have not been realized so far. Also, little is known about the genetic control of grain-quality parameters and their relationships with the main component of sorghum productivity.

Improving drought tolerance is an important objective in a sorghum-breeding program. Early breeding for host plant resistance to sorghum midge, shoot fly, and stem borers brought about worthwhile resistance in sorghum; however, fast evolving races require incorporation of multiple resistance genes, which has not been possible through classical breeding efforts.

The genetic improvement of sorghum through classical plant breeding has resulted in the successful development and deployment of highly adapted high-yielding cultivars that are stable across years.

Table 5. List of released sorghum hybrids

No.	Name	Parentage	Year of release	Duration (d)	Plant ht (cm)	Grain yield (q/ha)	Fodder yield (q/ha)
1.	CSH 1	CK 60A × IS 84	1964	105	150	28–31	80
2.	CSH 2	CL 60A × IS 3691	1965	110	150	30–32	95
3.	CSH 3	2219A × IS 3691	1970	110	145	33–35	105
4.	CSH 4	1036A × Swarna	1972	110	175	34–35	90
5.	CSH 5	2077A × CS 3541	1975	115	185	35–38	95
6.	CSH 6	2219A × CS 3541	1977	100	155	32–35	75
7.	CSH 7R	36A × 168	1977	110	130	27–29	24
8.	CSH 8R	36A × PD3-1-11	1977	110	120	33–35	97
9.	CSH 9	296A × CS 3541	1981	115	190	38–40	95
10.	CSH 10	296A × SB 1085	1984	110	235	36–38	130
11.	CSH 11	296A × MR 750	1986	110	190	38–40	95
12.	CSH 12R	296A × M 148-138	1986	115	205	25–28	50
13.	CSH 13R	296A × RS 29	1990	115	180	31–32	55
14.	CSH 14	AKMS14A × AKR-150	1989	103	178	30–32	75
15	CSH 15R	104A × RS 585	1996	110	195	32–33	56
16	CSH 16	27A × C 43	1997	110	210	42–45	90
17	CSH 17	AKMS 14A × RS 673	1999	105	205	42–45	105
18	CSH 18	IMS 9A × INDORE 12	1999	115	210	40–44	130
19	CSH 19R	104A × R 354	2000	125	165	25–28	45

However, to further enhance productivity, quality, and resistance to the constraints such as drought, s*triga*, grain mold, and insect pests that are so common on farm fields in the tropics, much more needs to be done. The resistance level available in cultivated sorghum types is not adequate to build durable resistance to some of the constraints, especially those caused by insect pests.

Therefore, biotechnological tools like DNA markers, genome mapping, identification, characterization and expression of genes, and genetic engineering have been adopted from the crop improvement perspective to address limitations of classical breeding efforts. It will accelerate identification and incorporation of useful genes into cultivars, facilitate positional cloning of genes, provide new opportunities for assessing and expanding the gene pool in sorghum through comparative mapping of related and unrelated taxa, and contribute to the understanding of the biological basis of complex traits and phenomena important to crop improvement and in the development of transgenics.

7.2 Construction of Genetic Maps

7.2.1 First-Generation Genetic Maps

Construction of a linkage map is the most fundamental step required for a detailed genetic study and marker-assisted breeding approach in any crop (Tanksley et al. 1989). Sorghum genome mapping based on DNA markers began in the early 1990s, and since then several genetic maps of sorghum have been constructed. All the sorghum molecular maps generated to date are summarized in Table 6. Initially, the genetic maps of sorghum were based largely on DNA probes previously mapped in the maize genome (Hulbert et al. 1990; Binelli et al. 1992; Whitkus et al. 1992; Melake-Berhan et al. 1993; Pereira et al. 1994). Later, three more maps were constructed using mainly sorghum genomic DNA probes (Chittenden et al. 1994; Raghab et al. 1994; Xu et al. 1994). Another sorghum map published was based on both maize and sugarcane probes (Dufour et al. 1997). All of these were developed using RFLP markers, and most of the mapping populations were F_2, with the exception of the maps of Dufour et al. (1997) and Peng et al. (1999). Dufour et al. (1997) used two recombinant inbred line (RIL) populations for the construction of a composite map, which was later extended by Boivin et al. (1999) with the addition of a large number of RFLP and AFLP markers to the map of Dufour et al. (1997). Tao et al. (1998a) constructed a sorghum map using an RIL population and variety of probes, including sorghum genomic DNA, maize genomic DNA and cDNA, sugarcane genomic DNA and cDNA, cereal anchor probes, and eight SSR loci. They attempted to review and compare their map with other published maps, which is supposed to enhance the effectiveness of mapping information and facilitate efforts to map agronomically important traits in sorghum. However, Subudhi and Nguyen (2000) completely aligned all ten linkage groups of all major sorghum RFLP maps using a common RIL population and sorghum probes from all three sources (Chittenden et al. 1994; Raghab et al. 1994; Xu et al. 1994) along with many cereal anchor and maize probes.

Kong et al. (2000) mapped 31 polymorphic SSR loci obtained from 51 clones isolated from a size-fractionated genomic DNA library of *S. bicolor* (L.) Moench that had been probed with four radiolabeled di- and trinucleotide oligomers using an RI population BT × 623 × IS3602C. Taramino et al. (1997) have characterized a total of 13 SSR loci in *S. bicolor* and mapped seven of these using an existing sorghum RFLP map.

Haussmann et al. (2004) have mapped molecular markers for resistance of sorghum to the hemiparasitic weed *Striga hermonthica* in two recombinant inbred populations (RIP-1, -2) of $F_{3.5}$ lines developed from the crosses IS9830 × E36-1 (1) and N13 × E36-1 (2). The resistant parental lines were IS9830 and N13; the former is characterized by a low stimulation of striga seed germination, the latter by "mechanical" resistance. The genetic maps of RIP-1 and RIP-2 spanned 1,498 cM and 1,599 cM, respectively, with 137 and 157 markers distributed over 11 linkage groups.

7.2.2 Integrated Genetic Maps

An integrated SSR and RFLP linkage map of the sorghum was reported by Bhattramaki et al. (2000) using 18 diverse sorghum lines. They designed SSR loci from clones isolated from two sorghum bacterial artificial chromosome (BAC) libraries, their enriched sorghum genomic DNA (gDNA), and sorghum DNA sequences present in public databases. The linkage

Table 6. Sorghum genetic maps developed to date

S. no.	Cross	Mapping population	Types of marker	Number of linkage group	Reference
1.	Shanqui Red × M91051	F2	Cloned maize DNA fragments from 14 characterized genes and 91 random fragments	8	Hulbert et al. (1990)
2.	Shanqui Red × M91051	F2	Maize DNA fragments	15	Melake-Berhan et al. (1993)
3.	S2482C × IS18809	F2	Isozymnes and maize nuclear sequebces	13	Whitkus et al. (1992)
4.	IS 18729 × IS 24756	F2	Maize DNA probes	5	Binelli et al. (1992)
5.	CK 60 × PI 229828	F2	Maize and sorghum DNA probes	10	Pereira et al. (1994)
6.	BSC35 × BTx623	F2	Sorghum and maize DNA probes	11	Ragab et al. (1994)
7.	IS 3620C × BT × 623	F2	Sorghum RNA probes	14	Xu et al. (1994)
8.	BT × 623 × *Sorghum propinquum*	F2	Sorghum DNA probes	10	Chittenden et al. (1994)
9.	IS2807 × 379	RILs	Maize, sugarcane, and cereal anchor probes	13	Dufour et al. (1997)
	IS2807 × 249	RILs	—do—	12	Dufour et al. (1997)
10.	IS2807 × 379	RILs	Sorghum cDNA probes, rice, oat, barley, pearlmillet, wheat and maize probes	12	Boivin et al (1999)
	IS2807 × 249	RILs	AFLPs	12	Boivin et al (1999)
11.	QL36 × QL41	RILs	Sorghum, maize, and sugarcane probes	21	Tao et al. (1998a)
12.	B35 × Tx430	RILs	Maize, sorghum, cereal anchor probes	14	Crasta et al. (1999)
13.	BT × 623 × 153620C	RILs	Sorghum, maize genomic DNA clones Rice, maize, barley, oat, and rice cDNA clones	10	Peng et al. (1999)
14.	CK60 × PI22898	F2	SSRs	–	Taramino et al. (1997)
15.	BT × 623 × IS3620C	RILs	SSRs	–	Kong et al. (2000)
16.	IS9830 × E36-1	RIPs	ALFP, SSR,RFLP, and RAPD	10	Haussmann et al. (2004)
	N13 × E36-1			12	
17.	BT × 623 × IS3620C	RILs	AFLP, RFLP, SSRs	10	Menz et al. (2002)
18.	BT × 623 × *S. propinquum*	F2	RFLP	10	Bowers et al. (2003)

map spanned 1,406 cM and consisted of 147 SSR loci and 323 RFLP loci. Klein et al. (2000) constructed an integrated genetic and physical map of the sorghum genome (750 Mbp). They have developed a new high-throughput PCR-based method for building BAC contigs and locating BAC clones on the sorghum genetic map. Subudhi and Nguyen (2000) attempted alignment and integration of all major molecular maps previously developed for sorghum. To achieve this objective, a genetic map of 214 loci with a total map of 1,200 cM was constructed using 98 F_7 sorghum recombinant inbred lines from a cross between B35 and T × 700. Five major restriction fragment length polymorphism (RFLP) maps independently developed were used for alignment purposes.

A high-density genetic map using AFLP technology was constructed by Menz et al. (2002). The 1,713-cM map encompassed 2,926 loci distributed on 10 linkage groups; 2,454 of those loci were AFLP products; 136 SSRs previously mapped in sorghum and 203 were cDNA and genomic clones from rice, barley, oat and maize. Besides, a comprehensive reference map of the sorghum genome (Fig. 3) was also constructed from two recombinant inbred populations using AFLP, SSR, RFLP, and RAPD markers (Haussmann et al. 2002a). Recently, Bowers et al. (2003) reported a genetic recombination map for sorghum of 2,512 loci spaced at average 0.4-cM (~300-kb) intervals based on 2,050 RFLP probes, including 865 heterologous probes from sugarcane, maize, *Oryza, Pennisetum* (pearl millet, buffle grass), the Triticeae (wheat, barley, oat, rye), and *Arabidopsis*.

7.2.3 Comparative Mapping

Geneticists and evolutionary biologists have a long-held interest in the mechanisms involved in chromosomal evolution. Until recently, the primary means of addressing questions surrounding this issue has been via cytological analysis of interspecific hybrids and surveys of naturally occurring chromosomal diversity within populations (Stebbins 1971; Jackson 1984; Grant 1987). Comparative genome mapping adds a powerful new technique for investigating the mode and tempo of chromosomal evolution. This approach involves the use of molecular markers such as restriction fragment length polymorphisms (RFLPs) to map the genomes of two species for a common set of markers (loci). Although a labor-intensive and expensive method, comparative genome mapping allows one to determine the extent and nature of chromosomal rearrangements between cross-incompatible species. This method thus opens up comparisons among distantly related genomes that are not amenable to analysis by traditional cytogenetic techniques. This approach was pioneered by Tanksley and coworkers using tomato RFLP probes to map the tomato (Tanksley et al. 1988). Recognition of the considerable conservation of features within sets of plants such as rice, wheat, and maize (Ahn et al. 1993); sorghum and maize (Pereira et al. 1994; Paterson et al. 1995b); wheat, barley, and rye (Devos et al. 1993); tomato, pepper, and potato (Tanksley et al. 1988, 1992); and *Arabidopsis* and *Brassica* (Teutonico and Osborn 1994) has inspired the suggestion of considering such groups as single genetic systems (Bennetzen and Freeling 1993; Helentjaris 1993). The recent discovery of small chromosomal regions retaining similar gene order in sorghum and two dicot species (*Arabidopsis* and cotton) suggests that comparative mapping may ultimately reach across a much greater "evolutionary distance" than has been spanned to date (Paterson et al. 1996). This concept should have considerable merit and mutual advantages for both breeders and geneticists.

The comparative mapping results between sorghum and closely related grass species are described below.

Sorghum, Maize, and Rice

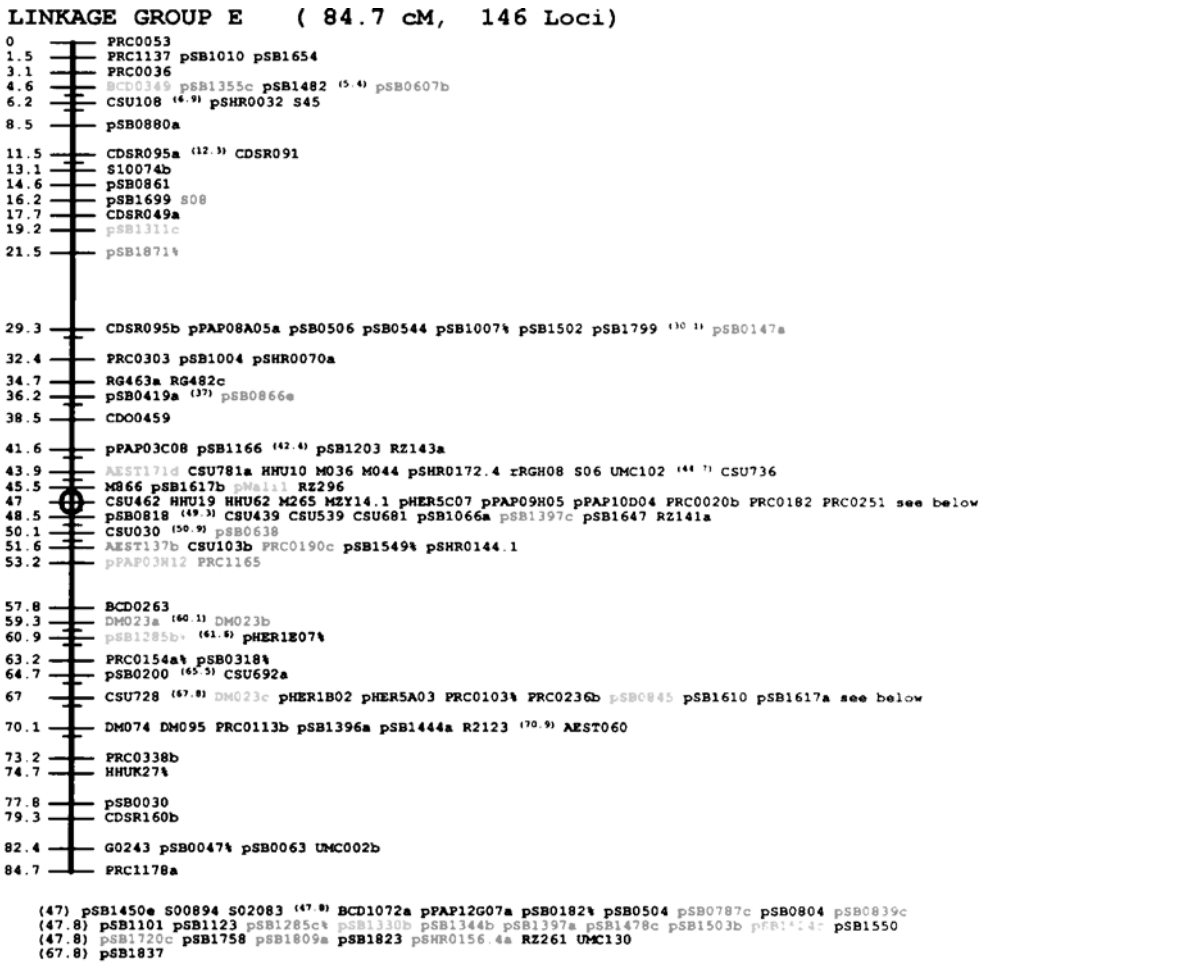
Within the tribe Andropogoneae, comparative mapping facilitates an understanding of sorghum genetics. Several groups established the relationship between the sorghum and maize genomes (Hulbert et al. 1990; Whitkus et al. 1992; Melake-Berhan et al. 1993; Grivet et al. 1994; Pereira et al. 1994; Paterson et al. 1995b; Dufour et al. 1997). Gene orders appear to be largely conserved between sorghum and maize; only a limited number of rearrangements have been identified. With the exception of major evolutionary translocations, which characterize the Panicoideae, extreme colinearity also appears to have been maintained with rice. An RFLP linkage map of *S. bicolor* (L.) Moench was constructed (Peng et al. 1999) in a population of 137 F_{6-8} recombinant inbred lines using sorghum, maize, oat, barley, and rice DNA clones. The map consisted of 10 linkage group and 323 markers. Comparison of the map with RFLP maps of maize, rice, and oat produced evidence for sorghum-maize linkage group rearrangements and homoeologies not reported pre-

Fig. 3. Sorghum genetic map (Reprinted, with permission of Genetics Society of America, from Bowers et al. 2003)

Fig. 3. (continued)

Fig. 3. (continued)

Fig. 3. (continued)



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viously. Comparative maps of rice and maize (Ahn and Tanksley 1993) may help to link rice and sorghum using maize as a bridge. This may be extended similarly to wheat (Ahn et al. 1993). Comparative maps should make it possible to begin uniting the genetics of these species and allow for transfer of mapping information (including centromere positions) and molecular-marker resources (e.g., RFLP probes) between species. In addition, such maps should shed light on the nature of chromosome evolution that accompanied the radiation of grasses in the early stages of plant diversification.

The extent of colinearity and other aspects of genome structure in cereals were investigated by cloning *Sh2* homologs from sorghum and rice using the maize *Sh2* gene as a probe in screening rice and sorghum bacterial artificial chromosome libraries (Woo et al. 1994; Zhang et al. 1996). In maize, the *Sh2* and *Al* loci are separated by about 140 kbp (Civardi et al. 1994). In both sorghum and rice, an *Al* homolog is near the *Sh2* homologs, but the *Al* and *Sh2* genes are about seven times closer together than in maize (Chen et al. 1997). In addition, the sorghum *Al* homolog was tandemly duplicated. Sequencing these regions indicated that the same genes were present in all three species, but the gene density was about one per 45 kb in maize and about one per 10 kb in sorghum and rice (Chen et al. 1998). A third gene encoding a putative transcription factor was located between these two loci, but no other sequences in the region were conserved except the genes. Comparative analysis of the orthologous *adh1* regions of sorghum and maize revealed the presence of nine known or candidate genes, including *adhl,* in a 225-kbp maize sequence, whereas the homolog of the same nine genes was identified in colinear order along with five additional genes in a 78-kbp space in sorghum (Tikhonov et al. 1999).

Significantly, it was discovered that only the genes cross-hybridized between these two colinear segments of the sorghum and maize genomes. Intergenic regions are likely to have accumulated species-specific sequences, which prohibit prediction of physical distances between homologous genes in related species. This made the genomic cross-referencing technique (i.e., cross-hybridization between homologous segments) (Avramova et al. 1996) a better method for gene identification than either transcript identification (Avramova et al. 1995) or enrichment for single-copy DNA (San Miguel et al. 1996). The combined *Al-Sh2* and *adh1* regions show that grasses often exhibit extensive colinearity and similar gene content at the 50- to 300-kbp level. Therefore, map-based cloning, genomic sequencing, and gene identification using the smaller rice and sorghum genomes will usually be simpler in these species than in maize, barley, or wheat. Thus, a successful and efficient way to find genes in a large region of a complex genome is to use a homologous colinear clone from another species.

To gain insight into the relationship between spatial organization of the genome and genome function, Avramova et al. (1998) identified the locations of the matrix attachment regions (MARs) in the colinear *sh2/a1* homologous chromosome segments of rice and sorghum (30 and 50 kbp, respectively), which could serve as anchors for individual structural units or loops. All identified genes were placed in individual loops of comparable size for homologous genes. Hence, gene composition, gene orientation, gene order, and the placement of genes into structural units have been conserved evolutionarily in this region. Their analysis demonstrated that the occurrence of various "MAR motifs" is not indicative of MAR location. However, most of the MARs discovered in the two genomic regions were found to colocalize with miniature inverted repeat transposable elements (MITEs), suggesting that MITEs preferentially insert near MARs and/or that they can serve as MARs.

The nature, timing, and lineages of most of the genic rearrangements that have differentiated the chromosome segment that is orthologous to the maize *adh1* region of sorghum, rice, and *adh1* homologous region of maize, a remnant of the tetraploid history of the *Zea* lineage over the last 60 million years, was described by Ilic et al. (2003). The rice genome has been the most stable, sharing 11 orthologous genes with sorghum and exhibiting only one tandem duplication of a gene in this region. The lineage that gave rise to sorghum and maize acquired a two-gene insertion (containing the *adh* locus), whereas sorghum received two additional gene insertions after its divergence from a common ancestor with maize. The two homoeologous regions of maize have been particularly unstable, with complete or partial deletion of three genes from one segment and four genes from the other segment. As a result, the region now contains only one duplicated locus compared with the eight original loci that were present in each diploid progenitor. Deletion of these maize genes did not remove both copies of any locus. This study suggests that grass genomes are generally unstable in local genome

organization and gene content but that some lineages are much more unstable than others.

Maize, probably because of its polyploidy origin, has exhibited extensive gene loss so that it is now approaching a diploid state. *Al* toxicity is a major constraint to crop production on acidic soils. To assess the possible ancestral relationship between *Al* tolerance genes in the grasses, Magalhaes et al. (2004) conducted a molecular genetic analysis of *Al* tolerance in sorghum and integrated their findings with those from previous studies performed in crop species belonging to different grass tribes. A single locus, AltSB, was found to control *Al* tolerance in two highly *Al*-tolerant sorghum cultivars. Significant macrosynteny between sorghum and the Triticeae was observed for molecular markers closely linked to putatively orthologous *Al* tolerance loci present in the group 4 chromosomes of wheat, barley, and rye. However, AltSB was not located within the homoeologous region of sorghum but rather mapped near the end of sorghum chromosome 3. Thus, AltSB not only is the first major *Al* tolerance gene mapped in a grass species that does not belong to the Triticeae, but it also appears to be different from the major *Al* tolerance locus in the Triticeae. Intertribe map comparisons suggest that a major *Al* tolerance QTL on rice chromosome 1 is likely to be orthologous to AltSB, whereas a rice QTL on chromosome 3 is likely to correspond to the Triticeae group 4 *Al* tolerance loci. Therefore, this study demonstrates a clear evolutionary link between genes and QTLs encoding the same trait in distantly related species within a single plant family.

To provide a phylogenetic context to two maize genes *r*1 and *b*1, which have been a rich source for studying transposition, Swigonova et al. (2004) sequenced orthologous regions from maize and sorghum (>600 kb) surrounding these genes and compared them with the rice genome. This comparison showed that the homoeologous regions underwent complete or partial gene deletions, selective retention of orthologous genes, and migration of nonorthologous genes.

Rp1 is a complex resistance (R) locus in maize conferring race-specific resistance to a fungal pathogen, common leaf rust (*Puccinia sorghii*). A 268-kb chromosomal segment containing sorghum (*S. bicolor)* genes that are orthologous to the maize (*Zea mays) Rp1* disease resistance (R) gene complex was sequenced (Ramakrishna et al. 2002a) to determine structural variation for an R gene cluster that has diverged at least since the ancestral divergence of maize and sorghum. A region of approx. 27 kb in sorghum was found to contain five *Rp1* homologs, but most have structures indicating that they are not functional. In contrast, maize inbred B73 has 15 *Rp1* homologs in two nearby clusters of 250 and 300 kb. As at maize *Rp1*, the cluster of R gene homologous in sorghum is interrupted by the presence of several genes that appear to have no resistance role, but these genes were different from those found within the maize *Rp1* complex.

Conservation of gene order between sorghum and rice is well documented, which helped to enhance our understanding of cereal genome structure and evolution (Moore et al. 1995; Shimano et al. 1995; Paterson et al. 1995a). Multani et al. (1998) demonstrated that in sorghum and rice, the homologs of a pair of unlinked duplicate genes *Hml* and *Hm2* conferring resistance to *C. carbonum race* 1 in maize map to two chromosomal regions that are syntenic with the regions in maize harboring these loci, indicating that they are related to maize genes by vertical descent. These results suggest that the Hm-encoded resistance is of ancient origin and probably is conserved in all grasses. A direct comparison of the genetic linkage maps of sorghum and rice was done by Ventelon et al. (2001). It was based on the mapping of a common set of 123 RFLP probes scattered on the genomes of both species. For each species a composite map was established by merging two individual maps comprising many common loci. This enabled them to confirm the global correspondence scheme that had previously been established between the chromosomes of sorghum and rice. Morishige et al. (2002) have developed a "gene-island" sequencing strategy that expedites the targeted acquisition of orthologous gene sequences from related species for comparative genome analysis. A 152-kb bacterial artificial chromosome (BAC) clone from sorghum (*S. bicolor*) encoding phytochrome A (*PHYA*) was fully sequenced, revealing 16 open reading frames with a gene density similar to many regions of the rice (*Oryza sativa*) genome. The sequences of genes in the orthologous region of the maize (*Zea mays*) and rice genomes were obtained using the gene-island sequencing method. BAC clones containing the orthologous maize and rice *PHYA* genes were identified, sheared, subcloned, and probed with the sorghum *PHYA*-containing BAC DNA. Comparative mapping of rhizomatousness between rice and *Sorghum propinquum*, a wild relative of cultivated *Sorghum*, indicated that each gene closely corresponds to two major quantitative trait loci (QTL) (Hu et al. 2003). Correspondence of these genes in rice

and sorghum, which diverged from a common ancestor ca. 50 million years ago, suggests that the two genes may be key regulators of rhizome development in many poaceae.

Sequence-based alignment of sorghum and rice chromosomes was attempted by Klein et al. (2003) for refining the sorghum genetic/physical map based on the rice genome sequence. A framework of 135 BAC contigs spanning ca. 33 Mbp was anchored to sorghum chromosome 3. A limited number of sequences was collected from 118 of the BACs and subjected to BLASTX analysis to identify putative genes and BLASTN analysis to identify sequence matches to the rice genome. Extensive conservation of gene content and order between sorghum chromosome 3 and the homologous rice chromosome 1 was observed (Fig. 4). One large-scale rearrangement was detected involving the inversion of an approx. 59-cM block of the short arm of sorghum chromosome 3. Several small-scale changes in gene colinearity were detected, indicating that single genes and/or small clusters of genes have moved since the divergence of sorghum and rice. Additionally, the alignment of the sorghum physical map to the rice genome sequence allowed sequence-assisted assembly of an approx. 1.6-Mbp sorghum BAC contig.

Using bacterial artificial chromosome sequence analysis Ramakrishna et al. (2002b) have studied four orthologous regions in barley, rice, sorghum, and wheat and observed general microcolineariry to shared genes in this region. However, three genic rearrangements were observed. First, the rice region contains a cluster of 48 predicted small nucleolar RNA genes, but the comparable region from sorghum contains no homologous loci. Second, gene 2 was inverted in the barley lineage by an apparent unequal recombination after the ancestors of barley and wheat diverged 11 to 15 million years ago (mya). Third, gene 4 underwent direct tandem duplication in a common ancestor of barley and wheat 11 to 29 mya.

A duplication or diploidization event that predates divergence of taxa from a common ancestor may account for some incongruence in "comparative maps". Specifically, if gene loss were still continuing at an appreciable rate after taxon divergence occurred, then differential gene loss in independent lineages would cause incongruities in their comparative maps. To test this possibility, Paterson et al. (2004) examined a sorghum–rice comparative map developed by BLASTing sequences from 2509 genetically mapped sorghum loci (Bowers et al. 2003) against the genome assembly. The positions of 1626 corresponding loci could be plotted based on the rice physical location and sorghum genetic location. This revealed much colinearity, with eight sorghum linkage groups (A, D, E, F, G, H, I, and J) corresponding to single rice chromosomes (1, 4, 12, 2, 5, 11, 6, and 8) and two sorghum linkage groups (B and C) differing from rice by translocations between chromosomes 7/9 and 3/10, respectively.

Sorghum and Sugarcane

The first comparison between the sorghum and sugarcane genomes was mostly indirect, in which maize was used as an intermediate, but it hinted at a large degree of synteny between the genomes of two species (D'Hont et al. 1994; Grivet et al. 1994; McIntyre et al. 2004). Grivet et al. (1994) determined the syntenic genomic regions in maize, sorghum, and sugarcane according to the existing bridge loci. The distribution of these synteny clusters closely matched the duplication pattern in maize. There appear to be common chromosome rearrangements between maize and sugarcane and between maize and sorghum. In this respect, sugarcane and sorghum appear to be more closely related than either is with maize. Distances between genes were similar in maize and sorghum, whereas sugarcane tended to display less recombination.

Existence of large colinear regions among the three species (sugarcane, maize, and sorghum) was also revealed in a study involving comparative genetic mapping between duplicated segments on maize chromosomes 3 and 8 and homologous regions in sorghum and sugarcane (Dufour et al. 1996). Their results emphasize that those duplications will considerably complicate precise comparative mapping at the whole genome scale between maize and other Poaceae. A more elaborate analysis by Dufour et al. (1997) revealed a straight synteny between two pairs of sorghum and sugarcane linkage groups and a large array of colinear probes with sugarcane along the other sorghum linkage groups (Fig. 5). Similarly, colocation of RFLP markers associated with stalk number and suckering in sugarcane with QTLs associated with tillering and rhizomatousness in sorghum was reported by Jordan et al. (2004). Guimaraes et al. (1997) also observed striking colinearity between *Sorghum* and *Saccharum* genomes.

Alignment of complex polyploid genomes of three *Saccharum* species with the compact diploid genome of sorghum ($2n = 2x = 20$) was also reported by

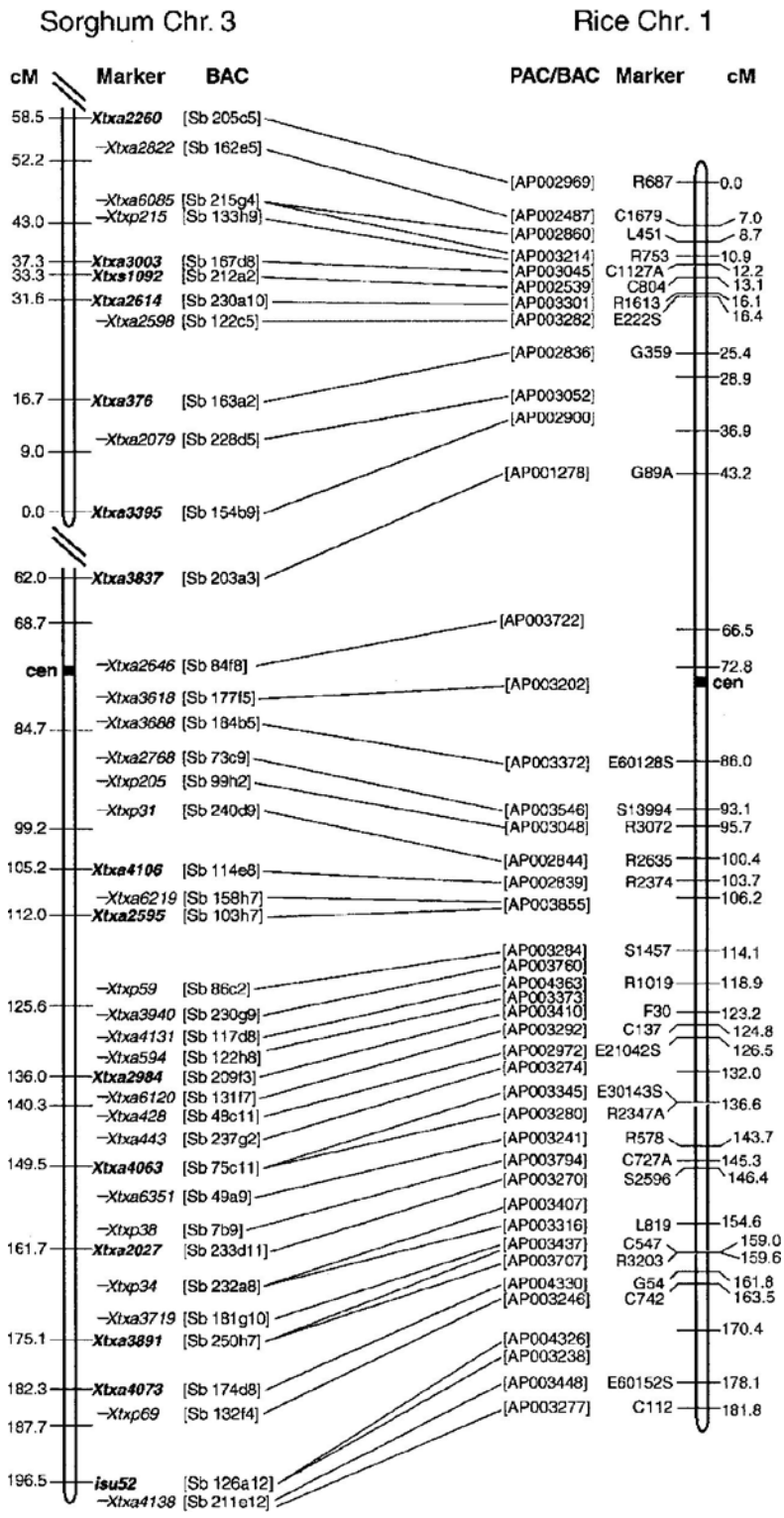

Fig. 4. Sequence-based alignment of sorghum chromosome 3 and rice chromosome 1 (Reprinted, with permission of Blackwell Publishing, from Klein et al. 2003)

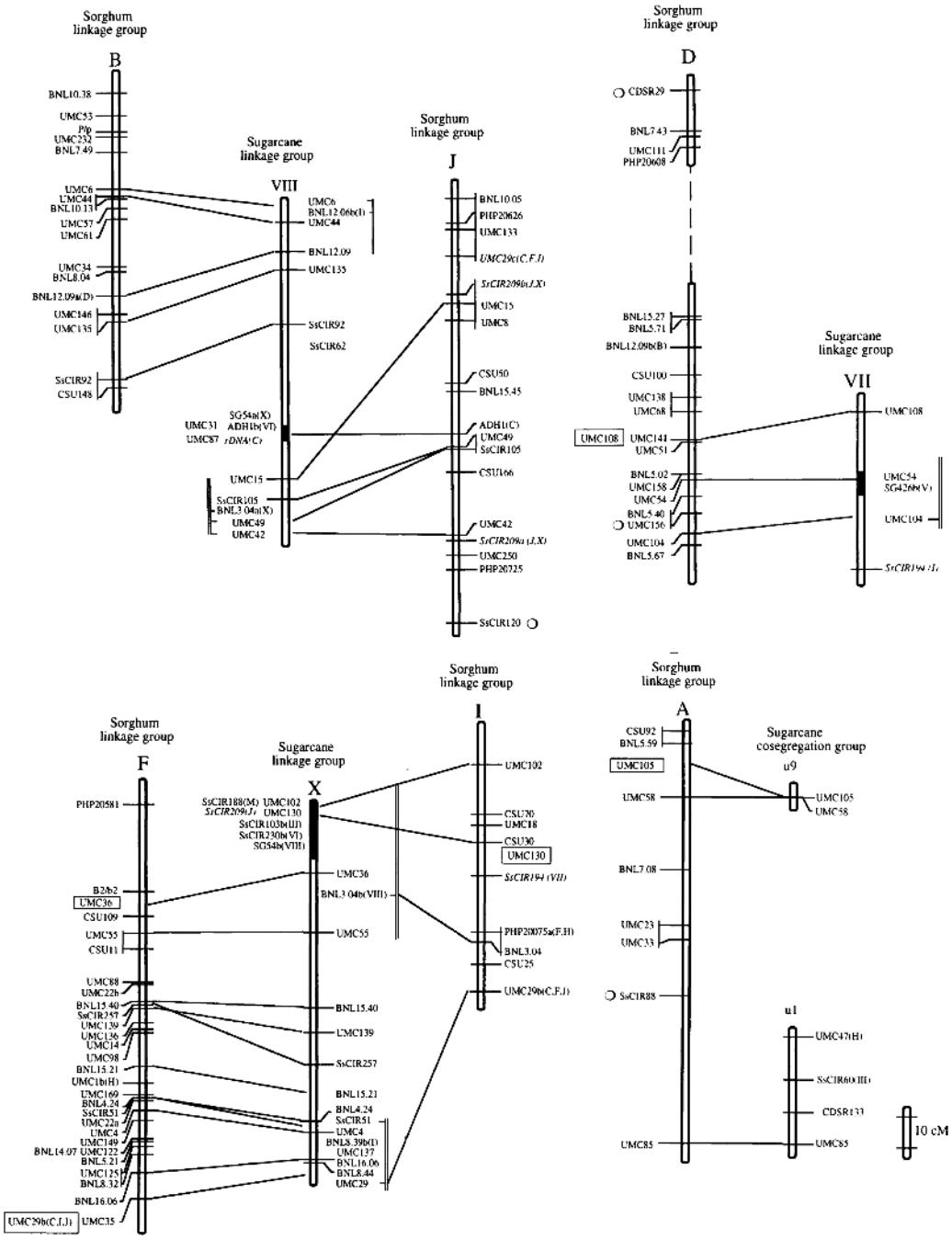

Fig. 5. Comparative mapping between sorghum and sugarcane (Reprinted, with permission of Springer, from Dufour et al. 1997)

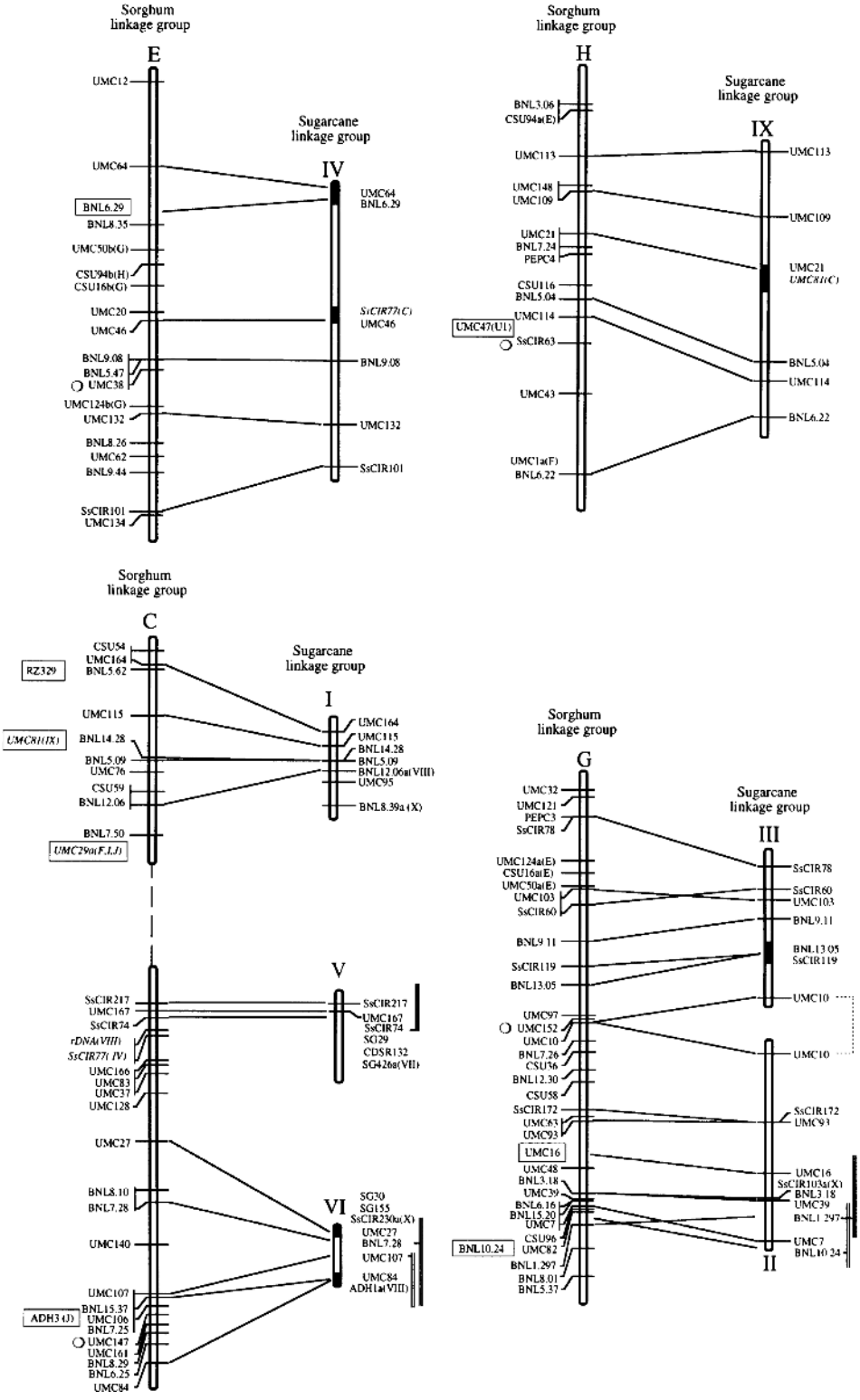

Fig. 5. (continued)

Table 7. Major genes tagged by molecular markers in Sorghum

Trait	Closely linked markers	Reference
Heat smut resistance	RFLP (TXS 560)	Oh et al. (1994)
Shattering	RFLP (PSB 766 and PSB 195)	Paterson et al. (1995b)
Organophosphate insecticide reaction	RFLP (TXS 713)	Toure et al. (1997)
Awn	RFLP (SSCIR 203)	Tao et al. (1998a)
Mesocarp thickness	RFLP (TXS 636)	Tao et al. (1998a)
Juicy midrib	RFLP (CSU6 and UMC34)	Xu et al. (2000)
Red coleoptile	RFLP (UMC 44)	Xu et al. (2000)
Red pericarp	RFLP (TXS 584)	Xu et al. (2000)
Leaf blight resistance	RAPD (OPD12)	Boora et al. (1999)
Male sterility	AFLP	Wen et al. (2002)
Pollen fertility	AFLP; SSR	Klein et al. (2001)
Yield	RFLP	Jordan et al. (2003)
Downy mildew resistance	RFLP	Gowda et al. (1995)
Acremonium wilt, downy mildew, and smut resistance	RFLP, RAPD	Oh et al. (1996)

Ming et al. (1998). Genetic maps of the six *Saccharum* genotypes, constituting up to 72 linkage groups, were assembled into homologous groups based on parallel arrangements of duplicated loci. About 84% of the loci mapped by 242 common probes were homologous between *Saccharum* and sorghum. One interchromosomal and two intrachromosomal rearrangements differentiated *S. officinarum* and *S. spontaneum* from sorghum, but 11 additional cases of chromosome structural polymorphism were found within *Saccharum.* Cross utilization of microsatellites or single sequence repeats developed from sugarcane ESTs between sugarcane and sorghum revealed lower level of polymorphism in sugarcane and a significantly higher level of polymorphism in a related genus *Sorghum sp.* (Cordeiro et al. 2001).

McIntyre et al. (2004) mapped a sugarcane cDNA clone with homoeology to the maize *Rp1–D* rust resistance gene in sorghum. The cDNA probe hybridized to multiple loci, including one on sorghum linkage group E in a region where a major rust resistance QTL had been previously mapped. Partial sorghum *Rp1–D* homologs were isolated from genomic DNA of rust resistance and susceptible progeny selected from a sorghum mapping population. Sequencing of the *Rp1–D* homologs revealed five discrete sequence classes: three from resistant progeny and two from susceptible progeny. Cluster analysis of these sorghum sequences and available sugarcane, maize, and sorghum *Rp1–D* homolog sequences showed that the maize *Rp1–D* sequence and the partial sugarcane *Rp1–D* homolog were clustered with one of the sorghum resistant progeny sequence classes.

Sorghum and Foxtail Millet

Comparative mapping revealed a very close relationship between foxtail millet (Setaria italica) with haploid chromosome $n = 9$ and sorghum with $n = 10$ (Devos and Gale 1997). The difference in chromosome number is accounted for by the synteny of foxtail millet chromosome III with sorghum chromosomes E and I (Devos et al. 1998; Wang et al. 1998). Elsewhere, only one inversion was detected in sorghum chromosome D and one translocation involving foxtail millet chromosomes III and VII, which differentiate the two species.

7.3 Gene Mapping

Determination of the relative positions of genes on a DNA molecule (chromosome or plasmid) and of the distance, in linkage units or physical units, between them is critical for marker-assisted selection, gene cloning, and elucidating the functions of these genes, thereby contributing to accelerated crop improvement. Sorghum is an important target of plant genomics because of its unusual tolerance to adverse environments, a small genome (750 Mbp) relative to most other grasses, a diverse germplasm, and util-

ity for comparative genomics with rice, maize, and other grasses. Efforts are under way for discovery and mapping of genes in sorghum (Table 7). Boora et al. (1999) analyzed the genetic basis for resistance to leaf blight, which revealed resistance was transmitted as a dominant single-gene trait. By combining the random amplified polymorphic DNA (RAPD) technique with bulked-segregant analysis, it was possible to identify PCR amplification products that segregated with disease response. Primer OPD12 amplified a 323-bp band (D12R) that segregated with resistance.

Molecular mapping of a gene for pollen fertility in *Al* (milo) type cytoplasm of sorghum using AFLP and SSR marker analysis was reported by Klein et al. (2001) that will facilitate the selection of pollen fertility restoration in sorghum inbred-line development and provide the foundation for map-based gene isolation. Fifteen AFLP markers were linked to fertility restoration from the initial screening with 49 unique AFLP primer combinations (+3/+3 selective basis). As many of these AFLP markers had been previously mapped to a high-density genetic map of sorghum, the target gene (*rf1*) could be mapped to linkage group H. Confirmation of the map location of *rf1* was obtained by demonstrating that additional linkage group-H markers (SSR, STS, AFLP) were linked to fertility restoration. The closest marker, AFLP *Xtxa2582,* mapped within 2.4 cM of the target loci, while two SSRs, *Xtxp and Xtxp250,* flanked the *rf1* locus at 12 cM and 10.8 cM, respectively. Wen et al. (2002) also reported three RFLP markers suitable for mapping rf4 linked to restoration of male fertility in the sorghum IS 1112 (A3) male sterile cytoplasm.

7.4 Detection of Quantitative Trait Loci (QTL)

Quantitative phenotypes have been a major area of genetic study for over a century because they are a common feature of natural variation in populations of all eukaryotes. They include commercially important traits in crop plants and domestic animals as well as in vital traits in humans from hypertension to intelligence (Kearsey and Farquhar 1998). The first attempt to study individual determinants of quantitatively inherited characters in plants date back to Sax (1923). The studies on quantitative variation suffered from a lack of precision in the absence of complete genetic maps (Thoday 1961). This limitation was overcome with the advent of DNA markers detected as restriction fragment length polymorphism (Paterson et al. 1988). The advent of RFLPs and subsequent PCR-based markers has revolutionized the field of genetic mapping and gene identification in both animals and plants. The basis of all QTL detection is the identification of association between genetically determined phenotypes and specific genetic markers. In sorghum several QTLs have been associated with plant height (Lin et al. 1995) and pre- and postflowering drought tolerance (Tuinstra et al. 1996, 1997). Later Tao et al. (1998b) mapped four regions, each in a separate linkage group, associated with rust resistance (Table 8).

Subudhi et al. (2000) determined the consistency of quantitative trait loci (QTLs) controlling stay-green in sorghum, which is characterized by the plant's ability to tolerate postflowering drought stress by reevaluating the recombinant inbred line (RIL) mapping population from the cross B35 × Tx7000 in two locations over 2 years and compared it with earlier reports. Analysis using the combined stay-green-rating means of seven environments and the expanded molecular map reconfirmed all four stay-green QTLs *(Stgl, Stg2, Stg3,* and *Stg4)* that had been identified earlier by Xu et al. (2000). Similarly, comparison of the stay-green QTL locations with earlier reported results indicated that all four stay-green QTLs showed consistency across different genetic backgrounds. Sanchez et al. (2002) also identified four genomic regions associated with the stay-green trait using an RIL population developed from B35 × Tx7000, whereas Kebede et al. (2001) reported nine QTLs located over seven linkage groups for stay-green using the method of composite interval mapping. In addition, three and four major QTLs responsible for lodging tolerance and preflowering drought tolerance, respectively, were detected. Haussmann et al. (2002b) reported five to eight QTLs for the stay-green trait in two recombinant inbred populations (IS 9830 × E 36-1 and N 13 × E 36-1), and three QTLs present on linkage groups A, E, and G were common to both crosses.

Preharvest sprouting (PHS), one of the important agronomic problems in the production of sorghum [*Sorghum bicolor* (L.) Moench] in humid climates, was studied by Lijavetzky et al. (2000). A molecular linkage map was developed using 112 molecular markers in an F_2 mapping population derived from a cross between IS 9530 (high resistance to PHS) and Redland B2 (susceptible to PHS). Two years' phenotypic data were obtained. By means of interval mapping analy-

Table 8. List of QTLs identified in sorghum

S. no.	Trait	Population	Marker type	No. of QTLs	Reference
1	Stay green	RILs (B35 × TX7000)	RFLP, SSR, RAPD	4	Subudhi et al. 2000
2	Plant height	*S. bicolor* × *S. propinquum*	RFLP	6	Lin et al. 1995
3	Flowering			3	
4	Pre-harvest sprouting	F2 (IS9530x Redland B2)	RFLP	2	Lijavetzky et al. 2000
5	Tiller number	BC1 and F2 (BTx623 × S. propinquum)	RFLP	4	Paterson et al. 1995a
6	Rhizomatousness			3	Paterson et al. 1995a
7	Ratooning ability			6	
8	Stay green	RILs (SC56 × TX7000)	RFLP	9	Kebede at al. 2001
9	Lodging tolerance			3	
10	Pre-flowering drought tolerance			4	
11	Flowering time	RILs (IS2807 × TS 7680)	RFLP	1	Chantereau et al. 2001
12	Photoperiod sensitivity			2	
13	Height of main culm	RILs (BTX623 × IS3620C)	RFLP & SSR	3	Hart et al. 2001
14	Tallest basal tiller height			2	
15	Number of basal tillers			2	
16	Panicle length			3	
17	Panicle width			7	
18	Leaf angle			3	
19	Maturity			2	
20	Awn length			1	
21	Greenbug resistance and tolerance	RILs (GBIK × Redlan)	SSR and RAPDs	9	Agrama et al. 2002
22	Staygreen	RILs (IS9830 × e36-1 and N13 × H36-1)	AFLP, RFLP, SSR, RAPD	5–8	Haussmann et al. 2002b
23	Staygreen	RILs (B35 × TX70000)	–	4	Sanchez et al. 2002
24	Midge resistance (Antixamosis)	RILs (ICSV 745 × 90562)	RFLP SSR	2	Tao et al. 2003
25	*Striga hermonthica*	RIPs (IS9830 × E36-1 and N13 × E36-1)	RFLP AFLP SSRs	11 (RIP1) 9 (RIP2)	Haussmann et al 2004
26	Grain mold	RTx430x Sureno	–	5	Rooney and Klein 2000
27	Rust Resistance	QL 39 × QL 41	RFLP	4	Tao et al. 1998b

sis, two significant QTLs were detected in two different linkage groups with LOD scores of 8.77 and 4.39. Each of these two QTLs individually explained ca. 53% of the phenotypic variance, but together, in a two-QTL model, they explained 83% of the phenotypic variance with a LOD score of 12.37.

The plant *vp1* gene, which encodes a transcription factor originally identified in maize, participates in the control of the transition from embryogenesis to seed germination. Different lines of evidence suggest that *vp1* participates in preharvest sprouting resistance in cereals. Carrari et al. (2003) studied the connection between *vp1* and formerly documented QTLs (Lijavetzky et al. 2000) for PHS in sorghum. Linkage analysis revealed that the sorghum *vp1* (*sbvp1*) locus is linked to markers on chromosomes 3 and 8 in maize, and this gene is not correlated with PHS.

Chantereau et al. (2001) investigated the genetic control of flowering time in sorghum using a recombinant inbred line population derived from a cross between IS 2807, a slightly photoperiod-sensitive tropical caudatum landrace, and TS 7680, a highly photoperiod-sensitive tropical guinea landrace. Emphasis was placed on identifying the most relevant

traits to account for basic vegetative phase (BVP) and photoperiod sensitivity *sensus stricto.* One QTL was detected on linkage group (LG) F for the traits related to BVP. Two QTLs were detected on LGs C and H for the traits related to the photoperiod sensitivity *sensus stricto.* For nine morphological traits, including the presence vs. the absence and the height of basal tillers, number of tillers, plant height, and time of anthesis, Hart et al. (2001) mapped a minimum of 27 unique QTLs.

For resistance and tolerance to green bug (*Schizaphids grami-num* Rondani) biotypes I and K, Agrama et al. (2002) mapped 113 markers (38 SSRs and 75 RAPDs) in 12 linkage groups covering 1,530 cM. In general, nine QTLs were detected affecting both resistance and tolerance to green bug (GB) biotypes I and K. The phenotypic variance explained by each QTL ranged from 5.6 to 38.4%. For green bug biotypes C, E, I, and K, Katsar et al. (2002) also reported at least nine loci, dispersed on eight linkage groups. Tao et al. (2003) identified two and one quantitative trait loci associated with two of the mechanisms of midge resistance, antixenosis, and antibiosis, respectively, in an RI population from the cross of sorghum lines ICSV745 × 90562. Haussmann et al. (2004) detected 11 and nine QTLs in two recombinant inbred populations IS9830 × E 36-1 and N13 × E36-1, respectively, for resistance to *Striga hermonthica*

Comparative Mapping of QTLs

Conversion of gene order along the chromosomes is well known to transgress species boundaries, but the extent of correspondence in the QTLs that account for variation in complex phenotypes has been a point of conjuncture. Paterson et al. (1995b) hypothesized that if QTLs in separate taxa mapped to corresponding locations more often than would be expected by chance, such a finding would strongly suggest that corresponding genes were involved in the evolution of the relevant phenotypes. They tested the hypothesis by assessing correspondence between QTLs that affect seed mass, temperate (day-neutral) flowering, and disarticulation of the mature inflorescence (shattering) in crosses between divergent sorghum, O*ryza* and Z*ea* taxa. Three QTLs that affect seed mass (size) correspond closely in sorghum, rice, and maize, and at least five additional QTLs correspond between two of these genera. Among seven QTLs that account for 52% of phenotypic variance explained (PVE) in sorghum seed mass, five (on linkage groups A, C, E, F, and I) correspond to five of the eight QTLs that account for 78% of PVE in rice. Four of the sorghum QTLs (on linkage groups A, B, C, and F) correspond to four of the eight QTLs that account for 69% of PVE in maize. Five maize QTLs correspond to rice QTLs. Only four QTLs (two on maize chromosome 2, one on rice chromosome 5, and one on sorghum LG J) showed no correspondence. The probability that seed mass QTLs in sorghum, rice, and maize would correspond so frequently by chance is conservatively estimated as 0.1 to 0.8%. QTLs that affect seed dispersal show similar correspondence across taxa. Shattering mapped to a single locus (ca. 100% PVE) in sorghum, three loci (24% PVE) in rice, and ten loci (60% PVE) in maize. The discrete sorghum locus corresponds to rice QTLs on chromosome 9 and to maize QTLs on duplicated regions of chromosomes 1 and 5. Rice QTLs on chromosomes 2 and 3 correspond to maize QTLs on chromosome 4 and 1. Six additional QTLs influence shattering in maize but not in rice or sorghum.

The ability of many cultivated cereals to flower in the long days of summer temperatures may be largely the result of mutations at a single ancestral locus. Sorghum LG D QTL (probably *Ma1*) explains about 86% of PVE in flowering time and accounts for the dichotomy of F_2 phenotypes in our day-neutral (*S. bicolor)* × short-day (*S. propinquum)* cross. It also accounts for short-day flowering in each of the five races of *S. bicolor* (Lin et al. 1995). Short-day flowering of sugarcane is closely associated with the DNA probe PSB188 (Paterson et al. 1995b), which lies near *Ma1*. The corresponding region of maize chromosome 10 accounts for up to 26% of PVE in the flowering of a temperate/tropical cross (Koester et al. 1993). The corresponding region in wheat and barley, the short arm of the group 2 homologs, all harbor photoperiodic flowering mutants (Laurie et al. 1994). In rice, the orthologous (directly descended from a common ancestral locus) region on chromosome 4 harbors no known flowering mutants; however, short-day flowering mutations *Se1* and *Se3* both map to a region of chromosome 6 (Mackill et al. 1993; Causse et al. 1994), that is, are orthologous to sorghum LG I and paralogous (derived by duplication and subsequent divergence from a common ancestral locus) to the sorghum LG D region of *Ma1*. The *Sel/Se3* region of rice corresponds to a region of maize chromosome 9 that harbors QTLs that affect flowering in at least four populations (Lin et al. 1995). This model implies an-

cient duplication of regions of maize chromosomes 9 and 10 and regions of rice chromosomes 4 and 6 equivocally supported by the correspondence of *Pi2* and *Pi5t* genes that influence rice blast reaction (Causse et al. 1994). These day-length-insensitive flowering mutations are not in any of at least three genes for phytochrome, a key regulator of photomorphogenesis (Paterson et al. 1995b).

Comparative mapping has provided the basis for parallel investigations of other genetic factors. The first report of detection of orthologous QTLs with the greatest effects on seed weight in mungbean and cowpea was provided by Fatokun et al. (1992). In a similar manner, comparative mapping in maize and sorghum has revealed four putatively orthologous regions for plant height (Pereira and Lee 1995; Lee 1996) and other possible instances of orthologous QTL included regions for maturity and tillering. The putative orthologous regions for plant height are on linkage group A and the long arm of chromosome 1, D and chromosome 5, E and the long arm of chromosome 6, H and chromosome 9 of the sorghum linkage map and maize chromosome, respectively. The regions of the maize plant height QTL also contain genetic loci defined by mutants with qualitative effects on stature, such as *br1* and *an1* on chromosome 1, *na1* and *td1* on chromosome 5, *py1* on chromosome 6, and *d3* on chromosome 9. The effects of some of these maize mutants strongly resemble those of the sorghum plant height QTL and *dw* loci. At least three of the maize loci, *an1*, *br1*, and *d3*, have been tagged with transposons or cloned by various laboratories. These sequences could be used to isolate the related gene from sorghum and further assess the degree and nature of conservation between these two genomes. In sorghum, each region has a major effect on that trait and on a unique suite of other traits (e.g., tillering, panicle dimensions, leaf length, and width), much like some of the *dw* loci in sorghum. Interestingly, plant height mutants at maize genetic loci in related regions have pleiotropic effects on some of the same combinations of traits as the sorghum QTL and the candidate *dw* loci. Possible duplication of QTLs that affect the height of sorghum and maize has also been reported (Lin et al. 1995).

Evidence for several other orthologous regions has also been provided through comparative QTL analysis (Lee 1996). For example, a region of linkage group A (*isu033* to *isul23)* was strongly associated with tillering and production of lateral branches. This region of the sorghum genome is most closely related to the long arm of chromosome 1 of maize. This region of the maize genome is the site of a genetic locus, *tb1*. The mutant phenotype at that locus is characterized by the production of many tillers and lateral branches in a manner strongly resembling the tillering QTL in sorghum. Other possible instances of orthologous QTL included regions for maturity. These observations suggest that the conservation of the maize and sorghum genomes encompass sequence homology, colinearity, and function despite their divergence millions of years ago and subsequent evolution in different hemispheres with contrasting ecogeographical conditions. Thus, comparative QTL mapping provides a means to unify, and thereby simplify, molecular analysis of complex phenotypes.

7.5 Marker-Assisted Breeding

7.5.1 Marker Conversions

Molecular markers help unravel patterns of diversity in crops and their wild relatives. DNA markers are used to evaluate the genetic variation in gene banks as well as to identify phylogenetic and molecular structure of crops and their associated wild species. Molecular-assisted genetic analysis provides a means to locate and select genes controlling important agronomic, pest-resistance, stress-tolerance, and food quality traits.

For leaf blight resistance, Boora et al. (1999) developed RAPD primer OPD12, and a 332-bp PCR band has been converted into SCAR, which resulted in the amplification of a single major band of the predicted size from all the resistant F_2 progeny and the resistant parent SC326-6, but not from BT $\times$ 623 or 24 of 29 susceptible F_2 progeny. The SCAR primers also amplified a single band with DNA from TS3620C, the female parent in a cross with BT $\times$ 623 that has been used to produce a recombinant inbred population for RFLP mapping. An equivalent band was amplified from all 137 recombinant inbred progeny, indicating that organelle DNA is the amplification target in this cross.

The gene *rf4* restores fertility in IS1112 (A3) male sterile cytoplasm, for which three AFLP markers were identified and subsequently converted to STS/CAPS markers, two of which are codominant (Wen et al. 2002). Markers LW8 and LW9 were used to screen sorghum BAC libraries to identify the genomic region

encoding *rf4*. A contig of BAC clones flanking the LW9 marker represents seed clones on linkage group E, from which fine mapping of the *rf4* locus and chromosome mapping can be initiated.

7.5.2 Marker-Assisted Selection

Conventional plant breeding is primarily based on phenotypic selection of superior individuals among segregating progenies resulting from hybridization. Although significant strides have been made in crop improvement through phenotypic selections for agronomically important traits, considerable difficulties are often encountered during this process primarily due to genotype-environment interactions. Molecular-marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers. With the development and availability of an array of molecular markers and dense molecular genetic maps in crop plants, MAS has become possible for traits governed by both major genes and by quantitative trait loci (QTLs).

Grain mold caused by *Curvularia lunata* (Wakker) Boedijn is a serious disease on sorghum especially when grain development coincides with wet and warm weather conditions. Rooney and Klein (2000) identified five QTLs on linkage groups D, E, F, G, and I using a mapping population consisting of 125 F_5 RILs from a cross between RT × 430 × Sureno. Five populations were developed using Sureno as grain mold resistant parent. From each cross, F_2 progeny were selected based on maturity and short plant height. A total of 1,000 $F_{2:3}$ lines were evaluated for agronomic desirability and grain mold resistance. From this evaluation, a total of 100 $F_{3:4}$ lines were selected and advanced. In the F_4 generation, an array of molecular markers linked to the sorghum grain mold QTL was screened. To test the effectiveness of MAS, lines from each population were classified for QTL marker alleles at each of the five loci. This comparison indicated that only one of the five QTLs enhanced selection for grain mold resistance. The presence of the Sureno allele in LG-F enhanced mold resistance. MAS was clearly effective in the population derived from crosses with RT × 430 since these QTLs were developed in this population (Rooney and Klein 2000).

Drought is another major limiting factor in sorghum productivity. Moisture stress during both pre- and postflowering stages reduces sorghum yield drastically. Therefore, improvement in both pre- and postflowering drought tolerance is necessary to improve and stabilize productivity of sorghum in stress environments. Subudhi et al. (2000) have identified QTLs for stay-green, postflowering drought tolerance trait using three random inbred lines (RILs). Near-isogenic lines (NILs) for stay-green QTLs have been developed using MAS to dissect the QTL regions and to determine the effect of QTLs in stress environments.

Jordan et al. (2003) investigated the value of molecular-marker-based distance information to identify high-yielding grain sorghum hybrids in Australia. Data from 48 trials were used to produce hybrid performance estimates for four traits (yield, height, maturity, and stay-green) for 162 hybrid combinations derived from 70 inbred parent lines. Each line was screened with 113 mapped RFLP markers. The researchers utilized the concept of using diversity on linkage groups to predict hybrid performance. Using data from just two linkage groups, 38% of the variation in hybrid performance for grain yield could be explained. A model combining phenotypic trait data and parental diversity on particular linkage groups explained 71% of the variation in grain yield and has potential for use in the selection of heterotic hybrids.

7.6 Physical Mapping in Sorghum

Molecular physical mapping will provide an invaluable, readily accessible system for many detailed genetic studies. The development of large DNA fragment (>100 kb) manipulation and cloning technologies, such as pulsed-field gel electrophoresis (PFGE), and yeast artificial chromosome (YAC) (Burke et al. 1987) and bacterial artificial chromosome (BAC) (Shizuya et al. 1992) cloning have provided the powerful tools needed to generate molecular physical maps for genomes of higher organisms. Once generated, the physical map will provide a virtually unlimited number of DNA markers from any chromosomal region for gene tagging, gene manipulation, and genetic studies. It will also provide an online framework for studies in genome molecular structure, genome organization, evolution, and gene regulation. The identification, isolation, characterization, and manipula-

tion of genes will become far more user feasible than ever before. The physical map, therefore, will become central to all types of genetic and molecular enquiry and manipulation, including genome analysis, gene cloning, and crop improvement.

The first construction and characterization of a 2.7 × BAC library from *S. bicolor* cultivar BT × 623 with 13,440 ordered clones and average insert size of 157 kbp was reported by Woo et al. (1994). Sorghum inserts of up to 315 kbp were isolated and shown to be stable when grown for over 100 generations in liquid media. No chimeric clones were detected as determined by fluorescence in situ hybridization of 10 BAC clones to metaphase and interphase *S. bicolor* nuclei. Lin et al. (1999) constructed and characterized a 6.6× BAC library of *Sorghum propinquum,* with 38,016 clones and average insert size of 126 kbp. This wild relative of sorghum has been utilized in RFLP linkage mapping and QTL analysis of many important traits related to domestication and productivity (Chittenden et al. 1994; Lin et al. 1995; Paterson et al. 1995a,b). Further, *S. propinquum* appears to have been the ancestor that conferred many "weediness" traits to johnsongrass (*S. helepense)* and so offers opportunities to pursue new dimensions in agricultural research (Paterson et al. 1995a). This *S. propinquum* library is a valuable complement to an established *S. bicolor* BAC library (Woo et al. 1994) for the cloning of genes associated with domestication and many other traits. Six traits related to domestication were analyzed in the F_2 of a cross between *S. bicolor* cultivar BT × 623 and *S. propinquum*. *S. propinquum* possessed most of the dominant alleles at five traits (grain shattering, plant height, flowering time, tiller number, and rhizomatousness). Dominant and additive alleles have an advantage over recessive alleles in physical mapping, and the testing of candidate DNA sequences for mutant complementation requires that the candidate sequence be genetically dominant or additive. Thus, BAC libraries of wild species offer unique advantages for map-based cloning that harbor dominant and additive alleles for many traits of agronomic importance. Bowers et al. (2001) reported their efforts toward the construction of two physical maps of sorghum based on a 6 × coverage BAC library of *S. propinquum* and 14× coverage BAC library of *S. bicolor*. Markers from a 2,600-loci RFLP-based genetic map of sorghum are being used to probe the BAC libraries either as individual plasmid probes or by using synthetically designed overgo probes. Attempts at constructing robust physical maps of sorghum using a high-density RFLP map as a framework were also reported by Draye et al. (2001); such a map is being assembled by integrating hybridization and fingerprint data with comparative data from related taxa such as rice and using new methods to resolve genomic duplications into locus-specific groups. By taking advantage of allelic variation revealed by heterologous probes, the positions of corresponding loci on the wheat (*Triticum aestivum*), rice, maize, sugarcane, and Arabidopsis genomes are being interpolated on the sorghum physical map. Bacterial artificial chromosomes for the small genome of rice are shown to close several gaps in the sorghum contigs. Characterwise positional cloning efforts are discussed below.

Seed dispersal via disarticulation of inflorescence, or shattering, is an important agronomic trait contributing to significant yield loss in many common cereal crops. Isolation of shattering genes can enhance our understanding of the seed dispersal process and perhaps help us to reduce grain losses. Lin (1998) focused on positional cloning of the sorghum shattering gene, *Sh1*, and used substitution mapping to narrow down the chromosome segment associated with *Shl* to 0.8 cM. Based on these data, *Shl* cosegregates with RZ474 and is flanked by pSB097 and BCD1072b. These three RFLP markers were used to screen the *S. propinquum* BAC library. Twelve BAC clones with an average size of 113 kbp were identified, and nine of them formed a contig spanning the region of pSB097 and RZ474 (*Shl*). Wise et al. (2002) also screened the *S. propinquum* BAC library with DNA markers closely linked to *sh1* for the fine mapping of a chromosomal segment associated with *sh1*. Interval mapping showed that *sh1* cosegregated with one marker, SOG0128, that is located between markers SOG0251 and SOG1273 in a genetic interval of 0.42 cM. Thirteen BACs that hybridized markers in the region formed one contig. One BAC, 39E21, spanned a large part of the contig with SOG0251 at one end, and the *sh1* cosegregation marker SOG0128 near the middle. Sequencing revealed this BAC to be 220 kb in size. But the researchers were unable to extend the BAC contig at satisfactory stringency to include the BAC hybridizing marker SOG1273.

Lin (1998) studied characteristics of photoperiodic-sensing genes in sorghum, a short-day plant, focusing on positional cloning of the sorghum photoperiodic flowering gene, *Ma*1. Previous work on comparative mapping of flowering-time QTLs in the Poaceae has revealed that *Ma1* may be homologous to sugarcane, maize, barley, and wheat

photoperiodic flowering genes and paralogous to rice photoperiodic flowering genes. Substitution mapping was used to narrow down the chromosomal segment containing *Ma1* to 0.5 cM. The two most closely linked RFLP markers, pSB1113 and CDSR084, were used to screen a *S. propinquum* BAC library. These two markers hybridized to ten BAC clones with an average size of 190 kbp, which set the stage for chromosome walking to clone *Mal*. Positional cloning and subsequent analysis of the sorghum photoperiodic flowering gene will pave the way to understanding how photoperiodic genes regulate flowering in response to day length.

Stay-green is an important postflowering drought resistance trait in sorghum. With the objective of isolating the drought resistance genes in sorghum, markers linked to stay-green QTLs (Xu et al. 2000) were used for screening the BAC libraries in Henry Nguyen's laboratory. Several positive BAC clones corresponding to the stay-green QTL 1 and 2 regions were identified, and these positive BACs fall entirely into five contigs. Simultaneously, large mapping populations have been developed using near-isogenic lines for the stay-green QTL regions for fine mapping. Identification of BACs in conjunction with the NIL mapping populations will be a useful starting point for chromosome walking toward the stay-green genes.

The liguleless (*lg-1*) linkage group is a highly conserved region of the rice and maize genome (Ahn and Tanksley 1993). Zwick et al. (1998) used fluorescent in situ hybridization (FISH) for physical mapping of BACs to analyze the *liguleless (lg-1)* linkage group in sorghum and compared it to the conserved region in rice and maize. Six *liguleless*-associated rice RFLP markers were used to select 16 homoeologous sorghum BACs, which were in turn used to physically map the *liguleless* linkage group in sorghum. Results show a basic conservation of the *liguleless* region in sorghum relative to the linkage map of rice. Selected BACs, representing RFLP loci, were end-cloned for RFLP mapping, and the relative linkage order of these clones was in full agreement with the physical data. Similarities in locus order and the association of RFLP-selected BAC markers with two different chromosomes were found to exist between the linkage map of the *liguleless* region in maize and the physical map of the *liguleless* region in sorghum.

Fertility restorer gene *Rf1* in sorghum is very important because of its critical role in hybrid seed production. Klein et al. (2004) utilized four BAC libraries from two unique sorghum genotypes to create an integrated genetic, physical, and cytological map of the sorghum genome targeting *Rf1* gene for positional cloning. Initial cytological examination of this genomic region suggested that the physical size of the trait locus was amenable to positional cloning. A minimum tiling path of BAC clones spanning the *Rf1* locus was subsequently assembled. A key feature in physical map closure in the *Rf1* region was the exploitation of the synteny between rice and sorghum to identify sorghum BACs that span gaps in the sorghum physical map. A 0.5-Mbp genomic region surrounding *Rf1* was sequenced. The development of a high-resolution map for the *Rf1* locus was accomplished in part by identifying sequence polymorphisms in overlapping BACs derived from two unique sorghum genotypes. The culmination of these efforts was the identification of a member of the pentatricopeptide repeat gene family that cosegregates with *Rf1*.

Development of modified cDNA selection protocol to aid the discovery and mapping of genes across an integrated genetic and physical map of the sorghum genome has been reported by Childs et al. (2001). BAC DNA from the sorghum genome map was isolated and covalently bound in arrayed tubes for efficient liquid handling. Amplifiable cDNA sequence tags were isolated by hybridization to individual sorghum BACs, cloned, and sequenced. Analysis of a fully sequenced sorghum BAC indicated that about 80% of known or predicted genes were detected in the sequence tags, including multiple tags from different regions of individual genes. Data from cDNA selection using the fully sequenced BAC indicate that the occurrence of mislocated cDNA tags is very low. Analysis of 35 BACs (5.25 Mb) from sorghum linkage group B revealed (and therefore mapped) two sorghum genes and 58 sorghum ESTs. Additionally, 31 cDNA tags that had significant homologies to genes from other species were also isolated. The modified cDNA selection procedure described will be useful for genomewide gene discovery and EST mapping in sorghum and for comparative genomics of sorghum, rice, maize, and other grasses.

7.7 Structural Genomics

Structural genomic resources for *S. bicolor* (L.) Moench were applied by Islam-Faridi et al. (2002) to target and develop multiple molecular cytogenetic

probes that would provide extensive coverage for a specific chromosome of sorghum. Bacterial artificial chromosome (BAC) clones containing molecular markers mapped across sorghum linkage group A were labeled as probes for fluorescence in situ hybridization (FISH). Signals from single-, dual-, and multiprobe BAC-FISH to spreads of mitotic chromosomes and pachytene bivalents were associated with the largest sorghum chromosome, which bears the nucleolus organizing region (NOR). The order of individual BAC-FISH loci along the chromosome was fully concordant with that of marker loci along the linkage map. In addition, the order of several tightly linked molecular markers was clarified by FISH analysis. The FISH results indicated that markers from the linkage map positions 0.0 to 81.8 cM reside in the short arm of chromosome 1 whereas markers from 81.8 to 242.9 cM are located in the long arm of chromosome 1. The centromere and NOR were located in a large heterochromatic region that spans ~60% of chromosome 1. In contrast, this region represents only 0.7% of the total genetic map distance of this chromosome. Variation in recombination frequency among euchromatic chromosomal regions also was apparent. The integrated data underscore the value of cytological data because minor errors and uncertainties in linkage maps can involve huge physical regions. The successful development of multiprobe FISH cocktails suggests that it is feasible to develop chromosome-specific "paints" from genomic resources rather than flow sorting or microdissection and that, when applied to pachytene chromatin, such cocktails provide an especially powerful framework for mapping. Such a molecular cytogenetic infrastructure would be inherently cross-linked with other genomic tools and thereby establish a cytogenomics system with extensive utility in development and application of genomic resources, cloning, transgene localization, development of plant "chromonomics", germplasm introgression, and marker-assisted breeding. In combination with previously reported work, the results indicate that a sorghum cytogenomics system would be partially applicable to other gramineous genera but recent publication by Kim et al. (2004) has changed this notion completely. They have used FISH-based karyotyping in metaphase chromosomes of elite inbred BT × 623 to estimate the molecular size and to establish a size-based nomenclature for sorghum chromosomes. This size-based nomenclature for BT × 623 represents a reasonable choice as the standard for a unified chromosome nomenclature. Adoption of such a common reference for nomenclature of sorghum chromosomes and a related nomenclature for linkage groups would definitely facilitate development of gramineous genomics, e.g., by enhancing communication between research groups and data usage across genome maps. The unified nomenclature system for chromosomes and linkage groups of line BT × 623 provides a reasonable basis for a genomic nomenclature for *S. bicolor* in that this line is readily available, highly inbred, and extensively used for genetic, breeding, and genomics research. However, caution must be exercised in applying the nomenclature to other mapping endeavors because the incidence of structural rearrangements in sorghum is inadequately studied, so it remains reasonably likely that genomes of mapping parents differ structurally (Kim et al. 2004)

7.8 Functional Genomics

The complete sequence of the Arabidopsis [*Arabidopsis thaliana* (L.) Hyenh.] and rice (*Oryza sativa* L.) genomes ushered plant biology into the postgenomic era. From being largely a genetic black box, the genome sequence is revealing all the possible genes that make up a flowering plant. Now the goal for plant biologists in the postgenome era is to understand the function of every gene and how individual gene products interact and contribute to major plant processes. This new challenge for plant functional genomics is destined to become the most difficult hurdle in plant biology and requires the systematic application of global molecular approaches integrated through bioinformatics. Several tools are now required to decipher gene function including the traditional methods of random mutagenesis, gene knockout and silencing, and the new high-throughput "omic" disciplines of transcriptomics, proteomics, and metabolomics. In the last few years, new techniques for the global analysis of gene expression (including microarrays and DNA chips) using thousands of sequences at a time have been rapidly changing the way to do research to determine gene expression and function for both basic and applied objectives. This shift from the analysis of one gene at a time to thousands at a time has created opportunities to dramatically increase the rate of gene discovery in higher plants and animals. For

an important agronomic crop such as sorghum, the traits of interest include preharvest sprouting, shattering, flowering and fertility, nutritional quality, disease and insect resistance, photosynthesis, drought tolerance, and many others.

7.8.1 Development of ESTs

Expressed sequence tags (ESTs) are currently the most widely sequenced nucleotide commodity from plant genomes in terms of the number of sequences and the total nucleotide count. ESTs provide a robust sequence resource that can be exploited for gene discovery, genome annotation, and comparative genomics (Rudd 2003). To date, 190,949 ESTs in *S. bicolor*, 21,387 in *S. propinquum*, and 1,641 in *S. halepense* (Johnsongrass) have been submitted to GenBank (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html; as of 26 November 2004) from various global EST sequencing projects.

7.8.2 Gene Function Analysis

With the advancement of bioinformatics, sequence analysis of molecular probes to assign function has been realized. Schloss et al. (2002) collected and analyzed DNA sequence data for 789 previously mapped RFLP probes from *S. bicolor* (L.) Moench. DNA sequences, comprising 894 nonredundant contigs and end sequences, were searched against three GenBank databases, nucleotide (nt), protein (nr), and EST (dbEST), using BLAST algorithms. Matching ESTs were also searched against nt and nr. Translated DNA sequences were then searched against the conserved domain database (CDD) to determine if functional domains/motifs were congruent with the proteins identified in previous searches. More than half (500/894 or 56%) of the query sequences had significant matches in at least one of the GenBank searches. Overall, proteins identified for 148 sequences (17%) were consistent among all searches, of which 66 sequences (7%) contained congruent coding domains.

The 3-deoxyanthocyanidins, a unique class of flavonoid phytoalexins, have been reported to be synthesized in sorghum in response to fungal infection. Lo et al. (2002) studied the biosynthetic pathways for 3-deoxyflavonoids, which are known to involve transcriptional activation of chalcone synthase (CHS). CHS, or naringenin CHS, catalyzes the formation of naringenin, the precursor for different flavonoids. They have isolated seven sorghum CHS genes, CHS1 7, from a genomic library on high-density filters. CHS1 7 genes are highly conserved and closely related to the maize C2 and Whp genes. Several of them are also linked in the genome. These findings suggest that they are the result of recent gene-duplication events. Expression of the individual CHS genes was studied *in silico* by examination of EST data available in the public domain. Analyses suggested that CHSl 7 genes were not differentially expressed in the various growth and developmental conditions represented by the cDNA libraries used to generate the EST data. However, a CHS-like gene, CHS8, was identified with significantly higher EST abundance in the pathogen-induced library. CHS8 shows only 81 to 82% identity to CHSl 7 and forms a distinct subgroup in the phylogenetic analysis. In addition, the active site region contains substitutions that distinguish CHS8 from naringenin CHS. The researchers proposed that CHS8 has evolved new enzymatic functions that are involved in the synthesis of defense-related flavonoids, such as the 3-deoxyanthocyanidins, during fungal infection.

Complete sequences of mitochondrial (mt) genomes or chondrions are now available from *Arabidopsis thaliana*. As a consequence of recombination, the order and localization of mitochondrial genes differ largely among plant chondrions. But cotranscripts for two mt genes, *nad*3 and *rps*12, are conserved within angiosperms and also in gymnosperms. The *nad*3 gene codes for a subunit of the mitochondrial NADH-ubichinonoxidoreductase complex, while the *rps*12 gene product is a protein of the mitochondrial small ribosomal subunit. Howad and Kempken (1997) have cloned and sequenced the *nad3-rps12* genes from *S. bicolor*. The DNA sequence was very similar to known sequences from wheat or maize. Both genes were cotranscribed. A total of 17 RNA editing sites in *nad3* and six editing sites in *rps12* were detected. Cotranscripts exhibited a low degree of RNA editing, which was the same in four different fertile and cytoplasmic male sterile lines. In contrast to *atp6* RNA editing, no cell-type specific loss of RNA editing was observed.

Photosynthesis depends upon the strict compartmentalization of the CO_2-assimilatory enzymes of the $C_4$and Calvin cycle in two different cell types, mesophyll and bundle-sheath cells. A differential accumulation is also observed for enzymes of other metabolic

pathways, and mesophyll and bundle-sheath chloroplasts of NADP-malic enzyme type C4 plants differ even in their photosynthetic electron transport chains. A large number of studies indicate that this division of labor between mesophyll and bundle-sheath cells is the result of differential gene expression. To investigate the extent of this differential gene expression and thus gain insight into the genetic basis of C_4 photosynthesis, Wyrich et al. (1998) cataloged genes that are differentially expressed in the mesophyll and bundle-sheath cells in the NADP-malic enzyme type C_4 grass *S. bicolor.* A total of 58 cDNAs were isolated by differential screening. Using a tenfold difference in transcript abundance between mesophyll and bundle-sheath cells as a criterion, 25 cDNAs were confirmed to encode mesophyll-specific gene sequences, and eight were found to encode bundle-sheath-specific sequences. Eight mesophyll-specific cDNAs showed no significant similarities within GenBank and may therefore represent candidates for the elucidation of hitherto unknown functions in the differentiation of mesophyll and bundle-sheath cells. The chromosomal location of 50 isolated cDNAs was determined by RFLP mapping using an interspecific sorghum cross.

Bak et al. (1998) have isolated a cDNA encoding the multifunctional cytochrome P450, CYP71EI, involved in the biosynthesis of the cyanogenic glucoside dhurrin from *S. bicolor* (L.) Moench. A PCR approach based on three consensus sequences of A-type cytochromes P450 – (V/T) KEX (L/F) R, FXPERF, and PFGXGRRXCXG – was applied. Three novel P450 cytochromes (CYP71E1, CYP98, and CYP99), in addition to a PCR fragment encoding sorghum cinnamic acid 4-hydroxylase, were obtained. Reconstitution experiments with recombinant CYP71E1 heterologously expressed in *Escherichia coli* and sorghum NADPH-cytochrome P450-reductase in L-a-dilaurylphosphatidyl choline micelles identified CYP71E1 as the P450 cytochrome that catalyzes the conversion of p-hydroxyphenylacetaldoxime top-hydroxymandelonitrile in dhurrin biosynthesis. In accordance with the proposed pathway for dhurrin biosynthesis, CYP71E1 catalyzes the dehydration of the oxime to the corresponding nitrile, followed by a C-hydroxylation of the nitrile to produce p-hydroxymandelonitrile. In vivo administration of oxime to *E. coli* cells results in the accumulation of the nitrile, which indicates that the flavodoxin/flavodoxin reductase system in *E. coli* is only able to support CYP71E1 in the dehydration reaction and not in the subsequent C-hydroxylation reaction. CYP79 catalyzes the conversion of tyrosine to p-hydroxyphenylacetaidoxime, the first committed step in the biosynthesis of the cyanogenic glucoside dhurrin. Reconstitution of both CYP79 and CYP7 IE1 in combination with sorghum NADPH-cytochrome P450-reductase resulted in the conversion of tyrosine to p-hydroxymandelonitrile, i.e., the membranous part of the biosynthetic pathway of the cyanogenic glucoside dhurrin. Isolation of the cDNA for CYP71E1 together with the previously isolated cDNA for CYP79 provided important tools necessary for the tissue-specific regulation of cyanogenic glucoside levels in plants to optimize food safety and pest resistance.

Preharvest sprouting (PHS) in sorghum is related to the lack of a normal dormancy level during seed development and maturation. Carrari et al. (2001) used a PCR-based approach to isolate two *S. bicolor* genomic and cDNA clones from two genotypes exhibiting different PHS behavior and sensitivity to abscisic acid (ABA). The two 699 amino-acid-predicted protein sequences differ in two residues at positions 341 (Gly or Cys within the repression domain) and 448 (Pro or Ser) and show over 80, 70, and 60% homology to maize, rice, and oat *vp1* proteins, respectively. Expression analysis of the sorghum *vp1* gene in the two lines shows a slightly higher level of *vp1* mRNA in the embryos susceptible to PHS than in those resistant to PHS during embryogenesis. However, timing of expression was different between these genotypes during this developmental process. Whereas for the former the main peak of expression was observed at 20 d after pollination (DAP), the peak in the latter was found at later developmental stages when seed maturation was almost complete. Under favorable germination conditions and in the presence of fluridone (an inhibitor of ABA biosynthesis), sorghum *vp1* mRNA proved to be consistently correlated with sensitivity to ABA but not with ABA content and dormancy.

Sorghum is attacked by *Colletrotrichum sublineolum,* which causes leaf blight. Goodwin et al. (2004) analyzed the types of genes being expressed and their level of expression by conducting single-pass, partial sequencing of cDNA clones to generate expressed sequence tags (ESTs). They compared expressed sequence tag redundancy between EST collections from resistant and susceptible *S. bicolor* inoculated with *C. sublineolum.* Differences in expressed sequence redundancy between interactions included a greater

abundance of heat shock protein ESTs in the susceptible interaction and a greater abundance of cystine proteinase ESTs in the resistant interaction.

7.9 Future Prospects

Population trends predict increasing food needs, while progress in developmental and genomic plant sciences offer new opportunities for crop improvement. Sorghum is an important target for molecular genetic studies because of its adaptation to harsh environments, diverse germplasm collection, smaller genome size, and value for comparing the genomes of grass species such as corn, rice, and sugarcane. Concerted efforts over the past one and a half decades have greatly helped in the construction of integrated and highly saturated molecular maps in sorghum, and the majority of the agronomically important genes have been tagged. Successful utilization of this information in sorghum genetic improvement has not yet been realized. This is largely due to lack of application of marker information in marker-assisted breeding. Molecular breeders must reassess their strategies and design efficient MAS programs to augment efforts in breeding for better plant types to meet the growing needs of modern agriculture.

The most noted accomplishment is in the filed of comparative genomics as sorghum stands central in the Andropogoneae tribe. Sorghum has also served as a model to bridge the comparative analysis between the grass relatives. Conservation of gene order across cereal genomes is evident from several studies. However, very little information is available on chromosome walking and positional cloning of agriculturally important genes in sorghum to facilitate isolation of orthologous genes in the related crop species and vice versa. Physical mapping efforts were initiated (Woo et al. 1994; Lin 1998; Klein et al. 2000; Bowers et al. 2001) and are near completion, which will eventually provide innumerable number of DNA markers from any chromosomal region for map-based gene isolation and a better understanding of genome organization, evolution, and gene regulation.

Recent programs to understand the function of every gene and how individual gene products interact and contribute to major plant processes resulted in the development and deposition of 190,949 sorghum ESTs in GenBank. Utilization of corresponding cDNA clone libraries in large-scale expression profiling will prove to be a valuable resource for gene discovery implicated in plant development processes, disease and insect resistance, drought tolerance, and nutritional qualities.

With the availability of these efficient molecular biology tools in hand, there is a great potential for the exploitation of large genetic diversity as yet untapped so far in sorghum. Furthermore, application of novel gene-combining techniques has the potential to meet the challenges of increasing the productivity of sorghum.

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