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Amaranthus: A Promising Crop of Future

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Preface

Pseudocereals are promising crops for coming decades keeping in view the global food security. Amaranths are a leading group of plants among the pseudocereals that have the great potentiality to prevent malnutrition especially in the low-income food-deficient countries. Though its cultivation and use as food grains have great antiquity, but later its cultivation was ignored by a larger section of people of the world. It lagged behind the conventional cereal crops in spite of being nutritionally very much competitive to those. In the last few decades, the nutritive potentialities and other unique features of amaranths have been rediscovered in different corners of the world. Research done so far on amaranths has surfaced its unique and unparallel nutritive value, wide adaptability, herbicide resistance property and vast germplasm variability. Its germplasm variability has opened up a new avenue to evolve much improved varieties or cultivars. Little efforts have been devoted to improve its genetic background by applying biotechnological methods. But that yielded significant achievements. Keeping in view its tremendous potentiality to become a super crop of coming decades, much more attention is to be given to it especially when the conventional crops are overburdened with a task to feed the huge world population. To increase the food production at a global level and at a sustainable basis, the importance of amaranths cannot be ignored.

In this book, attempts have been made to accumulate updated information, significant research achievements and knowledge about amaranths also to revive interest about amaranths and to explore them comprehensively.

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West Bengal, India; and the head of the Department of Vegetable Crop, TNAU, Coimbatore for their necessary Cooperation.

The effort devoted in this book will be successful if the book gets recognition and appreciation. The author welcomes relevant comments and suggestions for future improvement of this book.

Taki, West Bengal, India Saubhik Das

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Introduction 1

1.1 General

Global food security and struggle against malnutrition are going to face a strong challenge in the coming decades from population outburst specially in the developing and Third World countries. Only a handful of crops have to feed nearly nine billion people all over the world by 2030 as against 5.7 billion at present. The condition is much more threatening due to the declining number of plant species and genetic erosion of overexploited plant species. Scientists all over the world are engaged in exploring the plant biodiversity to broaden the crop list. There are many neglected and underutilised species which are capable of satisfying the demand for food, nutrition and energy. They can function as alternative crops, not competitive to other major crops, and can be adapted to fragile environment and marginal areas needing least agronomic requirements.

1.2 Agricultural Development and Global Food Security

Food is one of the basic needs of human being. The bulk of the food consumed in the world are procured from a very limited number of crop species. Today by and large, 25–30 food-yielding species supply food to mankind, viz. wheat, rice, maize, barley, oats, sorghum, millets, soybean, bean, pea, chickpea, peanut, banana, citrus, tomato, sugarcane, cassava, potato, sweet potato, yams and five oil seed types and a few beverages (Harlan 1975), of which only three crop types, viz. rice, wheat and maize, supply 60 % of food requirements for the world human population. Though over 7000 species either partly or fully domesticated have been used so far from time to time for food production, today only 30 species are bearing the herculean responsibility of providing 95 % of the huge world food requirements. Many of the neglected and underutilised species are of great significance as a source of food in low-income food-deficient countries (LIFDCs). They are extremely important because of their wide adaptability to the marginal areas and contribution of a significant part of the local diet with useful nutritional supplements. In comparison with major crops, these neglected and underutilised species require relatively low input and therefore help in sustaining agricultural production. These regional traditional crops are often low yielding and are not competitive to conventional major crops, even though many of them have the potential to become economically viable. Very often narrow genetic diversities in important agronomic traits as well as lack of genetic improvements prevent the development of these crops. Other constrains include lack of adequate knowledge on the taxonomic delimitation, the genetics of agronomic and quality traits and reproductive biology.

Many plant species with significant food and/or industrial value are yet to be utilised properly due to lack of appropriate and adequate

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programmes for their evaluation and development and remained underutilised. Some of them have been so neglected and erosion of their gene pool is so severe that they are often considered as 'lost crops'. A vast majority of these plants have the capability to meet the increasing demand for food and nutrition, healthcare, medicine and industrial needs. Many of these species are involved in traditional localised farming especially in marginal remote areas, and quite often these crops act as life-savers for millions of poor people in the regions where food security and malnutrition are a problem. The rural community knows very well the cultivation practices of the underutilised crops and prepare food from them and use them in their daily life, for health care, shelter, forage and fuel particularly during drought, famine and the dry seasons (Campbell 1987). These crops include cereal, pseudocereals, fruits and nuts, pulses, vegetables, root and tubers, oilseeds and other industrial, forage and fodder species.

Global food security and economic growth is certainly going to face a stiff challenge in the coming few decades due to population outburst especially in the developing and Third World countries. In an estimate it appears that nearly 1.2 billion people in the world are not lucky enough to get adequate food to meet their daily nutritional requirement and a further 2 billion people are deficient in one or more micronutrients (Azam-Ali and Battcock 2002). The scale of food shortage has been estimated by the Food and Agriculture Organization; Jacques Diouf, its secretary general, says that 800 million people in the world (20 % of the population of developing countries and up to 37 % of sub-Saharan Africa) suffer from shortage of food, and 192 million children have chronic malnutrition. By 2030 the humanity has to perform a task of feeding 9 billion people as against 5.7 billion at present. Nearly 30 % of the total population of Africa is suffering from chronic hunger and malnutrition which compelled the stakeholders in and outside the region to look into possible measures to solve food crisis. The gradually emerging threat of worldwide food shortage compelled the US National Academy of Science (NAS) to issue reports on underutilised tropical plants with promising economic value (1975). In recent times NAS issued further reports (e.g. NAS 1989, 1996) and FAO issued many (FAO 1988; Hernandez Bermejo and Leon 1994).

An autonomous international scientific organisation, International Plant Genetic Resources Institute (IPGRI), was established by the Consultative Group on International Agricultural Research (CGIAR) in 1994, and it is situated in Rome, Italy, at the Food and Agriculture Organization of the United Nations. The prime role of IPGRI is to monitor research activity and to promote and coordinate an international network of plant genetic resource, germplasm management conservation, evaluation, documentation and utilisation of useful plant germplasm globally and also the collection and exchange of plant genetic resources. The functioning of IPGRI and other such institutes is monitored by CGIAR which was established in 1972. The joint efforts of Food and Agriculture Organization (FAO), the World Bank and United Nations Development Programme (UNDP) resulted in the creation of CGIAR to establish international research institutes and subsequently to look after their progress.

Global food security and economic growth is now being threatened by declining number of plant species. This decline has placed the future supply of food and rural income at risk. IPGRI has succeeded in promoting greater awareness regarding the important role that minor crops can play in securing and safeguarding the livelihood of people all over the world. Ethnobotanical surveys confirm the presence of hundred of such crops in many countries and different remote corners of the globe that represent a plentiful treasure of agro-biodiversity. Such underutilised mainly ethnic crops can play a vital role to improve income, food security and nutrition. The development of agriculture and food security depends partly on our ability to extend the agricultural species range in an effective and sustainable manner. This requires finding of ways and means to protect and enhance cultivation of the locally important species so that they can be employed more widely in agriculture and

environment management and finding of ways to explore the use of local crops in order to tap the hidden potential contained in them. Today global food security has become increasingly dependent on few conventional crops causing their over-exploitation. Even if mankind has used more than 10,000 edible species since the prehistoric period, today only 150 plant species are commercialised globally in a significant scale, 12 of which are supposed to provide approximately 80 % dietary energy from plants and only four plant types, viz. rice, wheat, maize and potato are supposed to supply over 60 % of the global requirements for protein and calories. Moreover the gradual decrease of the crop varieties are increasingly threatening the future supply of food and rural income and this has compelled research organisations and scientists worldwide to retrieve, research and disseminate the knowledge regarding production and utilization of neglected, underutilised new plant species or the so-called alternative crop (FAO 1996a, b, c, 2005).

1.3 Underutilised Crop

Underutilised crops can be defined as a class of crop that once grown more rapidly and intensely but lagged behind the conventional major crops in terms of cultivation and use for variety of agronomic, genetic, economic and cultural reasons. They are not properly utilised though not competitive with other crops. Neglected crops appear to be synonymous with underutilised crops. But neglected crops are those crops which are grown mainly in their centre of origin by traditional farmers for local communities and are ill documented and neglected for research and conservation. The benefits and features of these plants are as follows:

1. They are of local importance in consumption and production system.
2. They can grow well in fragile environment and help to stabilise the agroecosystem particularly in arid, semiarid, mountainous locality and tropical forest. They are virtually adapted to any environmental condition, soil types and specialised agro-ecological niche. They can tolerate difficult conditions and environment stresses.
3. Regarding achievement of success in various social objectives like poverty elimination and generation of employment and income opportunities in both rural and urban environment, they have a great role to play.
4. For the sustenance of livelihood through safeguarding food security and widening food basket, their role cannot be ignored.
5. They are the rich source of nutrients and can add nutrients to the diet. These nutrient-enriched foods are convenient for low-income people group.
6. They provide a wide range of crop species to meet new market demands.
7. They have attracted attention of the National Agriculture and Biodiversity Corporation policies, researches and development.
8. They are cultivated and utilised based on indigenous knowledge.
9. They are scarcely represented in ‘ex situ’ collection.
10. They are represented by ecotypes/or landraces.

Pseudocereals: An Efficient Food Supplement

2

2.1 General

Pseudocereals are defined as fruits or seeds of non-grass species that are consumed in very similar way as cereals. Pseudocereals are effective supplements to conventional cereals. The protein contents of pseudocereals like quinoa, amaranths and buckwheat are much higher than cereals, and the quality of proteins is also much improved containing higher amount of lysine which is limiting in cereals. From the angle of digestibility, bioavailability, available lysine and net protein utilisation, pseudocereal proteins are definitely better when compared to cereals. The nutritive value of pseudocereals is very much competitive to conventional crop, in most cases even better.

2.2 Floristic Composition of India

India is the seventh largest and tenth industrialised country in the world with a geographical area of about 3287,263 sq km situated between 804′ N to 3796′ N latitude and 6807’ E to 97025′ E longitude. The Indian subcontinent is divided into four climatological zones – equatorial, tropical, subtropical and warm temperate according to longitudinal variation.

India represents about 11 % of the world’s flora in spite of having only just about 2.4 % of the total landmass. India has two biodiversity hotspots, namely, Eastern Himalaya and Western Ghats, out of the 25 biodiversity hotspots (Fig. 2.1) identified in the world (Myers 1990). These two hotspots show most of the plant diversities in India. As far as species diversity is concerned, approximately 45,000 plant species are recorded in India (Khoshoo 1995; Sharma et al. 1997). In India the angiosperm flora is represented by approximately 17,500 species of which 5725 species belong to endemic category. In another estimation about 28 % of the total Indian flora and about 33 % of angiosperm flora occurring in India are endemic (Nayar 1996). In a rough estimate about 10 % of flowering plant species in India are threatened and 34 plant species have been declared to be extinct (Nayar and Sastry 1987–1990).

India is one of the versatile centres of diversity of cultivated plants. This region is characterised with enormous landrace diversity which is gifted with useful gene pools for tolerance to physiological and ecological stresses and resistance to disease, pest, etc. and good quality traits. Wild relatives and progenitors of cultivated plants are of particular importance. About 326 of such plants have been identified in India. Nearly 1000 wild plant species which are edible have been widely exploited by native tribal people (Arora and Nayar 1984; Arora 1985, 1987; Arora et al. 1991; Pandey 1998; Malik and Singh 2006).

All India Coordinated Research Project (AICRP) on underutilised crop (UUC) was initiated in 1982 with a headquarter at the National Bureau of Plant Genetic Resources (NBPGR),

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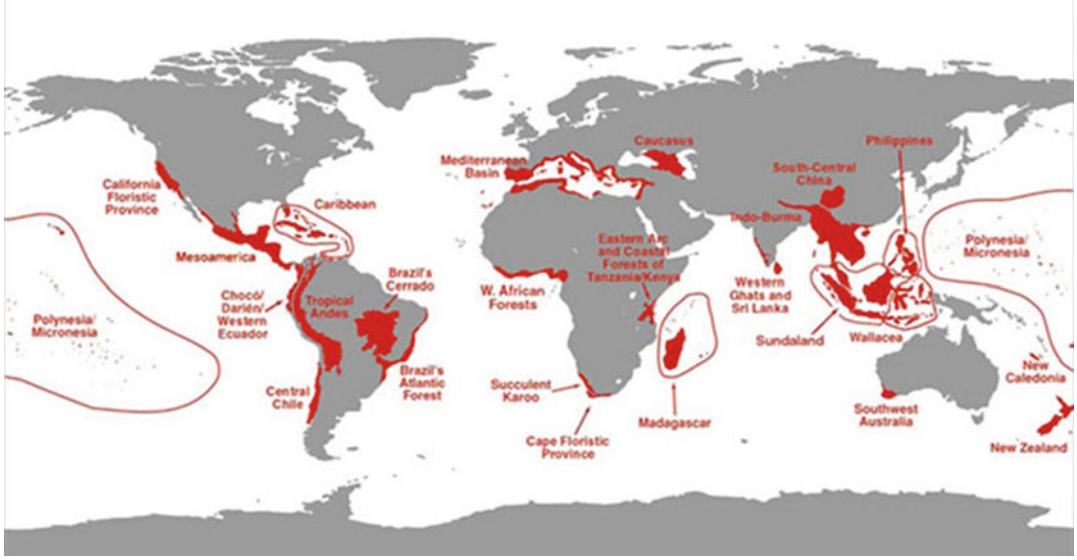

Fig. 2.1 Biodiversity hotspots identified worldwide with two biodiversity hotspot in India

New Delhi, with 15 main centres and 10 co-operating centres in different agricultural zones of the country.

AICRP has identified the following categories of underutilised crops which are to be considered for utilisation:

1. Pseudocereals – Grain amaranths, quinoa, buckwheat, Job's tear, etc.
2. Food legumes and pulses – Rice beans, winged beans, faba beans, etc.
3. Oilseed – Perilla, paradise tree, etc.
4. Vegetables – Kankora, winged bean, etc.
5. Fodder plant – Amaranth, salt bush, etc.
6. Energy, hydrocarbon and industrial plants – Jatropha, guayule, jojobe, etc.

The bulk of India's cereal supply (nearly 75 %) is provided by three major cereal crops like wheat, rice and maize, while minor cereals like *Avena sativa*, *Eleusine coracana*, *Echinochloa* sp., *Hordeum vulgare*, *Panicum miliaceum*, *Pennisetum* sp., *Secale cereale, Setaria italica* and *Sorghum bicolor* and pseudocereals like *Amaranthus*, *Chenopodium* and *Fagopyrum esculentum* provide only the remaining 25 %. More than 1200 cultivated and wild herbaceous plants are consumed as leafy vegetable. By the year 2020, the anticipated food grain demand would escalate up to nearly 250 million tonnes, which means an additional 72 million tonnes of food grains are to be procured.

2.3 Pseudocereals: An Alternative Food Resource

Cereals belong to the grass family (Poaceae) and cultivated for their starchy edible seeds. Pseudocereals are also grown for the same purpose, but they do not belong to the grass family. According to the definition proposed by Shewry (2002), pseudocereals are dicotyledonous species which are not closely related to each other or to the monocotyledonous true cereals. These are grouped artificially keeping in view the use only rather than the biology of plants and rapidly gaining popularity especially in the Third World countries. Cereals and pseudocereals are the primary suppliers of carbohydrates for the world's human population. The human population consume nearly half of the annual cereal production. The primary cereals comprise of rice, wheat, corn, sorghum, millet, oats, barley and triticale. The term millet refers to the small-seeded grain

obtained from few unrelated species. Beside conventional cereals human food resources also include a wide variety of other plants like minor cereals and pseudocereals which are of minor significance not cultivated extensively but not at all negligible. Such plants have some obvious advantages: firstly they are adapted to drought and stress condition, secondly they have the ability to grow on poor soils of marginal areas unfit for other major crops and thirdly local rural people know well how to cultivate and use them. Such plants provide better nutrition than the major crops. The black fonio (*Digitaria iburua*) in Nigeria, white fonio (*Digitaria exilis*) in the rest of the tropical Africa, *Brachiaria deflexa* var. *sativa* and *B. ramose* in certain areas of Africa and the staple cereal Teff grass (*Eragrostis abyssinica*) in Ethiopia have gained great importance and popularity and contributed a lot towards food security specially in Africa. Few dicotyledonous members like *Amaranthus caudatus*, *Amaranthus cruentus* and *Amaranthus hypochondriacus* of Amaranthaceae, *Chenopodium quinoa* (quinoa) of Chenopodiaceae and *Fagopyrum esculentum* and *F. tartaricum* (buckwheat) of Polygonaceae produce starch-rich seeds that can be consumed as cereals, known as pseudocereals (Fig. 2.2).

2.4 Nutritive Value of Pseudocereals

The nutritive value of pseudocereals is much superior than cereals. As far as protein content and protein qualities are concerned, the pseudocereals are much better than the cereal species. Amino acids like tryptophan, lysine particularly lysine which is limiting in cereals is found to be present in high amount in pseudocereals. The amino acids arginine and histidine both proved essential for infant and child health present in significant amount in seeds, which projected amaranth and quinoa as an appropriate food supplement for child nutrition. Net protein utilisation (NPU) or protein efficiency ratio (PER), protein digestibility or bioavailability of protein and available lysine are some of the prime indicators of nutritional quality of a protein. From this view point, the values for pseudocereal proteins are definitely higher in comparison to cereals and are close to those of casein. The protein composition of pseudocereals is typical for dicotyledons in having 2S albumins, 11S and 7S globulins and therefore similar to protein of legumes, crucifers and composites (Marcone 1999). Due to lower prolamine content, the pseudocereal proteins are suitable for the person suffering from celiac disease. The fat content of pseudocereals is also higher compared to most cereal species and that fat is characterised with a high content of unsaturated fatty acids (e.g. linolenic acid). The mineral content in amaranth and quinoa is about twice as high as in other cereals.

The genus *Amaranthus* comprises many edible species besides many weedy members which grow worldwide but prefer the tropical climate. Amaranths are one of the earliest known crop plants extensively cultivated and utilised by the Aztec people, who considered it as a ‘superfood’. Its exceptionally high nutritive value was explored long before. In Africa the leaves of the vegetable amaranths are consumed like spinach. The presence of amaranth seeds in the prehistoric period was evidenced through archaeological excavation at a cave in Tehuacan, Puebla, Mexico. The seeds of *Amaranthus cruentus* collected from Puebla, Mexico, dates back nearly 6000 years. Records from Aztec civilisation indicated the cultivation, collection and use of grain amaranths and also indicated collection of grains in huge quantities along with corn and beans as annual tribute to ruling elite class. The people of Aztec, Mayan and Inca civilisation (Fig. 2.3) used to grow different species of *Amaranthus* and consume both leafy vegetable and cereal grains. In Central America during the Mayan and Aztec period, people used amaranths as principal food. After the colonisation of America, the use of amaranths significantly faded out and its utilisation drastically decreased. This crop is now cultivated only in some pockets of the world as merely traditional regional crop, though global interest in amaranths has been rejuvenated in the last couple of decades.

Enzyme inhibitors and allergens are known to be present in cereals. Protein isolated from wheat,

Fig. 2.2 Different types of pseudocereals (**a**) quinoa, (**b**) buckwheat, (**c**) *Amaranthus*

Fig. 2.2 (continued)

Fig. 2.3 Use of grain amaranths in ancient civilisations

rice, maize and barley may cause allergic reaction, a gliadin fraction isolated from wheat causes celiac disease. But these components are not available in pseudocereals and legumes such as soybean and amaranths (Kuhn et al. 1996). Furthermore, pseudocereals contain dietary fibre in high proportion, which improves lipid metabolism (Gorinstein et al. 1998; Oleszek et al. 1999). The nutritional value of pseudocereals is mainly due to its protein fraction. Natural vegetable proteins of leafy amaranths are useful for its high biocompatibility, nutritional value and low cost. All the pseudocereals have major group of 11S globulin storage protein while smaller amounts of 2S albumin and 7-8S globulins are found in buckwheat and amaranths.

Pseudocereal quinoa (*Chenopodium quinoa*) is considered as the mother of all grains by the Incas and originated in the Andean region of South America. It is now produced in Bolivia, Peru and Ecuador. Unlike many other grains, it has a significant amount of protein and a well-balanced amino acid profile including high concentration of lysine (generally low in most other cereals). Its recognition as grain of high nutritive value has not been changed much since the Inca period, and today it is sometimes considered as ‘supergrain’. It resembles amaranths in having a relatively high protein content of good nutritive value and great tolerance to arid condition (Taylor and Parker 2002). Possible utilisation of quinoa as food was studied by several research-

ers emphasising its use in the production of bread and cakes (Been and Fellers 1982; Lorenz and Coulter 1991; Chauhan et al. 1992) and pasta (Caperuto et al. 2001). Amaranths and quinoa both produce significant amount of edible grain, especially amaranth which is considered as the grain of the twenty-first century (Oleszek et al. 1999; Vetter 1994; Zheleznov et al. 1997; Gorinstein et al. 1996). Both amaranths and quinoa are very rich source of minerals and vitamins than most of the cereals (Vetter 1994; Gorinstein et al. 1996). Qualitatively and quantitatively the protein of grain amaranths is more superior than cereals and legumes (Singhal and Kulkarni 1988). The protein content of *Amaranthus* is about 16 %, and proteins are also enriched with high content of arginine, lysine, tryptophan and sulphur containing amino acids (Oleszek et al. 1999; Vetter 1994). The lysine content of amaranth is twice that of wheat and thrice that of maize, and the nutritive value is about 75 compared to 44, 57 and 62 for maize, wheat and barley, respectively (Zheleznov et al. 1997). It has also been observed that the globulin fraction of oat and amaranth is highly homogeneous and shares similarity with the legume 11S storage protein (Bressani and Garcia-Vela 1990; Segura-Nieto et al. 1994; Gorinstein et al. 1999). Main storage protein fractions, such as prolamins in cereals and globulins in pseudocereals were investigated by several workers (Konishi and Yoshimoto 1989; Ker et al. 1993; Gorinstein et al. 2002). A correlation between nutritive value of protein and amino acid composition revealed a close identity between soybean and amaranth. The suitability of amaranths as a nutritive substitute for cereals is well substantiated by its rich protein and amino acid composition.

Buckwheat, not a type of wheat but another pseudocereal, has also gained attention as a prospective crop. It also contains protein of high nutritional value and the protein is relatively rich in lysine and other essential amino acids. It is enriched with high amount of phenolics, iron, chromium, calcium, magnesium, selenium and polyunsaturated fatty acids. Buckwheat originated in the Middle Asia and was transported to Central and Eastern Europe by the nomadic people. Within the thirteenth century, buckwheat gained some importance in Germany, Austria and Italy, which was eventually lost due to the cultivation of other cereals. Today, due to the demand of gluten-free diet, the interest in buckwheat has been revived (Tables 2.1a and 2.1b).

According to the recent APG classification, both the genus *Amaranthus* and *Chenopodium*

Table 2.1a Percentage based dry weight of chemical components of amaranth, quinoa and buckwheat

Components	Amaranth (*Amaranthus cruentus* L.)	Quinoa (*Chenpodium quinoa* L.)	Buckwheat (*Fagopyrum esculentum*)	Wheat (*Triticum aestivum* L.)
Protein	15.2	13.3	10.9	11.7
Fat	8.0	7.5	2.7	2.0
Starch	67.3	69.0	67.2	61.0
Ash	3.2	2.6	1.59	1.8

Souci et al. (2000)

Table 2.1b Comparative account of nutritive value of grain amaranths and other cereals

Crops	Protein	Fat	Carbohydrate	Calcium	Iron	Phosphorus	Food energy
Amaranth	16.0	3.1	60.0	0.49	17.5	0.60	391
Rye	12.1	1.7	73.4	0.38	10.5	0.37	334
Buckwheat	11.7	2.4	72.9	0.12	15.5	0.28	335
Chenopod	12.0	5.0	68.0	0.20	12.6	0.50	342
Wheat	13.3	2.0	71.0	0.41	10.5	0.37	333
Maize	9.2	3.9	73.7	0.20	3.5	0.25	355
Rice	7.0	1.0	78.0	0.20	3.5	0.18	345

Souci et al. (2000)

are now included in the family Amaranthaceae under the order Caryophyllales, whereas buckwheat (*Fagopyrum* sp.) is included in the family Polygonaceae under the order Polygonales. Polygonales and Caryophyllales are closely linked, though Drzewiecki et al. (2003) found significant genetic divergence between Caryophyllales and Polygonales.

Amaranths: The Crop of Great Prospect

3

3.1 General

Amaranthus is a widely distributed herbaceous genus of herbs comprising approximately 70 species collectively called amaranths or pigweeds. Most of them are annual weeds; few are known as nutritive vegetable and graceful ornamentals, while protein-rich grains of few species are consumed as pseudocereals known as grain amaranths. The species number is still tentative due to misapplication of names and synonyms applied to the misidentified names. Three species of *Amaranthus* are familiar for grain production – *A. hypochondriacus*, *A. caudatus* and *A. cruentus*. According to one school of thought, all the grain amaranths are of the New World origin, but other school of thoughts suggested that grain amaranths might have been cultivated in South Asia from prehistoric period and probably have domesticated there. A comparative study of grain amaranths in India and Central America indicated close similarity in species distribution, evolution, variety pattern and cultivation practices. Seeds of grain amaranths are very rich in crude protein with lysine and threonine. Average protein score is either equal or much greater than rice, wheat, soybean and maize. Seed oil contains squalene, trypsin inhibitor, tocotrienols, tannins, etc. Vegetable amaranths are the most popular vegetable crops in tropics especially in the tropical humid climate of Africa and Asia. Several species are known as vegetable amaranths of which two are most popularly grown, *A. tricolor* and *A. blitum*. The tender plant of grain species *A. cruentus* is also consumed as leafy vegetable. Green amaranths are rich source of lysine-rich protein, β-carotene, various vitamins, minerals and dietary fibres. Anti-nutrients like nitrates and oxalates are present in small amount that does not cause any nutritional problem under normal condition of consumption. The underutilised amaranths are projected as crop of the twenty-first century because of their health benefits and nutritive value. Vegetable amaranths provide energy, help to maintain proper mineral balance, reduce bad cholesterol, improve eyesight and prevent anaemia. Compared to wheat, rye, rice and oat, grain amaranths are gluten free and contain 30 % more protein with complete set of amino acid. Amaranth grain may be processed in various forms like flaked, popped, extruded and ground into flour and also can be used as a substitute in porridge, stirred into soup. Grain amaranths have several health benefits like lowering of plasma cholesterol level, protection of heart, stimulation of immune system, anticancer activity, control of blood sugar level, improved condition of hypertension and anaemia, anti-allergic and antioxidant activity, etc., due to the presence of some bioactive components. Like other vegetables, green amaranths go through cooking such as frying, simmering, boiling, steaming and blanching before consumption. Cooking has a significant effect on bioactive components either positive or negative depending on the process. Amaranths are also known for its weedy members known as

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pigweed. Approximately ten *Amaranthus* species are regarded as weeds. They are also capable of competing with other crop plants, express highly flexible adaptability to environmental changes and various ecoclimates and ensure their existence producing a large number of seeds.

3.2 Grain Amaranths: A Nutritive Supplement to Major Cereals

Amaranthus L. is a cosmopolitan genus of herbs of the family Amaranthaceae collectively known as amaranths or pigweed. It includes about 70 species (Costea et al. 2001a, b; Iamonico 2012) and 40 of which are considered native to America. In another estimation the genus *Amaranthus* is reported to include 87 species, of which 14 found in Australia, 17 found in Europe and 56 available in America (Jacobsen and Mujica 2001; Mujica and Jacobsen 2003). Among the American species, ten are dioecious and the remaining 46 are monoecious. Dioecious species are confined in North America. Distribution of monoecious species in American subcontinent is scattered: 13 species are endemic to North America and Mexico; 17 species are restricted to Antilles, Central America and South America; and the remaining 16 species are quite common to Americas. It is very difficult to decide which are distinct species and which are synonyms applied to the misidentified specimen. Same new species of considerable phenotypic differences from the existing species may turn out to be ecotypes or natural hybrids of complete sterility or marginal fertility, but such plants may be included as new species (Chan 1996). There is no general agreement on the taxonomy of amaranths and species number. Brenner et al. (2000) and Robertson (1981) mentioned about 60 species, while USDA, ARS included 86 species under the genus *Amaranthus*. Behera et al. (1974) and Brenan and Townsend (1980) included 50 species under *Amaranthus*, while Sauer (1993) considered 75 species under the genus *Amaranthus*. Over 400 varieties are included within *Amaranthus* found throughout the tropical and temperate regions of the globe. Approximately 25 species of *Amaranthus* are available in Asian region. The antiquity of amaranths in Indian subcontinents was evidenced by fossil record of *Amaranthus* pollen documented in several excavations in India that dates back to the Holocene and late Palaeocene periods.

The species of *Amaranthus* are mostly annual weeds; few are utilised as vegetables and ornamentals. Protein-rich grains or seeds of few species (*A. caudatus* L., *Amaranthus hypochondriacus* L. and *A. cruentus* L.) are consumed as pseudocereals; they are called grain amaranths. All the species fall roughly under any of the four categories – grain, vegetable, ornamental and weed. Amaranth is considered as one of the few multipurpose crops which supply seeds in huge quantities that can be used as pseudocereals, as tasty leafy vegetable of higher nutritional quality and also as food and animal feed. Some member has attractive inflorescence of various colours that made them valued as ornamental also. Although the crop was one of the sources of staple food in the pre-colonised South American civilisation, the cultivation and knowledge fell into oblivion, and thus nowadays it could be considered as a new, forgotten, neglected but prospective and alternative crop of immense nutritive potential. The grain amaranths growing in the Himalayan region show wide genetic diversity and morphological variability, which surely substantiate the speculation about probable spread of the crop in India from that region in the eighth century. Based on direct and indirect evidences regarding antiquity of the crop in India, Joshi and Rana (1991) suggested that the grain amaranths were prevalent in South Asia from the time immemorial (Joshi and Rana 1991). In Asia one can find a great ill-defined grain amaranth region extending all the way from Manchuria through interior China and Himalaya to Afghanistan and Persia. Wide scattering of the crop throughout Asia and popularity and traditional use of the grains in marginal areas are supportive to its antiquity in the area.

The word 'amaranth' originated from the Greek word 'amarantos' which means 'unwithering'. The term was applied to amaranth to signify its hearty characteristics symbolising immortality. Grain amaranths are grown all over India from

high slopes of Himalaya to coastlines. A large number of varieties are grown throughout the country, but Himalayan region is considered to represent the centre for diversity of grain amaranths in India because varietal and morphotypic variability which is found in Himalaya is unique and unparallel not comparable to other parts of the country. The grain amaranths also grow as native species in the Andean region of South America, including Argentina, Peru and Bolivia. Today in the Andes region, it is widely cultivated. This crop has been termed as 'Incan Wheat' because it was a principal food for the Incas. Today the grains are often familiar by the name *kiwicha*.

3.3 Different Species of Grain Amaranths

Grain amaranths are appropriate to be considered as an alternative and prospective crop in temperate climate. Due to globalisation and industrialisation of agriculture, the food supply gradually became dependent on only a handful of plant species. Grain amaranths may be utilised as an alternative source of food. Sauer's taxonomic key (1967) recognised three principal species of genus *Amaranthus* for grain production:

- *A. hypochondriacus* L. (sin. *A. leucocarpus* S. Watts, *A. frumentaceous*) - Prince's feather (Fig. 3.1);
- *A. cruentus* L., sin. *A. paniculatus* L. - bush greens, red amaranth (Fig. 3.2)
- *A. caudatus* L. of two subspecies: subsp. *caudatus*; and subsp. *mantegazzianus* Passerini syn.: *A. edulis* Spagazzini, named love-lies-bleeding and Inca wheat, respectively (Fig. 3.3)

Within each grain species, there are several grain types or races defined by their common branching pattern, height, inflorescence size and form, days to maturity, seed size and colour and other morphological characteristics (Kauffman 1992; Espitia-Rangel 1994; Brenner et al. 2000). Though cultivation of grain amaranths has a great antiquity, today it is cultivated in small scale in some discrete pockets of the world like in parts of Mexico, Guatemala, Peru, India and Nepal. It has a bright prospect for further cultivation in the USA and tropical countries. The grain amaranths have been described as native crops of low profile that could be cultivated easily by native people in

Fig. 3.1 Morphology of *Amaranthus hypochondriacus* L.

Fig. 3.2 Morphology of *Amaranthus cruentus* showing different morphotypes with massive colourful terminal inflorescence

rural areas for several reasons: (i) They can be harvested easily, (ii) they grow very fast and produce a lot of seeds which are used as pseudocereals (iii) tolerant to arid environment and suited for subtropical and tropical region and (iv) seeds contain large amount of protein and essential amino acids.

Although grain *Amaranthus* is a crop of the Americas, *Amaranthus hypochondriacus* migrated to Asia. During the last century, increasing popularity in its cultivation has been observed among the hill tribal areas in India, Pakistan, Nepal, Tibet and China. In India grain amaranths is an important crop of the Himalayan region. Besides upland

Fig. 3.3 Morphology of *Amaranthus caudatus* with characteristic drooping inflorescence

Asian region in the Himalayan belt, grain amaranths are also gaining popularity among the people in northwestern plains of India as well as in the hilly regions of Southern India with a common name Rajgira ('king seed'), Ramdana ('seed sent by God') and Keerai. It often occupies more than half of the non-irrigated crop land of the higher elevation in the hills of North-West. It is now a prominent crop in a few local areas in India where a kind of bread made from its seed flour is a popular food. Popped grain are used to make confectioners and also taken soaked in milk.

According to Sauer (1950), there are four major grain amaranth regions in the Americas, each having its own particular cultivated species: the Mexican centre which is dominated by *A. hypochondriacus*, Guatemala with its main crop of *A. cruentus*, the Andes with *A. caudatus* and Argentina with *A. edulis.* In subsequent analysis of the genus, it was concluded that *A. edulis* was a variety of *A. caudatus*, and thus, the latter two centres, the Andes and Argentina, could be considered one large region where *A. caudatus* dominates. Sauer concluded that all the grain amaranths are of the New World origin. He acknowledged the observations of other investigators like deCandolle (1883), Hooker (1885), Ames (1939), Vavilov (1949), Darlington and Janaki-Ammal (1945) and Merrill (1950) who opined that grain amaranths have been cultivated in South Asia from time immemorial and probably originated there. This concept has been consolidated by the fact that the grain amaranths growing in the Old World are indistinguishable both taxonomically and morphologically from few specific members of grain amaranths cultivated in the New World. The similarity was observed both at species level and subspecific level. It is speculated that the Old World specimens represent nothing but a small sample of diversity available in the American grain amaranths. Ancient literature of the Old World which has not been searched for evidence of grain amaranths indicates short gaps in the history of grain amaranths in the Old World. The first record of *A. hypochondriacus* in Asia was surfaced through Linnaeus' description of an Indian form called *A. flavus.* The grain amaranths in Asia were first studied by Francis Buchanan Hamilton. In the early nineteenth century, he found grain amaranths in South India and in the Himalayan region. He named the white-seeded grain of South India *A. frumentaceus.* A later observer, Wight (1843), wrote that seeds of *A. frumentaceus* were the principal food of the wild inhabitants of the hill areas in Coimbatore, Salem and Madurai (South India). Sauer noted that the name of the crop recorded by Buchanan Hamilton was

Kiery which is currently grown under the name *A. hypochondriacus*. Amaranths are an important field crop in the foot hills and mountains of the entire Himalayan region. The extensive research on the grain amaranths of the Himalayan region was carried out by Joshi (1978–1988). They are cultivated as a minor crop in Mexico, Guatemala, Peru, Bolivia, Ecuador, Argentina, Sierra Leone, Nigeria, Zambia, Kenya and Egypt, Afghanistan, Persia, China, Manchuria, Nepal, Bhutan and India. Its maximum cultivation and distribution at present is observed in the Himalayas.

On the basis of his detailed study of the Himalayan grain amaranths, Joshi concludes that the cultivation of grain amaranths in India is very ancient. A comparison of collections and evaluation studies of grain amaranths in India by Joshi (1981a, b) and in Central America by Hauptli et al. (1979) indicated close similarity in species distribution, evolution, variation pattern and cultivation practices in two widely separated geographical regions of the world (Table3.1). The study further suggests that either of the grain amaranths, i.e. *A. hypochondriacus* and *A. caudatus*, have independently originated or were introduced in the Old World before 1500 AD. Fossil records of pollens have been found in several digs in India from Holocene and Late Pleistocene periods (Possehlo 1993).

Table 3.1 Comparative study of grain amaranths in India and in Central America

(A) Name of the species	Similarity observed
1. *Amaranthus hypochondriacus*	Distribution from lower to higher altitude in both regions
2. *A. cruentus*	Distributed in lower to middle altitude in both the regions comparatively less adaptable to higher hills than *A. hypochondriacus* and *A. cruentus*
3. *A. caudatus*	Confined to higher elevation in both India and Central America, indicates specific adaptations
4. A new genotype close to *A. edulis*	Found in both the regions in the plant population of amaranth field
(B) Crop characteristics	
1. Seed colour	Seven seed colour types were collected by Hauptli (1979) in Central America and Joshi (1981a, b) in Himalayas
2. Inflorescence types	Similar variation in inflorescence pattern and other plant parts in both regions
3. Harvestability	Presence of circumscissile utricle
4. Cultivation process	Mixed cropping with maize and French beans, indicating close ecological relationship

Joshi and Rana (1991)

3.3.1 *Amaranthus hypochondriacus* L.

Among the grain amaranths, it is the most robust and the highest-yielding grain types. It was probably domesticated in Central Mexico and further north much after the domestication of *Amaranthus cruentus* and reached the USA in prehistoric time, but later became extinct there. The pale seeds of *A. hypochondriacus* of 1500 years back were discovered in the Tehuacan caves. Though it originated in Central America, today it is mostly cultivated in India, particularly in the Garhwal and Kumaon regions of Uttar Pradesh and in the Sutlej Valley of Himachal Pradesh. It shows diversity in habits, and some types of *Amaranthus hypochondriacus* are bushy; others are tall and unbranched. The species is suitable for the tropical areas, high altitudes and dry conditions. It is of greatest potentiality to be used as food ingredient due to its excellent seed quality. The grain mills and pops well that tastes and smells pleasant. The grain reached Europe with the help of the Spanish who took seeds to Europe, and by the sixteenth and seventeenth century, the plants spread through European gardens as graceful ornamentals. Around 1700, it reached Central Europe and Russia as a minor grain crop and consumed as mush and groats. By the early nineteenth century, it was taken to Africa and Asia, and at present it is cultivated as

grain crop in widely scattered regions of the world like the mountains of Ethiopia, the hills of South India, the Nepal Himalaya and the plains of Mongolia (Fig. 3.4).

Amaranthus hypochondriacus is known variously as Prince's feather, Prince-of-Wales-feather and Prince's feather amaranth. It is an annual, erect, herbaceous plant, glabrous or moderately pubescent in distal portion, at maturity often becoming glabrescent, attaining a height up to 3 m. Stems are green or reddish purple in colour, branched, mainly in inflorescences, to nearly simple proximally, coarse. Leaves are simple, petiolate, petioles of the distal leaves equaling or slightly shorter than blade, become longer proximally. Leaf blades are rhombic-ovate to broadly lanceolate or elliptic or ovate-oblong, 4–14×2–8 cm, base cuneate to broadly cuneate, narrowly cuneate in distal leaves, margins entire, apex acute or acuminate or indistinctly emarginate, mucronulate. Inflorescences are enormous, robust, predominantly terminal or lateral thick erect panicle or spikes, dark red or deep beet-red, purple, less commonly yellowish or greenish, leafless at least in distal part, prickly and stiff. Bracts are lanceolate to linear-subulate, spinescent, two times as long as tepals and style branches, texture rigid. Pistillate flowers have five tepals and tepals are slightly recurved, proximal ones lanceolate, distal ones narrowly ovate-elliptic to elliptic, unequal to occasionally subequal, inner tepals shorter than outer tepals, apex acute to acuminate; tepals are comparatively larger than other grain species. Style branches are spreading recurved, meeting in a sharp cleft at thick bases, stigmas 3. Staminate flowers are clustered at tips of inflorescence branches, tepals 5, stamens 5. Fruits are compressed, elongate-ovate to ovoid, circumscissile utricles with broad cap, (1.5 -) 2–3 mm long, equaling tepals or nearly so, smooth or lid slightly rugose or minutely verrucose, dehiscence regular. Seeds are white, pinkish white or black to dark reddish brown, subglobose to lenticular, 1–1.4 mm in diameter, smooth and shiny (Fig. 3.5).

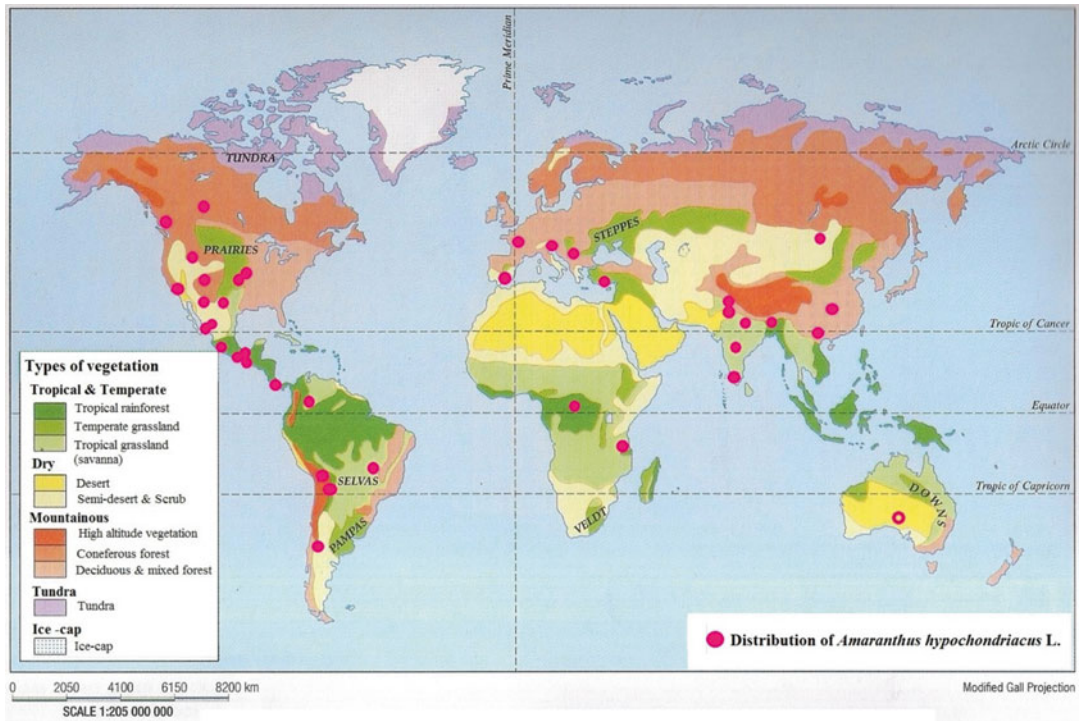


Fig. 3.4 Worldwide distribution of *Amaranthus hypochondriacus* L.

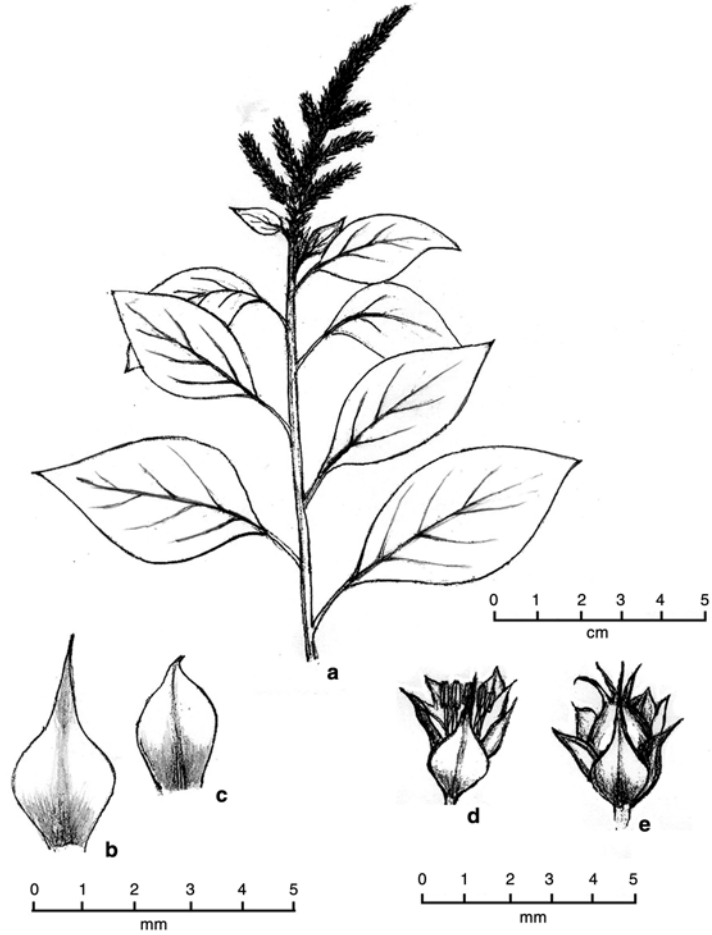


Fig. 3.5 *A. hypochondriacus* L. (**a**) Habit, (**b**) bract, (**c**) tepal, (**d**) male flower, (**e**) female flower

3.3.2 *Amaranthus cruentus* L.

The species was domesticated in Central America (Mexico and Guatemala) much earlier than *A. hypochondriacus*. It is useful both as a source of pseudocereal and leafy vegetable and consists of two grain types – the white-seeded type used as pseudocereal and brown-seeded vegetable type used as vegetable. The latter type is also used to extract red dye. The remains of pale grains and bundle of plants for threshing at a dozen levels which date back to 5500 years have been dug up through archaeological excavation from the renowned Tehuacan caves in Central Mexico. In a few Indian village of Guatemala and Southern Mexico, *A. cruentus* is still cultivated as grain crops, and seedcakes made up of popped amaranth seeds are sold on streets. In the arid Southwestern USA, light brown grain type of *A. cruentus* is used as a source of dye for colouring corn-based food in the Indian Pueblos where it probably became established in prehistoric times. Among the *Amaranthus* species, it is probably the most adaptive and can bear flowers under a wider range of day lengths. *Amaranthus cruentus* L. with yellowish white or pale brown seed is traditionally grown as pseudocereal crop in Latin American countries like Mexico, Guatemala, Ecuador and Columbia. It is also cultivated for commercial purpose in dry and hot regions of the USA, Argentina and China (Fig. 3.6).

Amaranthus cruentus is commonly known as blood amaranth, purple amaranth and caterpillar amaranth. It is an erect, annual herb and almost glabrous or slightly pubescent at distal part, especially when young, attaining a height of

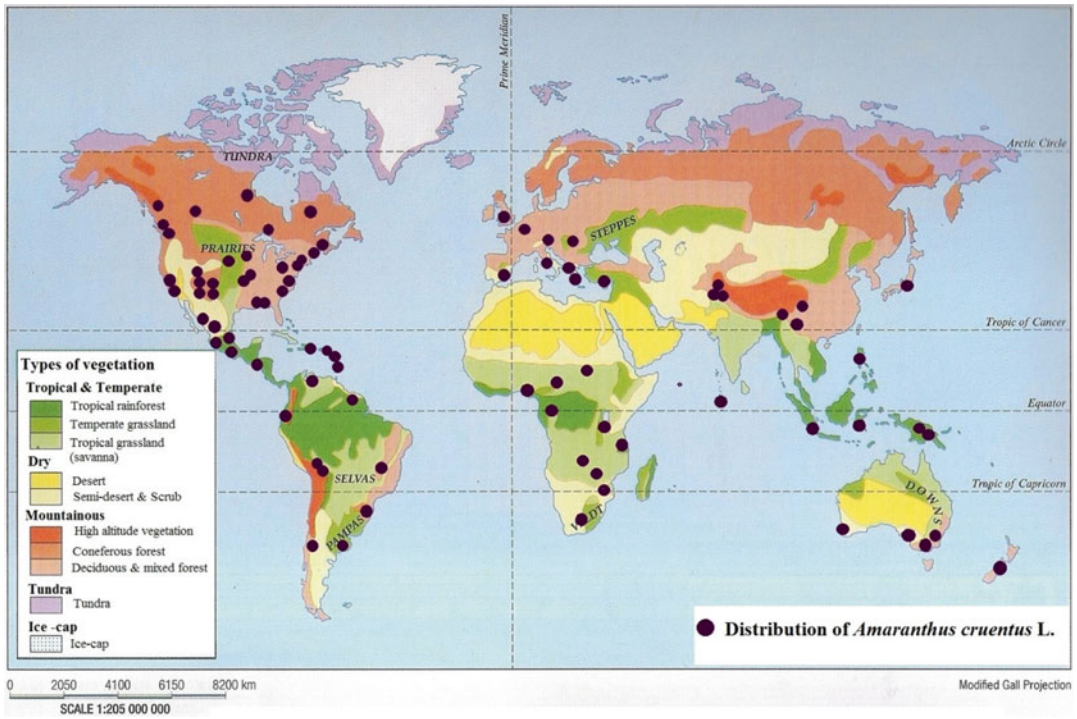


Fig. 3.6 Worldwide distribution of *Amaranthus cruentus* L.

2.0 mt, smaller than *A. hypochondriacus*. Stems are erect, branched distally, mostly in inflorescence, to nearly simple, green or reddish purple. Leaves are petiolate, petiole one-half as long as to nearly equaling blade, rhombic-ovate or ovate to broadly lanceolate, 3–14(−20) × 1.5–8(−15) cm; in robust plants it is occasionally larger, cuneate to broadly cuneate, entire, acute to acuminate or subobtuse to slightly emarginate with small mucro. Inflorescences are both terminal and axillary, erect, reflexed or nodding, usually dark red or deep beet-red, purple less commonly, almost green or greenish red, leafless at least distally, huge and robust, lower inflorescence forming lax and soft spikes and higher-forming panicles. Bracts are spatulate, 2–3 mm long, equaling or slightly longer than tepals, with short-spinescent apex, nerve as long as style branches. Pistillate flowers have five tepals: tepals straight, oblong to oblong-obovate or lanceolate, not clawed, equal or subequal, the inner tepals shorter than outer tepals, 1.5–3 mm long, with acute apex. Style branches are slender, erect, stigmas 3. Staminate flowers are situated at tips of inflorescences, tepals 5, stamens 5. Fruits are obovoid to elongate-obovoid, circumscissile utricle, tapering into a tower at apex, 2–2.5 mm long, smooth or distally slightly rugose, dehisce regularly. Seeds are broadly lenticular to elliptic-lenticular, showing various colour morphs like white or ivory with reddish or yellowish tint, occasionally dark brown to dark reddish brown, 1.2–1.6 mm in diameter, smooth or indistinctly punctate (Fig. 3.7).

3.3.3 *Amaranthus caudatus* L.

This species originated in the Andean highlands of Argentina, Peru and Bolivia where common potato originated. The Spanish conquerors termed it as ‘Inca wheat’, but it appeared and domesticated much earlier than Inca civilisation. Pale seeds of *A. caudatus* more than 2000 years old were discovered from the tombs, where it was kept as food for the dead. Continuing the

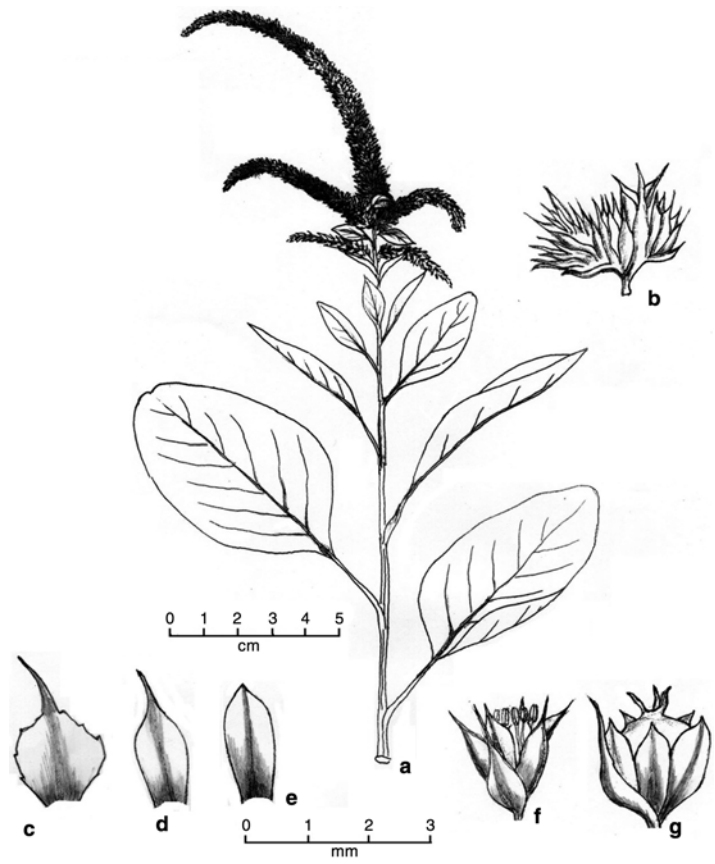


Fig. 3.7 *A. cruentus* L. (**a**) Habit, (**b**) a small part of inflorescence, (**c**) bract, (**d**) bracteole, (**e**) tepal, (**f**) male flower, (**g**) female flower

tradition, the plants are still grown in the Andean highlands mostly by the Indians to maintain the traditional custom. The plants are grown not in a large scale as a staple crop but in a small patch adjacent to houses. The species is characterised with pendulous, blazing-red, elephant hoodlike inflorescences, commonly sold in European and American countries as an ornament with a nickname 'love-lies-bleeding' or 'red-hot-cattail' . Other forms of the species give much better grain yields. The crop shows a great deal of genetic variability in South America, only a small sample has been introduced to other continents (Fig. 3.8). The grains are ground into flour, or boiled for gruel, toasted and popped to be used in seedcakes. It is considered useful for children and invalids. In India, germplasms collected from the Northern part showed much genetic diversity.

Amaranthus caudatus L. is an annual, erect herb, attaining a height of 2.0 mt, and is commonly reddish or purplish throughout. Stems are rather stout, branched sparsely and glabrous or finely covered with rather long, multicellular hairs which are increasingly numerous upwards. Leaves are simple, long petiolate (petiole up to 8 cm long but not longer than the lamina), lamina broadly ovate to rhomboid-ovate or ovate-elliptic, lanceolate, 3.0–15 × 1–8 cm, apex acute to subacute or obtuse, base shortly cuneate to attenuate, glabrous, sparingly pilose along the margins and lower surface of the primary venation. Inflorescences are extremely long, soft and lax (up to 1.5 m.) of drooping spikes or panicles, with knobby appearance due to large glomerules spaced relatively far apart. Flowers are arranged in axillary and terminal spikes formed of increasingly aggregated cymose clusters; the terminal inflorescences vary from a single, elongated, tail-like, pendulous spike to a panicle with the ultimate spike so formed. Male and female flowers are intermixed throughout the spikes. Bracts are

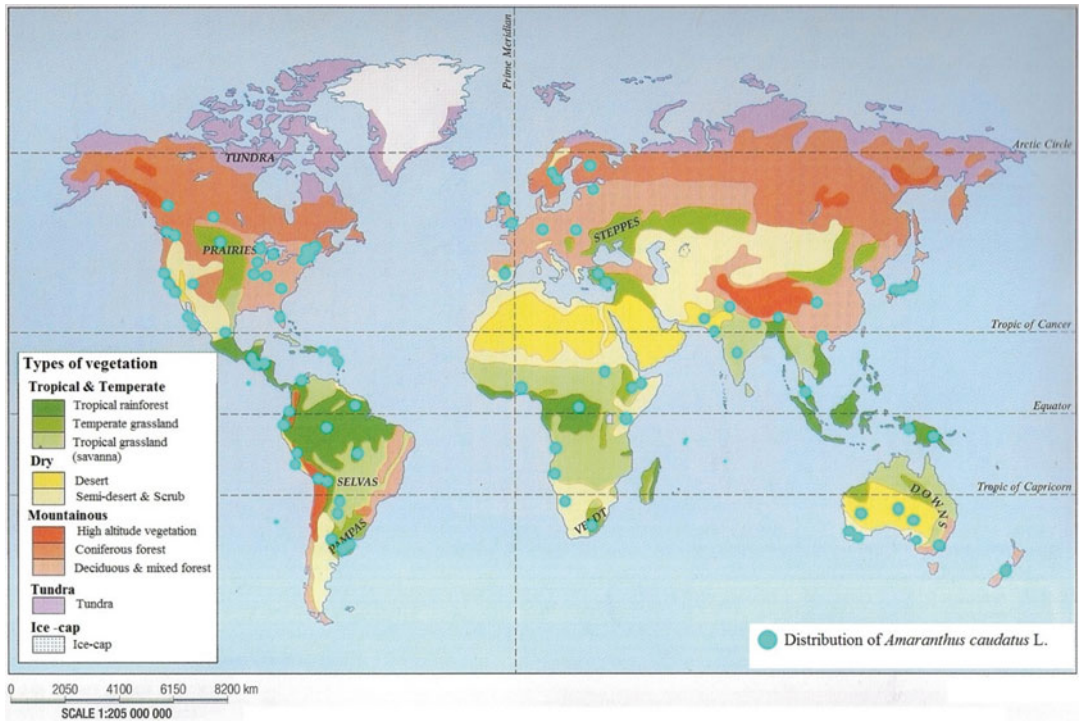


Fig. 3.8 Worldwide distribution of *Amaranthus caudatus* L.

deltoid-ovate, pale-membranous, acuminate with a long, pale or reddish, rigid, erect arista formed of excurrent midrib, the longest one up to twice as long as the perianth, as long as style branches. In perianth with five tepals, those of the male flowers are oblong-elliptic, acute, aristate, 2.5–3.5 mm long, and those of the female flowers are broadly obovate to spatulate, abruptly narrowed to a blunt or sometimes faintly emarginate, mucronate tip, 1.75–2.5 mm long, inner tepals shorter than outer tepals. Staminate flowers are mostly at the tips of inflorescence, stamens 5. Style branches spreading meeting in a saddle at base, stigmas 3. Fruits are ovoid-globose, circumscissile and utricle, not forming tower at the *apex*; the lid is smooth or furrowed below, abruptly narrowed to a short, thick neck, 2.0–2.5 mm long. Seeds are compressed, shiny, creamy white or grey coloured with a thick yellowish or pink rim and translucent centre, 0.75–1.25 mm in diameter (Fig. 3.9).

3.4 Potentiality of Grain Amaranth as Food

The seeds of the grain amaranths on average are composed of 13.1–21.0 % of crude protein, 5.6–10.9 % of crude fat, 48–69 % of starch, 3.1–5.0 % of dietary fibre and 2.5–4.4 % of ash. The 'protein component of grain amaranth and its quantity and quality (as far as amino acid composition is concerned) are very close to the levels recommended by FAO/WHO (Table 3.2). Protein efficiency ratio (PER) of grain amaranths ranges from 1.5 to 2.0 (value is 2.5 for Casein); for cooked grains, proteins have high digestibility (approx. 90 %) and are rich with lysine (0.34 g Lys/g N). Extremely balanced amino acid composition is due to the fact that in amaranth, 65 % of the proteins are found in the embryo and only 35 % in the perisperm, while in other grains amino acids are mainly concentrated in the endosperm (85 % in average) and embryos are poorer

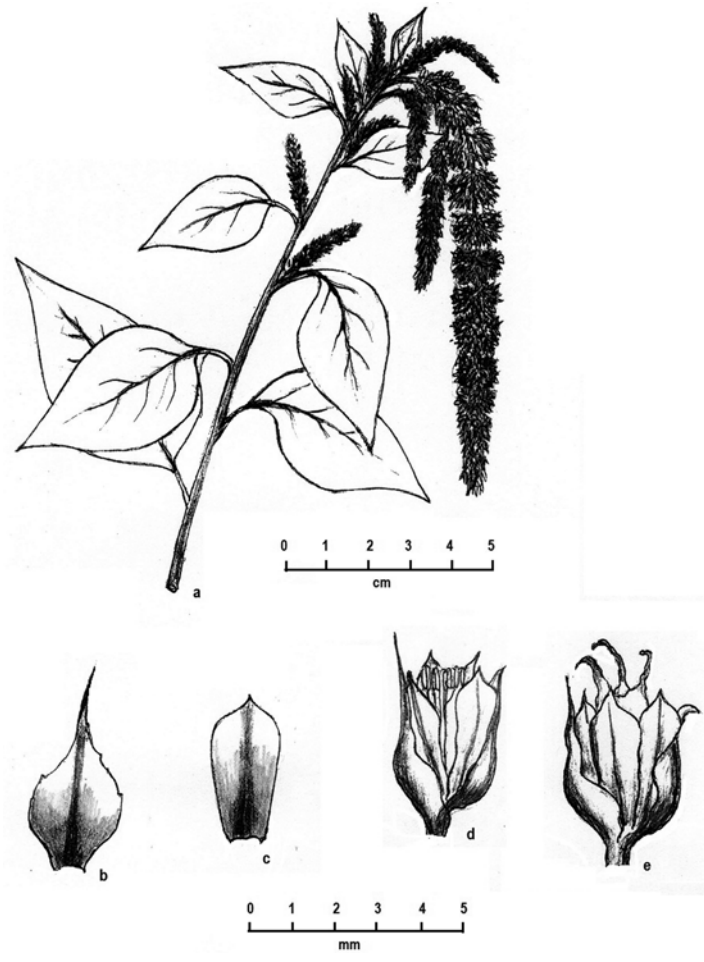


Fig. 3.9 *A. caudatus* L. (**a**) Habit, (**b**) bract, (**c**) tepal, (**d**) male flower, (**e**) female flower

Table 3.2 Seed composition in different grain amaranths

Components	*A. cruentus*	*A. hypochondriacus*	*A. caudatus*
Crude protein	13.8–21.5	15.0–16.6	13.1–21.0
Lysine (g100 g^{-1} on dry basis)	4.9–6.1	4.9–6.0	5.9
Crude fat	5.6–8.1	6.1–7.3	5.8–10.9
Crude fibre	3.1–4.2	4.9–5.0	2.7–4.9
Carbohydrate	63.1–70.0	67.9	63.7–76.5
Ash	3.0–3.8	3.3–3.4	2.5–4.4
Squalene (% in oil)	2.2–6.9	1.9–4.6	3.8–6.7

Sources: Becker et al. (1981), Sanchez-Marroquin et al. (1986), Lyon and Becker (1987), Pederson et al. (1987), Singhal and Kulkarni (1988), Ayorinde et al. (1989), Gorinstein et al. (1991), Prakash and Pal (1992), Bressani (1993), Dodok et al. (1994), Zheleznov et al. (1997), Marcone and Yada (1998) and Leon-Camacho et al. (2001)

Table 3.3 Distribution of essential amino acids in seeds of different grain amaranths and other cereals (g 100^{-1} of protein)

Protein sources	Amino acids									
	Trp	Met/Cys	Thr	Isl	Val	Lys	Phe/Tyr	Leu	LAA[A]	EAA[B]
FAO/WHO (1973)	1.0	3.5	4.0	4.0	5.0	3.5	6.0	7.0	–	–
Amaranth (average)[a]	1.3	4.4	2.9	3.0	3.6	5.0	6.4	4.7	67	87
A. cruentus[b]	–	4.1	3.4	3.6	4.2	5.1	6.0	5.1	84	89
A. cruentus[c]	0.9	4.6	3.9	4.0	4.4	6.0	7.9	6.2	88	95
A. cruentus[c]	–	4.6	3.9	4.0	4.5	6.1	8.5	6.1	87	96
A. caudatus[c]	1.1	4.9	4.0	4.1	4.7	5.9	8.1	6.3	90	98
A. hypochondriacus	1.82	0.6	3.3	2.7	3.9	5.95	8.42	4.2	34	78
A. cruentus[e]	1.4	4.1	3.4	3.6	4.2	5.1	6.0	5.1	73	91
Amaranth (average)[a-e]	1.3	4.5	3.5	3.6	4.2	5.6	7.3	5.4	75	94
Barley[a]	1.2	3.2	3.2	4.0	4.7	3.2	8.2	6.5	83	97
Buckwheat[a]	1.4	3.7	3.9	3.8	5.2	5.9	5.8	5.8	83	97
Maize[a]	0.6	3.2	4.0	4.6	5.1	1.9	10.6	13.0	35	86
Oat[a]	1.2	3.4	3.1	4.8	5.6	3.4	8.4	7.0	62	92
Rice	1.0	3.0	3.7	4.5	6.7	3.8	9.1	8.2	69	94
Soya[a]	1.4	3.1	3.9	5.4	5.3	6.3	8.1	7.7	89	98
Wheat[a]	1.2	3.5	2.7	4.1	4.3	2.6	8.1	6.3	47	86

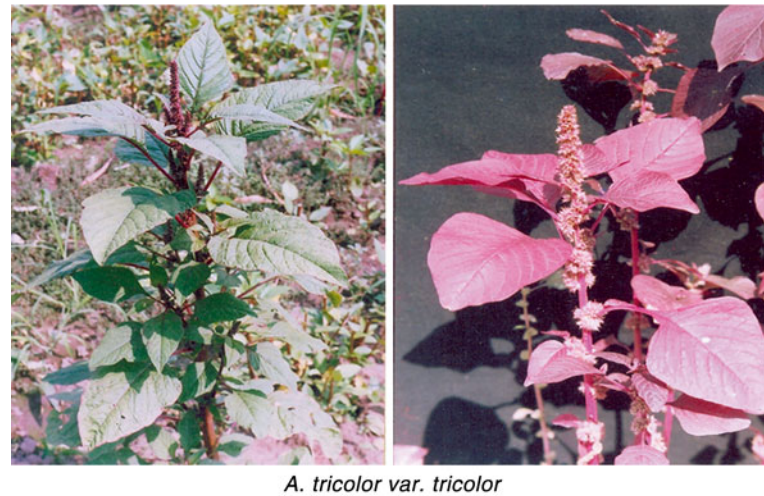
Sources: [a]Senft (1979), [b]Betschart et al. (1981), [c]Becker et al. (1981), [d]Dodok et al. (1997) and [e]Sanchez-Marroquin et al. (1986)

with essential amino acids (Table 3.3). The compilation of maize and amaranth grain flour in 50:50 ratio nearly reaches the perfect score of 100 on the nutritionist's scale (Segura-Nieto et al. 1994; Saunders and Becker 1984; Grobelnik et al. 2009a, b). In grain amaranths the saturated and unsaturated fatty acid ratio ranges from 0.29 to 0.43. The shares of linoleic acid, oleic acid, palmitic acid, stearic acid and linolenic acid are 25–62 %, 19–35 %, 12–25 %, 2–8.6 %, and 0.3–2.2 %, respectively. Amaranth seed oil has been reported to contain large amount (7–8 % and 11 %) of squalene which is often used in cosmetics and medicine, where olive oil contains only 1 % of squalene. Amaranth oil is also a rich source of tocotrienols which is very effective to lower the LDL cholesterol (Becker et al. 1981; Plate and Areas 2002). Anti-nutritive components like saponins, trypsin inhibitor and tannin are present in amaranth grain keeping parity with legumes and some other grains like sorghum. Now these components are not considered as nutritional hazard.

3.5 Vegetable Amaranths

Vegetable amaranths are considered as the most popular vegetable crops grown in the tropics for their protein, vitamin and mineral-rich leaves and stems. Vegetable amaranths are grown in the hot, humid regions of Southeast Asia (especially Malaysia and Indonesia), Africa, Southern China, India and Caribbean islands. Leaves of most *Amaranthus* species are edible, but few are very popular, e.g. vegetable amaranths such as *A. tricolor* L., *A. blitum* L., *A. dubius*, *A. cruentus* and *A. viridis* L., the first two being the most popularly grown. Mild spinach-like flavour of vegetable amaranths, high yields, ability to grow in hot weather and high nutritive value are few reasons for their popularity. They are probably the most widely consumed leafy vegetables in the humid tropical lowland of Africa and Asia (Schnetzler and Breene 1994). *Amaranthus tricolor* is very rich in morphological diversity, represented in a number of different morphotypes (Fig. 3.10). Vegetable amaranths are very rich in protein; calcium; iron; vitamin A, C

A. tricolor var. tricolor



A. tricolor var. tricolor

Amaranthus tricolor var. tristis Amaranthus tricolor var. acutus

Fig. 3.10 Different varieties of *Amaranthus tricolor* L.

and K; riboflavin (B2); niacin (B3); vitamin B6; and folate which have attributed to their high nutritive value. Due to the presence of a number of male flowers per glomerule, terminal inflorescence and development of axillary glomerules (Rajan and Markose 2007), self-pollination is the predominant phenomenon in vegetable amaranth. In spite of the fact that vegetable amaranth is used as a cheap source of protein and staple food crop in many parts of the world, negligible efforts have been made for its genetic improvement.

3.6 Species of Vegetable Amaranths

3.6.1 *Amaranthus tricolor* L.

It has been recognised as good as or superior in taste to spinach and considerably rich in carotenoid (90–200 mg/kg), protein (14–30% on dry weight basis) and ascorbic acid (nearly 28 mg/100 g) (Makus 1990; Prakash and Pal 1991; Shukla et al. 2006b). This underutilised plant with significant nutritive value has been recognised by the US National Academy of Science (1984). This popular vegetable is supposed to have originated in tropical South Asia (Grubben and Van Sloten 1981) and spread throughout the tropical and temperate regions of the globe (Martin and Telek 1979). In South and Southeast Asia, it is a major leafy vegetable. It is occasionally cultivated in East, West and Southern Africa. It was domesticated in prehistoric times from the wild progenitor which is not clearly known. Weedy plants of *Amaranthus tricolor* can be found occasionally as an escape from cultivation. It is far from competitive with true weeds. *Amaranthus tricolor* occurs as a rare exotic vegetable in several African countries, thought to have been introduced by Indian immigrants and occasionally cultivated especially in East and Southern Africa. Its cultivation has been reported from Benin, Nigeria, Kenya and Tanzania. In many tropical regions of India, Southeast Asia and South Pacific Islands, *Amaranthus tricolor* is extensively grown (Fig. 3.11).

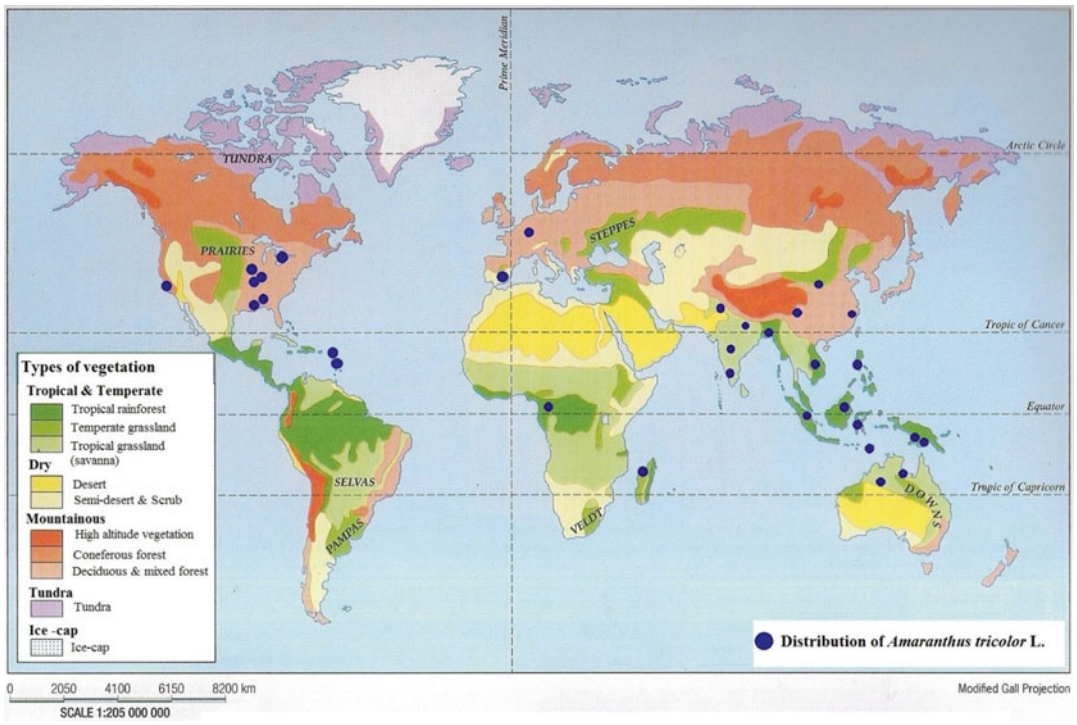


Fig. 3.11 Worldwide distribution of *Amaranthus tricolor* L.

Amaranthus tricolor is an annual, ascending or erect herb, attaining a height of 1.0–1.25 m or more in cultivation. Stem is usually much-branched and stout and the branches are angular, glabrous or furnished in the upper parts with sparse (or denser in the inflorescence), more or less crisped hairs. Leaves are simple, petiolate, the lamina broadly ovate, rhomboid-ovate or broadly elliptic to lanceolate-oblong, lamina tip emarginate to obtuse or acute, base shortly cuneate to attenuate, decurrent along the petiole, glabrous or thinly pilose on the lower surface of the primary venation, green or purplish, very variable in size (up to 18 cm long). Flowers are green to crimson in more or less globose clusters of 4–25 mm in diameter; all or only the lower parts are axillary and distant, with the upper sometimes without subtending leaves and increasingly approximate to form a thick terminal spike of variable lengths. Male and female flowers are intermingled. Bracts and bracteoles are deltoid-ovate, bracteoles subequalling or shorter than the perianth, pale-membranous, broadest near the base and narrowed upwards to the green midrib, which is excurrent to form a long, pale-tipped awn usually at least half as long as the basal portion and not rarely equalling it. Perianth consists of three tepals: tepals 3–5 mm long, elliptic or oblong-elliptic, narrowed above, pale-membranous, the green midrib excurrent into a long, pale-tipped awn. Male flowers have three stamens, female flowers with the tepals slightly accrescent in fruit, stigmas 3, erect or recurved, 2 mm long. Fruits are ovoid-urceolate utricle with a short neck below the style-base, 2.25–2.75 mm, circumscissile, membranous, obscurely wrinkled. Seeds are blackish- brown, lenticular, shiny, 1–1.5 mm in diameter, spermoderm very faintly reticulate (Fig. 3.12).

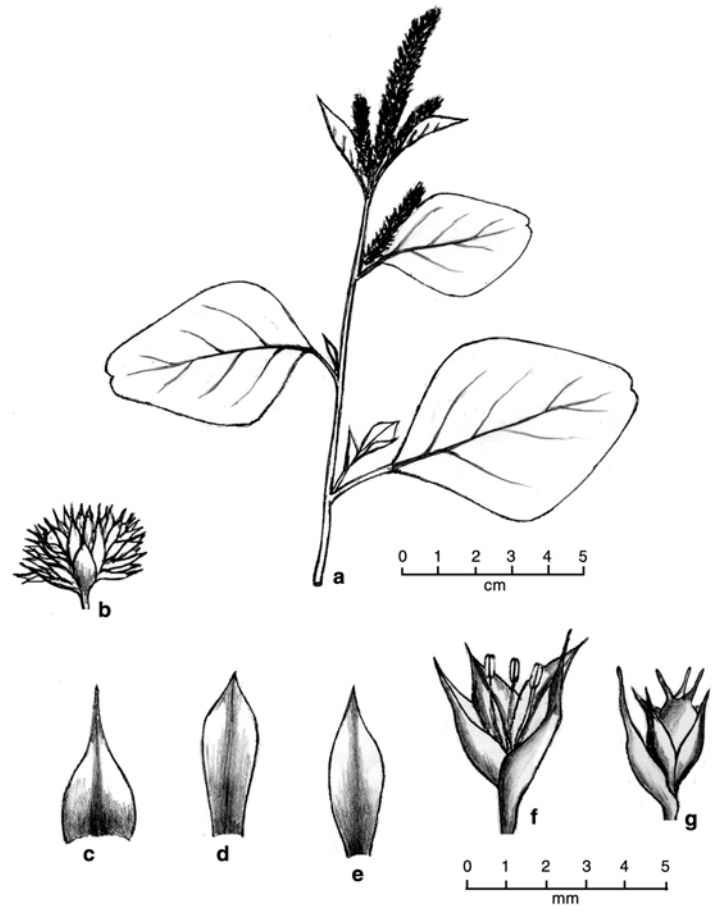


Fig. 3.12 *Amaranthus tricolor* L. (**a**) Habit, (**b**) a part of inflorescence, (**c**) bract, (**d**) bracteole, (**e**) tepal, (**f**) male flower, (**g**) pistillate flower

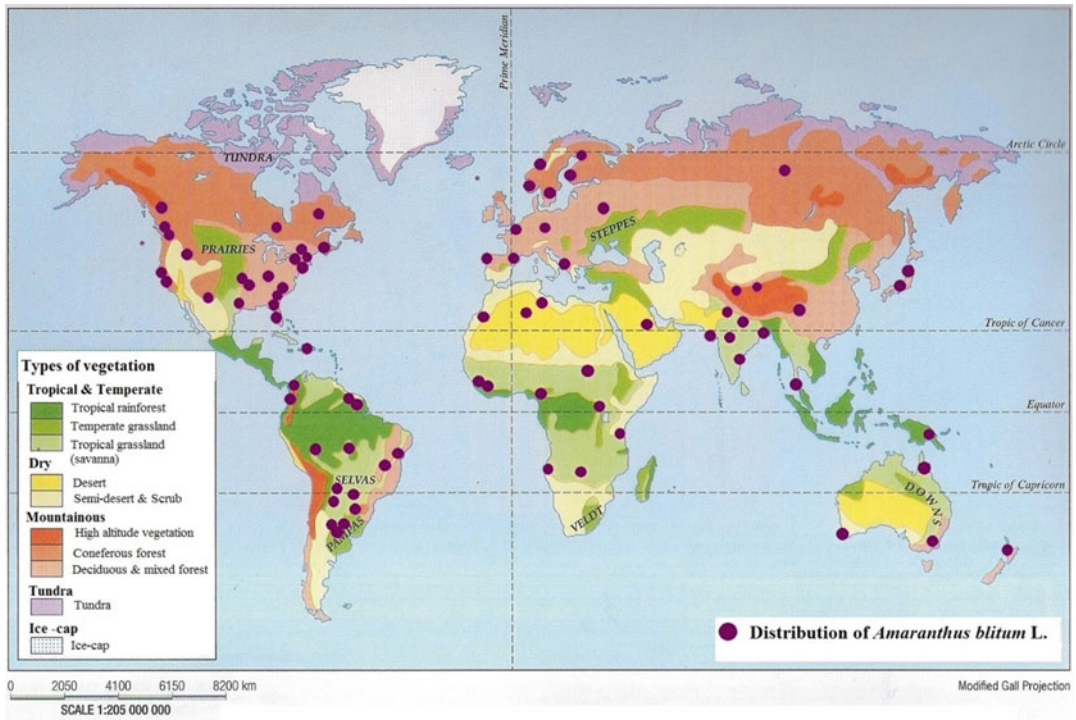


Fig. 3.13 Worldwide distribution of *Amaranthus blitum* L.

3.6.2 *Amaranthus blitum* L.

Though it is supposed to have originated in the Mediterranean region, now it is found worldwide ranging from the tropical to temperate region. It is one of the most popularly cultivated vegetables in India, and its cultivation has been reported from East and Central Africa. It has also been recorded from many African countries and probably occurs throughout tropical Africa, from Senegal to Ethiopia, South Africa and the Indian Ocean islands. It is generally a protected weed in backyards and home gardens and occasionally produced for sale in the market. In Greece, Japan and Western Europe, it is a popular green vegetable and is used as a substitute for spinach (*Spinacia oleracea*) during the hot summer season (Fig. 3.13).

Amaranthus blitum is an annual herb with light green or purple, ascending stem, branched from base, glabrous, attaining a height of 10–30 cm. Leaves are simple, petiolate, petiole 1–3.5 cm, leaf blade ovate or ovate-rhombic, with notched apex with a mucro, cuneate base, entire or slightly undulate margin, dimension 4.0–7.5 × 3.0–6.0 cm. Flowers are arranged in slender terminal spikes or panicles and also in axillary clusters; spikes are erect or sometimes reflexed. Bracts and bracteoles are oblong, shorter than 1 mm. Perianth has three tepals, tepals are elliptic, spatulate, oblong or lanceolate, with an adaxial midvein, equal or subequal, apex acute, light green, length 1.5–2.0 mm. Staminate flowers are clustered at the tip of spikes, stamens 3, slightly shorter than tepals. Style branches are erect, stigmas 3, fall off when utricle matures. Fruits are compressed-ovoid, utricle, exceeding tepals, indehiscent, slightly rugose to nearly smooth, 3 mm long. Seeds are black to brownish black, circular in outline, 1.2 cm in diameter (Fig. 3.14).

Fig. 3.14 Morphology of vegetable amaranths, *A. blitum* L.

3.6.3 *Amaranthus dubius* L.

It may have evolved from weedy ancestors in tropical Asia (Indonesia and India) and may have been introduced later to Africa and Central America by immigrants. This weedy species is occasionally used as a green leafy vegetable in West America and the Caribbean islands and is found in Java and other parts of Indonesia as a home garden crop (Fig. 3.15). One of the best varieties of this species, known as the cultivar 'claroen', is particularly popular in Benin and Suriname. Its seeds are extremely small (4500 seeds per g). It is a fast-growing, high-yielding plant with considerable morphological variability. It has characteristic dark green, broad, ridged leaves. This is the only known tetraploid (2n = 64) species in the genus *Amaranthus* so far.

Amaranthus dubius (Spleen amaranth) is an annual erect herb, glabrous or slightly pubescent in distal portion. Stems are green and branched. Leaves are simple, petiolate, petioles of proximal leaves are equal or longer than blade, becoming shorter distally, lamina rhombic-ovate or ovate to elliptic, 3–12 × 2–8 cm, with entire margin, slightly acuminate to obtuse and faintly emarginate, mucronate tip and broadly cuneate base. Inflorescences occur as dense terminal panicles and axillary spikes, and panicles are erect or often drooping, green, branched, leafless at least distally. Bracts are lanceolate, less than 2 mm, shorter than tepals with spinescent tip. Pistillate flowers have five tepals; tepals are oblong-spatulate to oblong, with acute apex often shortly mucronate, 1.5–2 mm long. Style branches are strongly spreading, shorter than fruit, stigmas 3. Staminate flowers are generally clustered at tips of inflorescence branches, sometimes aggregated in proximal glomerules (as in *A. spinosus*) with five tepals equal or subequal and five stamens. Fruits are ovoid or subglobose, circumscissile utricle,1.5–2 mm long, slightly shorter than tepals, smooth to irregularly wrinkled, dehisce regularly. Seeds are dark reddish brown to black, subglobose or lenticular, 0.8–1 mm in diameter with shiny, smooth spermoderm (Fig. 3.16)

3.6.4 *Amaranthus cruentus* L.

The vegetable form of *Amaranthus cruentus* was probably introduced in the tropics and subtropics of the Old World during the colonial period. Now *Amaranthus cruentus* is familiar as a widespread traditional vegetable in all countries of tropical Africa. It is the principal leafy vegetable in Benin, Togo and Sierra Leone and very important in many lowland areas like in Southern Nigeria, DR Congo, Kenya and Tanzania. It is more popular in humid lowland than in highland or arid areas. It is also an important leafy vegetable in many tropical countries outside Africa, e.g. in India, Bangladesh, Sri Lanka and the Caribbean islands. The Bangladesh type has big fleshy stems and is consumed with the leaves. *Amaranthus cruentus* is grown throughout Southeast Asia as leafy vegetable, although to a lesser extent than *Amaranthus tricolor* L. During thinnings of young seedlings

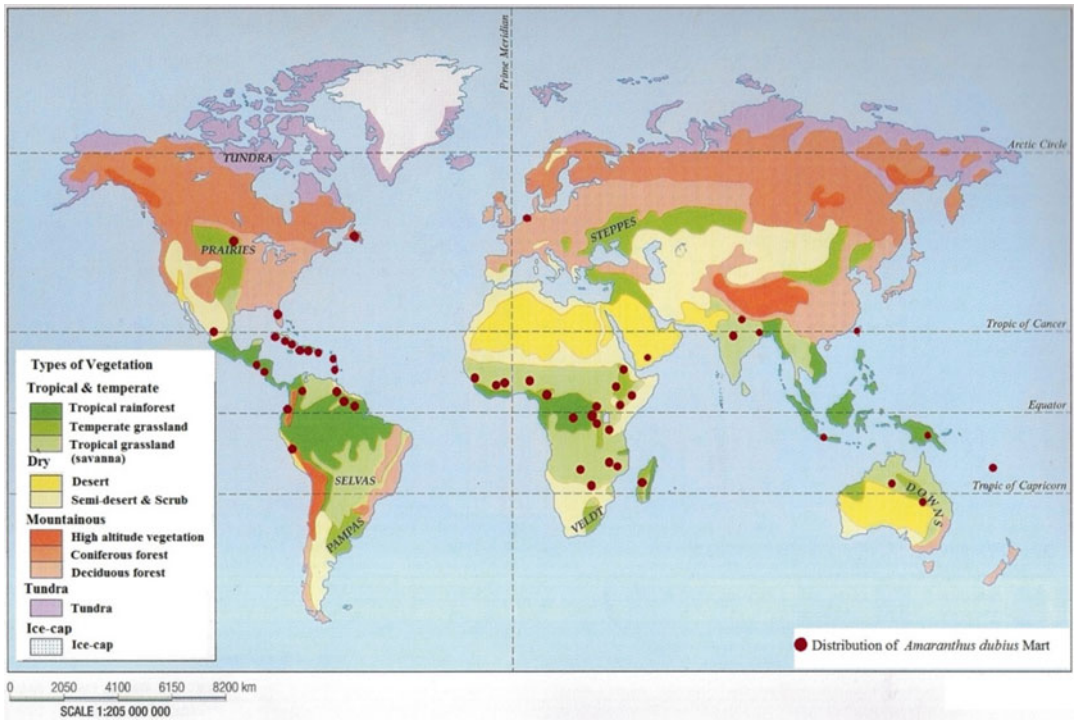


Fig. 3.15 Worldwide distribution of *Amaranthus dubius* Mart. ex Thell

Fig. 3.16 Plant morphology of *Amaranthus dubius* Mart. ex Thell

in the cultivation field of the grain crop, uprooted seedlings are frequently used as a vegetable. A very deep red coloured, dark-seeded morphotype of the species, sometimes known as 'blood Amaranth', is often sold as ornamentals. During the nineteenth century, this deep-red vegetable form was adopted as green vegetable by gardeners throughout the tropics. It soon appeared as a more important crop in tropical Africa than anywhere else. Like corn, sweet potatoes, peanuts and other American Indian crops, *Amaranthus cruentus* was evidently introduced in Africa by Europeans. After being introduced it was quickly transferred from tribe to tribe, probably as a weed in millet and sorghum seed. Now, it has colonised a large area in Africa and is being planted and gathered year-round in the humid regions of America. The tender young seedlings are pulled up by the roots and sold in town markets by the thousands of tons every year in parts of West America.

3.6.5 *Amaranthus viridis* L.

Amaranthus viridis is possibly of Asian origin, but now it has become a cosmopolitan weed in the tropical and subtropical regions of the world, also reaching far into temperate regions (e.g. in Europe, North America, Asia and Australia). In tropical Africa it is a widespread common weed. It is occasionally cultivated in Nigeria, Gabon and DR Congo.

It is an annual herb, erect or more rarely ascending, attaining a height of 75–100 cm. Stems are rather slender, sparingly to considerably branched, angular, glabrous or more frequently increasingly hairy upwards (especially in the inflorescence) with short or longer and rather floccose multicellular hairs. Leaves are glabrous or shortly to fairly long-pilose on the lower surface of the primary and most of the venation, long-petiolate (petioles up to 10 cm long and the longest commonly longer than the lamina), lamina is deltoid-ovate to rhomboid-oblong, the margins entire, occasionally obviously sinuate, apex obtuse and obscurely to clearly emarginate at the tip, minutely mucronate, base shortly cuneate to sub-truncate below, 2–7 × 1.5–5.5 cm in length. Flowers are green, arranged in slender, axillary or terminal, often paniculate spikes, 2.5–12 cm long and 2–5 mm wide, or in the lower part of the stem is in dense axillary clusters. Male and female flowers are intermixed but the latter is more numerous. Bracts and bracteoles are deltoid-ovate to lanceolate-ovate, white-membranous with a very short, pale or reddish awn formed by the excurrent green midrib, bracteoles are shorter than the perianth (c. 1 mm.). Tepals are three in number, very rarely four, those of the male flowers are oblong-oval, acute, concave, 1.5 mm long, shortly mucronate, while those of the female flowers are narrowly oblong to spatulate, 1.25–1.75 mm long, minutely mucronate or not, with white-membranous borders, midrib green and often thickened above. Male flowers are inconspicuous, mostly at the tips of inflorescence, with three stamens. Stigma 2–3, short, erect or almost so. Fruits are subglobose utricle, not or slightly exceeding the tepals, indehiscent or rupturing irregularly, very strongly rugose throughout, 1.25–1.5 mm. Seed are round, slightly compressed, black with an often paler thick border, 1–1.25 mm in diameter, more or less shiny, reticulate and with shallow scurfy verrucae on the reticulum (Fig. 3.17a).

Fig. 3.17 General plant morphology of (**a**) *A. viridis* L. (**b**) *A. graecizans* L.

In Asia, domestication of amaranths took place for use as pot herbs, and a variety of cultivated amaranth races belonging to *A. lividus* and *A. tricolor* are common pot herbs in Eastern and Southern Asia which subsequently have been introduced into the New World. *A. tricolor* might have originated in the Indian subcontinent especially in India and have spread to neighbouring countries and other parts of the world by the immigrants. Vegetable amaranths like *A. tricolor, A. melancholicus, A. gangeticus,* etc., are important group of cultivated amaranths which are obviously natives of Asia. However, these are grown exclusively as pot herbs or ornamentals, apparently never for seeds. This entire Asiatic potherb group is clearly distinguishable from the grain group.

3.6.6 *Amaranthus graecizans* L.

Amaranthus graecizans occurs scattered throughout tropical Africa. It is well distributed in Southern Europe and in tropical and subtropical Asia and has also been introduced to the USA. It is very popular as a vegetable in parts of Kenya, Uganda, Tanzania, Malawi and elsewhere in Southern Africa and sometimes appears as a protected weed in backyards, cultivated locally on small scale in home garden and for market sale in Nebbi, Uganda and in Tanzania.

Amaranthus graecizans is an annual erect, decumbent or prostrate herb, branched, pubescent in distal parts or become glabrous at maturity, attaining a height up to 45 cm (rarely to 70 cm). Stems are slender to stout, angular, glabrous or finely to moderately hairy; hairs are short or long, often crisped multicellular, which increase upwards, especially in the inflorescence. Leaves are simple, long-petiolate (petiole length 3–4.5 cm, sometimes longer than the lamina), lamina broadly ovate or rhomboid-ovate to elliptic-ovate or narrowly linear-lanceolate or linear, 4–55 × 2–30 mm, with generally entire margins, subacute to obtuse or emarginate, mucronulate apex and cuneate to broadly cuneate base, glabrous or occasionally the lower surface of the principal veins is sparingly covered with very short, gland-like hairs. Flowers are arranged all in axillary cymose clusters, male and female flowers are intermixed, and male flowers are generally situated mostly in the upper whorls. Bracts and bracteoles are narrowly lanceolate-oblong, membranous, acuminate, with excurrent green midrib forming a pale or reddish arista, bracteoles are subequalling or usually shorter than the tepals, bract length 1.5–2 mm. Perianth consists of three tepals, all 1.5–2 mm; those of the male flowers are lanceolate-oblong, acute or subacute, pale-membranous with a narrow green midrib excurrent in a short, pale arista, and those of the female flowers are lanceolate-oblong to linear-oblong, gradually to abruptly narrowed to a very short to rather long mucro, the midrib often bordered by a green vitta above and apparently thickened, the margins pale whitish to greenish. Staminate flowers are intermixed with pistillate flowers, male flowers have three stamens and female flowers have three stigmas; stigma is slender, usually pale, flexuose, 0.5 mm long. Fruits are subglobose to shortly ovoid utricle, 2–2.25 mm, usually strongly wrinkled throughout with a very short, smooth neck, slightly exceeding the perianth, circumscissile or sometimes not, even on the same plant. Seeds are shiny, compressed, black and 1–1.25 mm in diameter; spermoderm is faintly reticulate (Fig. 3.17b).

Amaranthus graecizans is mainly used as cooked leaf vegetable. In many countries it is collected from the wild as a potherb. Though it tastes slightly bitter, such slight bitterness is liked by the older people. A major drawback is that the leaves are small, so the collection is time taking. The plant has axillary clusters of flowers; the people don't like to cook the whole shoot, but they have to pluck the individual leaves which is time taking. This is one of the reasons of its low market value.

In some regions it is consumed by mixing with other leaf vegetables collected from the wild, as, for example, the Okiek people in Western Kenya, who mix it commonly with *Solanum* or *Rumex* species and *Urtica massaica* Mildbr. *Amaranthus graecizans* is also used as a fodder for livestock. In Mauritania the seed is baked into thin cakes, while in the Western USA,

it is ground into flour. The whole plant of *Amaranthus graecizans* is used in East and West Africa to manufacture a local salt. For this purpose, the plants are first dried and then burned to ashes, the filtrate is evaporated and the residue is used as a substitute for common salt. In Uganda, the leaves are chewed and the liquid extract is swallowed for the treatment of tonsillitis. In Senegal, the leaves are used as an anthelmintic.

3.7 Nutritive Value of Vegetable Amaranths

Prakash and Pal (1991) reported high nutritive value of vegetable amaranth. It is rich in protein (14–43 g kg^{-1} in fresh matter), lysine (40–56 g kg^{-1}), carotenoids (60–200 mg kg^{-1}) and different types of vitamins and minerals (Table 3.4). According to different authors, oxalate and nitrate concentrations in fresh matter vary from 4.1 to 9.2 g kg^{-1} and from 3 to 16.5 g kg^{-1}, respectively. Compounds are generally associated with forage and vegetable application, and they strongly depend upon genotype and fertilisation practice (Williams and Brenner 1995).

The vegetable amaranths are cultivated or collected from wild in so many regions of the world, but few references are available regarding their culture. This indicates the facts that their cultivation practice is easy and optimal conditions for maximum yields are unknown due to their wide adaptability. Often seeds are not at all sown, fall naturally in the cultivation field and raise the crop of the next year. The plants grow rapidly, so the time interval between planting and harvest of the tender foliage and stems is short (generally only 3–6 weeks). In Tamil Nadu (South India), plants

Table 3.4 Nutritional value of raw and cooked amaranth leaves, in comparison with raw cabbage, Chinese cabbage and spinach

Nutrients	Cabbage raw (value/100 g)	Chinese cabbage raw (value/100 g)	Spinach raw (value/100 g)	Amaranth raw (value/100 g)	Amaranth cooked (value/100 g)
Proteins (g)	1.28	1.20	2.86	2.46	2.11
Minerals					
Calcium(mg)	40	77	99	215	209
Iron (mg)	0.47	0.31	2.71	2.32	2.26
Magnesium (mg)	12.0	13.0	79.0	55.0	55.0
Phosphorus (mg)	26.0	29.0	49.0	50.0	72.0
Potassium (mg)	170.0	238.0	558.0	611.0	641.0
Sodium (mg)	18.0	9.0	79.0	20.0	21.0
Zinc (Zn)	0.18	0.23	0.53	0.90	0.88
Copper (mg)	0.019	0.036	0.136	0.162	0.158
Manganese (mg)	0.160	0.190	0.897	0.885	0.861
Vitamins					
Vitamin C (mg)	36.6	27.0	28.1	43.3	41.1
Riboflavin (mg)	0.040	0.050	0.189	0.158	0.134
Niacin (mg)	0.234	0.400	0.724	0.658	0.559
Vitamin B6	0.124	0.232	0.195	0.192	0.177
Folic acid (mcg)	43.0	79.0	194.0	85.0	57.0
Vitamin A RAE 1 (mcg)	5.0	16.0	469.0	146.0	139.0
Vitamin K	76.0	42.9	482.9	1140.0	–
Lipids					
Total saturated fatty acids (g)	0.034	0.043	0.063	0.091	0.050

Source: USDA National Nutrient Database for standard Reference, Release 23 (2010) http://www.nal.usda.gov/fnic/foodcomp/search

are pulled out 3 weeks after sowing and used as 'tender greens'. Certain varieties of vegetable amaranths remain succulent for longer period and can be harvested even up to 5 weeks of growth. Certain varieties are suitable for periodical cutting (clipping). The first cutting is done at 20 days after sowing and subsequently followed by weekly cuttings up to 10 cuttings. At a later age generally after flowering, the foliage and stems become fibrous, brittle, pithy and unpalatable.

3.8 Uses of Amaranths

Amaranths are the source of highly nutritious food. With the ever-increasing population and fast-growing depletion of natural resources, it became necessary to explore the possibilities of using newer plant resources, new crop and new usage of old ones that hold promise to restore the balance of trade and meet the growing needs of food, clothes and industrial products for human population. The underutilised amaranths are good options and projected as a future crop of the twenty-first century. Amaranths are categorised into three – vegetable, grain and weed. Vegetable amaranths are widely consumed as leafy vegetables in India and other Asian and Southeast Asian countries, also in African countries but not so familiar in North and South America, whereas grain amaranths are widely consumed as highly nutritious pseudocereals.

3.8.1 Uses of Vegetable Amaranths

The leaves, shoots and juicy tender stems of many cultivated species of vegetable amaranths are consumed as a potherb in sauces or soups, cooked with other vegetables and taken with a main dish or by itself. Young leaves of grain amaranths are also used as leafy vegetable. Amaranth leaves are a good source of high amount of protein, vitamins, minerals and dietary fibre. Chopped plants can also be used as forage for livestock.

Vegetable amaranths have the following beneficial role on human health:

1. *Provides energy:* Vegetable amaranths are very rich in carbohydrates, proteins, vitamin K, folate, riboflavin, vitamin A, vitamin B6 and vitamin C. Amaranth leaves boost energy in the body. The crude protein content in the leaves ranges from 20 to 32 %, on a dry weight basis.
2. *Prevents electrolyte imbalance:* Amaranth leaves are very good source of elements like manganese, iron, copper, calcium, magnesium, potassium and phosphorus necessary to maintain adequate electrolyte balance in the body.
3. *Excellent gluten-free diet:* Vegetarians with gluten intolerance or those suffering from celiac diseases can get daily recommended dose of protein from amaranth greens. Compared to other plant sources, namely, wheat, rye, rice and oats, amaranths are gluten-free and contain 30 % more protein with complete set of amino acids.
4. *Improves digestion:* It can improve the digestive system and reduce constipation due to the high content of dietary fibre which is three times that of wheat. It is easily digestible, so it is good for both the young ones and elder people.
5. *Aids in weight management:* The protein in the leaves helps to reduce insulin levels in the blood and also releases a hormone that lessens hunger pranks and prevent 'binging catastrophe'.
6. *Reduces bad cholesterol:* One of the key health benefits of vegetable amaranth leaves is their cholesterol-lowering capacity. Due to the high fibre content, this leafy vegetable is effective in reducing LDL levels in the blood and promotes weight loss. Tocotrienols, a type of vitamin E available in vegetable amaranths, also contributes to its cholesterol-lowering ability.
7. *Good for anaemic patients:* Iron-rich (five times that of wheat) red amaranth leaves promote coagulation and increase haemoglobin content and red blood cell counts. It is also an excellent source of folic acid which is necessary to increase the blood haemoglobin level.

8. *Decreases risk of cardiovascular disease:* Amaranth leaves are excellent dietary source of phytosterols that lowers blood pressure and prevents heart ailments including stroke.
9. *Fights off cancer:* The presence of lysine (an essential amino acid) along with vitamin E, iron, magnesium, phosphorus, potassium and vitamin C helps to fight against free radicals responsible for ageing and formation of malignant cells.
10. *Ayurvedic treatments:* Juice extracted from fresh amaranth leaves is good for treating diarrhoea and haemorrhage.
11. *Stops hair loss and greying:* Amaranth leaves have wonderful cosmetic benefit. Besides regular consumption, application of leaf juices prevents brittle hair from falling. This wonderful cosmetic benefit of amaranth leaves also retards the onset of premature greying.
12. *Prevents calcium-deficiency ailments:*Calcium present in amaranth leaves (two times that of milk) is helpful to reduce risk of osteoporosis and other calcium deficiency-related disorders.
13. *Improves eyesight:* Amaranth leaves are excellent source of β-carotene. Daily inclusion of vegetable amaranth in the diet can help to prevent vitamin A deficiency which is responsible for blindness. It is reported that the incidence of blindness in children due to malnutrition has been reduced with the consumption of 50–100 g of amaranth leaves per day.

Amaranth leaves, like some other vegetables, contain rather high amounts of oxalic acid and nitrates. The amount of oxalic acid is roughly the same as that found in spinach (*Spinacia oleracea*) and chard (*Beta vulgaris* var. *cicla*). Excessive amounts of oxalic acid may reduce the availability of certain minerals in the body, particularly calcium. This could be of great concern especially when the calcium intake levels are low or if foods containing high amount of oxalic acid are consumed on a regular basis for a long period of time. Nitrates present in amaranth leaves are also of concern because it is speculated that nitrates may be chemically changed into poisonous/carcinogenic nitrosamines in the digestive tract, though evidence supporting this is not available at present. Boiling of the amaranth leaves like spinach or chard for 5–10 min and then discarding the water are proven effective to remove both oxalates and nitrate problems, though research has shown that consumption of 200 g of cooked amaranth per day does not create any health problem.

The present level of nitrate and oxalate in amaranths does not create a nutritional problem under normal condition of consumption, but considering this aspect, Devadas et al. (1984) analysed 25 amaranth genotypes for oxalate and nitrate content. The oxalate content ranged from 0.94 to 1.29 % and the nitrate content from 0.55 to 1.0 %. The soluble oxalate content of *A. gangeticus* was reported as 4.4 % and 7.4 % in the leaves and stems, respectively, on a dry weight basis. The mean percentage of nitrate over two growing seasons were 0.51 %, 0.19 %, 0.39 %, 0.54, 0.29 % and 0.65 % and those for oxalates were 5.37 %, 5.59 %, 3.52 %, 6.95 %, 2.45 % and 4.33 %, respectively, in the leaves of *A. gangeticus*, *A. blitum*, *A. dubius*, *A. cruentus*, *A. caudatus* and *A. hypochondriacus*.

3.8.2 Uses of Grain Amaranths

Amaranth grains contain more protein than corn, and the protein is also of an unusually high quality, rich in amino acid lysine, which is the limiting amino acid in cereals like maize, wheat and rice, and sulphur-containing amino acids, which are normally limiting in the pulse crops. The total 'protein package' of amaranth grain is very near to the levels recommended by FAO/WHO. Grain amaranths have high protein scores. Protein scores are determined by taking the ratio of the essential amino acids to the level for those amino acids recommended by FAO/WHO and multiplying by 100. The protein score of grain amaranths is 67–87, while those of wheat (containing 14 % protein), soybean (containing 37 % protein), rice (containing 7 % protein) and maize (containing 9 % protein) are 47, 68–89, 69 and 35, respectively. Although amaranth is theoreti-

cally very close to the ideal level, combining it with another grain will increase its quality very close to the FAO/WHO standards. However, the actual nutritive value is less than expected from the above considerations due to the presence of anti-nutritional factors in the raw amaranth grain. Cooking procedure can reduce the toxic effects. The problems of unpalatability are caused by saponins and phenolic compounds in the amaranth grain (Cheeke et.al. 1981). It is a very nutritious animal feed, but the raw amaranth grains contain toxins and anti-nutritional factors that can lower its acceptability as an animal feed. Protein-rich grains of grain amaranths are easy to digest and are commonly given to those who are recovering from illness or fasting period. In India *A. hypochondriacus* is known as the 'king grain' and is often popped to be used in confections. In the Third World countries, the seeds are generally ground into gluten-free flour and used for bread making with wheat or rye flour proportionately. Considering its significantly high nutritional properties, it is called the 'third millennium grain'. However, it has a high glycemic index due to its readily digestible starch. To obtain maximum benefits, it should be best combined with nuts, seeds, pulses, legumes and vegetables. In addition, amaranth is relatively a good source of cholesterol-lowering soluble fibre, calcium, iron, magnesium, zinc, vitamins A and C and several B vitamins. Flavonoids (such as rutin) and some phenolic acids (such as gallic acid, p-hydroxybenzoic acid and vanillic acid) with antioxidant effects are also found in amaranth seeds and sprouts. Many consumers prefer amaranth because they want a wheat- and gluten-free product, like the nutritional profile of amaranth, or enjoy 'exotic' foods in their diet (Brenner et al. 2000).

Amaranth grain may be processed in various ways, like grains can be popped, flaked, extruded and ground into flour. In Mexico Alegria, a confection made from popped amaranth grains is a popular and favourite food item among local peoples and tourists. Popped amaranth can be enjoyed on its own or can be served with milk or soymilk and fruit for a healthy breakfast. Amaranth grain is very often used in the poppy machine, which made a poppy amaranth by the thermal shock. Poppy amaranth is used in the cereal muesli, for the muesli bars and also for bakery products to cover bread and rolls. The most positive properties of poppy amaranth seeds are the low weight and the water absorption capacity. So the breads and rolls remain soft and supple for longer time. The defatted amaranth grain flour was the most often used raw material in food industry in the Czech Republic in the year 2008.

Amaranth can be used as a substitute in porridge, stirred into soups; Amaranth grains can be cooked whole in a pot, rice cooker or pressure cooker to prepare breakfast porridge or savoury 'polenta'. The grain flour or flaked grains are combined with wheat or other flours to make cereals, cookies, bread and other baked goods. As per general recommendation, amaranth grain flour should contribute only 10–20 % of the mixed flour blended with wheat flour. But studies have shown that if amaranth grain flour blended up to 50–75 % of the mixed flour, it will still retain functional properties as well as flavour. Coarsely ground amaranth grain makes a tasty and nutritious porridge cooked by itself or by mixing with other grains and pseudocereals such as oats (*Avena* spp.), wheat (*Triticum* spp.), milled flax seed (*Linum usitatissimum*), wheat germ and cañihua (*Chenopodium pallidicaule*). Other seed components with significant potential include anthocyanin (red) pigments to produce nontoxic natural dyes, microcrystalline starch for food and industry and squalene, specialised oil used in skin cosmetics, computer and pharmaceutical industries. Lipid fractions from amaranth seeds contain high levels of unsaturated fatty acids and possess a high antioxidative activity. Amaranth oil also contains a unique squalene component, an intermediate of steroid synthesis, which is discussed as immunomodulator and is supposed to play a role in the rate of cholesterol synthesis (prevention of cardiovascular diseases). Amaranth starch is of promising use. The features of starch like high solubility and digestibility are due to its uniquely small size which is about one-tenth the size of cornstarch and therefore offer new possibilities for food processing, pharmacology and cosmetics (Resio et al. 2000).

Amaranth starch shows unique gelatinisation and freeze/thaw properties which could be beneficial to the food industry. Furthermore, amaranth-originated products may reduce dietary fibre insufficiency, vitamin deficiency as well as deficiency of bioactive compounds (antioxidants, folic acid). Amaranth grain oil is very special in the content. The amaranth oil is used in cosmetics in the pure form or with added tocopherol and it is given under argon for stabilisation. The advantage of amaranth oil for cosmetic industry is due to its squalene content. It is nonallergic on the skin and has a protective function for very sensitive skin and hair against sun and radiation. So, it can be utilised in various sun cream and lotions

Food specialists have evaluated the amaranth seed flour as an additive to wheat flour. To increase the palatability, different amounts of amaranth grain flour were mixed with the wheat flour and baking ingredients (1 % salt, 2.5 % fat, 1.5 % yeast, 10 % sugar and 52–74 % water), fermented, moulded, pan-proofed and baked. The baked products were evaluated for loaf volume, moisture content, colour, odour, taste and texture. The bread containing amaranth grain flour products were then compared with the bread made exclusively from wheat flour. The loaf volume decreased by 40 % and the moisture content increased from 22 to 42 % with the increase in amaranth grain flour. The study showed that the sensory levels of taste, odour, colour and texture decreased or degraded with increasing amounts of amaranth flour. Significant deviation in sensory qualities was detected when more than 15 % of amaranth grain flour was added, and products containing high amount of amaranth grain flour were found to be of unacceptable palatability to the population as appeared from the sample survey of baked product. The Organic Farming and Research Center (Rodale) has successfully used a 50:50 ratio of wheat flour and amaranth grain flour and suggested that the percentage of amaranth could be increased. As per their view, 'amaranth flour contributes to the sweetness and moistness of a baked good'. In a number of African nations, amaranth is gaining prominence as an important nutritious food especially for those suffering from HIV/AIDS. It is known that the antiretroviral drugs function poorly or not at all on a poor diet. When the diet is poor in nutrients, often the drug even becomes a toxin in itself. Amaranth grain porridge (1 cup) combined with *Moringa* leaf powder (1 Tbsp) from *Moringa* leaves (*Moringa oleifera*) provides an excellent nutritious food not only for the AIDS sufferer but also for those who are taking antiretroviral drugs without any complications.

Several health benefits like decrease in plasma cholesterol levels, protection of heart, stimulation of the immune system, anticancer activity, reduction of blood glucose levels, improvement of hypertension and anaemia, etc., are provided by grain amaranths. In addition, it has been reported to have anti-allergic and antioxidant properties. Most of these properties are due to the presence of bioactive compounds. The cholesterol-lowering effects in amaranth may be due to unsaturated fatty acids. Being a good source of magnesium which is effective to relax blood vessels and prevent constriction and rebound dilation, it helps to fight migraines. Both the vegetable and grain amaranths are equally significant from nutritional point of view (Table 3.5)

Table 3.5 Comparison of nutritive components of vegetable and grain amaranths (100 g portion)

Components	Vegetable amaranths	Grain amaranths
Moisture	86.9 g	9.0 g
Protein	3.5 g	15.0 g
Fat	0.5 g	7.0 g
Total carbohydrate	6.5 g	63.0 g
Fibre	1.3 g	2.9 g
Calories	36	391
Phosphorous	67 mg	477 mg
Iron	3.9 mg	–
Potassium	411	–
Vitamin A	6100 IU	–
Riboflavin	0.16 mg	0.31 mg
Niacin	1.4 mg	1.0 mg
Ascorbic acid	80 mg	30 m
Thiamin	0.08 mg	0.14 mg
Ash	2.6 g	2.6 mg
Calcium	267 g	490 g

Compiled from J. N. Cole Amaranth: from the past, for the future, Rodale Press, Emmus, PA, USA (1979)

3.9 Culinary Properties of Amaranths

The word culinary means 'related to cooking'; it is the art of the preparation, cooking and presentation of food, usually in the form of meals. People working in this field are also known as 'culinary artist' and 'culinarian'.

Amaranth is one of the most commonly consumed green leafy vegetables in Asia and Southeast Asia. Most people eat vegetables without getting the proper amount of nutrients from them. This is primarily due to the differences in the methods of preparation. It should be cooked in a proper way so that all the nutrients remain preserved. A few precautions should be adopted to reduce the cooking-related losses. Amaranth leaves should be consumed rapidly and washed quickly in freshwater before cutting. While cooking, steaming or stewing is advisable rather than boiling or blanching. The water produced from steaming should not be discarded, but instead used in cooking to make soup or sauce. Generally, cooking for a long time or keeping vegetables hot for a long time can destroy all the vitamins. During cooking all these parameters should be kept in mind to promote nutrition and communicated among women (Funke 2011).

Most vegetables including amaranth go through cooking such as frying, simmering, boiling, steaming and blanching before consumption. Cooking has a significant effect on chemical compositions such as bioactive components, antioxidant activities and physical characteristics in terms of colour, texture and flavour. However, the effect can be either positive or negative depending on the processing methodologies, vegetable species and shapes (Bernhardt and Schich 2006). Amaranth is one of the commonly consumed leafy vegetables in Guangdong Province, China. The vegetables are consumed after home cooking procedures such as simmering, frying, boiling, steaming and blanching. Nevertheless, knowledge about its bioactive components, for instance, ascorbic acid, polyphenol, anthocyanins, carotenoids and antioxidant activities, is limited. It is not clear how home cooking affects these bioactive components and their antioxidant capacities. The health beneficial antioxidant activities are related to their bioactive components.

3.10 Bioactive Components and Medicinal Properties

It is widely accepted that vegetables play an important role in preventing the development of cardiovascular diseases, ageing-related diseases, obesity and cancers and improving human memory (Wayne et al. 2000). The health-promoting effects of vegetables are attributed to their natural dietary antioxidants. Dietary antioxidants prevent free radicals related to ageing such as reactive oxygen species in the human body (Nilsson et al. 2004). The free radical theory of ageing involves cumulative damage through natural free radical oxidative changes, which over time results in increasing antigenicity, protein changes and oxidative DNA damages (Edelstein and Sharlin 2009). Vitamin C is a powerful antioxidant contributing to the normal function of the immune system (Melvin 2010). Polyphenolic compounds have most antioxidant function acting as electron donors, electron acceptors, decomposer of peroxides and hydroperoxides, metal activators and deactivators and UV absorber (Svobadva et al. 2003). Anthocyanin is demonstrated to have powerful antioxidant properties against low-density lipoprotein oxidation to reduce the risk of the coronary heart disease (Wallace 2011). The carotenoids are yellow to red pigment available in the diet mostly as α-carotene, β-carotene, lutein, cryptoxanthin, zeaxanthin and lycopene. The carotenoids are effective in scavenging reactive oxygen species or ROS formed in physiological processes. Biological molecules such as DNA, protein, lipids and carbohydrates can be damaged by ROS (Kumpulainen and Salonen 1999).

As a kind of vegetable, Amaranth is ranked as one of the top five vegetables in antioxidant capacities (Walter 2001). It contains plenty of bioactive components, such as L-ascorbic acid, betacarotene, polyphenol, anthocyanins and lutein (Walter 2001). It has been used as an antipyretic to reduce labour pain in Indian and Nepalese traditional medicine, as astringent,

diuretic, haemorrhage and hepatoprotective agent (Kirtikar and Basu 1987). Amaranths have also been used to treat bladder distress, piles, toothache, blood disorders and dysentery (Madhav et al. 2008). The health beneficial antioxidant activities are related to their bioactive components. Cooking had no deleterious effect on total bioactive component except for the reduction of anthocyanin content. Home cooking increases the antioxidant activities and the contents of carotenoids, especially by steaming. Steaming has a positive effect on the polyphenol and L-ascorbic acid, which are degraded seriously after simmering procedure. Both simmering and blanching increased the betacarotene and lutein in the cooked amaranth (Han and Xu 2014).

A significant part of the current pharmacological research is devoted to anticancer drug designing customised to fit new molecular targets. The identification of new antioxidants represents a highly emphasised research area, because antioxidants have the capacity to reduce the risk of various chronic diseases caused by ROS. Plants are some of the most attractive sources of new drugs and have been proven effective in the treatment of a number of disorders. Plants contain a wide array of natural antioxidants that might serve as a promising source for the development of new drugs (Badami et al. 2003). Secondary metabolites of plant such as flavonoids, terpenes and alkaloids have received considerable attention of research in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects. The growing intention in the gradual substitution of synthetic food antioxidants by natural antioxidants and wide health implications of antioxidants in nutraceuticals has accelerated the research on vegetable sources and the screening of raw materials like vegetable amaranths for identifying antioxidants. The antioxidants can terminate the oxidative damage of a tissue indirectly by triggering the natural defences of the cell and/or directly by scavenging the free radical species (Xia et al. 2004). One of the best approaches in search for anticancer agents from plant resources is the selection of plants based on their ethnomedical claims. Among the huge number of medicinal plants that are claimed to be anticancer, *Amaranthus paniculatus* is the one of them.

Several studies have shown that the possible benefits of antioxidants from plant sources lie in changing, reversing or forestalling the negative effects of oxidative stress. Antioxidant-rich foods or natural antioxidants may be used as effective substances to prevent diseases of higher age (Paredes-Lopez et al. 2010). Adequate amounts of phenolics, flavonoids and ascorbic acid are found in the leaves of *A. paniculatus* (Linn.). Intake of food containing high flavonoid content is helpful to prevent diseases. The leaf extracts are very rich in antioxidant phytochemicals like polyphenols, ascorbates and flavonoids which have free radical scavenging potentials. Amaranth leaves can be used as a rich source of natural antioxidants with health protective potentials. Earlier studies have also identified strong antioxidant and free radical scavenging activities of *Amaranthus paniculatus* seeds (Bhatia and Jain 2003). Antioxidant-containing foods may exhibit the carcinogenic potential by modulating carcinogen detoxification, by inhibiting lipid peroxidation or by enhancing antioxidant defence mechanism (Davis and Kuttan 2001). The preliminary phytochemical studies indicated the presence of flavonoids, saponins and tannins in the *Amaranthus paniculatus* leaf extract. Many such compounds are known to have antitumor properties (Kintzios 2006). Any of these phytochemicals especially the flavonoids may have attributed to the cytotoxicity and antitumor properties of the *Amaranthus paniculatus*. The extracts of *Amaranthus paniculatus* leaves possess chemopreventive potential and can therefore be exploited as antitumor agents.

The amazing health benefits of amaranths have always been recognised by homoeopathic and Ayurvedic experts. Both the seeds and leaves of amaranth that are used as herbal remedies have nutraceutical value. According to Manikandaselvi and Nithya (2011), nutraceuticals are nonspecific biological agents to promote wellness, prevent malignant processes and control symptoms. In Ayurvedic medicine amaranth seeds and leaves have been used effectively as an astringent to arrest diarrhoea, haemorrhagic problems like

bloody stools and urine and excessive menstruation. It is an excellent wash for skin problems such as acne and eczema to psoriasis and hives. It is used as a mouthwash for sore mouths, gums, teeth and throat and as an enema for colon inflammation and rectal sores (Vietmeyer 1983). Amaranth is used to fight against gastroenteritis, stomach flu and contraception. It can reduce tissue swelling from sprains and tick bites when applied externally, but it should not to be used by pregnant or lactating women. The amaranth oil has been shown to prevent and treat those affected with hypertension and cardiovascular disease. Regular consumption of amaranth can reduce cholesterol levels and lower blood pressure. Amaranth has been found to boost the body's immune system. Amaranth leaves have been used as a good home-made remedy for hair loss and premature greying. Application of the fresh leaf juice of amaranth helps hair to retain its colour and softness.

Amaranthus paniculatus is considered as the world's most nutritious plant. It is best for eye-related problems. The aqueous extract of leaves of *Amaranthus paniculatus* was detected to have radioprotective effect in whole body gamma radiation (Krishna and Kumar 2005). It can also be used to overcome the psychological stress-related problems, and this health benefit has been examined in stress-induced memory dysfunction problem and found to improve learning after radiation stress (Bhatia and Jain 2003). *Amaranthus spinosus,* a weedy amaranth, is used as cooling, laxative, diuretic, antipyretic, stomachic, astringent, diaphoretic, emollient, febrifuge and galactagogue. It can improve appetite and reduce burning sensation and is effective in treatment of hallucination, leprosy, piles, bronchitis, leucorrhoea, constipation and flatulence. Herb decoction can be used as a mouthwash for toothache. The root is used as heating and expectorant and is useful in the treatment of leucorrhoea, leprosy and eczema, considered specific in gonorrhoea. *Amaranthus spinosus* Linn. leaves significantly reduced aspirin-induced ulcer index. A gastric anti-secretary effect is created by the leaves by reducing gastric volume and acidity. The seed is used as a poultice for broken bones. It is used internally for the treatment of internal bleeding, diarrhoea and excessive menstrual flow and snake bites. Externally, it is used to treat mouth ulcers, nosebleeds and wounds. The plant can be used fresh or it can also be harvested before commencement of flowering and dried for later use. The root is emmenagogue and galactagogue; a paste of the root is used in the treatment of menorrhagia, gonorrhoea and eczema and helps to remove pus from boils. In Nepal the root juice is used for the treatment of fevers, urinary troubles, diarrhoea and dysentery. The root juice can also be used to treat indigestion and vomiting due to eating of unusual foods and in combination with root juice of *Dichrophela integra*, and *Rubus ellipticus* is often used to treat stomach disorders. *A. blitum* is used as stomachic, as good emollient also in the treatment of roundworm, in biliousness, haemorrhagic-diathesis and blisters. Another weed *A. viridis* is used as cooling, appetiser, laxative, stomachic, alexiteric, and antipyretic and is used in burning sensation, hallucination, bronchitis, leprosy, piles, constipation and leucorrhoea. The leaves are used as an emollient and the root as a warming agent and expectorant that help to lessen the menstrual flow and treat leucorrhoea and leprosy. A fairly high concentration of potassium, copper and iron makes it a potential nutraceutical suitable for fortification of foods. These plant organs might be evaluated as a significant supplement and rich source of dietary minerals in human food to fight various diseases. It is rich in ascorbic acid which is a known antioxidant. A popular vegetable *A. tricolor* is used as cooling, alexiteric, laxative, stomachic, appetiser and antipyretic and also used in burning sensation, leprosy, bronchitis, piles, hallucination, leucorrhoea and constipation. Many compounds and extracts from amaranths showed antidiabetic, anti-hyperlipidemic, spermatogenic, anti-cholesterolemic effects (Sangameswaran and Jayakar 2008; Girija et al. 2011), antioxidant and antimicrobial activity (Alvarez-Jubete et al. 2010; Tironi and Anon 2010).

A future promise of vegetable amaranths is the preparation of leaf-protein concentrates. Compared with most other species, amaranth leaf

protein is highly extractable in comparison with other species. In one experiment, amaranth had yielded the highest amount of extractable protein among 24 plant species tried. During the extraction of leaf protein, other nutrients are also extracted, for example, provitamin A (betacarotene), polyunsaturated lipids (linoleic acid) and iron. Heating or treating the extract with acid precipitates the nutrients as leaf-protein concentrate and most of the harmful compounds are eliminated, as they remain in the soup. The green cheeselike coagulum is washed with water and slightly acidified with dilute acetic acid (vinegar) to further minimise the amounts of anti-nutritive factors if any. The prepared leaf-nutrient concentrate is especially useful for young children and other persons requiring particularly high protein, vitamin A and iron. The fibrous pulp left after extraction of leaf-protein concentrate is a suitable feed for animals. The protein quality of the amaranth leaf-nutrient concentrate in terms of amino acid composition, digestibility and nutritional effectiveness is excellent. The quality is however species dependent probably due to the presence of secondary metabolites present in varying amounts in some species (Carlsson 1982).

Sanchez-Marroquin (1983) has given in detail agro-industrial uses of grain amaranths. A new food product called Amarlac and Amarmeal of high nutritional quality has been released in Guatemala by the Amaranth Food Company. It represents the first output of research towards processing and utilisation of amaranth grain and its conversion into high-quality human food particularly for young children and pregnant women (Amaranth news Letter 1987). These products can be tried in India.

3.11 Weed Amaranths

Human interests, mostly in economic perspectives, determine whether a plant is a weed or not. The weed can be defined in many ways, like any plant that is objectionable and interferes with the activities or welfares of man (Anonymous 1994), a plant out of place or growing where it is not wanted (Blatchley 1912) or a herbaceous plant, not valued for use and beauty, growing wild and rank and regarded as cumbering the ground or hindering the growth of superior vegetation. Generally, a weed can be defined as a member of undesirable, useless plant communities, thriving in a habitat disturbed by human, possessing competitive behaviour, capable of mass movement from one place to another and can interfere with the activities or welfare of human being.

The genus *Amaranthus* not only includes crop and ornamental species; it also includes many weedy species, known as pigweed. Pigweed is the common name applied for several closely related summer annuals that behave as major weeds of the vegetable and row crops and acknowledged among most damaging weeds in agricultural field. They are highly competitive with crop plants, express high flexibility in response to environmental changes and adaptability to diverse ecoclimate and ensure their existence by producing a huge number of seeds. Pigweeds are the natural inhabitants in parts of North and Central America. Cultivation of crop and commercial germplasm exchange has opened new niches, allowing pigweed to invade agricultural ecosystem throughout the America and parts of Africa, Europe, Asia and Australia. Approximately ten *Amaranthus* species are recognised as weedy member. These are either monoecious (male and female flowers are present on the same plant) or dioecious (have separate male and female plants) species. Monoecious category comprises redroot pigweed *(A. retroflexus),* smooth pigweed *(A. hybridus),* Powell amaranth *(A. powellii),* tumble pigweed *(A. albus),* prostrate pigweed *(A. blitoides)* and spiny amaranth *(A. spinosus)*, and dioecious category includes the common waterhemp *(A. rudis),* tall waterhemp *(A. tuberculatus),* Palmer amaranth *(A. palmeri)* and sandhills amaranth (*A. arenicola*) (Fig. 3.18). Weedy *Amaranthus* species (pigweeds) are regarded as the major problem in agriculture and the notable weed *A. retroflexus* is considered one of the world's worst weeds.

Amaranthus retroflexus is thought to be a native riverbank pioneer of the central and eastern USA and adjacent regions of southeastern Canada and northeastern Mexico (Sauer 1967). It

Fig. 3.18 Few notable pigweeds (**a**). *A. retroflexus* L. (**b**) *A. tuberculatus* (Moq.) J. D. Sauer (**c**) *A. quitensis* Kunth. (**d**) *A. palmeri* S. Watson (**e**) *A. powellii* S. Watson (**f**) *A. blitoides* S. Watson (**g**) *A. arenicola* I.M. Johnston (**h**) *A. albus* L.

Fig. 3.18 (continued)

has become naturalised throughout the temperate regions of the Northern and Southern Hemispheres. It is an annual herb, erect or with ascending branches, simple or branched (especially from the base to about the middle of the stem). Stems are stout, sub-terete to angled and densely covered with multicellular hairs. Leaves are long-petiolate (petioles up to 6 cm, in robust plants not rarely equalling lamina), lamina ovate to rhomboid or oblong-ovate, with obtuse to subacute, mucronulate tip, shortly cuneate or attenuate base, multicellular hairs along the lower surface of the primary venation and often on the lower margins, 3–6 × 5-11 cm. Flowers are arranged in greenish or rarely somewhat pink-suffused, stout, axillary and terminal spikes, which are usually shortly branched to give a lobed appearance, more rarely with longer branches. The terminal inflorescence is paniculate and very variable in size, with intermixed male and female flowers. Bracts and bracteoles are lanceolate-subulate, pale-membranous with a prominent green midrib excurrent into a stiff, colourless arista, longer bracteoles subequalling to twice as long as the tepals. Perianth have five tepals; tepals of the male flowers are lanceolate-oblong, blunt to subacute,1.75–2.25 mm long; those of the female flowers are narrowly oblong-spatulate to spatulate, obtuse or emarginate, 2–3 mm long, more or less green-vittae along the midrib, which ceases below the apex or is excurrent in a short mucro. Number of stamens 5 and stigmas 2–3, flexuose or erect, 1 mm long. Capsules are subglobose, usually shorter than the perianth, circumscissile, with an indistinct neck, rugose below the lid, 2 mm in length. Seeds are black, compressed, 1 mm in diameter, almost smooth centrally, faintly reticulate around the margins (Fig. 3.18a)

Spiny amaranth (*A. spinosus*) is a native of the tropical Americas, but now it is available on most of the continents as an introduced noxious weed species. It can be a serious weed in the ricefield in Asia. Spiny amaranth has spread through tropical and subtropical latitudes around the world and found in cultivated fields, waste places, roadsides, garbage heaps and abandoned fields. It grows both in wet or dry sites, but grows best when soil moisture levels are below field capacity. It originated probably in tropical lowland of South and Central America and from their introduced into other warmer parts of the world.

Amaranthus spinosus Linn. (sp. Pl. ed. 1: 991. 1753) is an annual erect or slightly decumbent herb, simple or much-branched and bushy, attaining a height of up to 1.5 m. Stems are stout, sometimes reddish and usually branched, angular, glabrous or increasingly furnished above (especially in the inflorescence) with long, multicellular hairs. Leaves are long-petiolate (petioles up to 9 cm, sometimes longer than the lamina), lamina ovate to rhomboid-ovate, elliptic,

lanceolate-oblong or lanceolate, with subacute or more commonly blunt or retuse apex having a distinct, fine, colourless mucro, entire margin and cuneate or attenuate base, surface glabrous or thinly pilose on the lower surface of the primary nervation, having a dimension of 0.8–6 × 1.5–12 cm. Each leaf-axil bears a pair of fine and slender to stout and compressed spines up to 2.5 cm long. Flowers are green, in the lower part of the plant arranged in axillary clusters, 6–15 mm in diameter; towards the ends of the stem and branches, the clusters are leafless forming simple or sometimes (especially the terminal) branched spikes usually up to 15 cm long and 1 cm wide. Lower clusters are entirely female, as are the lower flowers of the spikes, while upper flowers of the spikes are male, mostly covering the apical one-fourth to two-thirds of each spike. Bracts and bracteoles are deltoid-ovate, pale-membranous, with an erect, pale or reddish awn formed of excurrent green midrib. Bracteoles are shorter than, subequalling or little exceeding the perianth, commonly smaller than the bracts. Number of tepals is five; those of the female flowers are narrowly oblong or spatulate-oblong, obtuse or acute, mucronulate, frequently with a greenish dorsal vita, 1.5–2.5 mm long, while those of the male flowers are broadly lanceolate or lanceolate-oblong, acute or acuminate, with green midrib. Number of stamens 5 and stigmas 2–3, flexuose or reflexed, 1–1.5 mm in length. Capsule are ovoid-urceolate with a short inflated neck below the style base, 1.5 mm in length, regularly or irregularly circumscissile or rarely indehiscent, the lid rugulose below the neck. Seed are black, shiny, compressed, 0.75–1 mm in diameter (Fig. 3.19).

Fig. 3.19 General plant morphology of spiny amaranth (*Amaranthus spinosus* L.)

As reported by Sauer (1967), *A. hybridus* originated as a riverbank pioneer of Eastern North America, with earlier range expanding throughout milder and moister regions to Mexico, Central America and Northern South America. The earliest record of the species in Europe dates back to approximately 300 years. The spread of *A. hybridus* in Europe took place primarily in the Mediterranean region. The spread of *A. hybridus* was quite slow in comparison with other *Amaranthus* weeds, especially *A. retroflexus*. The presence of this species in the Western North America, Eastern Asia, Australia and South Africa has been reported since the mid-1900s. Today, *A. hybridus* is distributed worldwide as a weed of agricultural fields and other disturbed habitats, and it ranks among the 18 most noxious weeds in the world (Holm et al. 1991) (Fig. 3.20).

Amaranthus hybridus L. is an annual, erect or less commonly ascending herb, attaining a height up to 2 m. not infrequently reddish-tinted throughout. Stems are stout, branched, angular, glabrous or thinly to moderately hairy with short or long multicellular hairs (increasingly so above, especially in the inflorescence). Leaves are long-petiolate (petioles up to 15 cm but even then scarcely exceeding the lamina), lamina broadly lanceolate to rhomboid or ovate elliptic, ovate or oblong, with gradually narrowed to blunt or acute to subacute mucronulate tip, attenuate or shortly cuneate base, dimension 19–30 × 3–10 cm. Inflorescences are moderately developed in comparison with three grain species. Flowers are arranged in yellowish, green, reddish or purple axillary and terminal spikes formed of cymose clusters, which are increasingly closely packed upward. The terminal inflorescence varies from a

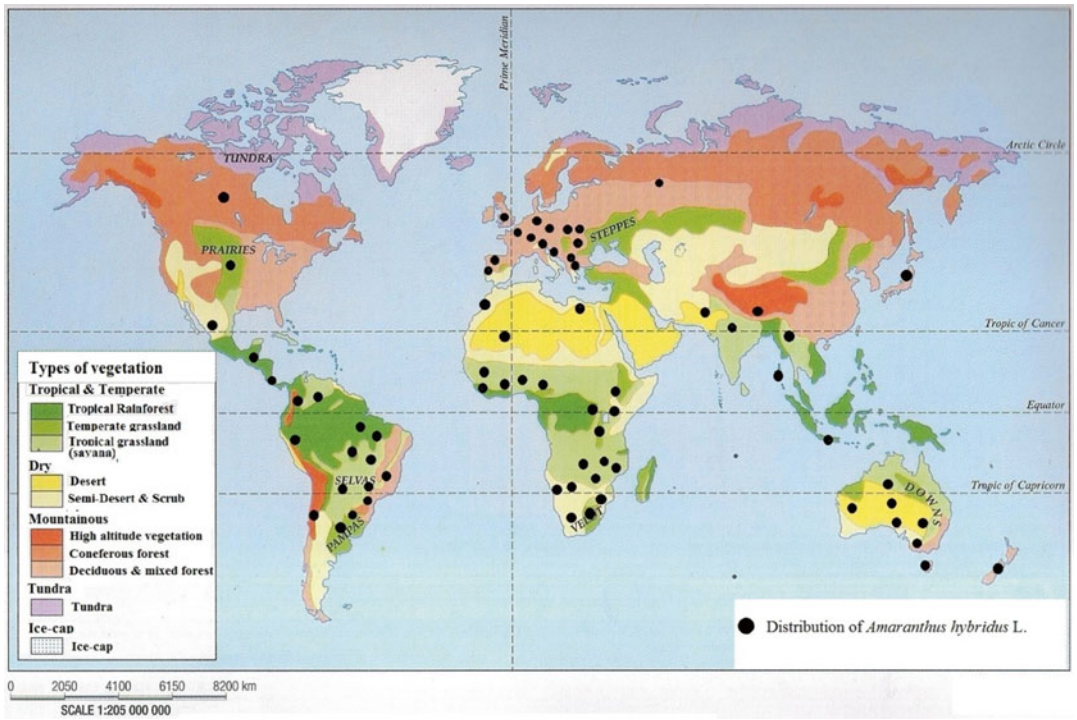


Fig. 3.20 Worldwide distribution of *Amaranthus hybridus* L.

single spike to a broad, much-branched, panicle up to 45×25 cm; male and female flowers are intermingled throughout the spikes. Bracts are deltoid-ovate to deltoid-lanceolate, pale-membranous, acuminate with a long, pale to reddish-tipped, erect arista formed by the stout, excurrent, yellow or greenish midrib, twice as long as tepals, exceeding style branches. Tepals are five in number, lanceolate or oblong, acute-aristate or the inner ones sometimes blunt in the female flowers, shorter than utricle and slightly recurved, inner tepals are shorter than outer tepals. Number of stamens 5 and stigmas 2–3, erect, flexuose or recurved, 0.75–1.25 mm in length. Fruits are subglobose to ovoid or ovoid-urceolate, circumscissile utricle. 2–3 mm in length, tapering into towers at apex, with a moderately distinct to obscure 'neck', smooth lid, longitudinally sulcate, or sometimes rugulose below the neck. Seeds are black and shiny or pale, compressed, 0.75–1.25 mm in diameter, with spermoderm smooth centrally, faintly reticulate in the margins (Fig. 3.21).

A. retroflexus, like *A. hybridus* and many other amaranths, is a riverbank pioneer. Its earliest distribution expanded from the Central Eastern USA to adjacent Canada and Mexico. Linnaeus is blamed for introducing the weed in Europe, where the species quickly spread (Sauer 1967). By the early 1800s, the species became a common weed in the temperate regions of the Old World. Today, the species is existing as an introduced or naturalised weed worldwide, ranking among the most widely distributed weeds of the world (Holm et al. 1997). Initial distribution of *A. powellii* was recorded in canyons, desert washes and other open habitats west of the Cordilleran system of America, with wide gaps in wetter regions of Central America. The earliest record of this species in Europe is found in German herbarium specimens from the late 1800s and later introduced in Southern India and South Africa which are evidenced by the samples of Southern India and South Africa. Migration of *A. powellii* to Eastern North America took place only during the last century.

Fig. 3.21 General plant morphology and inflorescence of *Amaranthus hybridus* L.

Amaranthus tuberculatus is an annual erect herb that bears flowers during the summer or fall. It has a long terminal inflorescences in the forms of linear spikes to panicles. Female flowers generally don't have any tepals, although rarely one or two rudimentary tepals may be observed. Sauer (1972) separated *Amaranthus rudis* (formerly *Amaranthus tamariscina*) as distinct from *A. tuberculatus*, giving emphasis on the features like dehiscence of utricle and absence of female tepals. Sauer's *A. rudis* was first described in Oklahoma in the 1830s, and since then it has shown continuous northward and eastward migration into midwestern states, overlapping with *A. tuberculatus*, distributed in the sandy and muddy streambanks, lakeshores and pond margins, along the Missouri, Mississippi and Ohio River systems (Sauer 1957, 1972), where both *A. tuberculatus* and *A. rudis* coexisted. The former was recorded on average 40 years prior to that of the later. Many of the samples collected from these areas were classified as putative hybrids between *A. tuberculatus* and *A. rudis*, with a higher ratio of hybrids to non-hybrids in artificial habitats compared to natural settings. According to Sauer's evaluation of dioecious amaranths (1957), *A. tuberculatus* and *A. rudis* represent the most abundant hybrid combination. The author also mentioned that actual hybridisation among these species may be underestimated due to the nature of morphological determinations based on character intermediacy, which is often diluted after a few generations of backcrossing with the predominant genotype. More recent work applying molecular and morphological markers suggested the abolition of different species status and considered them to be one and the same (Pratt and Clark 2001). At present a single polymorphic species, *A. tuberculatus*, has got recognition (Mosyakin and Robertson 2003). Costea and Tardif (2003a) however recognised the difference between *A. tuberculatus* and *A. rudis* at the variety level and introduced to varieties: *A. tuberculatus* var. *rudis* with more weedy tendencies than *A. tuberculatus* var. *tuberculatus*. Over the last 20 years, *A. tuberculatus* has appeared as the most significant weed problem in the midwestern USA (Steckel 2007), one of the world's premier agricultural regions. The success achieved by *A. tuberculatus* to become a notorious weed can be attributed mainly to its remarkable ability of herbicide resistance. Studies on the herbicide resistance of *A. tuberculatus* are discussed in detail in a later section. The capacity of this species to respond to selection and adaptive diversity identified may be of potential benefit to less orthodox crop-breeding pro-

gramme though from view point of weed management *A. tuberculatus* is of great concern.

The prime reasons for the noxious nature of these weedy species lie in their ability to withstand and adapt efficiently to the modifications in agricultural management practices which are specifically designed to control and prevent weed colonisation. For example, numerous populations of pigweeds have evolved herbicide resistance (Drzewiecki 2001; Rayburn et al. 2005).

Correct identification of pigweed species may be controversial, especially during early vegetative phase because of their almost similar morphological look (many look very similar). Significant variation may occur within a species which may lead to occasional intraspecific hybridisation (Sellers et al. 2003). Kansas State University extension has published an excellent guide on pigweed identification with illustrations and a key to discriminate nine different weed amaranths (Horak et al. 1994). Monoecious species are primarily self-pollinating (Murray 1940; Weaver and McWilliams 1980), while dioecious species are obligatory allogamous. Dioecism introduces genetic diversity by encouraging outcrossing, while monoecism supports colonisation of new areas by a single plant. Both the monoecious and dioecious pigweeds are highly successful invader.

Infrageneric Classification of Amaranths

4

4.1 General

Genus *Amaranthus* represents a cosmopolitan assemblage of species showing vast morphological variability and wide geographical distribution. Proper taxonomic delimitation of taxa is a prerequisite for it's involvement in any breeding programme. In case of *Amaranthus* it is much more applicable due its taxonomic ambiguity and presence of a large number of synonyms. Several attempts have been made from time to time to classify the genus infragenerically. The genus has been classified traditionally into three subgenera, viz. subgenus *Acnida* (comprising dioecious members), subgenus *Amaranthus* and subgenus *Albersia*. Grain amaranths are included in subgenus *Amaranthus* and vegetable amaranths are included in subgenus *Albersia*. Infrageneric classification in subgen. *Amaranthus* is to some extent satisfactory but, in case *Albersia* is far from conclusive, needs further studies. The works done so far mostly employed morphological data. But with the advent of molecular and biochemical evidences, existing taxonomic delimitations should be evaluated keeping in view phylogenetic relationship.

4.2 Synopsis of Infrageneric Classifications of Amaranths

The classification of *Amaranthus* is ambiguous due to the absence of specific and qualitative species-defining characteristics, the extremely wide range of phenotypic variability among species as well as the introgression and hybridisation among weedy and crop species (Hauptli and Jain 1978). There is no general agreement on taxonomy of *Amaranthus*. At present the best option to be adopted is to understand the taxonomic problem of *Amaranthus* compiling the available informations in the literature and studying the phenotypic variability in a comprehensive manner. This genus is nomenclaturally and taxonomically problematic both for its morphological flexibility and frequent hybridisation. Sauer (1967, 1976, 1993) made a taxonomic and geographic survey on the grain amaranths and their putative wild relatives employing morphological features and designated two subgenera – *Acnida* (which included the dioecious species) and *Amaranthus* (which included the monoecious species). Later Mosyakin and Robertson (1996) recognised three subgenera – subgen. *Acnida*

S. Das, Amaranthus*: A Promising Crop of Future*, DOI 10.1007/978-981-10-1469-7_4

(Linnaeus 1753: 1027) Aellen ex K. R. Robertson (Robertson 1981: 283), subgen. *Amaranthus* and subgen. *Albersia* (Kunth 1838: 144) Grenier and Godron (1856: 3) on the basis of the inflorescence and floral features. Traditionally, the subgen. *Amaranthus* has been divided into two sections (Thellung 1919; Aellen 1959; Robertson 1981) sect. *Amaranthus* [= sect. *Amaranthotypus* Dumortier (1827: 19)] and sect. *Blitopsis* Dumort. s.l. (1827: 19). Carretero (1985) further splitted the sect. *Blitopsis* into two groups: sect. *Blitopsis* s.s., including species having indehiscent fruits and $n = x = 17$, and sect. *Pyxidium* Moq. in De Candolle (1849: 262) comprising species with dehiscent fruits and $n = x = 16$. Recently Mosyakin and Robertson (1996) recognised four sections under the subgen. *Albersia*, of which three sections [sect. *Blitopsis*, sect. *Pentamorion* (Beck 1909: 24) Mosyakin & K. R. Robertson (1996: 280) and sect. *Goerziella* (Urban 1924: 301) Mosyakin & K. R. Robertson (1996: 280)] include species with indehiscent fruit and remaining one (sect. *Pyxidium*) includes species having dehiscent, circumscissile utricle.

4.2.1 *Amaranthus* Subgen. *Acnida* Aellen ex K.R. Robertson

The subgenus *Acnida* includes nine dioecious species which are native to North America and don't have any immediate phylogenetic affinity with the amaranth crop. However, recent studies (Trucco et al. 2005a, b) have indicated that gene exchange might have occurred between *Amaranthus tuberculatus*, an infamous member of *Acnida*, and *A. hybridus,* a probable progenitor of domesticated grin amaranths. In fact, studies on gene exchange between *A. tuberculatus* and *A. hybridus* provide interesting clues regarding applicability of the genetic diversity and novel gene pool of the dioecious taxon for crop improvement. Moreover, as *A. tuberculatus* is increasingly appearing as a model organism for the study of weeds (Trucco et al. 2009), a wealth of genomic resources are being generated that may be used for programmes designed for the crops.

4.2.2 *Amaranthus* Subgen. *Amaranthus*

The subgenus *Amaranthus* consists of 20 species of annual monoecious herbs (Mosyakin and Robertson 2003). The species are mostly native to the Americas (Mosyakin and Robertson 2003). As the female and male flowers are arranged in close proximity (Murray 1940), the monoecious amaranths are generally self-pollinating. Stems are generally erect, and both the axillary and terminal inflorescences are arranged in cylindrical spikes or panicles (Mosyakin and Robertson 2003). Attempts to discriminate or differentiate taxa within this group faced much difficulty because during taxonomic delimitation, emphasis was given on pigmentation and growth forms which are extremely variable within amaranths (Sauer 1967). However, examination of floral parts can provide a constant set of characters from which discontinuities can be explored to define taxa. In this regard, tepal number and morphology are significant in preparing taxonomic keys. *Amaranthus hybridus* is a fundamental and basal species in this crop subgenus and conforms an interbreeding complex involving two other *Amaranthus* weeds, *A. retroflexus* and *A. powellii*. Partially fertile hybrid swarms between these species can be found in the USA, in areas where their distributions overlap, and in Europe, where all three species are recent immigrants. *Amaranthus hypochondriacus*, one of the three grain amaranths, is cultivated as an alternative crop in North America and Asia. Initially the species was thought to have an Asian origin, but now it is believed that distribution in Asia is secondary in nature, and the species is supposed to have derived from *A. powellii* through domestication in North America. Hybridisation has played a significant role in the evolution of *A. hypochondriacus*, with several hybrid races cultivated by American aborigines. Sauer (1967) identified stable hybrid cultivars derived from crosses presumably between *A. hypochondriacus* and local admixtures of *A. cruentus* – an *A. hybridus* domesticated form originating in Southern Mexico or Guatemala – and its progenitor. For instance, in the region of Los Reyes (Michoacan),

a cultivar is grown to make special 'dark' tamales which was a putative hybrid between *A. hypochondriacus* and *A. hybridus*. Likewise, a Warihio Indian crop from Rancho Trigo (Chihuahua) was identified as a hybrid between *A. hybridus* and *A. powellii*. Another putative hybrid between *A. cruentus* and *A. hypochondriacus* is cultivated in the Oaxaca region of Southern Mexico, and the same crop is grown in small gardens in Madras, India. Another important grain species *A. caudatus*, the grain amaranth of South America, is presumed to have originated through the domestication of *A. quitensis* in the Andean region (Sauer 1967). *A. quitensis* originally distributed as riverbank pioneer of South America, in the mountains of the North-West and at the lower altitude of temperate south is a weedy member of hybridus complex. Cultivation of *A. quitensis* type of plant with incipient domestication can be found from Ecuador to Northern Argentina, mainly for the production of pigments needed for colouring of chicha and other maize dishes. The South American amaranths are not supposed to hybridise readily with the North American members of this complex

4.2.3 *Amaranthus* Subgen. *Albersia* Grenier & Godron

The subgenus *Albersia* includes all the species traditionally included by many earlier authors in *Amaranthus* sect. *Blitopsis sensu lato.* It still represents a rather polymorphic group. All the vegetable and weed *Amaranthus* except *A. hybridus* are included in subgenus *Albersia*. Carretero (1985), following Moquin-Tandon (1849), favoured the separation of dehiscent-fruited species in a section *Pyxidium.* But after this separation, the resulting infrageneric taxa of this subgenus still appear to be too polymorphic and widespread geographically to be considered as a natural group. Taxonomic delimitation of vegetable amaranths more particularly those included in sect. *Pyxidium* of subgen. *Albersia* (Mosyakin and Robertson 1996) requires more study from a morphological point of view. Dehiscence or indehiscence of the fruit is not an ultimate conclusive feature for segregation of infrageneric taxa.

4.3 Infrageneric Classification After the Modification by Mosyakin and Robertson (1996)

Recently Mosyakin and Robertson (1996) introduced four sections under the subgen. *Albersia*, of which three include species having indehiscent fruits [sect. *Blitopsis*, sect. *Pentamorion* (Beck 1909: 24) Mosyakin & K. R. Robertson (1996: 280) and sect. *Goerziella* (Urban 1924: 301) Mosyakin & K. R. Robertson (1996: 280)] and one (sect. *Pyxidium*) includes species with dehiscent fruits.

The Infrageneric Classification After the Modification by Mosyakin and Robertson (1996)

Genus *Amaranthus* L.
- Subgen. *Acnida* (L.) Aellen ex K. R. Robertson
  - Sect. *Acnida* (L.) Mosyakin & K. R. Robertson
  - Sect. *Saueranthus* Mosyakin & K. R. Robertson
  - Sect. *Acanthochiton* (Torrey) Mosyakin & K. R. Robertson
- Subgenus *Amaranthus*
  - Sect. *Amaranthus*
    - Subsect. *Amaranthus*
    - Subsect. *Hybrida* Mosyakin & K. R. Robertson
    - Nothosect. *Dubia* Mosyakin & K. R. Robertson
  - Sect. *Centrusa* Griseb.
- Subgenus *Albersia* (Kunth) Gren. & Godr.
  - Sect. *Blitopsis* Dumort
  - Sect. *Pentamorion* (G. Beck) Mosyakin & K. R. Robertson
  - Sect. *Goerziella* (Urban) Mosyakin & K. R. Robertson
  - Sect. *Pyxidium* Mosyakin & K. R. Robertson

Key for the Infrageneric Delimitation of Taxa

- Plants normally dioecious .... Subgen. *Acnida*
  - Leaves linear-lanceolate; margins of both bracts and leaves are crisped and minutely denticulate; pistillate flowers have very broad, deltate or rhombic-deltate foliaceous bracts..... ..... subgen *Acnida* sect. *Acanthochiton*
  - Both bracts and leaves with entire margin; bracts normally elliptic to subulate, linear not foliaceous:
    Pistillate flower without tepal or rarely with 1–2 reduced linear or linear-lanceolate tepals less than 2.mm long; utricle generally indehiscent (dehiscent only in *A. rudis*) .... subgen *Acnida* sect. *Acnida*
    Pistillate flower with five (rarely four) spathulate or obovate elliptic tepals, utricle generally dehiscent (indehiscent only in *A. greggii*) .... subgen *Acnida* sect. *Saueranthus*
- Plants usually monoecious
  - Plants ascending, prostrate or erect; tepals usually 2–3, but in some species 4–5; in most cases inflorescence axillary, glomerate or short spiciform; in some species terminal inflorescence is also developed, but in such cases utricles indehiscent or tepals less than five .... *Amaranthus* subgen. *Albersia*
  - Plants normally erect, always with more or less long terminal, spiciform, cylindrical, inflorescence; tepals usually five, rarely 4–3, fruit transversely dehiscent (erect *A. bouchonii* and some forms of *A. spinosus*) .....*Amaranthus* subgen. *Amaranthus*
    Bracts modified into firm spines; fruit transversely or irregularly dehiscent ..... *Amaranthus* sect. *Centrusa*
    Bracts not modified into spines; fruit dehisces transversely in the equatorial part (fruit indehiscent in *A. bouchonii*)..... *Amaranthus* sect. *Amaranthus*
      At least inner tepals spathulate, apex obtuse, truncate, emarginate or broadly triangular, occasionally abruptly narrowed into a protruding arista or bristle formed of midvein ..... sect. *Amaranthus* subsect. *Amaranthus*
      Tepals gradually narrowed into acute, often spinulose or bristle-like tip .... sect *Amaranthus* subsect. *Hybrida*

4.3.1 *Amaranthus* Subgen. *Acnida* (L.) Aellen ex K. R. Robertson

Acnida L. Sp. Pl. 1027. 1753. – Type: *Acnida cannabina* L. (= *Amaranthus cannabinus* (L.) Sauer). The only species originally assigned by Linnaeus in the genus *Acnida*.

Acnida L. was formerly recognised as a separate genus by most of the authors until 1955. But Sauer (1955) on the basis of convincing evidences included it within *Amaranthus*. Sauer did not provide any formal nomenclatural combination for this taxon either at the subgeneric or sectional level. Aellen (1959) suggested the combination *Amaranthus* subgen. *Acnida* (L.) Aellen without any citation of basionym. Later the combination was validated by Robertson (1981). This dioecious Amaranth represents a natural and morphologically distinct group originally endemic to North America.

The subgenus includes nine dioecious species, distributed into three sections each having a rather distinct combination of characters related to fruit (dehiscent or indehiscent), bract (foliaceous or not) and tepals of pistillate flower.

- *Amaranthus* subgen. *Acnida* sect. *Acnida* (L.) Mosyakin & K. R. Robertson, comb. nov.

Acnida L. sect. *Acnida* are autonym created by publication of *Acnida* L. sect *Montelia* Moquin in DC, Prodr.13/2: 277. 1849 – Type: *Acnida cannabina* L. (= *Amaranthus cannabinus* (L.) Sauer).

This section includes dioecious species characterised with greatly reduced tepals in pistillate flower and mostly indehiscent fruits. Three species belong to this section, viz. *Amaranthus cannabinus* (L.) Sauer, *Amaranthus australis*

(A. Gray) Sauer and *Amaranthus tuberculatus* (Moquin) Sauer. The other two species *Amaranthus rudis* and *Amaranthus floridamus* show a morphological transition towards this section. Besides dehiscence of fruit, all other characteristics of both the species like greatly reduced non-spathulate tepals, shape of inflorescence and general habit, provide ample reasons for their inclusion in section *Acnida*.

Sauer (1972) separated *A. rudis* (formerly *A. tamariscina*) as distinct from *A. tuberculatus*, primarily on the basis of utricle dehiscence and absence of female tepals, though more recent work using molecular and morphological markers suggested the merger of both the species into a single polymorphic species *A. tuberculatus* (Pratt and Clark 2001).

- *Amaranthus* subgen. *Acnida* sect. *Saueranthus* Mosyakin & K. R. Robertson, sect. nov.

  Type: *Amaranthus palmeri* S. Watson

  This section is named after J. D. Sauer with pioneering contribution on grain amaranths. Plants included in this section are dioecious. Flowers have five (rarely four) tepals spathulate in shape (occasionally only inner spathulate tepals), with rounded truncate or acuminate apex and usually dehiscent utricle. The section includes four species, viz. *Amaranthus palmeri* S. Watson, *Amaranthus watsoni* Standley, *Amaranthus arenicola* I. H. Johnson and *Amaranthus greggii* S. Watson. The species included in this section are characterised with five well-developed spathulate tepals in pistillate flowers and dehiscent fruit (except *A. greggi* having indehiscent utricles).

- *Amaranthus* subgen. *Acnida* sect. *Acanthochiton* (Torrey) Mosyakin & K. R. Robertson, comb. nov.

  Type: *Acanthochiton wrightii* Torrey (= *Amaranthus acanthochiton* Sauer)

  This is a monotypic section comprising the only one species *Amaranthus acanthochiton* Sauer (often incorrectly mentioned as *A. acanthochiton* (Torrey) Sauer. The plant possesses several features uncommon in amaranths like – extremely broad, deltate, foliaceous bracts of female flowers and narrow lanceolate to linear leaf lamina with crisped margin.

4.3.2 *Amaranthus* Subgen. *Amaranthus*

Type: *Amaranthus caudatus* L., a lectotype designated by Britton and Brown (1913) and supported by Hitchcock and Green (1929).

The presence of massive terminal inflorescence and dehiscent utricle is not the absolute identifying feature for subgen. *Amaranthus*. These features in combination with other features should be considered. Mosyakin and Robertson (1996) divided this subgenus into several sections like sect. *Amaranthus* and sect. *Centrusa* Griseb. and one additional sect. nothosect. *Dubia* Mosyakin & K. R. Robertson. Subsequent segregation of the sect. *Amaranthus* was recommended, two new subsects. were proposed, i.e. subsect. *Amaranthus* and subsect. *Hybrida* Mosyakin and Robertson subsect. nov. Proper subsectional delimitation is yet to be achieved. Placement of few species like *A. brandegei* Standley, *A. bigelowii* Uline & Brey, *A. viscidulus* Green, *A. scariosus* Bentham (Sauer 1967) and *A. asplundii* Thell. (Hunziker 1965) is questionable and requires further study. Sauer (1967) included the Australian species *A. pallidiflorus* F. von Muell. closely resembling *A. clementii* Domin in the section *Amaranthus*, but on the basis of floral morphology, it appears to be closely related to another Australian species *A. mitchellii* Bentham having axillary inflorescence typical of subgen. *Albersia* (Kunth) Gren. & Godr. Overlapping of morphological features and new findings through molecular approach regarding phylogenetic affinity have emphasised the need of evaluation of this section *Amaranthus* and supported the introduction of more new subsects. under sect. *Amaranthus*.

- *Amaranthus* subgen. *Amaranthus* sect. *Amaranthus*

Amaranthus sect. *Amaranthus* Dumort. (Florula Belgica : 19. 1827.)

Amaranthus sect. *Euamaranthus* Moquin in DC, Prodr. 13. 2: 255. 1849 (excluding *A. gangeticus* L. and *A. mangostanus* L.)

This section is divided into two subsections:

(1) *Amaranthus* subsect. *Amaranthus* comprising species like *A. quitensis* Kunth, *A. caudatus* L., *A. retroflexus* L., *A. celosioides* Kunth? and *A. asplundii* Thell.
(2) *Amaranthus* subsect. *Hybrida* Mosyakin & K. R. Robertson subsect. nov.

Type: *Amaranthus hybridus* L.

This subsection comprises species like *A. powellii* S. Watson, *A. hybridus* L., *A. hypochondriacus* L., *A. cruentus* L. and *A. bouchonii* Thell.

- *Amaranthus* subgen. *Amaranthus* nothosect. *Dubia* Mosyakin & K. R. Robertson nothosect. nov. (*Amaranthus* sect. *Amaranthus* x *Amaranthus* sect. *Centrusa*)

The allopolyploid species *Amaranthus dubius* Mart. is very closely related with *A. spinosus* L. (of sect. *Centrusa*) and members of sect. *Amaranthus*. This stabilised allopolyploid species are supposed to have evolved as a result of natural hybridisation between *A. spinosus* and either *A. hybridus* or *A. quitensis* of sect. *Amaranthus*.

- *Amaranthus* subgen. *Amaranthus* sect. *Centrusa* Griseb.

Type: *Amaranthus spinosus* L. (Fl. British West Indian Islands 68; 1859)

Amaranthus sect. *Acanthophora* G. Beck in Reichenb Icon. Fl. Germanicae et helveticae 24: 177. 1909.

Amaranthus spinosus is the only species included in this section. It is probably a native to South America. This polymorphic species is widespread in the tropics and subtropics of both the hemisphere. It shows some degree of morphological progress towards developing dioecious habit.

4.3.3 *Amaranthus* Subgen. *Albersia*

Type: *Albersia blitum* (L.) Kunth (= *Amaranthus blitum* L.)

This subgenus represents a polymorphic group comprising all species conventionally included in *Amaranthus* sect. *Blitopsis sensu lato* by many earlier authors. Relative closeness among the species included in this subgenus is less in comparison with subgenus *Amaranthus* and subgenus *Acnida*. Further infrageneric delimitation in subgen. *Albersia* is very inconsistent and inconclusive as well. Following Moquin-Tandon (1849), Carretero (1985) supported the segregation of section *Pyxidium* having dehiscent fruit, but still this group represents a group too polymorphic and geographically widespread to be considered as natural. The feature like dehiscence or indehiscence of fruit was not found to be a conclusive feature for delimiting infrageneric taxa. Justifying the situation Mosyakin and Robertson (1996) correctly segregated the subgen. into several narrow sections considering overall morphological characters:

1. *Amaranthus* subgen. *Albersia* sect. *Blitopsis* Dumort.

   The section includes species having indehiscent utricles and trimerous flower such as *A. blitum*, *A. viridis*, *A. emarginatus*, etc. Study on seed coat sculpturing and anatomy may demand further segregation of this section into narrower subsections (Kowal 1954).
2. *Amaranthus* subgenus *Albersia* sect. *Pentamorion* (G. Beck) Mosyakin and K. R. Robertson, comb. nov.

   The species included in this section have indehiscent utricle and generally five (occasionally four) spathulate or at least distinctly obovate tepals. Most of the species are native to South America and Australia.

3. *Amaranthus* subgen. *Albersia* sect. *Goerziella* (Urban) Mosyakin and K. R. Robertson, comb. nov.

   This monotypic section includes *Amaranthus minimus*, the Cuban endemic species with peculiar floral morphology that differs from all other taxa of *Amaranthus*.
4. *Amaranthus* subgen. *Albersia* sect. *Pyxidium* Moquin in DC.

This section includes species with dehiscent utricles like *A. tricolor* aggregates, *A. capensis*, *A. thunbergii*, etc. It appears to be most ambiguous grouping of taxa in the whole genus. It traditionally includes monoecious species, but recently Das (2014) described and identified a gynomonoecious member named *A. parganensis* from the Lower Gangetic Plain of West Bengal, India, that closely resembles *A. tricolor*. This new species demand further subdivision of this section into two subsections at least comprising monoecious and gynomonoecious members, respectively. The proposed classification does not appear to be conclusive and ultimate, new taxa at section, or varietal level can be incorporated as per taxonomic demand (Mosyakin and Robertson 1996).

The inclusion of grain amaranths in *Amaranthus* subgen. *Amaranthus* along with their wild progenitors appears to be beyond any confusion. But subcategorisation of cultivated grain and weed amaranths is a difficult job for the taxonomists that has been addressed by many workers (Sauer 1967; Mosyakin and Robertson 1996; Hanelt 1968). A comprehensive delimitation at subsection level is yet to be achieved. The genus as a whole is known to be taxonomically difficult. Moreover naturally occurring aneuploids in the plant populations of the farmer's field (Joshi1981a, b) and frequent synonymy have aggravated the taxonomic and nomenclatural ambiguity specially in the Himalayan region. Attempts towards classification and nomenclature have been made by Thellung (1914), Standley (1917), Schinz (1934), Kowal (1954) and Sauer (1950, 1955, 1957). Keeping in view the intermixing and segregate range among all the three grain species, suitable criteria are to be developed for proper taxonomic delimitation (Joshi 1981a, b). Infrageneric classification and inclusion of various species in it should reflect not only the species delimitation but also phylogenetic affinity between them. Keeping in view the available information achieved through various studies employing morphological and molecular parameters, infrageneric classification of all the subgenera of *Amaranthus* especially subgen. *Albersia* is to be evaluated.

4.4 Provisional Key to Some Edible Species of *Amaranthus*

Feine-Dudley (1980) compiled the informations from floras and monographs on *Amaranthus* represented as follows:

A. Flower unisexual
B. Three tepals
C. Tepals equal to longer than utricle; utricle circumscissile ..... 1. *A. tricolor*
C. Tepals shorter than utricle; utricle indehiscent
D. Utricle smooth ..... 2. *A. blitum*
D. Utricle rugose ..... 3. *A. viridis*
B. Five tepals
E. Tepals approximately equal in length and incurved against utricle
F. Plants armed; inflorescence with upper cymes staminate and lower cymes pistillate .... 4. *A. spinosus*
F. Plants unarmed; cymes with initial flower staminate reminder pistillate .... 5. *A. dubius*
E. Inner tepals shorter than outer tepals, straight or recurved from utricle
G. Bract exceeding style branches; inflorescence either short and thick or moderately developed; always dark seeded
H. Tepals longer than utricle; inner tepals with apex obtuse or emarginate; utricle not forming tower at apex; inflorescence short and thick ..... 6. *A. retroflexus*
H. Tepals shorter than utricle; inner tepals with acute apex; utricle narrowing into tower at

apex; inflorescence moderately developed .... 7. *A. hybridus*

G.Bract not exceeding style branches; inflorescence fully developed enormous (domesticated species) seeds usually light, sometimes dark

I.Bracts equalling style branches; inflorescence stiff; style branches forming sharp cleft at base; tepals with acuminate apex ..... 8. *A. hypochondriacus*

I.Branches shorter than style branches; inflorescence lax

J.Utricle narrowing into tower at apex; style branches erect; tepals with acute apex .... 9. *A. cruentus*

J.Utricle not forming tower; style branches spreading meeting in saddle at base; tepals broad, often overlapping; inner tepals with obtuse apex ..... 10. *A. caudatus*

Much of the problems in taxonomic delimitation of *Amaranthus* species have been generated from the attempts of recognising taxa on pigmentation or growth forms, which are extremely variable within amaranths. Delimitation of infrageneric taxa and placement of various grain amaranth species along with their weedy relatives as envisaged by Mosyakin and Robertson (1996) were pioneering. Overlapping features and new findings through molecular and biochemical approach regarding phylogenetic affinity have emphasised the need of evaluation of the section *Amaranthus* and supported the introduction of more new subsects under sect. *Amaranthus*. Sauer's key (Sauer 1967) and derived keys (Covas 1992; William and Brenner 1995; Hendrickson 1993; Sanchez-Del Pino et al. 1999) for separating species of grain amaranths could be very handy.

The infrageneric classification of the genus *Amaranthus* proposed by Mosyakin and Robertson (1996) is well substantiated by morphological and biomolecular data but challenged by AFLP-based UPGMA analysis. AFLP-based UPGMA analysis (Wassom and Tranel 2005) indicated that Sandhill amaranths (*A. arenicola*) was genetically more similar to waterhemp (*A. tuberculatus*) than to Palmer amaranths (*A. palmeri*) but differing from morphology-based taxonomy proposed by Mosyakin and Robertson (1996). They grouped all the dioecious species in the subgenus *Acnida*. This indicates the polyphyletic nature of the subgenus *Acnida* and the three-part classification of the genus *Amaranthus.* All the three grain amaranths and their wild progenitor though included in the subgen. *Amaranthus* but their distribution in different sections are yet to be ratified by biomolecular data.

Taxonomy and Phylogeny of Grain Amaranths

5

5.1 General

Species interrelationship and phylogenetic linkage among different species especially grain amaranths have been evaluated by many authors applying morphological, biochemical, molecular and cytogenetical parameters. Morphological evidences have played a significant role in solving taxonomic ambiguities in amaranths which are rich in morphological diversity. Besides conventional morphological features like inflorescence pattern and floral features, several other features like morphology and anatomy of bracteole, seed surface architecture, phyllotaxy, course of vascular supply and pollen morphology have played a key role in differentiating taxa in many cases. Biochemical and molecular evidences were used to validate the interpretations based on morphological data. In most of the cases those were concomitant with morphological data. Electrophoresis of seed proteins; isozyme polymorphism; different molecular markers like RAPD, AFLP, ITS, ISSR, etc.; leaf phenolic chromatogram; single nucleotide polymorphisms (SNPs); and tubulin-based polymorphism were applied to have a comprehensive idea about interrelationship of different species of grain, vegetable and weed amaranths. Most of the molecular techniques used yielded a common inference that all the grain amaranths have evolved from weed progenitor *A. hybridus*. Grain species showed close proximity with weed progenitor *A. hybridus* than with other weed species. The cultivated grain species are closely related, but *A. hypochondriacus* and *A. caudatus* are more closely related with each other than to *A. cruentus*. Size of the chromosome has made the karyological study in the genus very difficult. Three gametic numbers have been reported in the genus ($n = 14$, 16 and 17). This tribasic nature of the genus has supposed to have originated from dysploidy or aneuploidy. Information on distribution and variability of constitutive heterochromatin, the number of active ribosomal organiser region and karyotype analysis are very significant to increase our knowledge about genetic variability and phylogenetic linkage among the species. Structural alterations of chromosomes have played an important role in the evolution of species. Wild species have comparatively more symmetrical karyotype than the cultivated species. The differentiation was caused by chromosomal repatterning, recombination and selection at subspecific level. These certainly have played a decisive role to their genetic evolutionary process. The grain and vegetable amaranths are two distinct groups that have evolved from their respective weed progenitor through unique domestication event in different regions of the world. Two hypotheses have been proposed regarding the origin of grain amaranths from their wild weed progenitor – monophyletic and polyphyletic. The monophyletic hypothesis based on plant and seed morphology suggests that all three grain amaranths have originated from a single progenitor, *A. hybridus*. The polyphyletic theory based on

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phytogeography suggests that all the three grain amaranths have evolved independently. A third hypothesis suggests that all the three grain amaranths have originated from genetically differentiated population of *A. hybridus* through independent domestication event. The validity of these hypotheses has been challenged due to lack of adequate sampling of all grain amaranths and putative weedy progenitors, and modified versions have been proposed.

5.2 Morphological Approach in Solving Taxonomic Disputes

Genetic diversity is defined as the variation of individual genotypes within and among species. It is an important trait for long-term survival of species and enables a population to adapt to new conditions brought by environmental change (Hamrick and Godf 1996). Genetic variabilities are estimated from experimental data derived from morphological, cytological, biochemical and molecular traits. Application of morphological and cytological trait to estimate genetic variability has the demerits of being influenced by both environmental and genetic factors. Therefore they may not reflect accurate result (Basu et al. 2004). The genetic profile of the whole populations typically varies from place to place across a species range. These differences may arise due to chance occurrences, such as the genetic composition of dispersing individuals that create a new population (founder effect), or changes in allele frequencies that result from chance matings in very small populations (genetic drift). Differences among the populations may also arise systematically, especially when the individuals are exposed in the environments of various places offering different optima for survival and reproduction (fitness). For these and other reasons, populations often show divergence from each other in their genetic composition. Such divergence is especially strong and rapid when gene flow among populations is limited (e.g. limited dispersal of seeds or pollen or limited movement of animals across physiographic barriers). Knowledge of the amount and distribution of genetic variability within a species is very crucial to plant breeders because it is an important consideration especially when germplasm is to be selected for involvement in a breeding programme.

Morphology contributed a significant part of characters used in taxonomic delimitation in genus *Amaranthus*. Grain amaranths are characterised with few salient features like apical, large to moderately large complex inflorescence comprising aggregates of cymes, unisexual flowers with five tepals, five stamens, circumscissile utricle, seeds with variable seed coat colour (pale ivory, pinkish, brownish other than black and brownish black) and well-defined flange. Due to variability in morphological features, accurate identification of amaranth genetic resources is not always possible (Transue et al. 1994). For preliminary identification of *Amaranthus* species, the number, thickness, orientation and density of branches in inflorescences could be the useful tool. The flowers are arranged in small, very contracted cymes, and the cymes are agglomerated, axillary and also additionally arranged in racemose or spiciform terminal, large and complex synflorescences. In spite of extreme variability in inflorescence, there is usually a tendency towards a morphological 'type' (Costea et al. 2001a). The inflorescence structure of *Amaranthus* spp. is very complicated and has been described by Murray (1940), Weaver and McWilliams (1980) and Costea et al. (2001a). Flowers are small, green and unisexual and develop in numerous dense clusters (dichasial cymes). Each cyme has a determinate main axis with a terminal male flower followed by pairs of opposite, or occasionally single, lateral branches of female flowers (Fig. 5.1a). Each flower is subtended by 1–2 spinescent bracteoles, which are responsible for the overall increases in the density of the inflorescence (Costea and Tardif 2003c). The cymes are further arranged in numerous spikes (spiciform branches) which grow acropetally by the addition of new cymes. Towards the end of the inflorescence branches, there are several newly developed male flowers that can pollinate female flowers of the lower cymes. The formation of new male flowers decreases and ceases towards the end of the growing season. Wide morphological variability

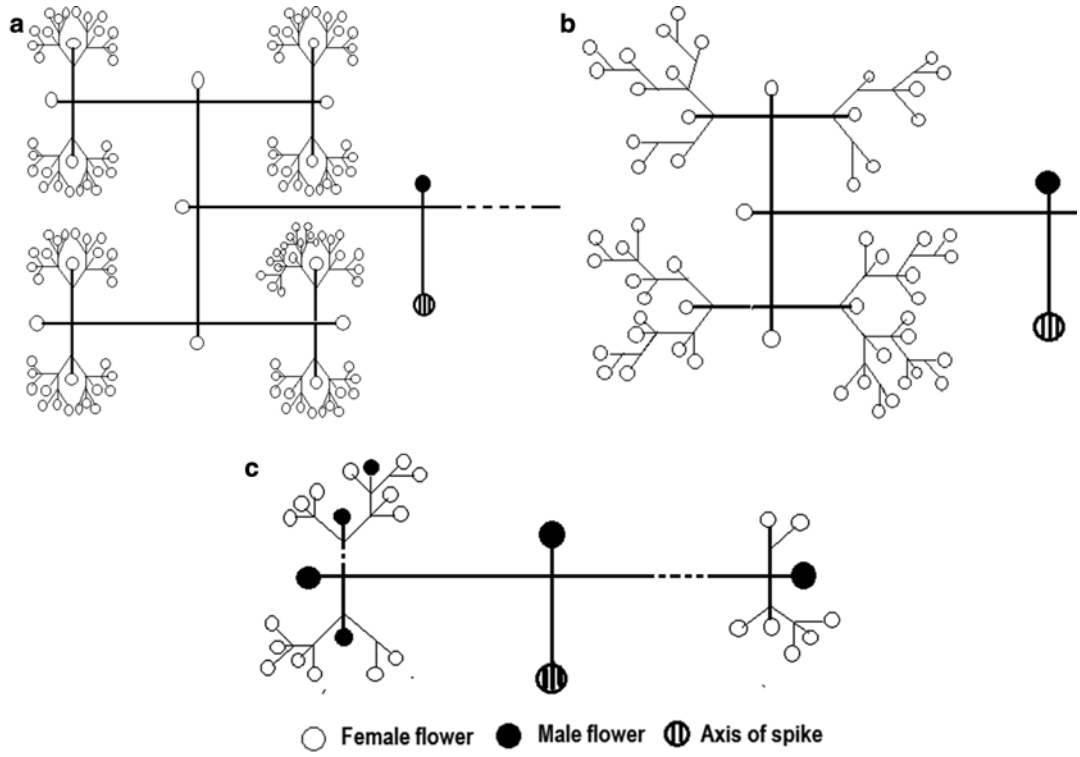

Fig. 5.1a Inflorescence pattern in amaranths. (**a**) Half glomerule of *A. caudatus,* (**b**) half glomerule of *A. hypochondriacus* and (**c**) full glomerule of *A. tricolor* (After Mohinder Pal 1972)

though with little taxonomic implications is shown by many *Amaranthus* species in response to several environmental factors like nutritional elements, water availability, light conditions, injurious factors, etc. (Costea et al. 2001a, b). The ratio of male/female flower in inflorescence is very important in reproductive behaviour of the species. As per estimation, the ratio of male/female flowers in the inflorescence of *A. powellii* was 7.6% (43 male to 524 female) and 9.7% for *A. hybridus* (26 male to 241 female). The female contribution to total reproductive effort, in terms of floral biomass, was estimated to be 98.4% for *A. powellii* and 95.6% for *A. hybridus* (Lemen 1980). All monoecious species of *Amaranthus* are self-compatible and probably self-pollinating (Brenner et al. 2000; Costea et al. 2001a, b). The morphology and anatomy of bracteoles were surveyed in 20 *Amaranthus* taxa in order to determine their taxonomic significance. Three types of bracteoles were distinguished: spinulose, foliaceous and membranous. Foliaceous and membranous bracteoles are considered symplesiomorphic, while spinulose bracteoles are considered as synapomorphic. The structure of bracteoles in the grain amaranths was found to be a reliable character that separates them from their weed relatives (Costea and Tardiff 2003b).

Morphotypes of *A. caudatus* are generally characterised by a distinctive apical, soft, dense, much branched thyrsoid long pendulous spike, like elephant trunk, mostly pinkish seed. Morphotypes of *A. cruentus* are characterised by an apical massive, lax, complex much branched, erect reddish, orange, pale-yellow inflorescence and brown or greyish-white seeds. The weed amaranths generally have small black seeds with undifferentiated flange. Wu et al. (2000) in a study on 229 genotypes from 20 *Amaranthus* species observed wide variability which was useful for improvement of cultivar in agronomic traits, such as plant height, seed, stem and leaf colour among genotypes within the same species and among different *Amaranthus* species. Similar results were also observed (Xiao et al. 2000; Varalakshmi 2004) in the evaluation of different

accessions of vegetable *Amaranthus*. Qualitative characters have been used for plant description. They are also useful in separating varieties especially when the range of quantitative characters is limited (Ghafoor and Ahmadt 2003).

The seeds of amaranths were described morphologically by many workers (Kowal 1954; Klopper and Robel 1989; Costea 1997). The seed coat colour is more constant in vegetable and weed amaranths ranging from black to blackish brown. But the seed coat colours in the grain species are more variable, may be dark or light coloured. Micromorphological features of seed surface are proved useful in identifying species even accessions of *Amaranthus*. The seeds of the grain amaranths are quite different from the other *Amaranthus* species in having well-differentiated folded flange, ill-defined or well-defined reticulation of ridges over spermoderm and presence of verucate processes. Vegetable and weed amaranths showed some commonality in seed surface features. Both have spermoderm with reticulation of elevated polygonal areas and are lacking well-defined ridges (Fig. 5.1b, 5.1c, and 5.1d). A large number of accessions of *Amaranthus cruentus* were studied that showed prominent variability in leaf shape, pattern of inflorescence, pigmentation and most significantly micromorphology of seed surface that demands for an evaluation and modification of existing infraspecific classification. *Amaranthus cruentus* have greyish-white or brown-coloured seeds. All the greyish-white-seeded accessions have rugulate spermoderm with irregular muriform arrangement of 'rugae'. But brown-seeded accessions of *A. cruentus* have reticulate spermoderm with reticulation of prominent ridges forming hexagonal or polygonal cavities. These seed surface features favoured the division of *A. cruentus* population into two varieties – *Amaranthus cruentus* (L.) var. *albus* S. Das, *var. nov.* (with rugulate spermoderm) and *Amaranthus cruentus* (L.) var. *cruentus* (with reticulate spermoderm showing reticulation of honeycomb-like cavities) (Das 2012b) (Figs. 5.2a and 5.2b).

Longevity of seeds in storage condition is a good parameter of seed quality and vigour in many crops. Kehinde et al. (2013) investigated physiological and genetic integrity of nineteen amaranth species during storage. It was found that amaranth seeds start to lose genetic integrity when germination capacity is below 40 %. For best performance of amaranth seeds in field storage under ambient condition should not exceed 3 months.

Phyllotaxy and leaf vasculature may be useful for species delimitation in amaranths. The phyllotaxy and nature of vascular supply were previously studied in *A. caudatus* (Gravis and Constantinesco 1907), *A. graecizans* L. and *A. hybridus* L. (Wilson 1924). Costea and DeMason (2001) extended this type of study to other common species. The results proved that a new set of characters including morphology, epidermal characters, phyllotaxy, complexity of leaf vascular supply and relative amount of secondary growth may be of taxonomic value that supported the segregation within the 'hybridus complex', i.e. separation of cultivated grain amaranths (*A. caudatus*, *A. cruentus* and *A. hypochondriacus*) from their presumed wild ancestors.

Pollen grains of *Amaranthus* are tectate pantoporate, generally with more than 18 sunken pores and supratectal granules and spinules (Eliasson 1988; Costea 1998a, b). This *Amaranthus* type of pollen grains (Erdtman 1952, 1966) is also found to be present in the other members of Amaranthaceae as well as in several other Centrospermous families (Nowicke 1993). Koracev (1969) reported that pollen diameter in the ten species of *Amaranthus* varies from 19.8 to 31.4 μ, and the number of pores in a pollen grain varies from 30 to 51, pore size from 2.2 to 5.5 μ and the distance between the pores 5.5 to 9.6 μ. Relationship between monoecious and dioecious amaranths as well as between members belonging to different ploidy level and interspecific hybrids can be estimated applying pollen grain features. Pollen grains of dioecious species have a greater number of apertures on the visible surface (Franssen et al. 2001a). The pollen grains of species belonging to lower ploidy level have comparatively narrow-sized pollen, and the size ranges increase with increase in the ploidy level. The surface ornamentation appeared more prominent in polyploids than in the diploid species (Chaturvedi et al. 1997).

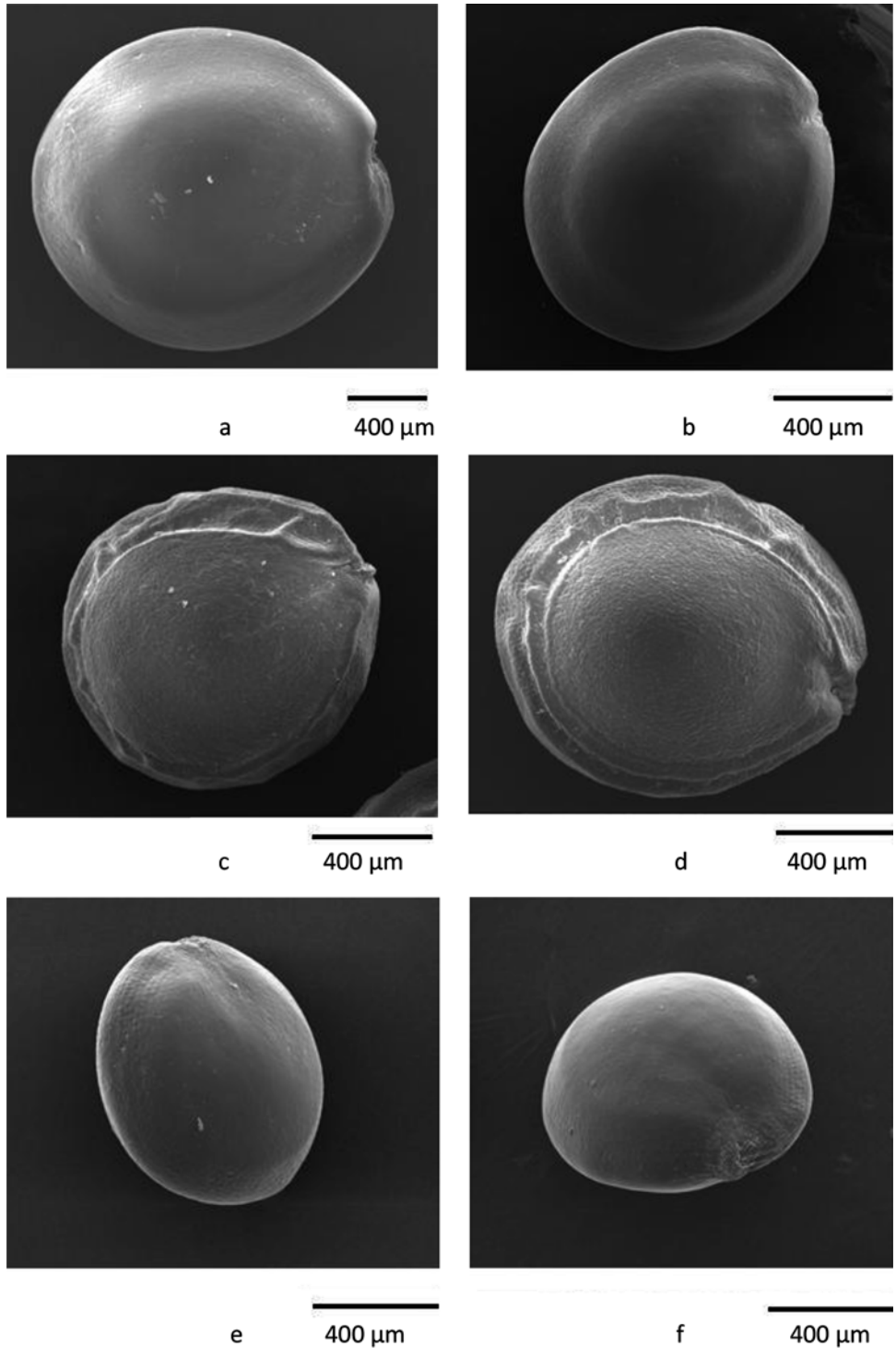

Fig. 5.1b Micromorphology of entire seed surface of amaranth seeds by SEM (**a**) *A. tricolor* (**b**) *A. blitum* (**c**) *A. caudatus* (**d**) *A. hypochondriacus* (**e**) *A. hybridus* (**f**) *A. retroflexus*

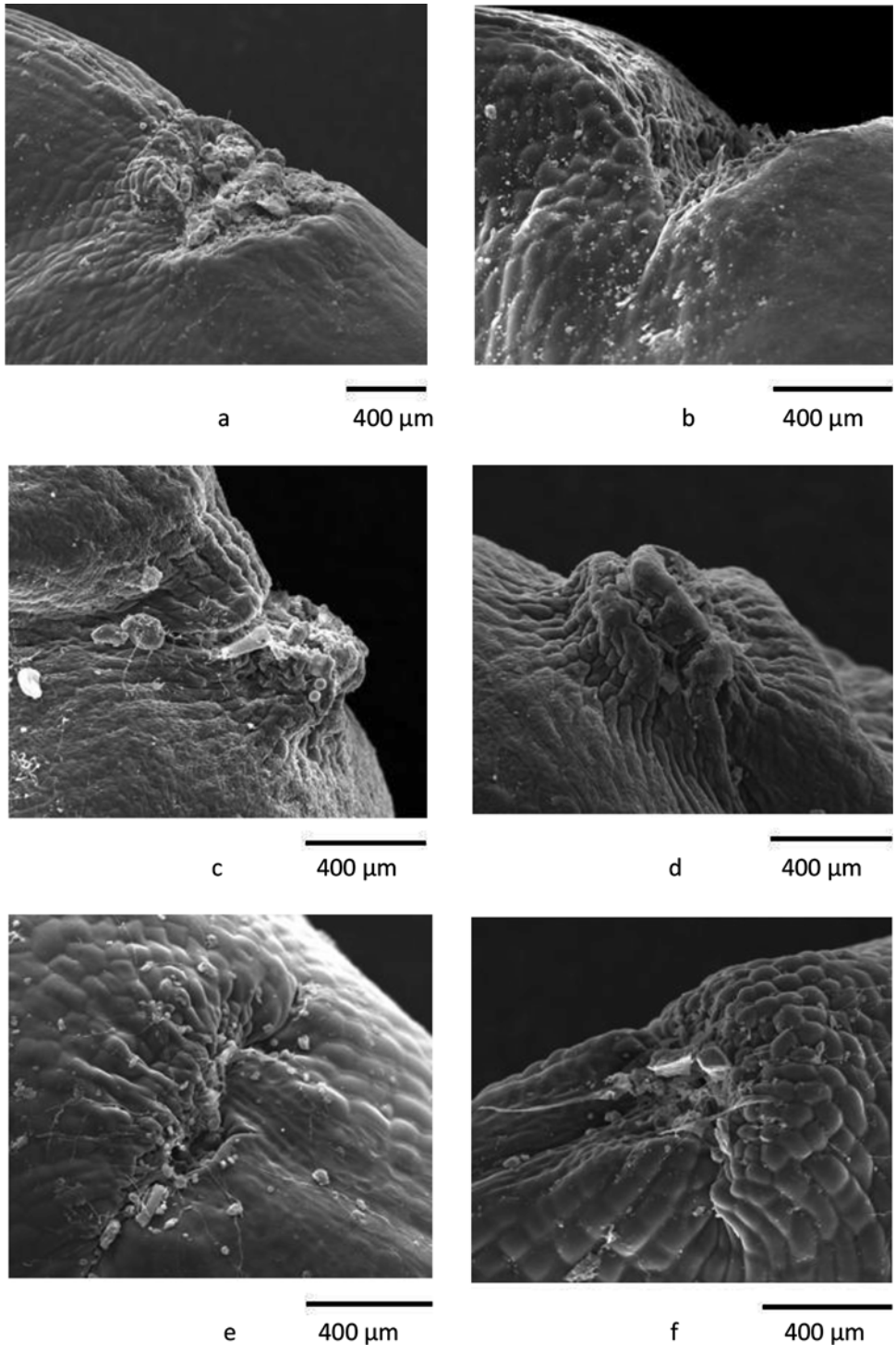

Fig. 5.1c Micromorphology of micropylar region of amaranth seeds by SEM (**a**) *A. tricolor* (**b**) *A. blitum* (**c**) *A. caudatus* (**d**) *A. hypochondriacus* (**e**) *A. hybridus* (**f**) *A. retroflexus*

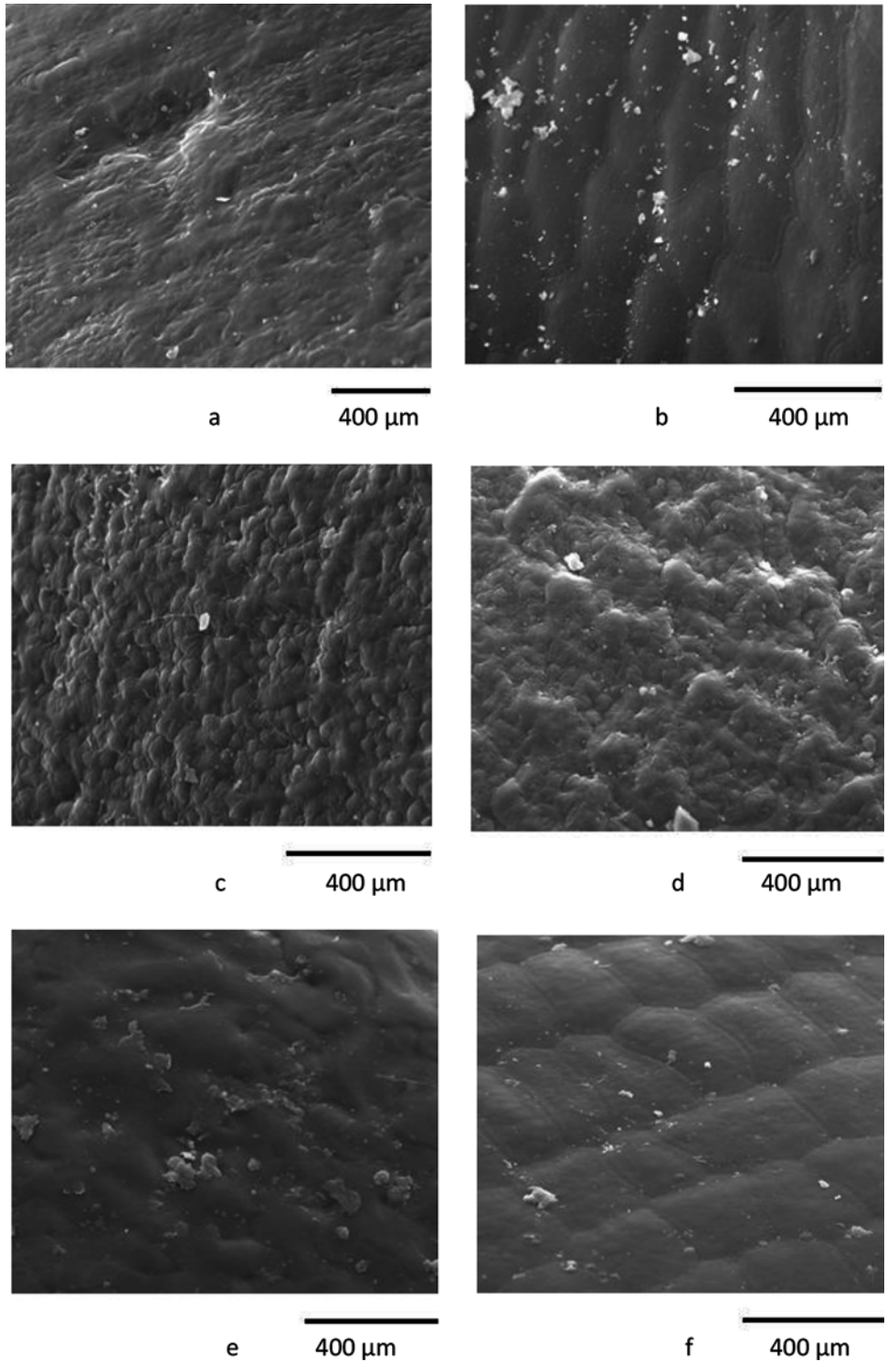

Fig. 5.1d Micromorphology of general seed surface of amaranth seeds by SEM (**a**) *A. tricolor* (**b**) *A. blitum* (**c**) *A. caudatus* (**d**) *A. hypochondriacus* (**e**) *A. hybridus* (**f**) *A. retroflexus*

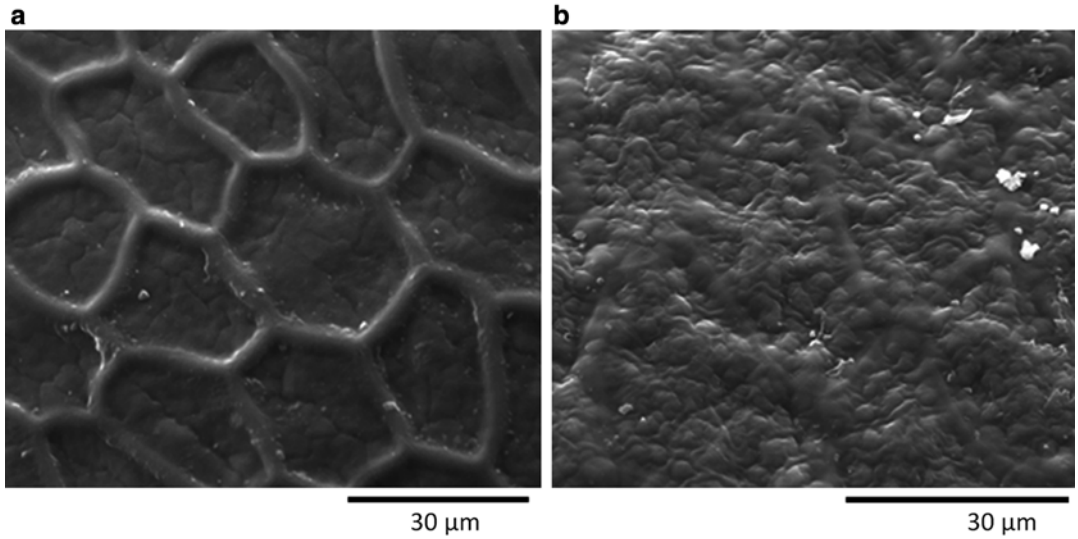

Fig. 5.2a Seed surface features delimited two varieties in *A. cruentus* L. (**a**). *A. cruentus* L. var. *cruentus* with reticulate spermoderm, (**b**) *A.cruentus* L. var. *albus* S. Das with rugulate spermoderm

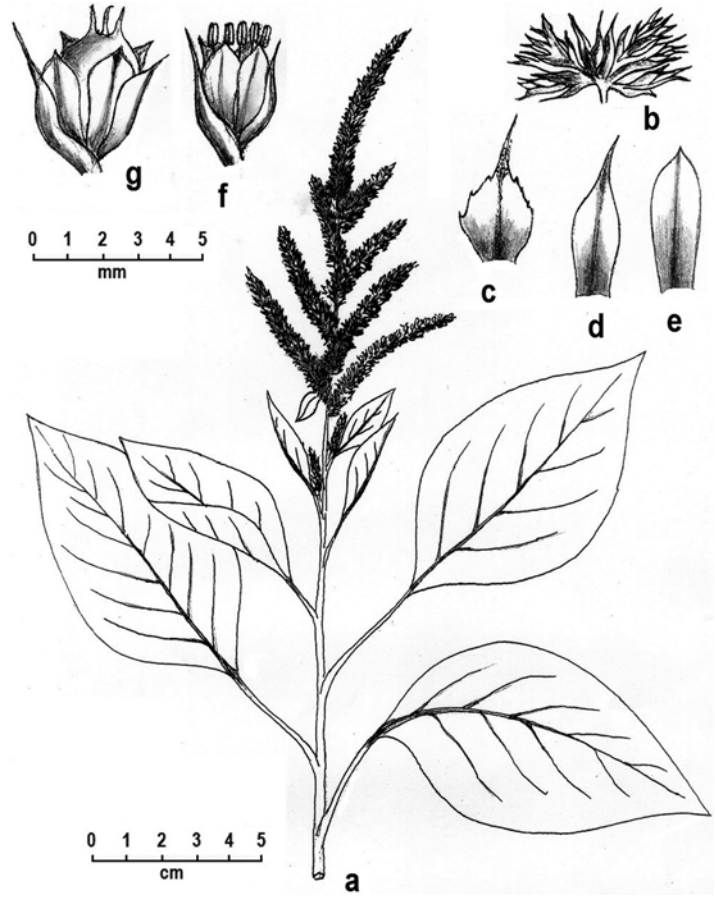

Fig. 5.2b *Amaranthus cruentus* var. *albus* S. Das (**a**) habit, (**b**) portion of inflorescence, (**c**) bract (**d**) bracteole, (**e**) tepal, (**f**) male flower, (**g**) female flower

5.3 Biochemical and Molecular Approach in Understanding Systematics and Taxonomy

Several attempts have been made to explore the putative relationship of the grain amaranths with their probable ancestor/ancestors employing biochemical and molecular parameters like seed storage protein, isozyme polymorphism, phenolic chromatograms and molecular markers, viz. RAPD, AFLP, ITS, SSR, ISSR, etc. Each and every parameters yielded data that were more or less concomitant not deviating from each other too much. Any observed variation in protein systems is considered as a mirror for genetic variations specifically seed proteins; they reflect the genetic history of the species without being affected by the environmental fluctuations. Electrophoretic analysis of native or denatured seed storage proteins was used to provide information concerning the genetic variability, which represent a source of information for assessing genetic and taxonomic relationships at the species level and below (Sammour et al. 2007; El-Esawi 2008; Drzewiecki 2001). Stebbins (1971) reported that polyacrylamide gel techniques provide clues to (1) explore interspecific or intraspecific variation, (2) screen a large number of cultivars or accessions for genetic purity, (3) verify whether or not two or more morphologically identical accession in the germplasm collection is also electrophoretically identical and (4) exploit the important traits of landraces and wild relatives to facilitate increase in crop production and stabilise crop yield. The seed storage proteins of 44 taxa of *Amaranthus* spp. were extracted in buffer solution and analysed on SDS-PAGE under reducing conditions. The result showed clear division in the studied amaranth taxa into two groups, one group containing basic chromosome number x = 17 and the other group containing basic chromosome number $x = 16$ (Sammour 1991). This information clearly indicated the relation between the chromosome number and the electrophoretic pattern. The data on seed storage protein also confirmed the separation of *A. cruentus* from *A. hybridus and A. sylvestris and A. sylvestris from A. graecizans*. Zheleznov et al. (1997) also studied the variation pattern in electrophoretic profile of seed proteins of wild and cultivated *Amaranthus* and identified seven biotype groups which revealed the close relationships between *A. cruentus* and *A. hybridus*, similarity between *A. caudatus* and *A. caudatus* var. *edulis* and distinctiveness of *A. caudatus* and *A. cruentus*. They reported that (1) the range of variation in seed protein content among both the wild and cultivated *Amaranthus* is rather wide; (2) *Amaranthus* seed proteins are highly nutritive and, on the whole, consist of easily digestable albumins and globulins (more than 50 % of total protein), alkali-soluble proteins-glutelins, which are close to albumins and globulins by their nutritive value (20.8 % of total protein), and alkali-soluble proteins-prolamines that are deficient in essential amino acids (12 % of total protein); (3) the seed protein of *Amaranthus* species was heterogeneous in nature consisting of 38 bands as appeared through SDS-PAGE with buffer pH 3.2. By decreasing electrophoretic mobility, these bands were conventionally assigned to four zones; and (4) the study of electrophoretic patterns of seed proteins is very promising to establish phylogenetic relationship among the species of genus *Amaranthus*. Electrophoretic characterisation of seed proteins is rapid, relatively cheap method, largely unaffected by the growth environment (Juan et al. 2007; Jugran et al. 2010), and has been applied by many workers to study the systematics of *Amaranthus* spp. Drzewiecki (2001) used SDS-PAGE of urea-soluble proteins of amaranth seeds to differentiate both – species and their cultivars. On the basis of similarity in protein profile, samples of seven species were classified into three groups. By protein patterns *A. tricolor* (leafy type of *Amaranthus*) clearly differs from other species. The study suggested a closer similarity between *A. caudatus* and *A. cruentus* species than between the pairs of species *A. hypochondriacus/A. caudatus* and *A. hypochondriacus/A. cruentus*. Only slight differences were seen among cultivars, especially of grain amaranths. According to Drzewiecki (2001) crossing rate can be evaluated based on electropherogram of urea-soluble protein extracted from

single seed. On the basis of solubility, Osborne (1907) divided proteins into four classes: albumins soluble in water, globulins soluble in high-salt concentration, prolamines soluble in aqueous alcohol and glutelins soluble in acid or alkaline solutions (Segura-Nieto et al. 1994). This classification into four protein fractions has enabled to differentiate various seed samples more clearly. Gorinstein et al. (1991) and Drzewiecki et al. (2003) generally characterised the protein fraction spectra of amaranth species. Dzunkova et al. (2011) established the methodology for clear identification of the amaranth species using glutelin protein fraction. The washing of water-, salt- and alcohol-soluble proteins in protein fraction separation process makes polymorphic peaks of amaranth glutelins to be distinguished very easily. SDS-PAGE analysis has been traditionally used to analyse glutelin subunit composition of wheat, but the procedure is slow, laborious and non-quantitative. The chip microfluidic technology based on capillary electrophoresis represents new approaches for the analysis of wheat HMW-GSs (high molecular weight glutenin subunits). This procedure is rapid and simple to apply and enables automatic and immediate quantitative interpretation. Other advantages over traditional gel electrophoresis method include lesser requirement of sample and reagents and a reduced exposure to hazardous chemicals (Bradova and Matejova 2008). The study of Janovská et al. (2008) on the seed protein profiles of 15 *Amaranthus* accessions from the Czech Gene Bank using both SDS-PAGE and chip electrophoretic profiles exhibited that (1) chip electrophoretic technique is highly sensitive and produces wider range of bands and (2) the obtained data confirmed the classification of *Amaranthus* species studied. The analysis of the total seed proteins was very useful to assess the genetic differences between two grain populations of *Amaranthus retroflexus* collected from field of the Maize Research Institute Zemun Polje, Serbia (Snezana et al. 2012). Two populations showed difference in protein profile; out of 18 protein fractions obtained in protein analysis, populations differed in four protein fractions of different molecular weight. Seed protein electrophoresis proved useful for genetic determination of *A. retroflexus* populations and identification of biotypes with a typical morphology.

Isozyme polymorphism analysis has been used for over 60 years as useful parameter in biological research to establish phylogenetic relationships, estimate genetic variability, identify cultivars and genes and study population genetics and developmental biology (Sammour et al. 2007; El-Esawi 2008; Rahman 2001). It was also utilised in plant genetic resource management, taxonomic delimitation and plant breeding. Furthermore, isozyme analysis was used in control of breeding, estimation of outcrossing, testing purity and in species delimitation and conservation (Ar'us et. al. 1985; Becker et al. 1992; Chamberlain 1998). Finally isozyme technique may be used by plant breeders to generate, evaluate and select desired genotypes in early stage of the breeding programme, which saves time, money and efforts of the breeders (Tanksley and Orton 1983). Thirty-four population of New World amaranth along with 21 weedy New World populations were screened for allozyme variability at electrophoretic loci using nine enzymatic system (Hauptli and Jain 1984). Eleven population of cultivated amaranth from Indian state of UP and six population from Nepal were also considered in survey for comparison. In the New World populations, heterozygosity was low, and polymorphic loci ranged from 0 to 44%. Diversity index was partitioned into the intra- and interpopulation as well as the interspecific components of variability. The crop versus weed genetic distances was the largest, whereas the intra- and interpopulation components of Diversity index were found to be equal. Genetic structure of all the three grain species of the New World amaranths together was described as a collection of distinct populations, each represented more or less a heterogeneous collection of highly homozygous individuals. The North Indian populations showed relatively less allozyme variability with the most common alleles same as those of Mexican landraces. Alleles at several loci proved to be diagnostic of the crop and weed groups and of the three individual crop species. Genetic distances based on pooled gene frequencies showed three crop species to be generally more closely

related than they were to their putative weedy progenitor species, respectively (with the exception of the weed-crop pair *A. quitensis* and *A. caudatus*). This consolidated the concept of single domestication event where *A. hybridus* performed as the common ancestor, rather than three separate domestication events. Close similarity between *A. caudatus* and *A. quitensis* might have resulted from transdomestication based on a weedy or semi-domesticated species having migrated from Meso-America to South America. Genetic variability and species relationships of a total of 23 species and 60 populations of cultivated and wild amaranths were studied using isozyme marker (Chan 1996). High degree of interspecific and intraspecific variations was observed between the investigated species and populations; 132 alleles were detected for 15 enzyme systems. Total gene diversity for grain amaranths and wild species was 0.39 and 0.72, respectively. The polymorphism analysis demonstrated the relationships of grain amaranths (*A. caudatus*, *A. cruentus*, *A. hypochondriacus*) with their putative ancestors (*A. hybridus*, *A. powellii* and *A. quitensis*), and the results also consolidated the idea about monophyletic origin of the grain amaranths. In addition, the genetic diversity and relationships of other species of amaranths were determined. Genetic diversity and relationships of 23 cultivated and wild *Amaranthus* species were examined using isozyme marker. A total of 30 loci encoding15 enzymes were resolved, and all were polymorphic at the interspecific level. High levels of inter-accessional genetic diversity were found within species, but genetic uniformity was observed within most accessions (Chan and Sun 1997). Iudina et al. (2005) examined the electrophoretic patterns of five isozyme systems in total 52 populations and two varieties (Cherginskii and Valentina). Allozyme variation of these materials was low. Irrespective of species affiliation, 26 populations and two varieties were monomorphic for five enzymes.

Genetic variability analysis with RAPD markers is a fast, technically less complex, less expensive methodology and involves no radioactivity and hybridisation. RAPD markers are usually scored as dominant alleles, since the amplified DNA product is present in one parent but absent in the other. For repositories with large collections, this technique represents an important advancement towards detailed characterisation and screening of individual accessions at the molecular level (Waycott and Fort 1994). RAPD analysis is a powerful tool in determining interas well as intraspecies genetic relationships (Hardys et al. 1992). Such studies have been done involving wild and cultivated species (Ranade and Sane 1995), among self- and cross-pollinated species (Sharma et al. 1995) and even within germplasms of a single-species population (Pammi et al. 1994). Earlier, germplasms of grain amaranth accessions were analysed by RAPD (Virk et al. 1995). However, this study did not reflect interspecific relationship.

The RAPD technique has been successfully used for evaluating variation within plant accessions and for establishing differences among lines of apparently closely related populations in germplasm collections, for example, American chestnut (Huang et al. 1998), barley (Russel et al. 1997, Hong et al. 2001), *Pinus longaeva* (Lee et al. 2002), strawberry (Zebrowska and Tyrka 2003), *Trigonella* (Dangi et al. 2004), *Morus* (Awasthi et al. 2004), *Orobanche* (Román et al. 2007), *Lactuca* (Yoo and Jang 2003), *Curcuma* species (Syamkumar and Sasikumar 2007) and *Amaranthus* (Yoshimi et al. 2007).

Genetic diversity and relative closeness among six *Amaranthus* species collected from eight phytogeographic regions were analysed using a random amplified polymorphic DNA (RAPD) marker. RAPD primers yielded a total of 262 amplicons, ranging from 250 to 3000 bp of which 254 amplicons (96.94 %) were polymorphic. The genetic similarity coefficient among all the *Amaranthus* species ranged from 0.16 to 0.97 with a mean value coefficient of 0.56, which indicated that variation exists in the genetic diversity of different populations (Yoshimi et al. 2007).

RAPD marker polymorphism tend to support a closer genetic relationship between *A. caudatus* and *A. hypochondriacus* species (Avise and Hamrick 1996), clustering of hybrids between *A. edulis* and *A. caudatus* with *A. caudatus* and clustering of hybrids between *A. hybridus* and *A.*

hypochondriacus with *A. hypochondriacus* (Khoshoo and Pal 1972). The low genetic distance values between these hybrids and other accessions of *A. caudatus* and *A. hypochondriacus*, respectively, indicated a weak genetic differentiation between them. Chan and Sun (1997) generated molecular progenies of cultivated and wild amaranth employing both isozyme and RAPD marker. They evaluated 23 different cultivated and wild *Amaranthus* species comprising the three cultivated grain species as well as accessions of all the species included in the *A. hybridus* aggregates. Fifteen isozyme systems encoded 30 loci that showed polymorphism at the interspecific level. Most accessions were monomorphic, but there was considerable diversity among the accessions as well. Out of 600 RAPD amplicons generated from 27 arbitrary ten-base RAPD primers, polymorphism percentage as revealed by RAPD amplicons within cultivated grain species, within progenitor species, within vegetable species and within other wild species were 39.9 %, 42.8 %, 51.0 % and 69.5 %, respectively. The evolutionary relationships between grain *Amaranthus* and their probable ancestors were investigated utilising both the RAPD and isozyme data sets that not only supported a monophyletic origin of grain amaranths and projected *A. hybridus* as the common ancestor but also indicated a closer relationship between *A. hypochondriacus* and *A. caudatus*. Dendrogram computed from RAPD and isozyme date showed difference. Information from both isozymes and RAPD when applied in a cumulative fashion appeared complementing each other and generated more accurate estimate of genetic diversity and relative closeness within and among crop species and their wild relatives, than data set when applied separately (Avise and Hamrick 1996). This interpretation was questioned by the facts that the number of accession considered was limited and the wild species analysed were not from the area of their origin in many cases. So, they were not the proper representatives of diversity. Mandal and Das (2002) demonstrated a high degree of genetic similarity between *A. hypochondriacus* and *A. caudatus* based on RAPD analysis which supports earlier observations of Chan and Sun (1997). The experiment was conducted using eight decamer primers. After evaluation of analytical data on interspecific hybridisation and hybrid fertility, it was also concluded that these two (i.e. *A. hypochondriacus* and *A. caudatus*) are the most closely related pair in the grain *Amaranthus* species group (Grant 1959b). From the similarity/dissimilarity percent and subsequent clustering in dendrogram computed on RAPD data, it was reasonable to apprehend that at least *A. hypochondriacus* and *A. caudatus* must have a common ancestor.

Transue et al. (1994) applying RAPD techniques also supported monophyletic origin of the grain amaranths from *A. hybridus* as suggested by Sauer. They classified 29 accessions of *A. caudatus*, *A. cruentus* and *A. hypochondriacus* into distinct groups and also classified and assigned 79 other accessions not previously assigned to species (all assigned to *A. cruentus* or *A. hypochondriacus*). The study showed that these three species could be classified unambiguously despite overlapping variations for morphological traits. Analysis of 282 polymorphic RAPD markers showed that *A. hypochondriacus* and *A. caudatus* are more closely related to each other than their individual closeness with *A. cruentus*. RAPD analysis was also successful in the investigation to establish relationships between four *A. hypochondriacus* varieties (Barba de la Rosa et al. 2009).

AFLP, ITS and ISSR markers were proved to be very efficient molecular parameters to study genetic diversity and phylogenetic relationships (Nolan et al. 2010), to assess the putative wild progenitor of grain *Amaranthus* (Costea et al. 2006; Xu and Sun 2001) and to resolve taxonomic confusion between the taxa of *Amaranthus* (Costea et al. 2006). The 'Morelos' accessions of *Amaranthus* from Mexico show taxonomic ambiguity at the basic morphologic level. Although basic morphological criteria are helpful for quick taxonomic delimitation and identification of specimens or germplasm collections, interpretation derived from morphological data alone often can be misleading. To determine the taxonomic identity of the 'Morelos' accessions and their speculative species affiliation to either *Amaranthus caudatus* or *Amaranthus cruentus*, Costea et al. (2006) conducted a comparative analysis of evolutionary link-

age among these taxa/accessions applying amplified fragment length polymorphism (AFLP) and micromorphology methods. AFLP data helped to assign all the controversial 'Morelos' accessions, consistently and unambiguously into a single *A. cruentus* clade. This *A. cruentus* clade containing 'Morelos' accessions was clearly separated from *A. caudatus* clade. This result clearly indicated that the 'Morelos' accessions are affiliated to *A. cruentus*. The AFLP-based phylogenetic relationship of 'Morelos' accessions and sharp delimitation of *A. cruentus* and *A. caudatus* are further consolidated by micromorphology, proving the fact that the combination of these techniques can provide more reliable information for germplasm identification than each method used separately. Qiang and Jin (2013) conducted a comprehensive investigation applying 14 simple sequence repeat (SSR) to assess the genetic variability and population structure of 70 amaranth accessions collected from South and Southeast Asia. In total, 67 alleles were detected, with an average of 4.79 per locus. A large portion of detected alleles (46.3 %) were raw alleles, while 29 unique alleles associated with rice accessions were also detected. The mean major allele frequency (MAF), genetic diversity (GD) and polymorphic information content (PIC) of the 14 SSR loci were 0.77, 0.36 and 0.34, respectively. A model-based structural analysis showed the presence of three subpopulations among the accessions studied. The genetic relationships configured by the neighbour-joining tree method were fairly concomitant with the structure-based membership assignments for most of the accessions. All 70 accessions showed a clear relationship to each cluster without any admixtures. Qiang and Jin (2013) observed a relatively low extent of genetic exchange within or among amaranth species from South and Southeast Asia. This result of genetic diversity analysis could be very useful to identify amaranth germplasms and also to facilitate their use for crop improvement.

Lanoue et al. (1996) studied restriction site variations in chloroplast DNA and nuclear ITS1 and ITS2 region of 28 *Amaranthus* species. The data revealed closer affinity between *A. caudatus* and *A. cruentus* than to their respective progenitor. *Amaranthus cruentus* could be separated from *A. caudatus* on the basis of micromorphology and AFLP analysis (Costea et al. 2006). Phylogenetic relationships of grain amaranths with their wild progenitor and taxonomic disputes that exist among three cultivated grain amaranths and their putative wild progenitors, *A. hybridus*, *A. quitensis* and *A. powellii*, were evaluated using ITS, AFLP and ISSR (Xu and Sun 2001). Phylogeny was poorly resolved due to low ITS divergence exhibited by gain amaranths and their wild progenitor, though extensive polymorphism exists within and among the species studied both at AFLP and ISSR loci. In the cladogram computed on either AFLP or ISSR or the combined data sets, nearly all intraspecific accessions could be clustered in their corresponding species clades, indicating these taxa as well separated species. The AFLP trees share many similarities with the ISSR trees, showing a close relationship between *A. caudatus* and *A. quitensis*, placing *A. hybridus* in the same clade where all grain amaranths are included also indicating *A. powellii* as the most divergent taxon in the *A. hybridus* species complex. This study has also demonstrated that both AFLP and double-primer fluorescent ISSR have a great potential for developing a large number of informative characters to explore phylogeny of closely related species especially when ITS polymorphism is insufficient. Most of the molecular techniques used to study the species relationship in amaranths yielded a common inference regarding the origin of grain amaranths. The grain amaranths are more closely related with wild progenitor *A. hybridus* rather than with other wild species. The cultivated grain species are closely related, but *A. hypochondriacus* and *A. caudatus* are more closely related than to *A. cruentus*.

Though molecular studies do not always support unanimous conclusion regarding the evolutionary origin and proximity of crop-wild allies, conventional studies considering hybrid fertility and chromosome number tend to support the hypothesis of independent domestication of grain amaranths and close relationship between *A. hypochondriacus* and *A. caudatus*. Gupta and Gudu (1990) identified closer affinity between *A. caudatus* and *A. hypochondriacus* applying hybridisation technique. Analysis of phenolic chromatograms has been successfully used in solving taxonomic disputes (Bate-Smith and

Lerner 1954; Baker and Ollis 1961; McClure and Alston 1966; Das and Mukherjee 1995) also as genetic marker to assess species affinity (Gornall and Bohm 1980). Das (2012a) successfully applied leaf phenolic compounds to assess the species relationship and taxonomic delimitation in amaranths. Methanolic extracts of leaf phenolics of different amaranth species were analysed by thin-layer chromatography. Phenolic spot profiles were viewed under UV light after spraying flavone reagent (diphenyl boric acid ethanolamine, Sigma). Variability in the distribution of coloured spots proved to be very useful in evaluating species relationship (Figs. 5.3a and 5.3b). Along with general morphological features, leaf phenolics (secondary metabolites) and isozyme polymorphism of acid phosphatase were applied to get a comprehensive idea about relative closeness among the species and morphotypes/accessions of both the vegetable, grain and weed amaranths. Twenty-two plant specimens affiliating to ten species of *Amaranthus* were included in the study (Das 2012a). The compiled chromatogram (Fig. 5.4) on phenolic spots was generated incorporating all phenolic spots detected in individual chromatogram of each species, and such compiled chromatogram showed 21 spots. Few spots were exclusive for vegetable amaranths and few for grain amaranths. The zymogram of acid phosphatase showed 29 polymorphic bands. Not only species, the morphotypes and varieties also showed apparent variability in band profile. Both

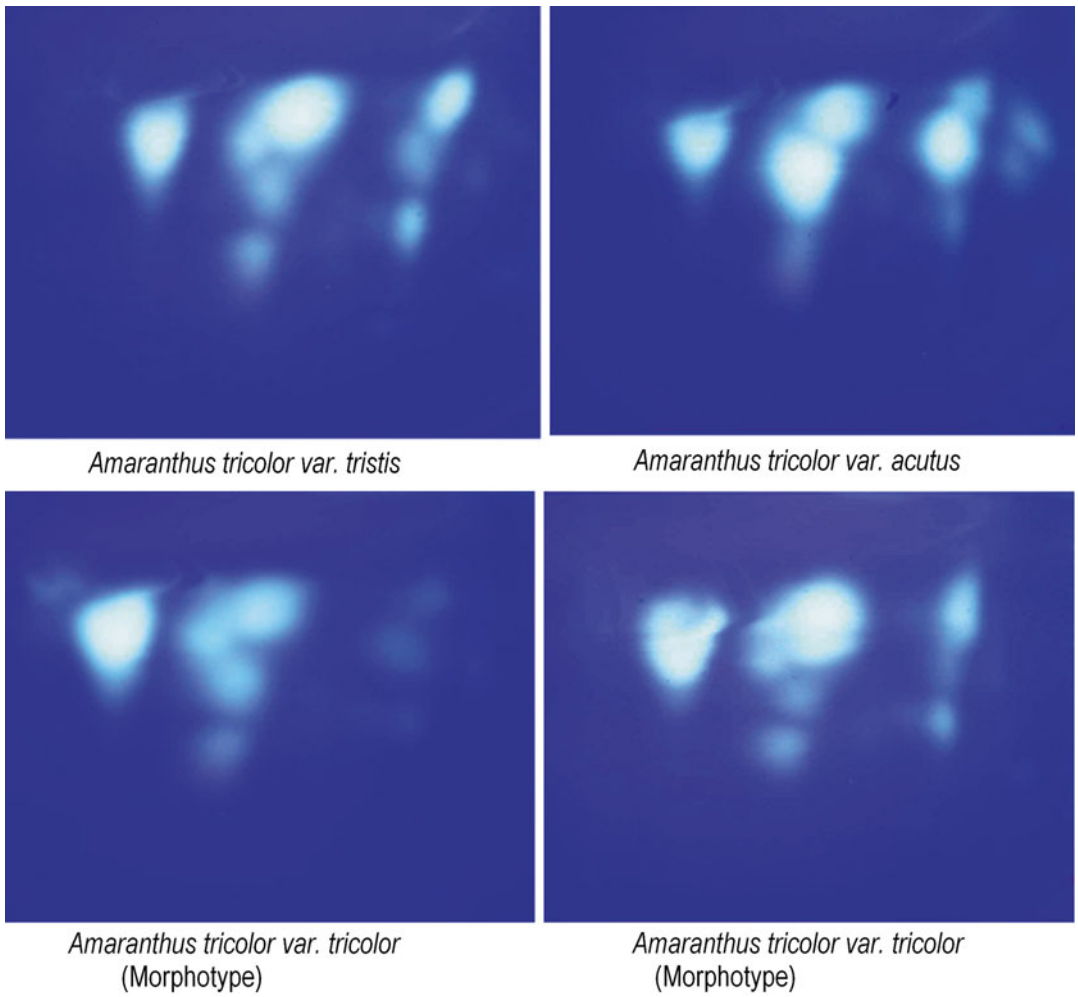

Fig. 5.3a Leaf phenolic chromatography differentiating varieties and morphotypes of *A. tricolor* L.

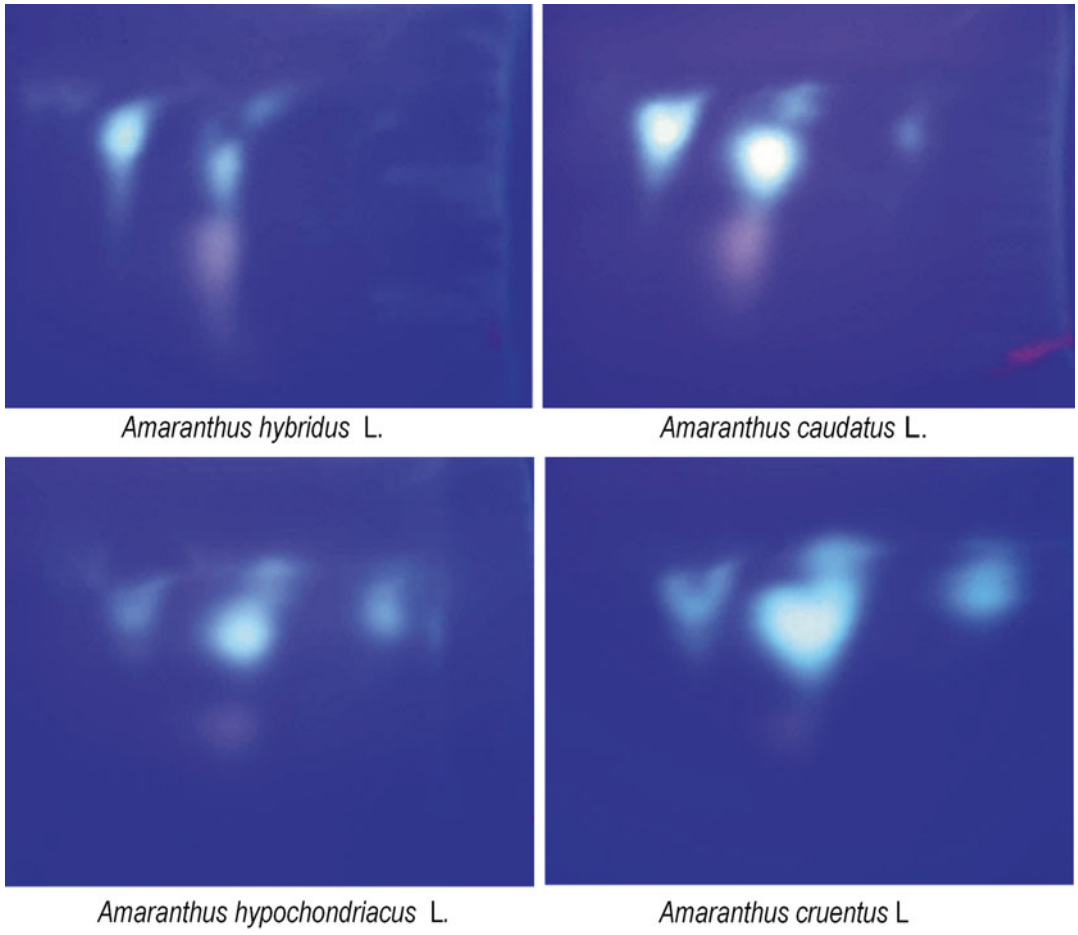

Fig. 5.3b Leaf phenolic chromatography differentiating grain amaranths

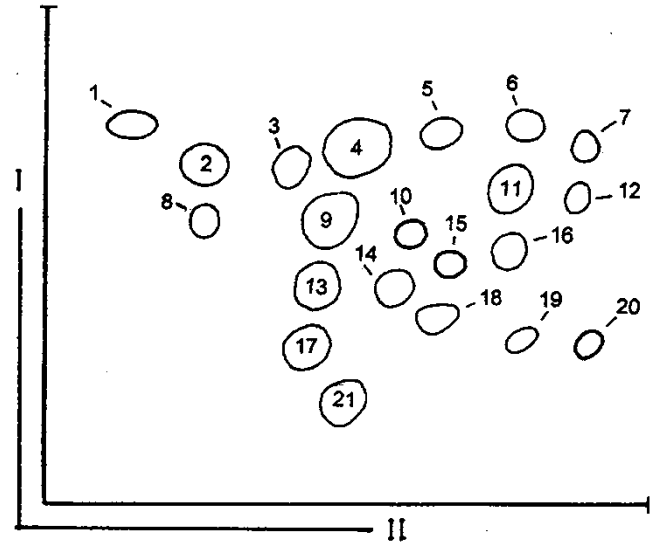

Fig. 5.4 Compiled phenolic chromatogram showing total number of phenolic spots found in all the species studied

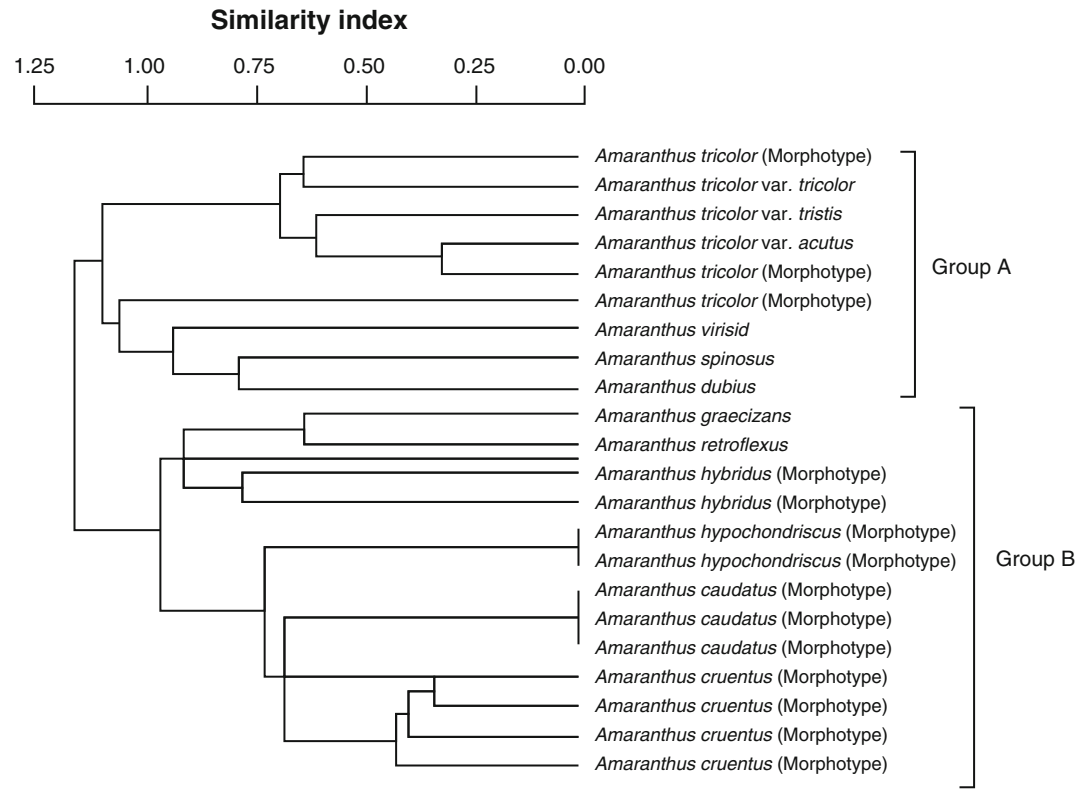

Fig. 5.5 Dendrogram computed from morphological data

the vegetable and grain amaranths are supposed to have originated from their respective weed progenitor through occasional outcrossing and gradual domestication. The study also strongly vouched the previous conclusion regarding interrelationships between grain amaranths, and their probable derivation from their putative progenitor also showed clear separate clustering of vegetable and grain amaranths. A sharp congruence in cluster pattern between morphological dendrogram (Fig. 5.5) and cumulative dendrogram computed on morphological and biochemical data set (Fig. 5.6) was apparent. Dendrogram on morphology as well as consensus dendrogram revealed clear separate clustering of accessions belonging to three grain amaranths. Accessions of *A. cruentus* formed a distinct cluster, while accessions of *A. hypochondriacus* and *A. caudatus* revealed closer relationship keeping parity with previous observations. The dendrograms computed from data also showed separate categorisation of vegetable and grain amaranths with their putative weed relatives and also consolidated the concept of hybrid origin of *A. dubius* from *A. spinosus* and *A. hybridus* and allopolyploid nature of *A. dubius* as well. Chan (1996) did not consider *A. spinosus* as one of the progenitors of *A. dubius*, according to him *A. hybridus* and *A. hypochondriacus* supposed to have undergone hybridisation and following doubling of chromosome gave rise to *A. dubius. A. dubius* appears to be an autotetraploid of *A. hybridus* and *A. hypochondriacus*. Stefu nova et al. (2014) utilised ISSR primers to study the intra- and interspecific variability of a large number of accessions of *A. caudatus*, *A. cruentus* and *A. hypochondriacus*. Highest intraspecific variability was shown by *A. hypochondriacus* in comparison with *A. caudatus* and *A. cruentus*. It proved that ISSR markers were significant enough to generate sufficient level of informative characters for intra- and interspecific molecular analysis of genus *Amaranthus*.

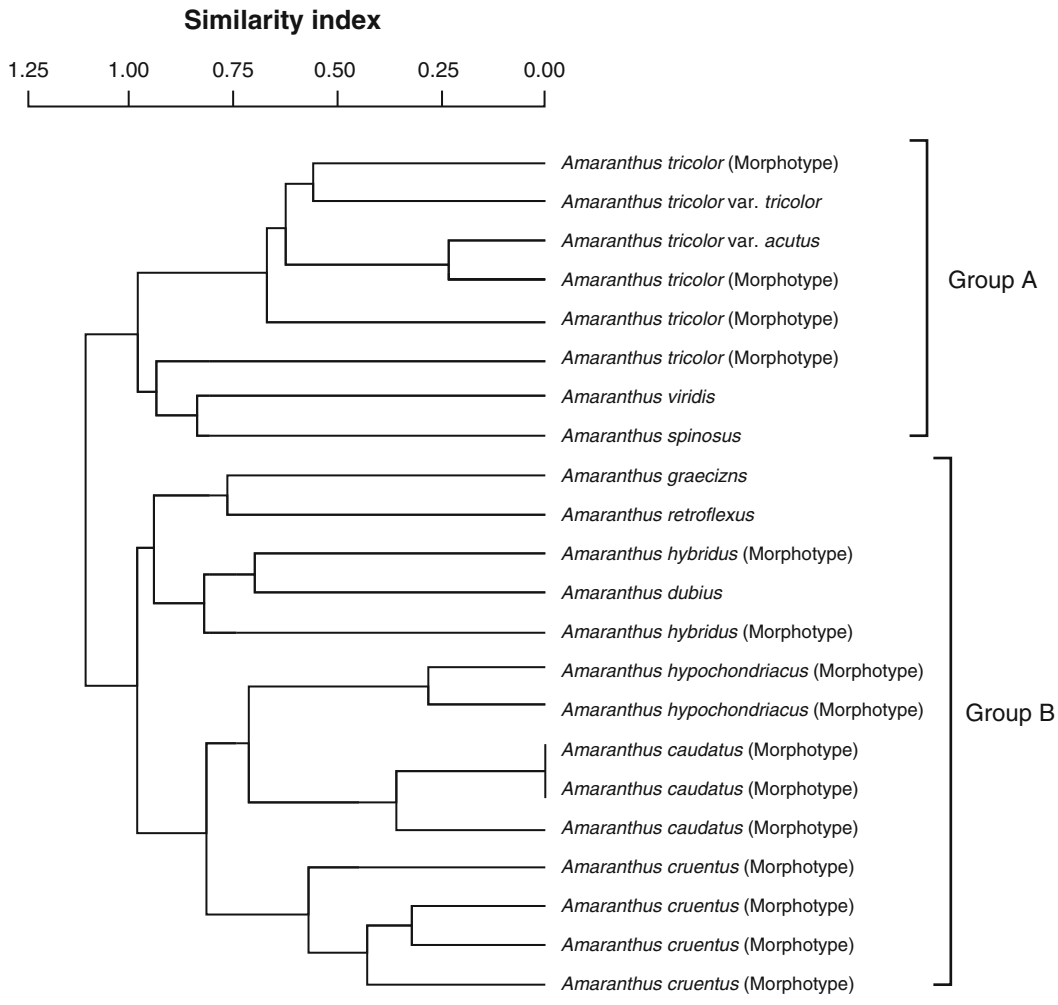

Fig. 5.6 Dendrogram computed from compiled date from all parameters

Many of the earlier molecular studies involving RAPD marker polymorphism yielded inconsistent results. Current studies are applying more reliable genetic marker like microsatellite marker and genome sequencing techniques to address evolution-related ambiguities. Mallory et al. (2008) applied microsatellite markers and genome sequencing to address evolutionary linkage among grain amaranths and identified and characterised 179 microsatellite markers for amaranths. The objective of the study was to generate and characterise a set of highly informative, reproducible microsatellite markers for the three grain amaranths. A total of 1457 clones were sequenced from three genomic libraries enriched for the microsatellite motifs AAC, AAT and AC. Of these, 353 (24 %) contained unique microsatellites. An additional 29 microsatellite loci were also identified among 728 BAC-end sequences of a newly developed amaranth BAC library. Flanking primers were designed for 319 of the microsatellite loci, and all were screened on a panel of diverse amaranths, including grain and weedy *Amaranthus* species. A total of 179 (56 %) microsatellites were found polymorphic across accessions screened from the three grain amaranths. Among these polymorphic microsatellite loci, a total of 731 alleles were identified with average of four alleles per locus. Heterozygosity values ranged from 0.14 to 0.83

with a mean value of 0.62. Thirty-seven (21 %) of the markers found were polymorphic between the parents of a segregating population and appeared to be inherited in a normal Mendelian fashion proving the applicability of these markers for linkage mapping of the amaranth genome. Phylogenetic tree computed from microsatellite marker data showed the placement of *A. hybridus* accessions in two of the three major grain amaranth clades, suggesting the polyphyletic origin of the three cultivated species from different *A. hybridus* ancestors. The microsatellite markers have been proved to be very promising for further evaluation of the relative closeness among the grain amaranths and their relatives also for use in marker-assisted breeding programmes, germplasm analysis and varietal identification. These markers may be useful to other species within the genus *Amaranthus*, including economically important weeds, vegetable amaranths and ornamentals due to the transferability of these markers to *A. hybridus*, *A. powellii* and *A. retroflexus*. Microsatellite loci generated for *Amaranthus hypochondriacus* were characterised, and their cross amplification in wild species was studied by Lee et al. (2008). Twelve polymorphic microsatellite markers for *Amaranthus hypochondriacus* were isolated and characterised in the study. A total of 92 alleles were detected from the 20 accessions, with an average of 7.7 alleles per locus. The observed heterozygosity (H_O) and expected heterozygosity (H_E) values ranged from 0 to 0.95 and from 0.49 to 0.92, respectively. At significance threshold ($P < 0.05$), nine loci deviated from Hardy-Weinberg equilibrium (HWE), whereas significant linkage disequilibria (LD) were observed between five pairs of loci. Twelve microsatellite loci were successfully amplified in 18 other amaranth species representing cultivated grain and vegetable categories, their probable progenitors and wild members. These results demonstrated great utility of these markers to study intra- and interspecific genetic variability as well as evolutionary relationships among cultivated and wild amaranths. A significant progress was achieved when Maughan et al. (2010) utilised a novel genomic reduction strategy linked with so-called next-generation sequencing technique to identify a huge number (27,658) of single nucleotide polymorphisms (SNPs) among four diverse amaranth accessions. *Amaranthus hypochondriacus* showed the highest total number of polymorphic SNP marker, while *A. cruentus* showed the lowest genetic diversity which may be indicative of the specialised domestication process, limited and uniform cultivation range (Mallory et al. 2008). On the other hand *A. hybridus*, the putative wild progenitor of grain amaranths, showed the highest genetic diversity of all the species studied which is explainable for its speculated progenitor status. Significant SNP polymorphism further consolidated the ancestral relationship between the grain amaranths and *A. hybridus.* One recently developed genotyping method is tubulin-based polymorphism based on intron-length polymorphism available in plant β-tubulin gene family (cTBP). The genes code for protein relevant for growth. The genomic structure of these genes is such that a multiple approach can be adopted to study genetic variability.

Bardini et al. (2004) developed the new technique called tubulin-based polymorphism (TBP) based on the length of the first intron present in the coding region of plant β-tubulin genes. Introns of plant tubulin genes function as important regulatory elements to support gene expression. Introns can either increase the level of expression through intron-mediated enhancement, or they can change the actual site of gene expression. Introns of tubulin genes are also useful for genotyping plant species and varieties, for assessment of parental status and also for assisting breeding programmes (Braglia et al. 2010; Breviario et al. 2007, 2008). Molecular markers are now the most widely used parameter to assess genetic diversity (Karp et al. 1998; Koebner et al. 2001). TBP method was successfully applied on a wide range of species like oilseed rape, coffee, *Lotus* (Bardini et al. 2004), *Brassica*, *Eleusine* and *Arachis* (Breviario et al. 2007). Popa et al. (2010) used TBP method on *Amaranthus* species for highlighting a possible polymorphism in the gene for β-tubulin. The results revealed an intraspecific polymorphism

in *A. cruentus* (Alegria) V1-R1, while no polymorphism was detected among the other studied species. However, this low level of polymorphism in these species may be indicative of high level of inbreeding in these *Amaranthus* species or the fact that the primers which they have used were not successful enough in amplifying bands in *Amaranthus* species under study (Bardini et al. 2004). Clear differentiation between two grain amaranths targeting a gene was achieved by Park and Nishikawa (2012). They delimited *A. caudatus and A. hypochondriacus* from each other at species level by sequencing and PCR-RFLP simple method of targeting two amaranth starch synthase genes.

The versatile polymorphism and genetic variability in amaranths especially in 'hybridus complex' created many misapplications of names and created disputes between synonymy and species delimitation. Besides these major taxonomic problems involving grain amaranths, there are few more diverse taxonomic disputes which are yet to be solved clearly like hybrid origin of *A. dubius* Mart., synonymy versus species delimitation of *A. quitensis* Kunth and *A. hybridus*, conspecific nature of *A. powellii* S. Watson and *A. bouchonii* Thell., taxonomy of waterhemp, etc. The widespread Northern races of *A. hybridus* can be distinguished from other *Amaranthus* species. But tropical races are morphologically intermediate between *A. hypochondriacus*, *A. cruentus* and other species (Sauer 1950). Sauer (1950) misapplied the binomial '*Amaranthus quitensis* H.B.K.' which should be the synonym of *A. hybridus* (Coons 1978). According to Coons (1975) *A. quitensis* Kunth as mentioned by Sauer (1950, 1967) is not a valid biological species but should be classified as *Amaranthus hybridus* var. *sangorache* which unfortunately has not been published validly. However the validity of *A. quitensis* as a biological species in Sauer's sense is supported by molecular data (Chan and Sun 1997) and cytogenetical observations (Greizerstein and Poggio 1992). Hybrids between these two species (*A. hybridus* and *A. quitensis*) yielded pollen grains with 60 % viability. The data showed closeness at the same time divergence between these two. *Amaranthus hypochondriacus* is a native of North America where its closest wild relative *A. powellii* is also existing. Sauer (1993) suggested that it may be of hybrid origin from *A. powellii* and *A. cruentus*. This relationship was also consolidated by some molecular information (Transue et al. 1994; Kirkpatrick 1995; Chan and Sun 1997). Thellung (1926) described a new species *A. bouchonii* in Europe. Costea et al. (2001a) considered it as a subspecies of *A. powelli*, i.e. *A. powellii* subsp. *bouchonii* (Thell.) Costea & Carretero. Though Sauer (1967) considered European taxon *A. bouchonii* to be conspecific to *A. powellii,* a study on isozyme polymorphism (Wilkins 1992) failed to establish separate identity of *A. bouchonii*, but cytological evidences supported their distinctiveness. To avoid confusion it would be better to consider both at subspecific level, i.e. *Amaranthus powellii* S. Watson subsp. *powellii* and *Amaranthus powellii* subsp. *bouchonii* (Thell.) Costea & Carretero.

5.4 Cytogenetical Approach in Understanding Species Relationship

Karyotypical studies in *Amaranthus* are limited, probably due to the small size of the chromosomes, which makes morphological analysis of chromosome very difficult (Grant 1959a, b, c). Updated data have indicated that there are two basic chromosome numbers in *Amaranthus*, $x=16$ and $x=17$, and it is diploid except for a solitary tetraploid ($2n=64$, $x=16$) species, *A. dubius* Mart ex. Thell., and in some cases, both numbers were mentioned for the same species (Grant 1959a; Pal and Khoshoo 1972; Pal 1972; Pal and Khoosho 1973a, b; Poggio 1988). Pal et al. (1982) suggested that the gametic number $n=17$ has originated from $n=16$ through primary trisomy. Greizerstein and Poggio (1992) supported this hypothesis analysing meiotic behaviour of species and interspecific hybrids. The existence of reproductive barrier during the crossing between *Amaranthus* species was suggested to be the contributory factor for variability

and suggested the possibilities of genic or chromosomal differences causing sterility in hybrids.

Studies carried out on chromosome morphology of some species of *Amaranthus* have indicated variation in number of chromosome pairs with satellites. Palomino and Rubí (1991) reported the karyotypic formula in some cultivars of *Amaranthus hypochondriacus* and *Amaranthus cruentus*, suggesting the existence of 6–10 pairs of chromosomes with satellites in different cultivars. Greizerstein and Poggio (1994) proposed karyotypic formulae of various accessions of cultivated species (*Amaranthus cruentus*, *Amaranthus hypochondriacus*, *Amaranthus mantegazzianus* and *Amaranthus caudatus*). In all the species studied, only one pair of chromosome was found with a satellite (Greizerstein and Poggio 1994). Kolano et al. (2001) reported the presence of one and two pairs of chromosomes with ribosomal hybridisation signals for two cultivars of *Amaranthus caudatus* applying FISH technique with 45 s ribosomal probes.

Basic chromosome number in Amaranthaceae is $x=8$ or 9 (Turner 1954). Three gametic numbers have been reported in the genus ($n=14$, 16 & 17), i.e. the genus is tribasic. In few cases $n=16$ and 17 occur in the same species. *Amaranthus dubius* is an exception, which is an allopolyploid with $2n=64$. The species might have originated as a result of interspecific hybridisation between *A. spinosus* L. and most probably *A. hybridus*. According to Pal and Khoshoo (1982), the basic chromosome number $n=17$ has evolved from $n=16$ through primary trisomy. On the basis of cytogenetic analysis, Greizerstein and Poggio (1994) supported the above idea and proposed that the species with somatic chromosome number $2n=32$ are actually polyploid (having basic chromosome no. $X=8$), and X_1 is a derived basic number. The basic chromosome no. $X_2=17$ might have originated by primary trisomy. Genus *Amaranthus* itself appears to be allotetraploid with chromosome count of $n=16$ or 17 (Greizerstein and Poggio 1994, 1995), and the genus behaves as diploid during meiosis. They reported the karyotype formulae and total DNA content of four species of *Amaranthus*. According to their observation *A. caudatus* with $2n=32$, *A. cruentus* with $2n=34$, *A. hypochondriacus* with $2n=32$ and *A. mantegazzianus* with $2n=32$ showed slight differences in karyotype formulae and asymmetry index (Table 5.1). The variation in karyotype of different species could be brought about by dysploidy, pericentric inversions, deletions, unequal translocation, etc. It suggests that the chromosome size is not constant, and it can vary according to selection factor or adaptive norms. Restricting their studies on four species of *Amaranthus*, viz. *A. viridis, A. spinosus, A. blitum* and *A. tricolor,* Srivastava and Roy (2012) concluded that by virtue of having more symmetrical karyotype than other species, *A. spinosus* is considered to be more primitive, and in having asymmetrical karyotype, *A.blitum* is considered more advanced.

Table 5.1 Genome formulas for grain amaranths and some other related wild species

Amaranthus species	Genome formulas	*N*
Amaranthus caudatus	$A_1A_1B_1B_1$	16
Amaranthus cruentus	$A_2A_2B_2B_2$	17
Amaranthus hypochondriacus	$A_4A_4B_4B_4$	16
Amaranthus mantegazzianus	$A_3A_3b_3B_3$	16
Amaranthus quitensis	AABB	16
Amaranthus hybridus	$A_5A_5B_5B_5$	16
Amaranthus spinosus	A_6A_6CC	17

Minor differences are expressed in the subscript for A and B genome. The genomes are $x=8$ except for B2 and C, which are $x=9$ (Greizerstein and Poggio 1995)

The genus *Amaranthus* is characterised by small chromosomes with indistinguishable secondary constrictions, satellites, etc. even at mitotic metaphase that act against a lucid and vivid working out of its karyotype. This fact has so far restricted the cytogenetical studies of this crop to base mainly on its male meiosis. However, to explore the genetic system of any organism, a precise knowledge of its karyotype is essential. Srivastava and Roy (2012) recorded the deviation in the karyotypic formulae of *Amaranthus* from the previous count. *Amaranthus spinosus* ($2n=34$) earlier showed the presence of one subtelocentric and ten telocentric pairs of chromosomes (Greizerstein et al. 1997), but later study (Srivastava and Roy 2012) showed presence of only five subtelocentric pairs of chromosomes. It

is reported that *A. viridis* has two metacentric, 20 submetacentric, eight subtelocentric and two telocentric chromosomes, and *A. tricolor* has six metacentric, six submetacentric, 16 subtelocentric and six telocentric chromosomes (Madhusoodanan and Nazeer 1983). According to later study (Srivastava and Roy 2012), *A. viridis* showed 14 metacentric, 12 submetacentric and eight subtelocentric chromosomes, and *A. tricolor* showed 14 metacentric, ten submetacentric and ten subtelocentric chromosomes. No satellite was found to be present in any species. On the basis of above observation, it could be presumed that the karyotype differences are probably due to divergence and frequent repatterning of chromosome over long periods of time and support the earlier reports as analysed in different accessions (Greizerstein et al. 1997; Madhusoodanan and Nazeer 1983). Srivastava and Roy (2012) reported the chromosome number in *A. blitum* ($2n=28$), which is similar to the new basic chromosome number ($x=14$), and this report was supported by observation of Pal et al. (2000). Pal et al. (2000) also reported chromosome number $2n=28$ in *Amaranthus tenuifolius*. This consolidated the possibility of new basic chromosome number ($x=14$) for the genus *Amaranthus*, and genus could therefore be tribasic ($x=14$, 16 and 17).

There are several groups of angiosperm having variable chromosome numbers as a result of dysploidy or aneuploidy (Stebbins 1971). *Amaranthus* is one such group of plants. The term dysploidy indicates the process by which the euchromatin of a genome is rearranged by translocation on to a greater or lesser number of centromeres, which is evidenced in morphology of chromosome. It is evidenced in the study of Srivastava and Roy (2014) that there is whole chromosome loss at diploid level in high number. It is assumed that dysploid reduction from relatively high chromosome number ($2n=34$) has taken place in *Amaranthus* due to repeated chromosome manipulation during hybridisation in between landraces. This type of changes in chromosome numbers represents a case of dysploidy rather than aneuploidy. Extensive dysploid reduction from high primitive chromosome number to lower chromosome number species associated with derived morphological and ecological condition may represent most common cyto-evolutionary pattern in angiosperm. *Amaranthus* represents a genus that shows a great variability in chromosome number and ploidy level (Table 5.2).

The distribution and variability of constitutive heterochromatin and the number of active ribosomal organiser regions were studied by Bonasora et al. (2013) in two different cultivars of the species *Amaranthus cruentus* ($2n=34$), *Amaranthus mantegazzianus* ($2n=32$), *Amaranthus hypochondriacus* ($2n=32$) and *Amaranthus caudatus* ($2n=32$). The aim of the study was to increase the knowledge about the genetic variability of genus *Amaranthus*. The distribution and variability of constitutive heterochromatin were studied using DAPI-CMA_3 banding techniques (Bonasora et al. 2013). The position of the nucleolar organiser (NOR) was observed using Ag-NOR banding and fluorescent in situ hybridisation (rDNA-FISH). All the cultivated *Amaranthus* species showed two active NOR regions except *A. caudatus* cv. EEA INTA Anguil. Furthermore the number of DAPI+/CMA3 bands allowed the characterisation and identification of heterochromatin in cultivars and species.

Madhusoodanan and Nazeer (1983) conducted a comprehensive morphological and karyotypical analysis of five species of sect. *Blitopsis*, viz. *A. tricolor, A. lividus, A. graecizans, A. viridis* and *A. albus*, of which all the species except *A. albus* were collected from India. The species of sect. *Blitopsis* are taxonomically well defined. Forms of *A. tricolor* are rather tall compared to other pot-herb species and their wild relatives. In general for all the phenotypic characters studied, namely, height of the plant, number of primary branches, leaf size, stomatal size, flower size, etc., variation pattern is similar for all the aspects indicating gigantic nature, thereby better yielding ability of *A. tricolor* compared to that of the other species. This may possibly be due to controlled evolution in *A. tricolor* resulting from recombination and selection in which all the desirable qualities for commercial growing are combined. In the members of other species, the morphological discontinuity is more pronounced which can be

Table 5.2 The number of chromosomes and ploidy levels in some amaranths

Species	No. of chromosomes (2*n*)	Ploidy level
Amaranthus albus	32 or 34	Diploid
Amaranthus blitum	28 or 34	Diploid
Amaranthus tricolor	34 or 35[a]	Diploid
Amaranthus viridis	34	Diploid
Amaranthus lividus	34	Diploid
Amaranthus aureus	34	Diploid
Amaranthus gangeticus	34	Diploid
Amaranthus hypochondriacus	32 or 34	Diploid
Amaranthus caudatus	30 or 32	Diploid
Amaranthus cruentus	32 or 34	Diploid
Amaranthus hybridus	32	Diploid
Amaranthus leucocarpus	32	Diploid
Amaranthus mangostanus	32	Diploid
Amaranthus mantegazzianus	32	Diploid
Amaranthus caturtus	64	Tetraploid
Amaranthus dubius	64	Tetraploid
Amaranthus edulis	32	Diploid
Amaranthus chlorostachys	32	Diploid
Amaranthus graecizans ssp. *graecizans*	34	Diploid
Amaranthus graecizans ssp. *sylvestris*	32	Diploid
Amaranthus giganteus	34 or 64	Diploid and tetraploid
Amaranthus retroflexus	32	Diploid
Amaranthus powellii	32 or 34	Diploid
Amaranthus quitensis	32	Diploid
Amaranthus paniculatus	32 or 34	Diploid
Amaranthus polygamous	34 or 68	Diploid and tetraploid
Amaranthus salicifolius	34	Diploid
Amaranthus spinosus	34	Diploid
Amaranthus sylvestris	32	Diploid
Amaranthus tenuifolius	28	Diploid
Amaranthus blitoides	32	Diploid
Amaranthus tuberculatus	32	Diploid
Amaranthus palmeri	32 or 34	Diploid
Amaranthus roxburghianus	34	Diploid

[a]Representing an aberrant form

Reference: 1. Behera and Patnaik (1974), 2. Greizerstein and Poggio (1994), 3. Kulakow and Jain (1990a, b), 4. Madhusoodanan and Nazeer (1983), 5. Mohideen and Irulappan (1993), 6. Sammour et al. (1993,) 7. Sauer (1976), 8. Sreelathakumary and Peter (1993), 9. Pal et al. (2000), 10. Srivastava and Roy (2014), 11. Rayburn et al. (2005).

attributed to their limited usage as greens. Moreover, unlike the cultivated forms of *A. tricolor,* most of them are usually collected from their wild habitat for use. They were found to be quite distinct, differing in height, mode of branching, shape as well as size of the leaf, inflorescence structure, nature of bract, utricle and dehiscence of utricle.

The karyotypes of the species analysed are highly asymmetric because all the chromosomes of the basic set vary in size as well as in form as a good number of them used to be subterminal and terminal, while the others are submetacentric and metacentric. However, there was no correlation between these two features in any of the species. It is evident from the karyotype formulae that none

of the five species have identical chromosome sets. As a whole, symmetrical karyotypes are usually considered as primitive and most asymmetrical karyotypes as derived. Hence, the presence of a large number of metacentric chromosomes and its reasonably symmetric nature in *A. albus* is indicative of its primitiveness, while an increase in the number of telocentrics as observed in *A. lividus* shows that the genome has undergone considerable structural modifications. The karyotypic variation detected in different species may be due to pericentric inversions, deletions, unequal translocation, etc. In *Amaranthus* both chromosome size and gross morphology are not constant, and it varies being influenced by the selection factors and/or adaptive norms. The results obtained by Madhusoodanan and Nazeer (1983) support the earlier finding (Madhusoodanan 1978) that in the members of sect. *Blitopsis*, neither hybridisation nor polyploidy seems to have played any role in their evolution, and species differentiation has been brought about by chromosomal repatterning, recombination and selection at the subspecific level which are regarded as the most important factors contributing to their genetic evolution. Sharma and Banik (1965) are also of the opinion that structural alterations of the chromosomes have played a very significant role in the evolution of the species. In the morphological and karyotypical analyses of five species of *Amaranthus* sect. *Blitopsis* (Madhusoodanan and Nazeer 1983) the species were found to be quite distinct, differing in height, mode of branching and shape as well as size of the leaf, structure of infloresence, nature of bract as well as utricle and mode of dehiscence of ripe utricle. Compared to the wild species, the cultivated ones showed advanced characters due to selection. The karyotypes of all the species are very much asymmetric with variation in size as well as in form of chromosome sets. No two species are karyotypically alike. Evidently, the species differentiation in members of the section is caused by chromosome repatterning, and in evolution of the species, structural alterations of chromosomes are playing a key role.

The trend in species relationship among the amaranths as revealed by RAPD analysis is consistent and supportive to their cytogenetic and evolutionary relationship (Ranade et al. 1997). On the basis of karyotype analysis, Sharma and Banik (1965) discussed species interrelationship in the family Amaranthaceae. Several authors have demonstrated the existence of compatibility barrier to the interspecific cross among *Amaranthus* species due to genic or chromosomal differences (Greizerstein and Poggio 1992; Pal and Khoshoo 1973a, b). It was observed that wild species have more symmetrical karyotype than the cultivated species. The karyotypic differentiation is caused by repatterning of chromosomes, recombination and selection at the subspecific level, and these are important contributing factors to their genetic evolutionary process (Srivastava and Roy 2012).

Interspecific hybridisation would be a confirmatory parameter for identification of *Amaranthus* specimens. Murray (1940) using controlled pollinations demonstrated that interspecific hybridisation in *Amaranthus* is possible. Interspecific hybridisation and transfer of herbicide resistance in *Amaranthus* have been demonstrated between two dioecious species, *A. palmeri* and *A. tuberculatus*, and between *A. tuberculatus* and a monoecious species *A. hybridus* (Tranel et al. 2002; Wetzel et al. 1999b). Interspecific hybridisation has also been reported in wild *Amaranthus* populations. Grant (1959b) described the karyotypes of putative interspecies amaranth hybrids. Hauptli and Jain (1984) described hybrids derived from interspecific crossing between cultivated grain amaranth (*A. caudatus*) and redroot pigweed growing wild near cultivated amaranth that showed intermediate morphology, but isozyme profile was more similar to cultivated grain amaranth with a low frequency of redroot pigweed isozymes.

Cultivated amaranths have relatively lower chiasma frequency in comparison with wild species. This phenomenon has attributed to repeated cycles of hybridisation and selection in cultivation which produced an inherent heterozygosity in the cultivated amaranths (Madhusoodanan and Pal 1981). Cultivated species are characterised by more chiasma per bivalent than semiwild species like *A. spinosus* also with bigger chromosomes and pollen grains (Sreelathakumary and Peter 1993).

5.5 Taxonomic Delimitation in Vegetable Amaranths

This group of plants are gradually feeding out of global crop directory, but it is true that at least 50 tropical countries grow vegetable amaranth and in quantities that are far from negligible. Nearly all the vegetable amaranths are included in the subgenus *Albersia*. Recently four sections have been recognised under subgen. *Albersia* – three (sect. *Blitopsis*, sect. *Pentamorion* and sect. *Goerziella*) comprising members having indehiscent fruit and one (sect. *Pyxidium*) including members having dehiscent fruit. Taxonomic delimitation and application of nomenclature in vegetable amaranths are still very tentative for its morphological variability and frequent hybridisation.

Few species of *Amaranthus* are known as vegetable amaranths like *A. tricolor, A. blitum, A. dubius, A. cruentus,* etc., of which the most significant are *A. tricolor* and *A. blitum. Amaranthus tricolor* is included in sect. *Pyxidium* and is a native species of tropical Asia. *A. tricolor* probably originated from weed progenitor in tropical Asia through outcrossing and domestication process. As a result several new taxa were described at subspecies, variety and form ranks. *A. tricolor* is the lectotype of the section *Pyxidium* and is a taxon of renewed taxonomic interest from the view point of misapplication of names, morphological variability, as well as due to the presence of a large number of synonyms. Several authors (Mathai 1978; Mosyakin and Robertson 1996; Das 2013) recognised *A. tricolor* as aggregate or complex comprising various synonyms like *A. gangeticus* L., *A. mangostanus* L., *A. polygamus* L., *A. melancholicus* L. and *A. tristis* L. These synonyms are creating confusion in delimitation of taxa. Infraspecific morphological variability of *A. tricolor* can be addressed by introducing taxa at variety level ignoring the synonyms. Lakshminarasimhan and Godbole (2001) recognised two varieties under *A. tricolor* – *A. tricolor* var. *tricolor* and *A. tricolor* var. *tristis* (L.) Thellung. During comprehensive field survey in West Bengal, India, a large number of plants were collected and studied morphologically (Das 2013). The morphometric study recognised three varieties (i.e. var. *tricolor*, var. *tristis* and var. *acutus var.nov.*) of which one (*Amaranthus tricolor* var. *acutus*) was new for the science. *Amaranthus tricolor* var. *acutus* differs from other varieties in having ovate-oblong leaves with emarginate apex, ovate-spathulate tepals with acute apex and tepals as long as or shorter than fruit (Fig. 5.7). Morphological characters delimiting three varieties of *A. tricolor* are mentioned in Table 5.3.

5.6 Key to the Varieties of *Amaranthus tricolor* L.

1. Leaf ovate-oblong, apex emarginate, tepal apex acute, as long as or shorter than fruit .... *A. tricolor* var. *acutus*
1. Leaf usually ovate-lanceolate or deltoid-ovate, tepal apex awned, tepal much longer than fruit(2)
2. Branching from base of the stem .... *A. tricolor* var. *tristis*
2. Branching from middle of the stem ....*A. tricolor* var. *tricolor*

Pan et al. (1992) studied 45 indigenous and exotic genotypes of *A. tricolor* taking into consideration ten quantitative traits like days to first clipping, number of clippings, diameter of stem, length of internode, leaf-stem ratio, length of lamina, width of lamina, days to flowering, duration of harvest and total yield. Variance analysis showed differences among the genotypes for all ten characters. In clustering pattern, genotypes were distributed in ten clusters, and the genotypes in these clusters showed significant divergence from each other. Clustering pattern did not reflect any parity with geographic distribution of the indegenous and exotic genotypes of *A. tricolor* studied. Devdas et al. (1992) investigated a total of 25 accessions belonging to *Amaranthus tricolor*, *A. dubius*, *A. spinosus* and *A. viridis* for 13 biometric characters. The accessions were classified into seven clusters. The study of inter- and intra-cluster differences revealed greatest variability in varieties of *A. tricolor*.

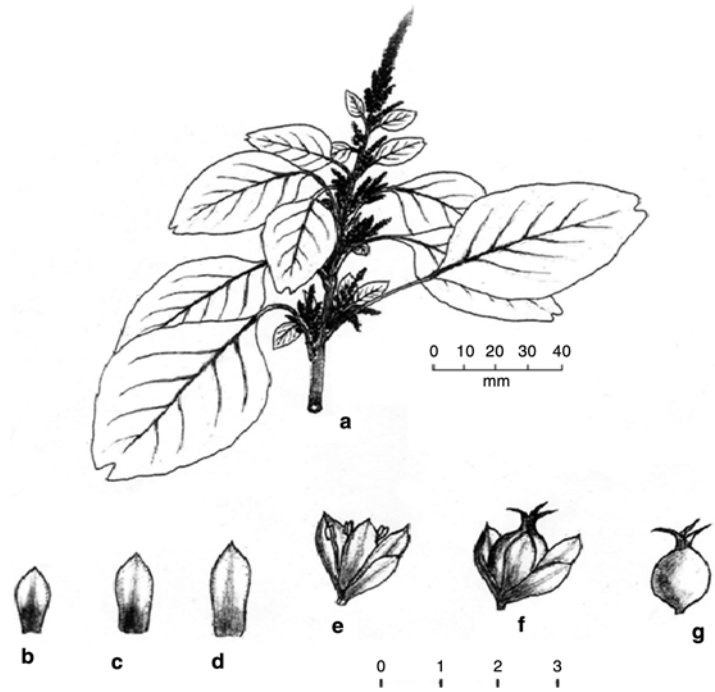

Fig. 5.7 *A. tricolor* var. *acutus* S. Das (**a**) habit, (**b**) bract, (**c**) bracteole, (**d**) tepal, (**e**) male flower, (**f**) female flower, (**g**) utricle

Table 5.3 Morphological characters delimiting three varieties of *A. tricolor*

A. tricolor var. *tricolor*	*A. tricolor* var. *tristis*	*A. tricolor* var. *acutus*
Small erect herb	Robust erect herb	Robust erect or procumbent herb
Branching from middle of the stem	Branching from base of the stem	Branching from middle of the stem
Leaf deltoid-ovate	Leaf ovate-lanceolate	Leaf ovate-oblong
Leaf apex acute	Leaf apex acute	Leaf apex notched or emarginate
Petiole shorter than the leaf blade	Petiole shorter than the leaf blade	Petiole equal or longer than the leaf blade
Bracteole ovate-lanceolate	Bracteole ovate-lanceolate	Bracteole ovate-spathulate
Bracteole apex long awned	Bracteole apex long awned	Bracteole apex acute
Bracte broad ovate	Bract broad ovate	Bract ovate-spathulate
Bract apex awned	Bract apex awned	Bract apex acute
Tepal ovate-lanceolate	Tepal ovate-lanceolate	Tepal ovate-spathulate
Tepal apex awned or setaceous	Tepal apex awned or setaceous	Tepal apex acute
Tepal length 4.5–5.0 mm	Tepal length 4.5–5.0 mm	Tepal length 2.25–2.5 mm
Fruit shorter than the tepals	Fruit shorter than the tepals	Fruit equal to longer than the tepals
Ratio tepal length/fruit length 0.9–1.0 mm	Ratio tepal length/fruit length 1.6–2.25 mm	Ratio tepal length/fruit length 2.0–2.25 mm

Amaranthus blitum L. included in sect. *Blitopsis* is a trailing ascending glabrous herb with light-green ovate-rhomboid emarginate, cuneate leaves widespread in warm tropical climate. It might have originated in the Mediterranean region. *A. blitum* known as livid amaranth (Britton and Brown 1896) is quite widespread in Indian tropics as well as other Southeast Asian countries with a large number of morphotypes. Gradual domestication process has resulted in the appearance of its cultivated form. The wild *A. blitum* has 1.5–2.5 cm long ovate-emarginate leaves, while

A. lividus L. is an erect cultivated form of *A. blitum* with large leaves up to 7.0–9.0 cm known as separate species. Both have long been known as synonyms (Hooker 1885; Thellung 1914).

A. blitum is a species with remarkable variability, and several subspecies have been described; some of them have been given species rank (Hugin 1987; Costea et al. 2001b). Keeping in view the morphological variabilities, it would be logical to treat *A. blitum* as a complex or aggregate comprising several taxa just like *A. tricolor* aggregate.

The *Amaranthus blitum* aggregate belonging to the subgenus *Albersia* includes four taxa: *A. blitum* Linnaeus (1753: 990) sensu stricto, *A. blitum* var. *oleraceus* (Linnaeus 1763: 1403) Costea et al. (2001b: 984), *A. emarginatus* (Moquin-Tandon ex Uline & Bray 1984: 319) sensu stricto and *A. emarginatus* var. *pseudogracilis* (Thellung 1914: 321) Iamonico (2014c).

As far as the revision of the genus *Amaranthus* is concerned, Iamonico (2010a, b, 2012, 2013, 2014a, b, c) contributed a lot in several projects, and Das (2012a, b, 2013) investigated morphological variability of the genus in India. They jointly contributed to the knowledge of the genus *Amaranthus* in India emphasising *A. blitum* aggregate (Das and Iamonico 2014).

Amaranthus blitum and *A. lividus* were first described by Linnaeus in the first edition of *Species Plantarum* (Linnaeus, 1753: 990). These two names have generated an interesting nomenclatural problem. Moquin-Tandon (1849) and Thellung (1914) partially included *A. graecizans* in the synonymy of *A. blitum*, leading Brenan (1961) to state *A. blitum* as nomen confusum. Brenan and Townsend (1980) agree with Brenan (1961) and proposed to list the name *A. blitum* as *nomen rejiciendum*. Few years later the Committee for Spermatophyta (Brumitt 1984) rejected this proposal, reporting '*…since the last century, and in the present century (A. blitum) was used in the correct sense of A. lividus or at not be used at all…*'. Filias et al. (1980) pointed out that the choice between the two names (*A. blitum* and *A. lividus*) was made by Hooker (1885) favouring *A. blitum* as the currently accepted name.

Concerning the infraspecific classification of *A. blitum*, several names (at subspecific, variety and form ranks) were published, and the situation is quite complicated.

Moquin-Tandon (1849: 265) accepted five varieties ($\alpha - \varepsilon$) under *A. blitum*, three of which (α-*sylvestris*, δ-*graecizans*, ε-*angustifolius*) referred to *A. graecizans* Linnaeus (1753: 990). On the other hand he recognised the genus *Euxolus* Rafinesque (1836: 42) and proposed the combinations *E. lividus* (L.) Moq. (≡ *A. lividus*), *E. oleraceus* (L.) Moq. (≡ *A. oleraceus*) and *E. viridis* (Linnaeus 1763: 1405) Moq. (≡ *A. viridis*), the latter of which has four varieties: β-*ascendens* (Loiseleur 1810: 141) Moq. (≡ *A. ascendens*), γ-*purpurascens* Moq. (new variety), δ-*rubens* Moq. (new variety) and ε-*polygonoides* Moq. (new variety). The name *A. emarginatus* Salzmann ex Moquin-Tandon (1849: 274) was published as synonym of *Euxolus viridis* L. var. ('ε') *polygonoides* Moq., so it is to be considered illegitimate under art. 36.1c of the ICBN (McNeill et al. 2012).

Roxburgh (1832: 605–606) recognised the taxa *A. lividus* and *A. oleraceus* as separate species; *A. blitum* was cited as similar, but not a well-defined species, under *A. polygamus* Willd. (Roxoburgh 1832: 603).

Hooker (1885: 721) accepted the name *A. blitum* and recognised three varieties under *A. blitum*: var. *blitum*, var. *oleraceus* and var. *sylvestris*. The latter is distinguishable from the other varieties in having dehiscent fruit, while the other two varieties have indehiscent utricles. The var. *sylvestris* is not part of the *A. blitum* aggregate and is currently accepted as *A. graecizans* Linnaeus subsp. *sylvestris* (Villars 1807: 111) Brenan (1961: 273) (see, e.g. Costea et al. 2001; Iamonico 2014d). The var. *oleraceus* was mentioned as *cultivated in India and elsewhere*.

Thellung (1914) accepted the name *A. lividus* and proposed the following classifications:

– *lividus* proles *polygonoides* (Moq.) Thell. [now *Amaranthus emarginatus*]: wild plants with prostrate or ascending stems, small leaves and fruit about 1.5 mm long. Two new forms were described: f. *pseudogracilis* Thell. ('*2*') and F. *axillaris* Thell. ('*3*'); the first one differs from the other in having the inflores-

cence in terminal spike (the flowers are arranged in axillary cluster in the F. *axillaris*)
- *lividus* proles *ascendens* (Loisel.) Thell. (now *A. blitum* sensu stricto): wild plants with prostrate or ascending stems, small leaves and fruit 2.0 – 2.5 mm long
- *lividus* proles *týpicus* (L.) Thell. (now *A. blitum* s. s.): cultivated forms with vigorous, erect or ascending stems and large leaves, red-coloured
- *lividus* proles *oleraceus* (L.) Thell. (now *A. blitum* var. *oleraceus*): cultivated plants, very much like proles *týpicus* but whitish-coloured

Hügin (1987) renamed *A. emarginatus* Moq. ex Uline & Bray and the combination *A. emarginatus* subsp. *pseudogracilis* (Thell.) Hügin was proposed.

Based on morphological and ecological observations, Costea et al. (2001b) recognised the taxon *emarginatus* at subspecies rank of *A. blitum*, also including two varieties: var. *emarginatus* and var. *pseudogracilis* (Thell.) Costea et al. (2001: 981).

Walter and Dobes (2004) carried out a morphometric study confirming the recognition of the classification proposed by Costea et al. (2001b).

More recently Iamonico (2014c) was in favour to the separation of the taxa *blitum* and *emarginatus* at species rank due to their different origins (Mediterranean Basin, Europe, North Africa and Tropical America, respectively). The taxon *oleraceus* was accepted at variety rank of *A. blitum*, the taxon *pseudogracilis* at variety rank of *A. emarginatus*.

Morphometric investigation on *Amaranthus blitum* complex in India and elsewhere showed that there exist two varieties of *A. blitum* in India (*A. blitum* var. *blitum* and *A. blitum* var. *oleraceus* (L.) Hooker filius (1885: 721). As pointed out in previous works (see, e.g. Costea et al. 2001b; Walter and Dobes 2004; Iamonico and Iberite 2012), the diameter of the seeds is an important character to distinguish the taxa belonging to the *A. blitum* aggregate. The analysis reveals two main groups: the first one including plant with seeds of 1.1–1.8 mm in diameter and the second group including plants with seeds of 0.7–1.1 mm in diameter. Also the size of leaves can be used to separate these two groups (see the diagnostic key below). The plants with larger seeds can be referred to *A. blitum* s.l., with two varieties (var. *blitum* and var. *oleraceus*, both occurring in India) that distinguish each other on the basis of the seed dimensions and surface. The population from West Bengal showed a clear distinctiveness having minute seeds, leaves with obtuse or obscurely emarginate apex, bract as long as tepals (ratio bracts/tepals about 1) and acute tepals. The populations from Europe are distinct from those of West Bengal having leaves with clearly emarginate to bilobed apex and bract always shorter than tepals (ratio bracts/tepals 0.5 – 0.6) and acute tepals. This European population should be assigned to a separate species *A. emarginatus* s.l. [containing two varieties – var. *emarginatus* and var. *pseudogracilis* – distinguished on the basis of the habitus and the synflorescence structure]. The Bengal population shows features that cannot be ascribed to any known *Amaranthus* species but certainly belong to the *A. blitum* aggregate but cannot be assigned to *A. emarginatus*, given a species status, named as *Amaranthus bengalense* Das & Iamonico *sp.nov.* In India specially in the lower gangetic plain of Bengal, the 'Blitum complex' is represented by three varieties of *Amaranthus blitum – A. blitum* var *blitum*, *A. blitum* var. *oleraceus* (L.) Hook and *A. bengalense* Saubhik Das & Iamonico (Das and Iamonico 2014).

Amaranthus blitum **var.** *blitum* A monoecious, annual, erect to prostrate herb attaining a height of 15–80 cm. Leaves are ovate (2.5–4.5 × 1.5–3.5 cm), entire, long petioled (petiole usually as long as the blade) apex emarginate to bilobed, often mucronate, base cuneate and green. Synflorescences are arranged in terminal spike-like fashion, erect or reflexed. Bracts are ovate to oblong shorter than the perianth. Staminate flowers have three tepals and three stamens; tepals are usually ovate. Pistillate flowers have three equal or subequal tepals and three stigmas; tepals are oblong-lanceolate or elliptic (1.2–2.0 mm long), membranous with acute apex and thick abaxial midvein. Fruits are reddish brown, compressed,

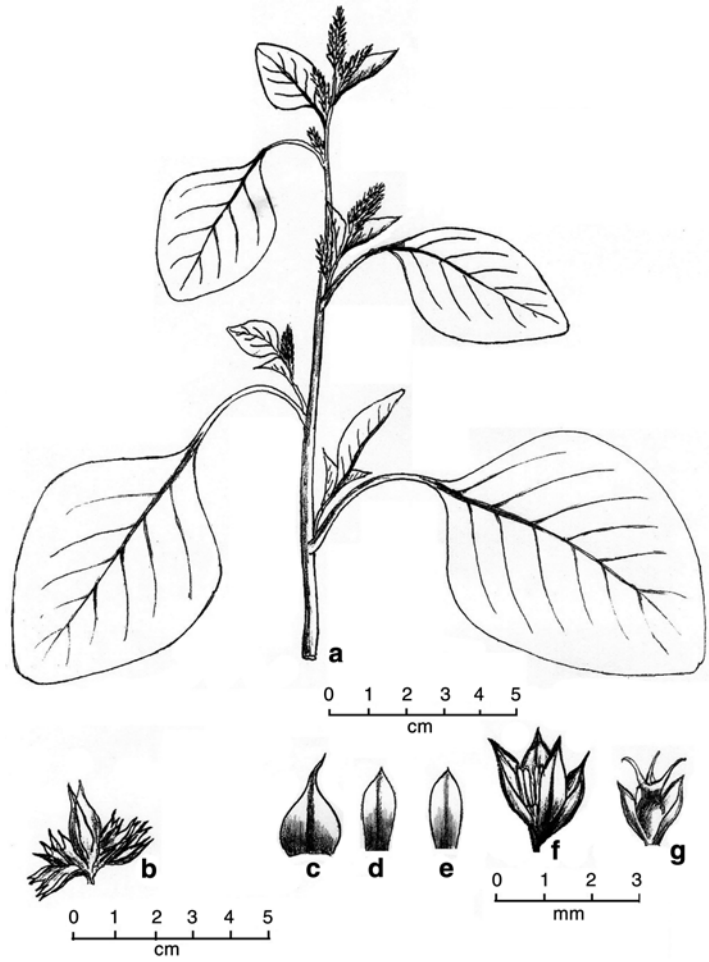

Fig. 5.8 *A. blitum* var. *oleraceus* (L.) Hooker f. (**a**) habit, (**b**) small portion of inflorescence, (**c**) bract, (**d**) bracteole, (**e**) tepal, (**f**) male flower, (**g**) female flower

subglobose to ellipsoidal utricle, 1.5–2.5 mm long, as long as or longer than the perianth, smooth to finely rugose and indehiscent; seeds are lenticular, black to dark reddish, with minutely punctiform surface 1.1–1.2 mm in diameter.

Amaranthus blitum* var. *oleraceus A monoecious, annual, erect, cultivated herb attaining a height of 25–90 cm. Leaves are green ovate-rhomboid (8.0–10.0 × 6.0–7.0 cm), mucronate, petiolate (petiole as long as or shorter than the blade) with entire margin and cuneate base. Synflorescences are arranged in terminal spike-like pattern and in axillary glomerules. Bracts are ovate, as long as or shorter than the perianth, membranous, with acute to acuminate tip. Staminate flowers have three tepals usually ovate and three stamens shorter than the tepals. Pistillate flowers have three equal or subequal tepals and three stigmas; tepals are oblong-lanceolate to spathulate (1.5–2.5 mm long), membranous with acute apex. Fruits are reddish brown, compressed, subglobose utricle (1.8–2.8 mm long), longer than the perianth, usually smooth and indehiscent. Seeds are broadly lenticular, black with smooth surface (1.2–)1.4–1.7(1.9) mm in diameter (Fig. 5.8).

***A. bengalense* Saubhik Das & Iamonico** Annual, monoecious, ascending, cultivated herb attaining a height of 30–40 cm. Leaves are green deltoid-ovate to ovate-lanceolate (4.0–4.5 × 3.0–3.5 cm), petiolate (petiole shorter than the blade) with obtuse to obscurely emarginate apex, entire margin and cuneate base. Synflorescences are

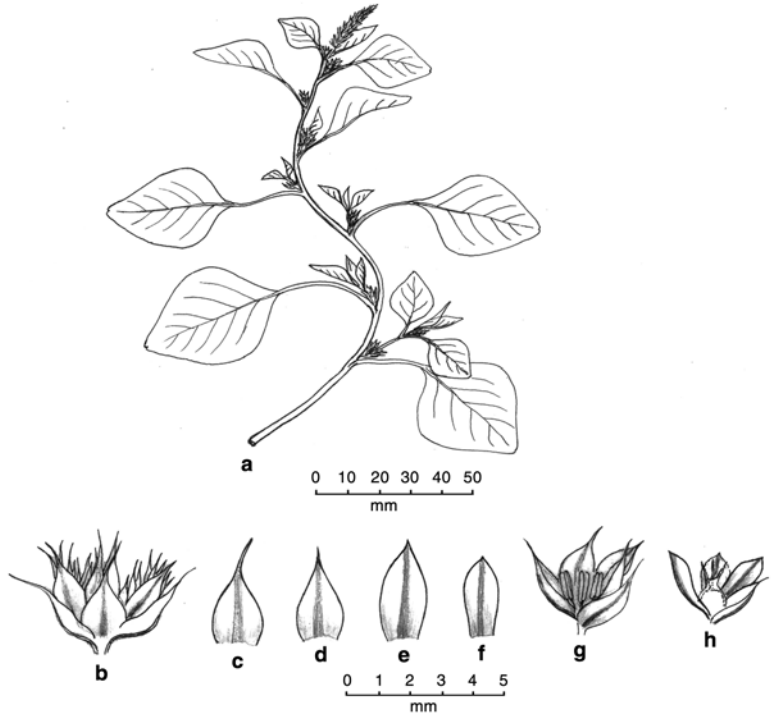

Fig. 5.9 *Amaranthus bengalense* Das & Iamonico (**a**) habit, (**b**) synflorescence part, (**c**) bract, (**d**) bracteole, (**e**) tepal of male flower, (**f**) tepal of pistillate flower, (**g**) male flower, (**h**) female flower

arranged in terminal spike-like fashion and in axillary glomerules. Bracts and bracteoles are ovate-lanceolate (4.0–4.5 mm long), as long as the perianth, with green adaxial midvein and acuminate apex. Staminate flowers have three tepals and three stamens; tepals are lanceolate (4.0–4.5 × 1.0–1.8 mm) and acuminate; stamens are shorter than tepals. Pistillate flowers have three equal or subequal, oblanceolate to spathulate (2.5–3.0 × 0.8–1.0 mm) tepals and three stigmas. Fruits are greenish brown, compressed, subglobose (1.5–1.8 mm long) utricle, slightly shorter than the perianth, smooth and indehiscent. Seeds are broadly lenticular, black with smooth surface, 0.7–1.0 mm in diameter (Fig. 5.9).

Key to the Varieties of *Amaranthus blitum* L.

A diagnostic key of all the taxa of the *A. blitum* aggregate is as follows:

1. Seed diameter 1.2−1.8 mm, length of the fruit 1.9–3.5 mm, leave blade size (3.0–) 3.5–9.0 × 1.5–6.2 cm ..... (2)
   – Seed diameter 0.7–1.1 mm, length of the fruit (1.2–)1.4=−1.8 mm, leave blade size 1−3.5 (−4.5) × (0.5−) 0.8−3.5 cm ..... (3)
2. Seeds with minutely punctiform surface and diameter 1.1−.2 mm ..... *A. blitum* var. *blitum*
   – Seeds with smooth surface and diameter (1.2−) 1.4 − 1.7(−1.9) mm..... *A. blitum* var. *oleraceus*
3. Bracts as long as tepals (ratio bract/tepal about one); tepals tip acuminate; leaves with apex obtuse or slightly emarginate ....*A. bengalense*
   – Bracts shorter than the tepals (ratio bract/tepal 0.5−0.6); tepals acute; leaves with apex clearly emarginate to bilobed ..... (4)
4. Stem ascending, synflorescence in axillary glomerules or short thickened terminal spike-like (up to 2 cm long) ..... *A. emarginatus* var. *emarginatus*
   – Stem prostrate, synflorescence in terminal long and slender spike-like (up to 10 cm long) often thin and flexuous (1.5–7.5 mm long) ..... *A. emarginatus* var. *pseudogracilis*

Das (2015) introduced a new vegetable amaranth, *Amaranthus parganensis* Saubhik Das *sp. nov.* from Lower Gangetic Plain of West Bengal, India. In general appearance and morphology, *Amaranthus parganensis* closely resembles *Amaranthus tricolor* L. Both the species are adapted to tropical climate, sympatric in distribution having similar ecology, distinguished by common features such as the erect habit, leaf size, bracts smaller than the tepal and the fruit as a dehiscent, circumscissile utricle with a smooth surface and blackish-brown seeds. It is further assigned to sect. *Pyxidium* under the subgen. *Albersia*. The new species shows a structural gynomonoecy with rudimentary gynoecia in bisexual flowers (Fig. 5.10). There is no functional gynomonoecy, and no seeds are found within the rudimentary carpels. Among the three subgenera as delimited by Mosyakin and Robertson (1996), subgen. *Acnida* comprises only dioecious species, and the other two, subgen. *Albersia* and subgen. *Amaranthus*, comprise only monoecious species. There are no records of any other amaranths having gynomonoecy or incipient gynomonoecy.

Amaranthus parganensis is a robust, erect, branched, 100–120 cm tall herb, appearing gynomonoecious but not functionally. Leaves are exstipulate, ovate to lanceolate, 13–17 cm × 5–9 cm, with acute or minutely notched apex having a small apicula. Flowers are arranged in axillary glomerules and a terminal compound inflorescence of cymes, terminal inflorescence massive but less branched. Flowers are either bisexual or pistillate, unequal in size, intermixed; tepals of the bisexual flowers are larger than those of pistillate flowers. Bract, bracteoles and tepals have apical awns. Bract and bracteoles are broadly ovate to lanceolate, 1.5–2.0 mm long. Perianth consists of three ovate to lanceolate or

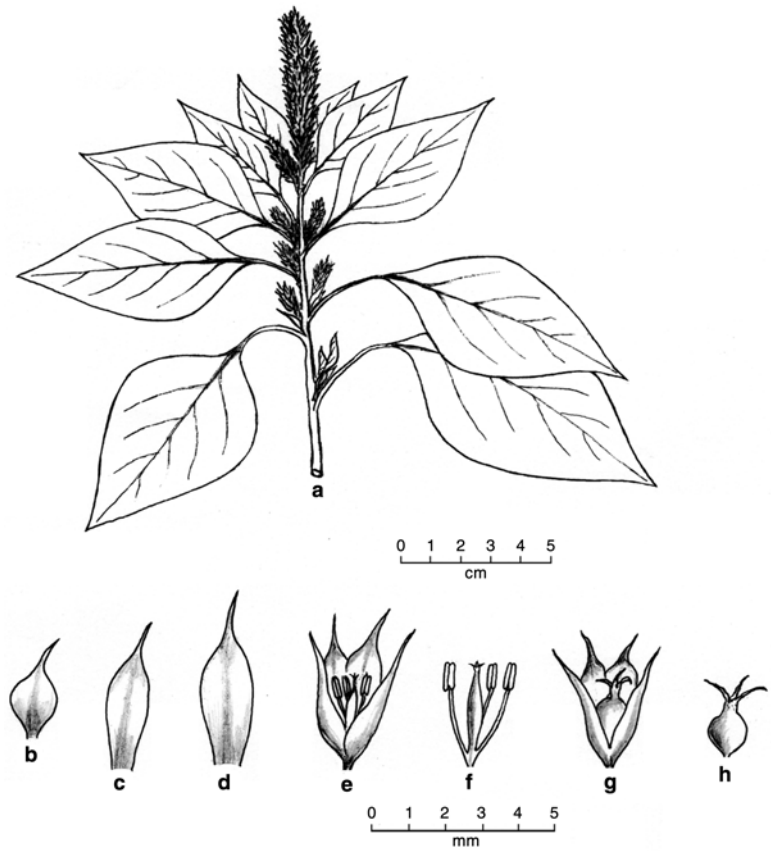

Fig. 5.10 *Amaranthus parganensis* Saubhik Das (**a**) fertile habit, (**b**) aristate bract, (**c**) aristate bracteole, (**d**) aristate tepal, (**e**) bisexual flower, (**f**) bisexual flower with stamens and rudimentary carpel, (**g**) pistillate flower, (h) gynoecium

oblong, membranous tepals with acuminate apex, 3.0–3.5 mm long in pistillate flowers. Bisexual flowers have three stamens, 4.5–5.0 mm long. Style is short and thickened, ovary ovoid, three stigmas, filiform and papillose. Gynoecium in the bisexual flower is rudimentary and has a short thickened gynophores, but gynoecium in the pistillate flowers lacks a gynophore. Fruits are circumscissile utricle, produced only by pistillate flowers, ovoid and 1.5–2.0 mm long. Seeds are blackish brown, shiny, compressed and 1.2–1.5 mm in diameter.

Morphological features separating the prime vegetable species of *Amaranthus* are mentioned in Table 5.4.

Genetic diversity and relationship among the cultivated and wild *Amaranthus* species were evaluated using protein and RAPD markers. Though high level of genetic diversity was common within species, genetic uniformity was observed within most accessions. Srivastava and Roy (2012) studied four species (*A. viridis, A. spinosus, A. tricolor* and *A. blitum*) and their accessions. The study revealed that the accessions of each species were very close to each other, but variation was observed at the species level. *A. spinosus* was shown to be the next most closely related to *A. tricolor* after *A. blitum*, though Zheleznov et al. (1997) reported no phylogenetic relationship between *A. tricolor* and *A. spinosus*; Chan and Sun (1997) also reported dissimilarity among *A. viridis*, *A. tricolor* and *A. spinosus*. Samour (1991) observed a definite protein profile, intermediate between *A. viridis* and *A. hybridus* in a population of *A. viridis* indicating a

Table 5.4 Comparative morphology of major vegetable amaranths

Morphological characteristics	*A. parganensis*	*A. tricolor*	*A. blitum*
Breeding system	Incipiently gynomonoecious	Monoecious	Monoecious
Stem	Erect, 100–120 cm	Erect, 90–100 cm	Erect or ascending, 35–45 cm
Leaf shape	Ovate-lanceolate or lanceolate, not rhombic	Deltoid-ovate or ovate to lanceolate or oblong	Ovate to lanceolate or rhomboid-ovate
Leaf sizes	13.0–17.0 × 5.0–9.0 cm	9.0–7.0 × 5.0–9.0 cm	2.5–8.0 × 1.5–6.0 cm
Ratio lamina/petiole length	2.1–2.6	0.89–1.66	0.5–1.5
Leaf apices	Acute or minutely notched	Acute or slightly emarginate	Emarginate
Inflorescence	Terminal inflorescence massive but less branched	Terminal inflorescence massive and much branched	Terminal inflorescence short
Flowers	Both bisexual and unisexual (female flower), bisexual and female flowers unequal	Unisexual, staminate and pistillate flowers are equal	Unisexual, staminate and pistillate flowers are equal
Bract vs. tepal	Bract < tepal	Bract < tepal	Bract ≤ tepal
Bract length	1.5–2.0 mm	1.5–2.0 mm	0.5–2.5 mm
Tepal shape	Ovate to lanceolate or oblong	Ovate to lanceolate or spathulate	Lanceolate to spathulate
Tepal tip	Acuminate	Acute or awned	Acute or awned
Tepal length	3.0–5.0 mm	2.25–2.5 (−5) mm	1.2–2.5 mm
Fruit vs. tepal	Fruit not exceeding tepals	Fruit may or may not exceed tepal	Fruit marginally exceeding tepals
Fruit surface	Smooth	Smooth	Faintly rugose
Dehiscence	Circumscissile utricle, dehiscent	Circumscissile utricle, dehiscent	Indehiscent
Seed diameter	1.2–1.5 mm	1.2–1.5 mm	1.1–1.8 mm
Seed colour	Blackish brown	Blackish brown	Black

probable hybridisation between the two taxa. These results confirmed the suggestions of Drzewiecki (2001) that the grain and leafy types of cultivated amaranths (from India) could be easily distinguishable as two genetic groups on their fixed allozyme alleles. The wild amaranths as a rule showed high RAPD polymorphism than the cultivars (Srivastava and Roy 2012). Highest polymorphism was shown by *A. viridis*, while *A. blitum* showed the lowest level of polymorphism. Dendrogram computed on RAPD and protein marker showed that cultivated amaranths are most closely related to each other than the wild members. A high degree of genetic variability shown by the wild species is expected and explainable because they are not subjected to any selection pressure of domestication; as such *A. viridis* showed highest polymorphism and *A. blitum* the least polymorphism. Observation of Chan and Sun (1997) was also concomitant to this interpretation as they found 39.9 % polymorphism in crop species, 51 % polymorphism in vegetable species and 69.5 % polymorphism in wild species of *Amaranthus*.

5.7 Taxonomic Delimitation in Weed Amaranths

Most of the people try to identify weeds based on 'how the plant looks', i.e. to recognise taxa on the pigmentation or growth forms which are extremely variable (Sauer 1967); more accurate identification needs vivid morphological study of the floral parts. Historically, taxonomic delimitation in *Amaranthus* species has been done mostly based on differences in floral characteristics, but new methods using molecular biological techniques are also gaining importance. Correct identification of *Amaranthus* weed species is necessary for efficient weed control (Horak et al. 1994; Sweat et al. 1998). Some molecular studies have been applied to weed species. Wetzel et al. (1999a) developed ribosomal ITS restriction site-based PCR-generated marker to identify common amaranth weeds which were otherwise difficult to be identified morphologically. Pratt and Clark (2001) used isozyme polymorphism to address the question whether *A. rudis* and *A. tuberculatus* would be merged and considered as a single species or their separate identity would be maintained. Wassom and Tranel (2005) used AFLP-based marker to highlight the phylogeny of both dioecious and monoecious amaranth. Eight weed species represented by 141 individuals from 98 different accessions were taken into consideration. Interestingly, the dioecious weed *A. rudis* and *A. tuberculatus* did not group together, perhaps indicating independent evolutionary ways and separate genetic entities as well. Correct identification of *Amaranthus* species is often difficult and frequent misidentification is common (Horak et al. 1994; Wax 1995; Sauer 1953; Arhens et al. 1981). For example, Ahrens et al. (1981) found that 13 of 14 accessions of weed amaranths that weed scientists earlier identified as redroot pigweed were actually smooth pigweed (*Amaranthus hybridus* L.) or Powell amaranth (*Amaranthus powellii* S. Wats.). As another example, Wetzel et al. (1999a) applying molecular marker analysis of ribosomal internal transcribed spacer (ITS) regions found that 12 of 92 *Amaranthus* accessions that had been collected and identified by weed scientists earlier were misidentified. In spite of accuracy it is not possible to apply molecular marker-based identification for routine use. It could be applied to verify the identity of troublesome biotypes with ambiguous or atypical morphology. Response to herbicides can be a very promising tool to delimit taxa. As for examples, Coetzer et al. (2002) reported significantly different responses to glufosinate herbicide among waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer and *Amaranthus rudis* Sauer), redroot pigweed (*Amaranthus retroflexus* L.) and Palmer amaranth (*Amaranthus palmeri* S. Wats.). Mayo et al. (1995) reported that Palmer amaranth was more difficult to control with various herbicides than were other *Amaranthus* species.

5.8 Different Phylogenetic Concepts on Grain Amaranths

It is generally considered that the vegetable and the grain amaranths are two distinct groups that have originated from their respective weed pro-

genitor. Both the groups have different centres of origin and unique processes of domestication. Weedy and grain *Amaranthus* spp. are inseparably linked phylogenetically and historically. The grain amaranths are supposed to have derived from their respective weed progenitors through frequent outcrossing and gradual unique domestication process. Two hypotheses have been proposed by Sauer (1967, 1976). The first hypothesis is based on geographical distribution, which suggests that all the three grain amaranths have evolved independently, i.e. origin is polyphyletic. *A. hypochondriacus* was domesticated in Mexico from *A. powellii*, *A. cruentus* from *A. hybridus* in Central America and *A. caudatus* from *A. quitensis* in South America (Fig. 5.11a). The second hypothesis based on plant and seed morphology suggested monophyletic origin of the grain amaranths. The three cultivated grain species have originated from a single weed progenitor *A. hybridus*, following subsequent introgressive hybridisation with two other wild species in different regions. According to this hypothesis, the first domesticated species was *A. cruentus*, originated from *A. hybridus* in Central America, followed by the domestication of *A. hypochondriacus* by repeated crossing between *A. cruentus* and *A. powellii* in Mexico and the domestication of *A. caudatus* by crossing between *A. cruentus* and *A. quitensis* (Fig. 5.11b). Since 1940 *Amaranthus powellii* has become a widespread and troublesome weed in Eastern North America. In Europe it was misidentified as *A. chlorostachys* Willd., a synonym of *A. hybridus*. The species is exceptional in producing flowers with tepal and stamen numbers varying from 3 to 5, even in a single plant. Individual plants with indehiscent utricle also occur sporadically in Native American populations and have formed the entire local population in Europe (Brenan 1961; Aellen 1961). The hybrids between other amaranth species may show such aberrant forms, but aberrations in *A. powellii* evidently arisen without hybridisation. *Amaranthus quitensis* is a riverbank pioneer of South America. The plant is semi-cultivated or tolerated as a source of food additives. Three grain amaranths along with *A. hybridus* are supposed to have formed a complex or aggregate ('hybridus species complex') in which taxonomic disputes and phylogeny are yet to be resolved with extreme clarity, especially because of apparent common hybridisation and misapplication of nomenclature.

More recently a third hypothesis was proposed by Mallory et al. (2008). He suggested

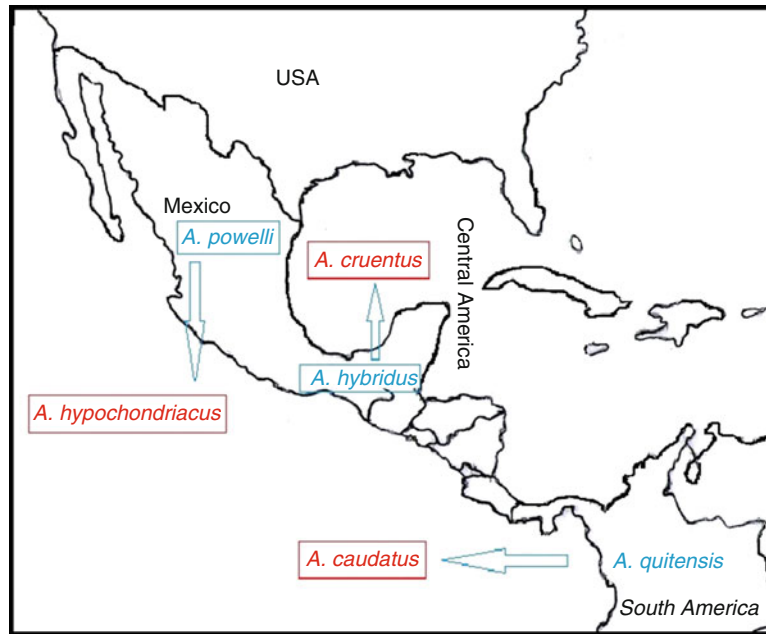

Fig. 5.11a Polyphyletic theory regarding origin of grain amaranths as proposed by Sauer (1967)

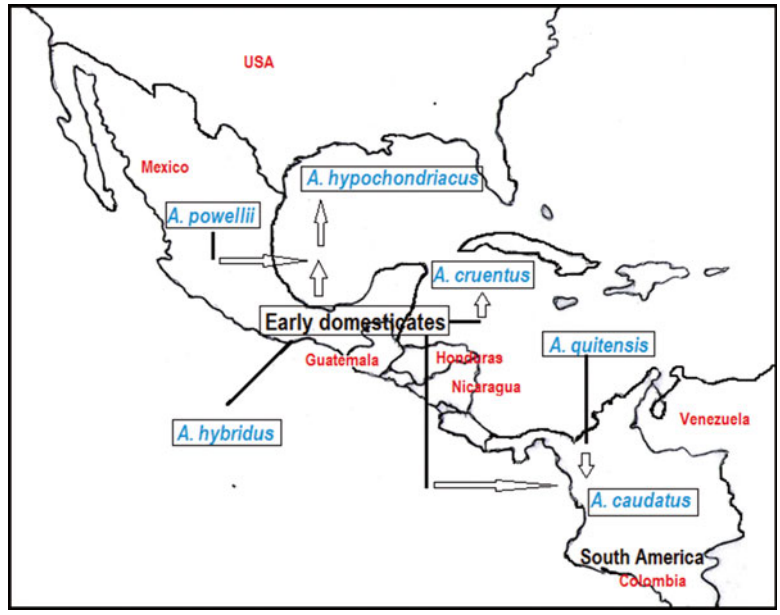

Fig. 5.11b Monophyletic theory regarding origin of grain amaranths as proposed by Sauer (1967)

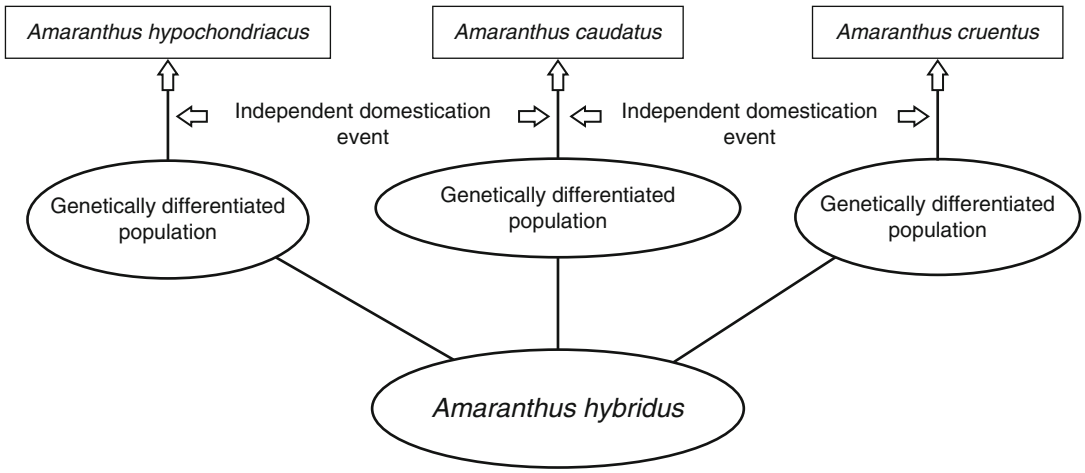

Fig. 5.12 Derivation of grain amaranths from ancestral *A. hybridus* through separate genetically differentiated populations as proposed by Mallory et al. (2008)

that *A. hybridus* is the progenitor species for all the three domesticated grain species, but each was derived through independent domestication events from genetically differentiated populations of *A. hybridus* (Fig. 5.12). The study designated *A. hybridus* as the only progenitor species of all the three grain amaranth species. According to neighbour-joining analysis, *A. caudatus*, *A. cruentus* and *A. hypochondriacus* were monophyletic, while *A. hybridus* was polyphyletic as it appeared from the presence of *A. hybridus* accessions in each of the three grain amaranth monophyletic clades. They also observed that *A. powellii* (a previously recognised progenitor of grain amaranths) has formed a monophyletic group distinct from any of the grain amaranths. It is obvious that a larger and more inclusive and comprehensive

investigation, involving substantially more diversified accessions of both the grain amaranth and progenitor species (*A. hybridus*, *A. powellii* and *A. quitensis*), is still needed to have a clear idea about origin and phylogeny of the grain amaranths. However, one can presume that taxonomic identification of these *Amaranthus* species may be ambiguous due to reciprocal gene flow that resulted from outcrossing in regions where the species are sympatric.

Progenitor status of *A. hybridus* is supported by the observation that it forms hybrid with all the other species in the 'hybridus complex' (Pal and Khoshoo 1972, 1973a, b, 1974). Study involving neighbour-joining method based on combined ITS, ISSR and AFLP data (Xu and Sun 2001) also supported the same monophyletic origin of the grain amaranths and close relationship between *A. caudatus* and *A. hypochondriacus*. Kirkpatrick (1995) utilised morphological features, isozyme polymorphism and nuclear ribosomal DNA analytical data to study interspecific relationship. Morphological features of *A. hybridus* were overlapping with that of cultivated grain amaranths, while *A. retroflexus* L., *A. spinosus*, *A. palmeri* S. Watson and *A. powellii* were morphologically distinct. *Amaranthus hybridus* was also grouped with grain amaranths, while the other weed species were distinct. All these evidences favoured the concept of monophyletic origin of grain amaranths from *A. hybridus*. Studies on allozyme variation in amaranths also proved helpful in the investigation of phylogenetic relationship among weedy and crop species (Hauptli and Jain 1984, 1985). Molecular marker analysis has contributed a lot to explore origin and evolution of cultivated amaranth and allied wild species, and Hauptli and Jain (1984) were among the pioneering workers who firstly used molecular marker to address evolutionary relationship among grain amaranths. In their study, they considered nine enzyme systems, and the study revealed that the grain species are related more closely to each other than they are to their respective wild progenitors. The study supported the monophyletic origin of the grain amaranths as proposed by Sauer and indicated that a single domestication event has occurred involving *A. hybridus* as the common ancestor rather than separate domestication event (Kulakow et al. 1985; Kulakow and Jain 1990a, b). But relative closeness between *A. caudatus* and *A. quitensis* shows some sort of deviation from Saure's perception. This suggests a separate domestication event of *A. caudatus* from *A. quitensis*. It is also presumed that *A. quitensis* may have also derived from *A. caudatus* through introgressive hybridisation with *A. hybridus*, or it is semi-domesticate which arose from the escape of *A. caudatus* from cultivation. *Amaranthus quitensis* is found to be ecologically a semi-domesticate in Ecuador occurring only in cultivated field (Coons 1982; Hauptli and Jain 1984).

Electrophoretic profile of total seed protein revealed that *A. cruentus* and *A. hypochondriacus* are closely related to *A. hybridus* (Sammour et al. 1993). Sammour concluded that *A. cruentus* and *A. hypochondriacus* are subspecies of *A. hybridus. Amaranthus cruentus* and *A. hypochondriacus* have more or less the same amount of DNA content, whereas *A. caudatus* differs significantly from other two species in DNA content (Greizerstein and Poggio 1994). On the other hand, studies involving interspecific fertility, electrophoretic data or RAPD data favoured much closer genetic affinity between *A. caudatus* and *A. hypochondriacus* than either one with *A. cruentus* (Gupta and Gudu 1991; Transue et al. 1994). Vegetable amaranths were studied for their morphology and karyotype (Madhusoodanan and Nazeer 1983). The study revealed that both, the chromosome size and gross morphology, vary according to the selection factor and/or adaptive protocols. Chromosome repatterning and structural modification played a key role in differentiation and evolution of *Amaranthus* species.

Investigation on morphological and genetic variability in Indian amaranth showed a high degree of morphological polymorphism but least allozymic variability, i.e. maximum monomorphism of allozyme loci. The lack of positive correlation between morphological polymorphism and genetic variability may be due to relatively recent introduction and rapid spread of grain

amaranths in India. Putative progenitor-derived species pair often shows high genetic identities, isolated by reproductive barriers such as chromosome repatterning or deviation in breeding system. Speciation can still proceed with little or no divergence at isozyme loci (Crawford 1983). The phylogenetic affinity of 28 species of *Amaranthus* were studied by Lanoue et al. (1996), applying restriction site analysis of PCR-generated chloroplast and nuclear DNA. Genetic variation found by the restriction site analysis was used to construct a cladogram of 28 species. The tree reflected *A. dubius* and/or *A. hybridus* as the possible progenitors of *A. cruentus* and *A. quitensis as* the probable progenitor of *A. caudatus*. Contradicting the previous observations (Hauptli and Jain 1984; Transue et al. 1994), Lanoue found much closer affinity between *A. caudatus* and *A. cruentus* than each of them with *A. hypochondriacus*. The study does not support the single origin hypothesis of the dioecious amaranths like *A. australis*, *A. cannabinus*, *A. floridanus*, *A. palmeri* and *A. rudis* as proposed by Sauer (1955). Lanoue suggested that *A. australis*, *A. cannabinus* and *A. rudis* are more closely related to each other, whereas *A. floridanus* and *A. palmeri* are more similar to other monoecious amaranths rather than other dioecious counterparts. The investigation by Chan (1996) involving RAPD assay and isozyme polymorphism supported Sauer's monophyletic hypothesis and projected *A. hybridus* as the progenitor of all the three grain amaranths but did not support the single origin hypothesis of the dioecious amaranths like Lanoue et al. (1996) (Fig. 5.13).

Previous phylogenetic studies investigating the origin of grain amaranth involved inadequate sampling of all the grain amaranths as well as the two putative weedy progenitors *A. hybridus* and *A. quitensis* across their native species range as well as inadequately investigated the genetic diversity component of both putative weedy species. Sampling errors like under-representation of weedy species compared to the cultivated species or over-representation of *A. hybridus* with exclusion of *A. quitensis* or involvement of weedy representatives collected from outside the species native range accounted for discordant interpretation between previous investigations (Kietlinski et al. 2013). Both the Sauer's hypotheses, i.e. single progenitor theory and independent domestication hypothesis, are based on morphology that is to be evaluated. The speculations made by Sauer based on morphological evidences validated by several workers in a

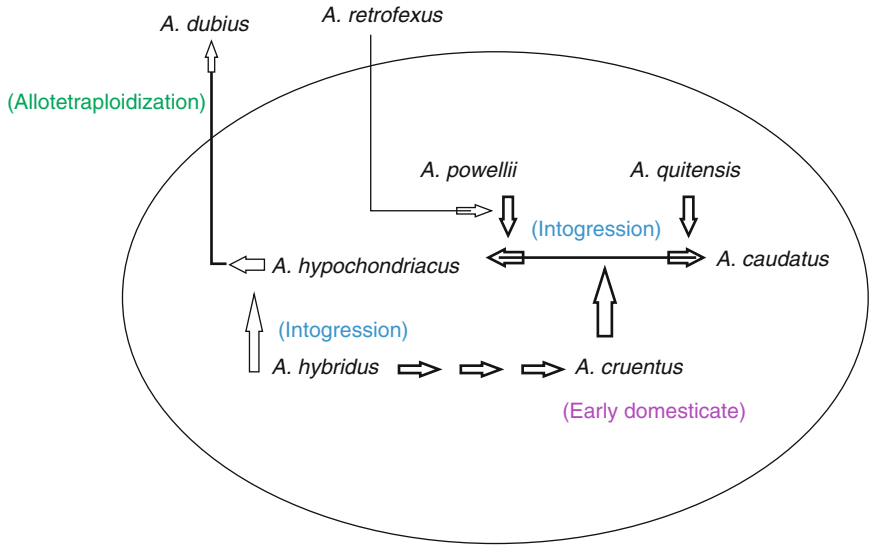

Fig. 5.13 Concept of Chan (1997) regarding origin of grain amaranths

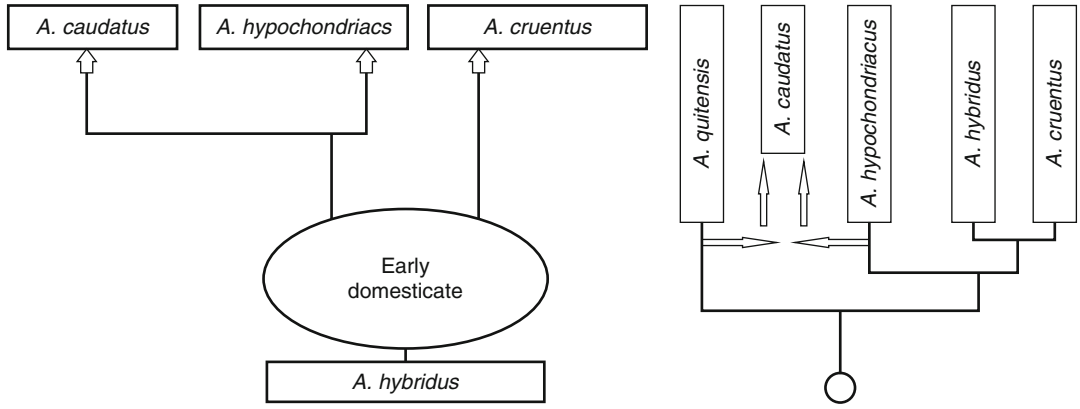

Fig. 5.14 Studies on evolutionary aspects of grain amaranths (**a**) phylogenetic relationship exclusively between three grain types and (**b**) phylogenetic relationship among all members of hybridus complex

fragmentary fashion not in a conclusive manner. All the studies were channelised in either of the two directions, i.e. (1) studies addressing the phylogenetic relationships exclusively between the three grain amaranths (Fig. 5.14a) and (2) studies addressing the phylogenetic relationships among all members of the hybridus complex (Fig. 5.14b). Kietlinski et al. (2013) considered 258 individuals from 56 taxa representing the three grain amaranths species and two putative weedy progenitor samples from their native species range and subjected to microsatellite analysis. Data analysed by PCA, clustering algorithms and genetic distance neighbour-joining tree dendrogram highlighted few focal points, viz. (1) the grain amaranths and *Amaranthus hybridus* were found to group together and all the grain amaranths have evolved from the progenitor *A. hybridus* via at least two domestication event. (2) *A. quitensis* is its own species, a distinct group not a variant of *A. hybridus*. (3) *Amaranthus caudatus* L. and *Amaranthus hypochondriacus* L. appear to be closely related though both the species are geographically separated. A strong inbreeding coefficient was found in all the studied taxa. *Amaranthus hybridus* may consist of two widespread morphologically cryptic species or may consist of a single highly variant species from which all grain amaranths arose. The study favoured two hypotheses regarding the domestication of *A. hypochondriacus* and *A. caudatus:*

1. Single domestication hypothesis – in which one domestication event occurring in Meso-America or the Andes in *A. hybridus*, followed by geographical separation giving rise to *A. hypochondriacus* and *A. caudatus*.
2. A dual-lineage domestication hypothesis – in which a single *A. hybridus* lineage spanning Meso-America to the Andes was independently domesticated at least twice giving rise to *A. hypochondriacus* and *A. caudatus. Amaranthus cruentus* appears to have originated separately from second widespread *A. hybridus* lineage spanning across Guatemala and Central Mexico. This species appeared to be more diverse relative to its cultivated counterparts as evidenced by high allelic diversity and numerous private allele (Kietlinski et al. 2013). The 'hybridus species complex' of *Amaranthus* comprises a group of cultivated grain species and weedy species from the New World where taxonomic delimitation and systematic are poorly understood. The 'hybridus species complex' comprises widespread agricultural weed *A. hybridus*, South American endemic *A. quitensis* and three grain amaranths. Adhikary and Pratt (2015) established an interrelationship between the com-

ponent species of this complex studying the floral variation pattern within the species complex. Twenty-one pistillate and 12 staminate floral characters from 41 specimens affiliating to five species were analysed morphologically. Results indicated that the hybridus complex to be divided vertically into two morphologically distinct larger groups by *A. hybridus* each comprising different cultivated taxa. One group includes *A. cruentus*, *A. caudatus*, *A. quitensis* and one morphotype of *A. hybridus* and another group comprises *A. hypochondriacus* and a different morphotype of *A. hybridus*. The result though supported the concept of considering *A. hybridus* as a progenitor species, but closer affinity of *A. hybridus* with both *A. cruentus* and *A. caudatus* was left controversial. In the study staminate morphological variation appeared to be more taxonomically informative than assumed before.

Stetter et al. (2015) investigated the domestication events of *A. caudatus* and its relationship with the two wild relatives *A. hybridus* and *A. quitensis* applying genotyping by sequencing (GBS) to genotype on 119 amaranth accessions from the Andean region. The population genetic analysis based on 9485 SNPs revealed very little genetic differentiation between the two wild species, negating their separate identity, but a strong differentiation between wild and domesticated amaranth *A. caudatus*. Earlier separate identity of *A. quitensis* and *A. hybridus* was ignored by Coons (1978). The taxonomic differentiation between these two wild amaranths is resting on a very fragile morphological trait namely the shape of the tepals which is very prone to misidentification (Sauer 1967). The genome size and phenotypic variation in two domestication-related traits like seed size and seed coat colour were determined and compared between *A. caudatus* and its two wild relatives by Stetter et al. (2015). *Amaranthus caudatus* has a higher genetic diversity than its wild relatives, and about 10 % of accessions showed a strong admixture between the wild and cultivated species suggesting recent gene flow, though genome size and seed size did not appear to be significantly different between wild and domesticated amaranth. It was concluded that *A. caudatus* is an incompletely domesticated species, which may be due to lack of strong selection process or due to high level of gene flow from its sympatric wild relatives preventing the fixation of key domestication traits in *A. caudatus*.

In grain crops, grain size and seed coat colour are important traits for selection and supposed to play a key role in domestication of various plants (Hake and Ross-Ibarra 2015). Key domestication traits such as shape of inflorescence, seed shattering and seed size were rather similar between wild and cultivated amaranth. Domesticated amaranth shows morphological differences from wild amaranth having large and more compact inflorescence (Sauer 1967). Trait like white seed, surely a domestication-related trait, is predominant in cultivated amaranth though other seed coat colour may have been preferred. Genes for white seed coat colour were not fixed. Lack of knowledge about genetic basis of domestication trait, lack of strong domestication syndrome and fixation of putative domestication trait in spite of long cultivation period may be due to genetic constraints and ongoing gene flow between wild and domesticated amaranth. The genotyping of wild and cultivated amaranth accessions revealed a strong genetic differentiation between wild and cultivated amaranths and a high level of genetic differentiation within domesticated *A. caudatus* due to ongoing gene flow making it incompletely domesticated. The history of cultivated amaranths represents a multiregional, multiple and incomplete domestication process with frequent and ongoing gene flow from sympatric wild relatives showing similarity to the history of rice, apple or barley (Londo et al. 2006, Cornille et al. 2012, Poets et al. 2015). Experimental data were consistent with the concept of Kietlinski et al. (2013) who proposed a single domestication of *A. caudatus* and *A. hypochondriacus* in Central America followed by migration of *A. caudatus* to South America.

Weed and Herbicide Resistance

6

6.1 General

Weed management in agriculture has become very problematic and costly due to the increase in the development of resistance to common herbicides by the obnoxious weeds. More than 180 different weed species showed resistance and amaranths amount more than 5 % of the total biotypes. Eleven weedy species of amaranths showed resistance. There are different groups of herbicides and amaranths showed variable response to those. Among the 60 photosystem II inhibitor resistant angiosperms recorded, amaranths contribute nine species. More than 90 weeds developed resistance against ALS inhibitor; pigweeds are represented by eight resistant species. *Amaranthus tuberculatus* is one of the three species that have developed resistance against protoporphyrinogen oxidase (PPO) inhibitor. It is a troublesome weed that shows multiple resistances to different herbicides. It has evolved resistance to five different classes of herbicides. In midwestern agricultural field of the USA, it shows two distinct groups of populations – agricultural and nonagricultural – with varying degrees of resistance against ALS. It is possible to transfer resistance trait from weed to crop through weed-crop hybridisation.

6.2 Resistance to Different Types of Herbicides

Herbicide resistance in crops is generated through a few mechanisms like enhanced metabolism, sequestration, target site resistance, reduced uptake and overproduction of herbicide target site. Resistance against the mechanism of ten herbicides has been confirmed. The most widespread resistances developed are against photosystem II (PS II) inhibitors, photosystem I (PS I) inhibitors, acetolactate synthase (ALS) inhibitors, EPSP synthase inhibitors (e.g. glyphosate), protoporphyrinogen oxidase (PPP or PROTOX) inhibitor, acetyl-coa carboxylase (ACCase) inhibitors, mitotic inhibitor and auxinic inhibitor. In each case resistance is induced by multiple amino acid changes within herbicide-binding domain.

A worldwide catalogue of herbicide-resistant weeds (Heap 2008) includes more than 300 resistant biotypes. A biotype is a group of plants within a species with a distinct genetic variation. A herbiside- resistant biotype is a particular weed species with resistance to a particular herbiside or a group of herbisides with same site of action. Herbiside-resistance is an inherited ability of a weed or crop species to survive a herbiside application. This catalogue of herbicide-resistant

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weed includes more than 180 different weed species, and amaranth is considered as the leading member in the list comprising more than 5 % of the total resistant biotypes. Among weedy amaranths, herbicide resistance has been detected in 11 species (Heap 2010). The first report of herbicide resistance shown by *Amaranthus* species came up from North America; *Amaranthus hybridus* showed resistance against the triazine herbicide atrazine in 1970 (Ryan 1970). The biotypes of *Amaranthus tuberculatus* have been reported to show multiple resistances against three herbicide actions (ALS, PPO and PS II) (Patzoldt et al. 2005). *Amaranthus palmeri* and *Amaranthus rudis* that have shown resistance against herbicide glyphosate and ALS inhibitor, respectively, are creating problems in the weed management system in cultivation field of soybean, maize and cotton in the USA. *Amaranthus* species provide ample scope to study herbicide resistance mechanism and may be treated as models to examine the evolution of herbicide resistance across broad geographical region.

6.2.1 Resistance to Photosystem II Inhibitors

Resistance to photosystem (PS) II inhibitor (e.g. triazine herbicide) in *Senecio vulgaris* is often regarded as the first major report of evolved herbicide resistance in a weed (Ryan 1970). More than 60 weed species have been identified to resist PS II inhibitor, of which nine species belong to genus *Amaranthus*. Early research on triazine resistance in *A. hybridus* contributed much to the understanding of the fitness penalty associated with this resistance (Arhens and Stoller 1983; Ort et al. 1983) and also to the exploration of mechanism of resistance (Steinbank et al. 1981; Hirschberg and McIntosh 1983). Triazine resistance in *A. hybridus* occurred due to glycine for serine codon change in the *psbA* gene of triazine resistance. The identification of this mechanism was the first ever characterisation of evolved herbicide resistance at DNA level (Hirschberg and McIntosh 1983).

6.2.2 Resistance to Acetolactate Synthase (ALS) Inhibitors

More than 90 weed species are known to have evolved resistance to ALS inhibitor. Eight species of pigweeds showed confirmed resistance against ALS inhibitor. Target site resistance is the most common mechanism of resistance against ALS inhibitors, and it is indicative of why ALS target site mutation occurs so frequently. In a survey of herbicide resistance among Illinois waterhemp population, several were observed with varied response to imazethapyr and thifensulfuron, two acetolactate synthase (ALS)-inhibiting herbicides. Multiple ALS mutations were a determining factor for herbicide resistance in waterhemp (Patzoldt and Tranel 2007). Due to the importance of ALS-inhibiting herbicide and the frequency at which weed population have developed resistance in them against ALS inhibitor, a wealth of data has been generated (Guttieri et al. 1996; Duggleby and Pang 2000; Tranel and Wright 2002).

6.2.3 Resistance to Protoporphyrinogen Oxidase (PPO) Inhibitors

Resistance to herbicide that target the enzyme protoporphyrinogen oxidase has been documented only in three species (Heap 2008). The first species which showed this type of resistance was *A. tuberculatus* (Shoup et al. 2003). The resistance to Protox-inhibiting herbicide in an *A. tuberculatus* biotype from Illinois was due to DNA mutation caused by codon deletion in the gene encoding the mitochondrial Protox isomer (PPX2) (Patzoldt et al. 2006).

The general characteristic that contributed to the efficacy of *Amaranthus* species as weed in modern agriculture is their well-exemplified ability to evolve herbicide resistance. Weed amaranths contribute over 5 % of herbicide-resistant biotypes distributed worldwide and has evolved resistance to diverse herbicide like that which inhibit photosystem II (PS II), acetolactate synthase (ALS), protoporphyrinogen oxidase (PPO) and 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) (Heap 2010; Tranel and Trucco 2009). In some cases resistance is evolved against more than one of these herbicides within a single population or even a single plant, making the control of weed like *A. tuberculatus* a real problem (Patzoldt et al. 2005). Frequent occurrence of herbicide resistance in weedy amaranths indicates the possibility of occurrence of the same trait in cultivated amaranth and the feasibility of possible transfer of this resistant trait from weed to crop via interspecific hybridisation. Nevertheless, the only *Amaranthus* species that has developed resistance against PPO inhibitors is *A. tuberculatus* and against EPSPS inhibitors are *A. tuberculatus* and *A. palmeri*. Dioecious nature of *A. tuberculatus* undoubtedly contributed to its ability to develop multiple resistances. Gene flow via pollen movement from one resistant biotype to another effectively combines different resistance traits, although two self-pollinating monoecious species *A. powellii* and *A. retroflexus* have also evolved resistance against multiple herbicides (Heap 2008). Tranel et al. (2002) examined introgression of herbicide resistance allele of ALS from *A. tuberculatus* to *A. hybridus*, and they also observed that *A. tuberculatus* allele could not be introduced into *A. hybridus* with monoecious background. Considering monoecism and dioecism as the taxonomic distinguishing character for both the taxa, it appears that gene flow between these species is unidirectional. Available informations have raised several queries regarding efficient multiple herbicide resistance of *Amaranthus* species:

(a) Whether the *Amaranthus* species possess 'innate genetic properties' like transposable element that preconditions the rapid evolution of herbicide resistance
(b) Whether hybridisation among *Amaranthus* species is responsible for its innate ability to evolve resistance
(c) Whether insertion/deletion (which is common in *Amaranthus* species) are contributing factors to the evolution of herbicide resistance

These are some of the prime questions that require investigation. Pigweeds have already offered a stiff challenge towards herbicidal control on weed through resistance evolution. A large number of herbicide-resistant varieties of weed amaranths have evolved in different parts of the world (Table 6.1).

The evolution of invasiveness of weeds has been extensively studied in natural ecosystems. In spite of its great economic impact on crop productivity, little is known about the evolution of agricultural invasiveness of weed. Evaluation of the population structure of recently arisen weeds can be very informative about evolutionary trends to the invasion of agroecosystems. Waselkov and Olsen (2014) addressed several questions about the origin of the Native North American agricultural weed waterhemp (*Amaranthus tuberculatus*), which invaded corn and soy fields in the midwestern USA in the twentieth century. Agricultural invasion by native, wild plant species can follow different evolutionary directions from weeds related to domesticated plants, which has great implications in evolutionary biology and weed control. *Amaranthus tuberculatus* has evolved resistance to five different classes of herbicides: (1) photosystem II inhibitors, (2) acetolactate synthase (ALS) inhibitors, (3) protoporphyrinogen oxidase (PPO) inhibitors, (4) p-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors and (5) EPSP synthase inhibi-

Table 6.1 Herbicide-resistant *Amaranthus* species

Herbicide	Species	Year of documentation	Country where resistance documented
PS II inhibitors	*A. hybridus*	1972	USA, South Africa, France, Italy, Switzerland, Spain, Israel
	A. powellii	1977	USA, Canada, France, Switzerland, Czech Republic
	A. lividus	1978	Switzerland, France
	A. retroflexus	1980	USA, Canada, France, Germany, Switzerland, Bulgaria, Czech Republic, Spain, Poland, Greece, Italy, Serbia, China
	A. blitoides	1983	Israel, Spain
	A. albus	1987	Spain
	A. cruentus	1989	Spain
	A. palmeri	1993	USA
	A. tuberculatus	1994	USA, Canada
ALS inhibitor	*A. blitoides*	1991	Israel
	A. palmeri	1991	USA
	A. retroflexus	1991	Israel, USA, Canada, Italy
	A. hybridus	1992	USA
	A. lividus	1993	USA
	A. tuberculatus	1993	USA, Canada
	A. powellii	1996	USA, Canada
	A. quitensis	1996	Argentina, Bolivia
Glyphosate	*A. palmeri*	2005	USA
	A. tuberculatus	2005	USA
Protoporphyrinogen oxidase inhibitor	*A. tuberculatus*	2001	USA
Bipyridyliums	*A. lividus*	1990	Malaysia
Dinitroanilines	*A. palmeri*	1989	USA

Heap (2008)

tors, glyphosate. The species has extremely large agricultural populations. Its constantly evolving resistance makes it one of the weeds difficult to control in midwestern agricultural field of the USA. The waterhemp has two distinct groups of populations, agricultural and nonagricultural, with varying resistance potentials against ALS inhibitor. Eleven Ohio populations of *Amaranthus tuberculatus* from both agricultural and natural habitat in the riverbanks were screened to evaluate the concept that agriculturally adaptive ALS inhibitor resistance alleles are found in natural habitat. The presence of the same alleles in natural and agricultural waterhemp population suggests that gene flow is responsible for its presence in natural habitat. It may be possible that allele arose independently in natural population and persisted at low frequencies. On average, agricultural waterhemp populations showed higher ALS inhibitor resistance than did natural population. It is possible that the mutation enhanced the performance of the plants in the fluctuating natural environment of riverbank habitat. The variation in resistance level among the agricultural populations may be due to herbicide rotation practices that vary from year to year and difference in rotation schedule as well (Waselkov and Olsen 2014).

Distribution and Maintenance of Amaranth Germplasm Worldwide

7

7.1 General

Amaranths show a wide geographical distribution, evolution of landraces and domestication in widely spaced areas. To carry out research on genetic improvement of the crop, proper collection, maintenance and periodical evaluation of germplasm are a prerequisite. There are quite a few amaranth research centres and germplasm collections all over the world which are maintaining a working germplasm collection. The germplasm collections are maintained in at least 61 collection centres. Prominent among them are Rodale Research Center, Pennsylvania; USDA Plant Introduction Center at Ames, Iowa; National Bureau of Plant Genetic Resources (NBPGR), New Delhi; etc. Amaranth genome may serve as a model system to study weediness. A bacterial artificial chromosome (BAC) library was constructed from *A. hypochondriacus* containing about 37,000 clones and an anticipated genome coverage of more than tenfold. Microsatellite markers developed from *A. hypochondriacus* and *A. tuberculatus* would be valuable for more detailed study on phylogeny and breeding efforts. These markers were shown transferrable to other cultivated as well as weedy amaranth species. Besides, shotgun sequence data from *A. tuberculatus* and collection of recombinant lines (RIL) derived from initial weed-crop cross between *A. hypochondriacus* and *A. hybridus* can also be very useful for the development of amaranth genetic map.

7.2 Germplasm Collection Centres in the World

The amaranths are characterised with wide geographical distribution that has resulted in the evolution of many landraces in widely separated areas. The wide gene pool of amaranths is to be assessed and characterised for future development of the crop. This needs systematic collection and maintenance of germplasm worldwide. Amaranth germplasm is catalogued and stored in germplasm banks in 11 countries (Toll and van Slotten 1982). A descriptive taxonomic key for all the cultivated species of the genus *Amaranthus* was developed by Feine-Dudley (Grubben and van Slotten 1981). Since 1977, Rodale Research Center (RRC) Pennsylvania, USA, is maintaining a working germplasm collection. The 1400 accessions in the amaranth germplasm collection include representatives from 12 amaranth species. The collection includes germplasm from banks in other countries, material from germplasm collection trips and materials which have been donated by collaborating researchers. The germplasm is catalogued according to 'grain type'. The grain type categories may actually be a regrouping of the germplasm according to landrace. The grain type categories have proven to be useful to manage the huge amount of variability that exists within each species. Descriptions of all species and the grain types can be found in the RRC germplasm catalogue (Kauffman and Reider 1986). To meet the problem of genetic

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erosion, *Amaranthus* germplasm has been collected for ex situ conservation (Grubben and Van Sloten 1981). Field studies have been conducted by Kauffman during 1978 and 1979. In 1978 yields were tested on 23 of the newly selected lines. Most of the lines were selected from the Mexican lines. The yield levels ranged from 5.8 to 18.8 q/ha. In California Davis Jain et al. (1984) reported grain yield of some selection as high as 36 q/ha (National Research Council 1984). Jorge Mario and Bressani (1987) while testing eight grain amaranth selections reported a grain-yield range from 20.3 to 38.q/ha in Guatemala. The highest grain yields were recorded in 20-USA (Rodale Selection of *A. cruentus*) followed by 28-USA (Rodale Selection). In China and Mongolia, maximum grain yields have been reported to be 5500 kg/ha and 3400 kg/ha, respectively. Four amaranth cultivars have been registered in the USA – 'Montana 3' (MT-3), 'Montata 5' (MT– 5), 'Amont' and 'Plainsman' (Schulz-Schaffer et al. 1991; Baltensperger et al. 1992). Several lines have been developed by the RRC, Nu-World Amaranth and American Amaranth that have been widely distributed and evaluated but never registered. All registered cultivars were developed by the RRC; 'MT-3' was a selection from RRC 1041, 'MT-5' was selected from RRC 425 and 'Amont' was a selection from 'MT-3'. 'Plainsman' was a selection from the cross RRC 1024 x RRC 1004 and widely distributed and treated as 'K 343' prior to release. Plainsman has become the most widely grown amaranth cultivar in the USA due to its relatively high yield potential, lodging resistance, limited seed shattering, seed colour and maturity range. Yield variation was high with Plainsman ranging from 2500 kg/ha in Colorado in 1991 down to 220 kg/ha in Missouri in 1990, typically in the range of 700 kg/ha to 1700 kg/ha considering all the entries over the year and locations tested. Several cultivars have been developed through the world including Russian, 'Pastevny 1', 'Turkestan' and 'Ural', and South America's *A. cruentus* genotype 'Anden' (Kaul et al. 1996). None of these cultivars have been widely tested in the USA. The main cultivars in China are five RRC lines (Corke et al. 1997) especially RRC 1011 (Yue and Sun 1993), but later three new lines have been developed in China (Wu 1998). Three cultivar lines, namely, 'Vietmeyer', 'Oscar Blanco' and 'Alan Garcia', were released through selection in Peru (Summar et al. 1992). Covas (1991) developed five cultivars in Argentina. Bansal (1996) described several cultivars in India, where 'Plainsman' replaced 'Annapurna' as the top yielding line.

The germplasm collections are maintained in at least 61 collections (IPGRI 1999). The collections are gradually becoming more and more important because of their accessibility and documentation through the Internet. Most collections have less than 100 accessions except six collections which are maintaining quite a large number of accessions (Table 7.1). *Amaranthus* is well adjusted to *ex situ* conservation due to their small and long-lived seeds (Kigel 1994). Brenner and Widrlechinar (1998) described an efficient protocol for regenerating seeds of *Amaranthus* germplasm and maintenance of its genetic integrity in ex situ situation.

A majority of the accessions in the RRC germplasm collection were collected as mass selections or single plant selections of cultivated landraces of those species which are commonly grown for their light-coloured seed. Many selections have been made from the segregating accessions in an effort to create uniform lines. Seed from the amaranth accessions has been distributed to thousands of researchers and farmers around the world. The germplasm collection is the backbone of the varietal improvement programme which is aimed at developing agronomically acceptable lines using classical plant breeding and selection methods.

Germplasm characterisation is being conducted at a number of locations around the world. Extensive, well-documented amaranth germplasm characterisation has been conducted by organisations in India, Peru and Mexico. Since 1982, the staff at RRC has collaborated with researchers who are conducting observations on 14 selected grain amaranth accessions (representing grain types from the species *A. hypochondriacus* L., *A. cruentus* L., *A. caudatus* L. and *A. hybridus* L.) to collect information on their

Table 7.1 Six largest ex situ *Amaranthus* germplasm collections in the world

	Institute	No. of accessions	Year updated	References
1.	Institute of Crop Germplasm Resources (CAAS)Beijing, China	438	1996	IPGRI (1999)
2.	Universidad Nacional del Altiplano Puno, Peru	440	1990	IPGRI (1999)
3.	Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP)	495	1993	IPGRI (1999)
4.	Univ. Nacional San Antonio Abad del Cusco (UNSAAC/CICA)	740	1990	IPGRI (1999)
5.	National Bureau of Plant Genetic Resources	3000	1995	Joshi (1985)
6.	North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa, USA	3380	1999	USDA, ARS (1999)

performance at distinctly different climates and latitudes (Bressani et al. 1987; Senthong 1986; Gupta 1986; Duriyaprapan 1986; Espitia 1986). Information generated at each location serves as a starting point to help determine which sources of germplasm should be exploited to develop improved lines of grain amaranth for any given area. Similar observations have also been made to characterise germplasm which was collected throughout India (Joshi 1986). Populations of landraces which were collected from Mexico to Argentina have been observed for their genetic structure (Hauptli and Jain 1984).

Germplasm enhancement is being conducted at RRC since 1977 using recurrent single plant selection and mass selection with an intention to develop ideotypes which would meet the needs of modern agriculture.

The USDA Plant Introduction Center at Ames, Iowa, has been working to characterise germplasms and to determine the amount of outcrossing that has occurred. This Plant Introduction Center is of great significance in maintaining a large number of amaranth germplasm collected from all over the world and their distribution to promote amaranth research (Tables 7.2 and 7.3). USDA, Ames, Iowa, USA, also published an amaranth catalogue of 2783 collections with origin and grouped them under respective species of the genus *Amaranthus* (Brenner 1990).

In India initial efforts to study grain amaranths were made in the 1960s by the Division of Plant Introduction, Indian Agricultural Research Institute, New Delhi (now National Bureau of Plant Genetic Resources or NBPGR), which then held a small germplasm collection (less than 50) at Shimla Station, assembled from indigenous sources as well as from a few foreign countries, viz. Nepal and the USA. It was only in 1978 onwards when more efforts were directed towards germplasm collection and evaluation of the existing genetic resources to enrich these collections and to analyse and study the potential of this dual-purpose crop (Joshi and Rana 1991). A great deal of variability was observed, especially in inflorescence, leaf and stem colour; inflorescence size and shape; inflorescence and stem branching pattern; axillary inflorescence branching pattern; plant height, spiny/glabrous nature of the bracts; seed colour, size, weight and transparency; terminal inflorescence length and shape; and maturity. The collected amaranth germplasm includes nearly 2722 indigenous accessions and 293 exotic accessions. Now the total amaranth germplasm collection of Shimla Regional Station is 3081 comprising mainly of grain amaranth (Table 7.4). Apart from exchange of germplasm within India, exchange of seeds under physiosanity vigilance between India and other countries are being encouraged by NBPGR making exchange links with about 70 different countries. It is linked with several international institutes like IRRI, ICARDA, IITA, CIAT, AVRDC, CSIRO, USDA and IBPGR.

Table 7.2 Various accessions of *Amaranthus* maintained at Plant Introduction Center at Ames, Iowa

Sl. no.	*Amaranthus* species	Number of accessions
1	*Amaranthus* spp. NC7-amaranth	41
2	*Amaranthus albus* NC7-amaranth	7
3	*Amaranthus hybridus* NC7-amaranth	154
4	*Amaranthus blitum* NC7-amaranth	10
5	*Amaranthus dubius* NC7-amaranth	43
6	*Amaranthus crispus* NC7-amaranth	1
7	*Amaranthus greggii* NC7-amaranth	2
8	*Amaranthus palmeri* NC7-amaranth	15
9	*Amaranthus pumilus* NC7-amaranth	7
10	*Amaranthus torreyi* NC7-amaranth	1
11	*Amaranthus viridis* NC7-amaranth	18
12	*Amaranthus caudatus* NC7-amaranth	557
13	*Amaranthus cruentus* NC7-amaranth	365
14	*Amaranthus deflexus* NC7-amaranth	5
15	*Amaranthus hybridus* NC7-amaranth	191
16	*Amaranthus powellii* NC7-amaranth	18
17	*Amaranthus spinosus* NC7-amaranth	24
18	*Amaranthus tricolor* NC7-amaranth	182
19	*Amaranthus watsonii* NC7-amaranth	1
20	*Amaranthus wrightii* NC7-amaranth	2
21	*Amaranthus arenicola* NC7-amaranth	7
22	*Amaranthus asplundii* NC7-amaranth	1
23	*Amaranthus australis* NC7-amaranth	2
24	*Amaranthus blitoides* NC7-amaranth	7
25	*Amaranthus crassipes* NC7-amaranth	2
26	*Amaranthus muricatus* NC7-amaranth	1
27	*Amaranthus quitensis* NC7-amaranth	51
28	*Amaranthus acutilobus* NC7-amaranth	2
29	*Amaranthus cannabinus* NC7-amaranth	3
30	*Amaranthus fimbriatus* NC7-amaranth	4
31	*Amaranthus floridanus* NC7-amaranth	1
32	*Amaranthus graecizans* NC7-amaranth	14
33	*Amaranthus* retroflexus NC7-amaranth	24
34	*Amaranthus tenuifolius* NC7-amaranth	1
35	*Amaranthus tucsonensis* NC7-amaranth	1
36	*Amaranthus californicus* NC7-amaranth	1
37	*Amaranthus polygonoides* NC7-amaranth	1
38	*Amaranthus standleyanus* NC7-amaranth	2
39	*Amaranthus tuberculatus* NC7-amaranth	51
40	*Amaranthus acanthochiton* NC7-amaranth	2
41	*Amaranthus tamaulipensis* NC7-amaranth	1
42	*Amaranthus hypochondriacus* NC7-amaranth	1523

Table 7.3 Various accessions of *Amaranthus* available at Plant Introduction Center at Ames, Iowa for distribution

Sl. no.	*Amaranthus* species	Number of accessions
1	*Amaranthus albus* NC7-amaranth 7	7
2	*Amaranthus hybridus* NC7-amaranth 153	153
3	*Amaranthus blitum* NC7-amaranth 10	10
4	*Amaranthus dubius* NC7-amaranth 31	31
5	*Amaranthus crispus* NC7-amaranth 1	1
6	*Amaranthus greggii* NC7-amaranth 2	2
7	*Amaranthus palmeri* NC7-amaranth 15	15
8	*Amaranthus viridis* NC7-amaranth 17	17
9	*Amaranthus caudatus* NC7-amaranth 543	543
10	*Amaranthus cruentus* NC7-amaranth 335	335
11	*Amaranthus deflexus* NC7-amaranth 5	5
12	*Amaranthus hybridus* NC7-amaranth 184	184
13	*Amaranthus powellii* NC7-amaranth 16	16
14	*Amaranthus spinosus* NC7-amaranth 23	23

Table 7.3 (continued)

Sl. no.	*Amaranthus* species	Number of accessions
15	*Amaranthus tricolor* NC7-amaranth 181	181
16	*Amaranthus watsonii* NC7-amaranth 1	1
17	*Amaranthus wrightii* NC7-amaranth 2	2
18	*Amaranthus arenicola* NC7-amaranth 6	6
19	*Amaranthus asplundii* NC7-amaranth 1	1
20	*Amaranthus australis* NC7-amaranth 2	2
21	*Amaranthus blitoides* NC7-amaranth 7	7
22	*Amaranthus crassipes* NC7-amaranth 2	2
23	*Amaranthus muricatus* NC7-amaranth 1	1
24	*Amaranthus quitensis* NC7-amaranth 49	49
25	*Amaranthus acutilobus* NC7-amaranth 2	2
26	*Amaranthus cannabinus* NC7-amaranth 2	2
27	*Amaranthus fimbriatus* NC7-amaranth 4	4
28	*Amaranthus floridanus* NC7-amaranth 1	1
29	*Amaranthus graecizans* NC7-amaranth 14	14
30	*Amaranthus retroflexus* NC7-amaranth 24	24
31	*Amaranthus tucsonensis* NC7-amaranth 1	1
32	*Amaranthus californicus* NC7-amaranth 1	1
33	*Amaranthus polygonoides* NC7-amaranth 1	1
34	*Amaranthus standleyanus* NC7-amaranth 2	2
35	*Amaranthus tuberculatus* NC7-amaranth 50	50
36	*Amaranthus acanthochiton* NC7-amaranth 2	2
37	*Amaranthus tamaulipensis* NC7-amaranth 1	1
38	*Amaranthus hypochondriacus* NC7-amaranth	1497

Table 7.4 Germplasm of amaranths maintained at Regional Station Shimla, NBPGR

Sl. no.	Species of *Amaranthus*	No. of accessions
1.	*Amaranthus hypochondriacus*	2452
2.	*A. cruentus*	556
3.	*A caudatus*	27
4.	*A. edulis*	08
5.	*A. dubius*	10
6.	*A. hybridus*	09
7.	*A. viridis*	05
8.	*A. retroflexus*	07
9.	*A. lividus*	03
10.	*A. tricolor*	04

Apart from NBPGR, Regional Station Shimla, collections have also been made by the Vivekananda Parvatiya Krishi Anusandhan Shala (VPKAS) Almora, GB Pant Agricultural University, Ranichauri, Tehri Garhwal, North Eastern Hill Complex, Shillong, Maharashtra and Gujarat State in India. Collection activities have also been reported for grain amaranth germplasm including IBPGR (1984)-supported explorations in Nepal, Bhutan, Thailand, Indonesia, Ethiopia, Zambia, Nigeria, Kenya, Argentina, Bolivia, Ecuador, Mexico, Guatemala and Peru. The global germplasm collections have been stored in the National Seed Storage Laboratory, Fort Collins, Colorado, USA, where IBPGR has designated NBPGR as a regional base centre for amaranth germplasm collection in view of pioneering work done on this crop by this station. The evaluation of indigenous and exotic germplasm collections of grain amaranths over several years led to the identification of strains/accessions which offer good opportunities for its utilisation and improvement (Annual Report 1981 to 1988, NBPGR, Regional Station, Phagli, Shimla). These have been multiplied and maintained sepa-

rately for distribution to the persons involved in crop improvement and those who are interested to take up inheritance studies. The most promising genotypes in indigenous and exotic material of interest to breeders include IC-38541, IC-38577, EC-170304 and EC-169626 (dwarf types, 68–81 cm); IC-38137, EC-38323 and EC-151544 (high number of spikelets, 65–95); IC-38052, IC-38508 and EC-157415 (high inflorescence length, 78–95 cm); IC-5626, IC-7934 and IC-38 l36 (hard threshability); IC-38133, EC-157413 andBDJ86–329 (early flowering 40–52 days); IC-38133, IC-38422 and EC-1574 l7 (early maturing 100–110 days); IC-38l3l, IC-386 l1, IC-38665 and BDJ-86–259 (bold seeded 1000 grain weight more than 1 gramme); and IC-38269, IC-38280, IC-42258–1, VL-21 and BDJ86–129 (high grain yield per plant). A very high yielding variety of grain amaranth *A. hypochondriacus* named 'Annapurna' was developed in the National Bureau of Plant Genetic Resources (NBPGR) Regional Station, Shimla, through screening germplasms of 2700 collections and multilocation trials after a continuous research of 14 years (Joshi et al. 1983). It has given an average grain yield of 22.3 q/ha. and 34 q/ha of seed yield in Shimla condition. The variety has few distinguishing features like tall habit, medium or late maturing, dark-green broad leaves, yellowish-green inflorescence with long terminal and lateral spikelet, about 74 cm long and creamish-white seed with 14.5 % protein content. The popping quality of the seeds is excellent, about six times of the seed size. The other significant varieties of grain amaranth like R 104, 20 USA, Jumla, VL-21 and S.K. Nagar have also been developed and employed in cultivation in different parts of the world. The variety 'Annapurna' has a wide adaptability and can be grown in rain-fed land in the hills as well as in the drought-prone areas and also under Himalayan watershed. It has given 68.9 % higher increase in grain yield over VL-21, another promising selection from Almora, Uttarakhand. All India Coordinated Research Project has recommended it as a national check (Joshi and Rana 1991). Nine promising varieties selected from landraces of grain amaranths were evaluated by repeated trials at Shimla and Almora during 1986. The highest grain yield (34.8 q/ha) was recorded for 'Annapurna' followed by VL-21 (32.5 q/ha) and IC-42290–17 (30.9 q/ha). Multilocation trials of 14 promising varieties were conducted at eight centres during 1988–1989. The varieties that appeared to be significant were S.K. Nagar, 'Annapurna', 'Akola local', *A. edulis* (ex Taiwan), IC-5564, etc. with varying yield amounts at different locations.

Table 7.5a Amaranth germplasm collection at Tamil Nadu Agricultural University, Coimbatore

Sl. no	Species of *Amaranthus*	No. of accessions maintained
1	*Amaranthus dubius*	18
2	*Amaranthus tricolor*	22
3	*Amaranthus tristis*	6
4	*Amaranthus hypochondriacus*	14
5	*Amaranthus cruentus*	19
6	*Amaranthus paniculatus*	18
	Total	97

In South India, Tamil Nadu Agricultural University, Coimbatore, is a prominent centre of amaranth germplasm collections, specially vegetable amaranths (Tables 7.5a and 7.5b), which released few improved varieties of vegetable amaranths.

7.3 Genome Resource Development of *Amaranthus*

Amaranth may serve as one of the best model systems of genome investigation of weediness (Basu et al. 2004; Chao et al. 2005). Amaranth possesses several characteristics which made them a desirable model system:

1. Most species are functional diploid having n = 16 or 17 (exception *A. dubius* is a polyploid with 2n = 64). *Amaranthus* species have detectable genome size 3 to 4 times that of model plant *Arabidopsis thaliana.*
2. *Amaranthus* species are generally easy to culture and manipulate under greenhouse or other experimental condition.

Table 7.5b Few new cultivars/varieties of vegetable amaranths were released from vegetable research station, TNAU

Sl. no	*Amaranthus* species	No. of accessions at VRS, Palur	Remarks
1.	*Amaranthus polygonoides*	8	Called Sirukeerai in Tamil
			A variety named PLR1 was released in 2013 from this university
2.	*Amaranthus tristis*	2	Called Araikeerai in Tamil
			A variety named CO3 was released in 2013 from this university
3.	*Amaranthus tricolor*	7	Called Mulaikeerai/Thandukeerai in Tamil
			Varieties: CO2 and CO5

3. As a typical weed, *Amaranthus* spp. exhibit plasticity in adaptability in diverse environmental condition.
4. The monoecious species are readily self-pollinated but outcross, and F1 hybrids are easily obtainable if selectable (herbicide resistance), detectable or scorable (pigmentation) markers are present in one of the parents.
5. Production of a large number of small seeds.

The first genome-based resource derived specifically from *Amaranthus* species is now available. A bacterial artificial chromosome (BAC) library was constructed from *Amaranthus hypochondriacus* (Maughan et al. 2008). It can be used as a tool for isolation and identification of the full-length gene sequence. This library contains about 37,000 clones and a presumed genome coverage greater than ten-fold. Thus any specific DNA sequence of *A. hypochondriacus* has greater than 99% chance of being represented in this library. A full-length genome sequence for desirable target site of the gene ALS and PPX2 can be obtained. Due to the sequence similarity among the *Amaranthus* species, the BAC library from *A. hypochondriacus* can be utilised for genome-based investigation of other weedy species as for example, transferability between *A. hypochondriacus* and other weed species. The BAC library can be used to generate a physical map of the *Amaranthus* genome, which ultimately can enable map-based cloning of *Amaranthus* genes of interest (Maughan et al. 2008).

The second genome resources available are a set of microsatellite marker. The microsatellite markers are one of the most robust and informative markers to study genetic variability. The microsatellite markers will be of great value to investigate queries related to gene flow, evolution and hybridisation within and among *Amaranthus* weeds. The microsatellite markers can also be used to construct genetic linkage map from mapping populations and for multilocus genome scanning to identify genetic target of selection (Smith and Haigh 1974). Microsatellite markers have been developed from *A. hypochondriacus* (Lee et al. 2008; Mallory et al. 2008), and markers were demonstrated to be transferable to the other cultivated as well as weedy amaranth species. Nearly 400 unique microsatellite markers were obtained primarily from microsatellite-enriched libraries but also from BAC-end sequence data. About 180 of these proved polymorphic among three grain amaranths. Many of these markers are transferable to the dioecious amaranth species as well. A preliminary phylogenetic analysis using some of these markers placed *A. hybridus* within multiple grain amaranth clades, suggesting multiple domestication events from *A. hybridus* (Mallory et al. 2008). Additional microsatellite markers were obtained recently from *A. tuberculatus* (Lee et al. 2009); collectively these microsatellite markers will be valuable for more detailed phylogenetic studies and breeding effects.

The third genome resource obtained recently is shotgun sequence data from *A. tuberculatus* (Lee et al. 2009). Random sequencing was done using massively parallel pyro-sequencing technology (Margulies et al. 2005). From a single pilot sequencing run, approximately 160,000 sequencing reads were obtained within an aver-

age read length of about 270 nucleotides, yielding a total of about 43 million nucleotides of *A. tuberculatus* sequence data. The data sheet included nearly a complete sequence of the chloroplast genome and partial sequence of most currently known herbicide target-site genes.

The fourth resource is a collection of recombinant inbreed lines (RILs) derived from an initial crop-wild cross between *A. hypochondriacus* and *A. hybridus*, selected based on herbicide resistance. They can be the useful source to provide ideal populations for development of an amaranth genetic map.

Breeding of Amaranths

8

8.1 General

Amaranths are characterised with remarkable germplasm diversity, adaptability to different growing conditions and unique matting behaviour ranging from obligate outcrossing (dioecious species) to greater outcrossing to greater self-pollination. The breeding mechanism in amaranths is variable due to variability and versatility of inflorescence, ratio and distribution of male and pistillate flowers in inflorescence. Breeding work in amaranths is to be adopted giving priority to traits like non-shattering seed, reduced plant height, high yield, increased seed size, synchronised drydown of plant, taste and nutritive value, improved pest resistance/or tolerance, low content of anti-nutrient factors, etc. The genetic improvement of grain amaranths has been done so far mostly applying conventional selection methods from local collections or landraces, and new varieties or improved lines are released. In amaranth breeding, hybridisation has been proved as the most effective breeding programme to produce new improved variant with useful trait. Naturally occurring hybrids between *A. caudatus* and *A. cruentus* and *A. hybridus* were identified by intermediate characters. One of the most popular and widely utilised grain amaranth varieties in the USA, *A. hypochondriacus* var. *Plainsman,* was obtained through a cross with *A. hybridus* accession from Pakistan having a feature of earliness. Interspecific hybridisation techniques have been employed to study the transfer of feature like herbicide resistance from a weed species to another weed species of amaranth. The crossability barrier between grain amaranths and their weedy relatives are very fragile showing variable frequency of cultivar-weed outcrossing. The use of interspecific amaranth hybrids appears to be promising to increase biomass productivity. In the case of vegetable amaranths used as parents, heterosis from interspecific hybridisation may double to quadruple the biomass of the parents. Crosses between grain species and their weedy relatives may yield sterile hybrids but with increased biomass. Grain yield heterosis has been estimated in *A. hypochondriacus*. Male sterility found only in *A. hypochondriacus* could be used to simplify hybridisation. High heterozygosity and low heritability in some traits are some of the problematic areas which breeders face in amaranth breeding that are to be solved. Wide range of genetic variability in cultivated landraces of some grain species offers much scope for its improvement through selection. Molecular markers are very useful for diversity analysis to distinguish genetically similar or distinct accessions or landraces. Marker-guided selection method can assist to select individual carrying molecular markers associated with the trait of interest. Mutation breeding has a great scope in amaranth to induce variability using radiation. Drought tolerance in *A. tricolor* and increased yield potential in *A. caudatus* were induced through gamma radiation. Three mutant varieties have been developed

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and registered in MVD, viz. centenario in Peru, New Asutake in Japan and Sterk in Russian Federation. Polyploidy was induced in *A. caudatus, A. tricolor, A. hypochondriacus* and *A. edulis* by colchicine treatment with positive effect. Tetraploid *A. caudatus* had 60 % more protein, more lysine and threonine than diploid. Polyploidy increased grain size and weight not decreasing the fertility or nutritive value. Amphidiploidy was induced in fertile F_1 hybrids between *A. caudatus* and *A. retroflexus* which were perfectly fertile and phenotypically alike. In plant breeding, genetic engineering involves artificial incorporation of foreign genetic materials followed by selection. *Agrobacterium*-mediated transformation protocol in *A. tricolor* and *A. hypochondriacus* has been standardised. This achievement made it possible to reduce the antinutrient oxalate level in vegetable amaranths through the insertion of cDNA of oxalate decarboxylase gene. The insertion of oxalate decarboxylase gene in tomato has been possible through *Agrobacterium*-mediated transformation. A storage protein gene *AmM1* isolated from *A. hypochondriacus* can be introduced in any other crop through genetic transformation as has been done in potato and wheat. Plant tissue culture can be very useful though not utilised so much in amaranth breeding. Variability in germplasms can be used for screening cell lines in search of plant more tolerant to stress, a potential source of herbicide resistance. Such traits can be introduced into grain and vegetable forms via protoplast fusion.

8.2 Breeding Behaviour in Amaranths

Amaranths have been subjected to a little domestication pressure as compared to major crops. It is notable for its remarkable germplasm diversity and adaptability to different growing conditions. Several characteristics are key attributes for success in the agronomic use of amaranths. According to Kauffman (1982), varieties of grain amaranths should have synchronised drydown of the entire plant at maturity. The characteristics like reduced plant height, reduced branching and non-shattering seeds facilitate easy grain harvesting. For vegetable amaranths branching is desirable. Vegetable cultivars with more branching are suitable for multiple cutting. This produce more total biomass yield compared to unicut cultivars (Sreelathakumary and Peter 1993).

In most of the species of *Amaranthus* investigated so far, nearly 40 have a diploid number of chromosome equal to 32 or 34, and the basic chromosome number is 16 or 17 (Madhusoodanan and Pal 1981). There are few tetraploid species like *A. caudatus* and *A. dubius* having basic chromosome number either 32 or 34. Natural polyploids occur in wild amaranths; *Amaranthus dubius* is a naturally occurring tetraploid. Induced polyploid forms of *A. dubius* and grain amaranth *A. hypochondriacus* have been obtained through colchicine treatment of the seeds and seedlings.

Broadly speaking, the genus *Amaranthus* shows three types of mating system, viz. (a) obligate outcrossing in dioecious species, (b) relatively greater outcrossing in monoecious member of sect. *Amaranthus* and (c) relatively greater self-pollination in members of sect. *Blitopsis*. Dioecious species are confined to a small area in North America (Sauer 1955, 1957), though sporadic appearance has been reported from time to time (Aellen 1961; Brenan 1961; Thakur 1964). The narrow geographic range of the dioecious species can be attributed to the fact that, for any long-distance dispersal, at least two propagules (male and female) are necessary before a particular species can successfully establish and colonise the new area. The second reason may be that few plants which supposed to have escaped their native place are often involved in interspecific hybridisation with monoecious amaranths and lead to the totally sterile hybrids. These perhaps were the reasons for reduction of their reproductive potential and ultimate elimination.

The monoecious species of *Amaranthus* are self-compatible. They are nearly cosmopolitan species, often weedy and seem to have achieved a significant balance between fitness and long-range flexibility. The breeding mechanism of *Amaranthus* is complex and variable due to

variability and versatility of inflorescence. The basic units of the inflorescence are little dichasial cymes, usually called glomerules, each ordinarily consisting of an initial male or staminate flower and an indefinite number of female or pistillate flowers. The glomerules are crowded on a leafless axis to form complex inflorescence, technically thyrses, which are generally called spikes. A completely developed glomerule may have as many as 250 flowers, while completely pollinated flowers are represented in lesser number. In all the grain species, each flower is subtended by a sharp-pointed bract. The perianth consists of five free 'tepals'; the male flowers characteristically have five stamens, and the female flowers have a single circumscissile utricle.

The main axis of the inflorescence is usually branched. The length and number of these branches and their angle with the main axis determine the shape of the inflorescence. The clusters of individual flower develop along the axis in an alternate fashion. The first or primary branch of inflorescence is terminated by the first flower. From the base of the primary branch, two branches arise, which are terminated by the second and the third flowers. At its base the next two flowers arise and the process continues until all the available space is occupied. The monoecious species exhibit two types of arrangements of the staminate and pistillate flowers. These types are important because of their different breeding behaviour. In the first type, the first flower of each flower cluster is a staminate and all the secondary ones are pistillate. There is only one staminate flower in each flower cluster of the inflorescence and that withers soon after shedding pollen. All the species, except *A. spinosus*, belong to this group. In the second type, all the pistillate flowers develop only in the axis of the branches and at the base of the terminal inflorescence, while the clusters of staminate flowers are borne terminally on the main axis and lateral branches. The species *A. spinosus* belongs to this type.

Species included in the sect. *Blitopsis* (the vegetable amaranths) have a number of staminate flowers per glomerule and small axillary inflorescence, resulting to predominant self-pollination of these species (Pal and Khoshoo 1973a, b). On the other hand, the single staminate flower per glomerule and big inflorescence should lead to more cross-pollination in the sect. *Amaranthus* (the grain amaranths). However, grain amaranths are also basically self-pollinated with 0–34.9 % cross-pollination which may be helped by insect pollinators (Mohideen and Irulappan 1993). The main inflorescence in sect. *Amaranthus* is a dense spike or panicle, which is usually profusely branched. The panicles or spikes may be either drooping (*A. caudatus*), semidrooping (*A. spinosus, A. cruentus,* etc.) or erect (*A. hypochondriacus* and *A. edulis*). The growth of the terminal inflorescence and its branches is indeterminate (racemose), and thus, it may reach a length of one metre or even more. However, *A. edulis* is the only exception in which the inflorescence is determinate due to the presence of a terminal polymerous male flower, and therefore, the elongation of the inflorescence in this species is due to intercalary growth of the axis. Individual flower clusters or glomerules develop alternately along the axis of the main inflorescence. In sect. *Amaranthus* glomerules develop chiefly in compact fashion on the terminal inflorescences and are rather feebly developed in the axils of the branches. In the *sect. Blitopsis,* due to the absence of prominent terminal inflorescence, axillary glomerules are well developed and resemble glomerules in the terminal inflorescence of sect. *Amaranthus*. The growth pattern of the individual dichasia, as described above, is common to all species irrespective of the sex. As expected in dioecious species, all the glomerules on a plant have the flowers of the same sex. The breeding pattern in grain amaranths varies from high rates of selfing (over 90 %) to mixed mating with as much as 30 % outcrossing (Jain et al. 1982). Outcrossing rates vary significantly with genotype and environmental factors between years. A bidirectional selection experiment based on male/female flower ratio per plant suggested that breeding system is under genetic control, and thus, it can be modified to suit breeding procedures for either inbreeding or outbreeding species. Kauffman (1979) while studying the floral morphology reported that each head of amaranth consists of hundreds of floret arranged in a

panicle. Each floret consists of three to six flowers with one staminate (male) flower surrounded by several pistillate (female) flowers. Occasionally, a floret consists of only female flowers. Anthesis begins when the stigmas of the female flowers exert. Available pollen does not come from the male flower in the same floret but from the staminate flowers of other florets. Due to the fact that flowers of both the sexes are located on the same inflorescence, the potential for selfing is increased. So breeding behaviour in amaranth, featured with variable sex ratio within glomerules and inflorescence as a whole, and variable outcrossing rate attributed to bidirectional selection in amaranth breeding. Such unique genetic features in breeding system have great impact on the evolution of amaranth species and domestication of landraces.

The outcrossing rate was found to be negatively correlated with the ratio of staminate to pistillate flowers within an inflorescence under strong environmental influence (Hauptli and Jain 1985). Interspecific hybrid is formed as a result of cross-pollination of two different species. Natural interspecific hybridisation occurs frequently in amaranths, eight naturally occurring interspecific hybrids have been reported in vegetable amaranth species, and they show varying degrees of sterility. Interspecific hybrid of *A. lividus* and *A. tricolor* showed an even higher degree of hybridisation defects, with about 90 % of pollen sterility, because of the occurrence of two or more interchanges of several chromosomes and subsequent meiotic abnormalities (Sreelathakumary and Peter 1993). On the other hand, interspecific hybridisations between grain amaranths and vegetable amaranths often produce hybrids with malformation, high pollen sterility and chromosome aberrations, suggesting the presence of a great genetic barrier between species included in the sections *Amaranthus* and *Blitopsis* (Mohideen and Irulappan 1993). The gene pool of *Amaranthus* provides an excellent source of materials for geneticists to work with. Amaranths are predominantly self-pollinating crop, with varying outcrossing rate (Hauptli and Jain 1985). By growing amaranth in isolation, it is possible to control the outcrossing rate and to raise true-to-type lines from segregating breeding lines involving only a few generations of selection.

The cultivated grain amaranths are monoecious. The basic emasculation and pollination techniques for breeding amaranths have been described (Murray 1938) and refined (Kauffman 1981a, b). Later concrete programmes are being followed at the University of California-Davis to identify genetic traits to be emphasised for improving grain amaranths (Jain et al. 1984). Efficacy of different breeding strategies has been determined and evaluated (Kulakow and Jain 1987; Ayiecho 1986). Applicability of additional techniques has been evaluated keeping in view the manipulation of plant height and increase in grain yields (Vaidya and Jain 1987).

Research programme carried out at the University of California-Davis and other locations related to the development of hybrids has enriched the breeding work by the identification of gene markers for the useful traits like time of flowering, determinate vs. indeterminate growth pattern, plant and seed pigment patterns and leaf characteristics (Kulakow et al. 1985; Kulakow and Jain 1985; Kulakow 1987). An additional marker like bract length has been identified in breeding trials at RRC. Okuno and Sakaguchi (1982) have identified the perisperm and grain starch of grain amaranths as potential gene markers. Markers are useful to trace and reject self-pollinated plants in the F_1 generation and also to allow breeders to avoid the complex process of emasculation in crossing event. The identification of male-sterile lines in *A. hypochondriacus* has further eased the way for getting hybrids as additional hybridisation techniques (Peters and Jain 1985; Peters and Jain 1987; Gudu and Gupta 1988a).

In India efforts have been devoted to learn more about traits which govern yield potential including harvest index, weight per 1000 seeds and yield per plant (Pandey 1984b). Investigations have also been directed to identify the inheritance pattern of trait that governs nutritional characters of grain. The factors regulating starch characteristic of grain were explored in Japan (Okuno 1985;

Konishi et al. 1985). In India also the inheritance pattern of grain protein percentage has been investigated (Pandey and Pal 1985).

A breeding programme was stated in 1978, in Rodale Research Centre (RRC), USA, to raise improved plant types for commercial production in the USA and Canada. A series of improved breeding lines (F_6 generation and beyond) was generated and grown at experimental station and commercial farm in the USA, applying classical breeding methodologies. The lines were included and distributed as a part of 'K-series'. One of such lines named 'K-343' is a derivation of crossing between white-seeded *A. hypochondriacus* from Mexico and a black-seeded weed *A. hybridus* from Pakistan. This single accession was widely utilised in the Great Plains of the USA in 1988 and grown on commercial scale. It is characterised with features like a short unbranched plant habit at high plant densities and slightly early maturity, as compared to 'MT-3' and other selected Mexican landraces which gained popularity among the farmers.

Scientists at RRC have identified a number of easy to isolate traits in order to proceed with the genetic improvement programme, which include the following:

- Reduced stature of plant (1.0–1.5 m)
- Flowering above the leaf canopy
- Lack of branching
- White or gold seed-coat colour
- Maturity between 100 and 120 days
- Higher yield due to traits like a large flower head with a high ratio of pistillate flowers that set seed

However, a number of other traits still need to be improved:

- Seed size
- Tolerance to insects and diseases
- Seedling vigour
- Reduced seed shattering
- Resistance to lodging
- Synchronous drydown of the plant and seedhead
- Easy threshability
- High protein content
- Functional attributes of the grain

Montana Agricultural Experiment Station first released an improved line of grain amaranth named 'MT-3' (Cramer 1988) selected from segregating accessions of *A. cruentus* L. originally collected in Mexico. Similar programmes were carried out in Mexico, Peru and Kenya. In Peru, the University of Cuzco has released three cultivars of *A. caudatus* L. named as 'Oscar Blanco', 'Noel Vietmeyer' and 'Alan Garcia'. These three cultivars are now being grown on commercial scale by the farmers in Peru (Sumar K., L. personal communication, 1988). A cultivar of *A. hypochondriacus* L., named 'Annapurna', has been released by NBPGR in India.

The concentration on only one source of germplasm is not helpful for improvement of the crop. Additional germplasm resources are to be explored and evaluated to initiate and expand applied breeding programmes for expected increase in grain amaranth production. The seed certification process initiated in 1988 will be helpful to address the need for sources of improved quality seed of grain amaranth.

The future improvement of amaranth is primarily dependent on the appropriate selection of genotypes from the existing germplasm collections. We have to explore unusual and minor sources of germplasm also to find traits such as early maturity and shorter plant stature. A dark-seeded accession ('African' grain type) of *A. cruentus* from West Africa, having a feature of greater branching, was utilised as a source for early maturity. A source for short plant stature trait was found among a few highly branched, shattering, dark-seeded *A. hybridus* L. ('Prima' grain type) accessions from Asia.

There are some traits such as plant height, days to reach maturity, plant architecture and drydown which are affected by environmental influence. For example, the Nepal grain type (accessions of *A. hypochondriacus* L.) when grown in a semiarid condition in Kenya produced short plants (<1 m), with multiple flowering stems that matured in 60 days with a high harvest index. When the same accession was grown in

Pennsylvania, it produced tall plants (>2 m) with a single stem that matured in >160 days and showed a low harvest index.

Information about incompatibility barriers preventing interspecific hybridisation are to be collected. Few barriers seem to exist between crosses of *A. cruentus L., A. hypochondriacus,* L. and *A. hybridus* L. However, crosses between *A. caudatus* L. and any of the above species often result in nonviable progeny (Pal and Khoshoo 1972).

8.3 Objectives in Amaranth Breeding

A systematic and comprehensive breeding work for improvement of amaranths is yet to be adopted. For long-term genetic improvement, few experimental approaches are essential. Experimental approaches and breeding objectives are quite different in vegetable and grain amaranths. The major objectives in improving cultivars of grain amaranths are to raise yield, increase pest resistance and improve harvestability. The modern cultivars tend to lodge, shatter and often mature early or late. Kauffman (1992) working at Rodale Research Centre (RRC), Pennsylvania, identified several useful breeding traits like increased seed size, synchronised drydown of plant and seedhead, resistance to seed shattering, improved pest resistance/tolerance and increased seed protein and functional traits. He also subsequently released several lines in the 1980s. Williams and Brenner (1995) emphasised three internationally recognised breeding objectives for grain amaranths, viz. reduced plant height and high yield, enhanced food quality and non-shattering of seed. A concise list of goal or objectives which should be considered in breeding programme of amaranth is mentioned below:

1. *Harvestability of grain*: The prime aspects which are associated with the proper and adequate harvest of grains include seed shattering, lodging, timing of maturity and uniformity of maturity and drydown of plant at seed maturity. The aspects with lesser significance are reduction of leafiness in the grain head and reduced plant height.

In the cultivation of grain amaranth, seed shattering is one of the prime reasons for significant loss in commercial grain production (Fitterer et al. 1996). Amaranth seeds are prone to shatter. Each amaranth seed is enclosed in a separate papery utricle. The grain amaranths have circumscissile utricle with a seam or abscission zone along the equator that opens at maturity. At maturity when utricles open, the papery utricle cap or lid gets separated along the abscission zone and fall off causing removal of seeds. Most of the seeds get trapped within the compact inflorescence. However when the grain heads are combined or bulk harvested, the brief shaking causes additional seed loss. Plant breeders could reduce shattering by developing cultivars that have indehiscent utricle cap lacking abscission zone and therefore do not separate from the lower part of the utricle (Brenner and Hauptli 1990; Joshi and Rana 1991). Taxonomists including Sauer (1967) have observed that some populations of *A. powellii* S. Watson have non-circumscissile utricles. *A. powellii* is a wild and weedy species closely related to the grain amaranths. Non-circumscissile utricles have also been observed infrequently in cultivated amaranths (Hauptli et al. 1980; Joshi 1981a, b), and few such populations are also indicated in the GRIN database (USDA-ARS 2001). Jain et al. (1984) used the symbol *Dh* and *dh* in *A. hypochondriacus*, with dh representing a recessive non-circumscissile allele and *Dh* representing the dominant circumscissile allele. They noted that the trait has potential for use in breeding programmes. The non-shattering trait from *A. powellii* (Pl 572261) has been possible to transfer to both *A. cruentus* and *A. hypochondriacus* applying traditional breeding procedures (CAD 2009). Three interspecific hybrid populations of grain amaranth with non-shattering (seed retaining) utricles, namely, DB 92226, DB 9350 and DB 98246, were developed at Agronomy Department, Iowa State University of the North Central Regional Plant Introduction Station (NCRPIS). These populations showed little or no abscission at the equator of the utricle or beneath. The first two are intended

to be crossed with standard cultivars and appeared as a source of shattering resistance in newly developed cultivars. The cultivar DB 98246 was developed with an intention of biomass production. The populations were released by Iowa State University during 1999 and 2000.

Lodging causes the whole plant to lean or fall over. This may or may not be related to head size. The RRC line that has been shown as the highest lodging resistant is D136 that may partially be due to its later maturity (Myers 1996).

Timing of seed maturity is an important trait. Inflorescences of the grain amaranths are highly branched. Flowering progressively proceeds from bottom to the top of the inflorescence so as the maturity of seeds. As a result seeds reach maturity at different times on a single plant. Adjacent plants in the field may also differ in maturity. Flowering over an extended period of time has an adverse effect on harvestability. However breeding for this trait should avoid developing amaranth cultivar dependent on timely rain during a narrow flowering span.

Amaranth grain matures much earlier and the plant dries up quite late. If the inflorescence heads are allowed to remain till the plants dry, heavy shattering can lead to grain loss. Vegetative drydown in relation to seed maturity affects harvest ability. Delay in drydown exacerbates lodging and seed shattering. Even after leaf drop, stems and heads may still contain too much moisture for efficient threshing. Care must be taken to avoid loss of stem strength for types that dry down too quickly.

Amaranth breeding lines lack uniformity for characters such as plant height and grain-head size. The height of individual plants in a field can be quite varied. Some plants that are nearly of full height have much smaller seedhead and thinner stem, while others usually have larger seedheads. Cultivars that are more uniform in height and grain size could improve the harvest ability of the crop. Breeding work at RRC succeeded (Kauffman 1992) in developing shorter stature line. Shorter stature is helpful to prevent lodging. Dwarfism has been recovered from several crosses though some of the RRC breeding lines can attain excessive height (≥2 m) when grown in fertile well-drained soil (Weber and Kauffman 1990). Dwarf plants are short due to reduced internode length. One source of dwarf statute was identified in *A. caudatus* (Fig. 8.1). In segregating progenies this was determined by a single recessive gene (Kulakow 1987). Reduced leafiness in the inflorescence head is an added attribute to improve harvestability. Most amaranth cultivar has leaves within inflorescence. Having no leaves in the inflorescence and also limiting flowering to the stalk terminus may be the appropriate goal for grain amaranths (Kauffman 1992). The dense inflorescence types can increase grain mould problems by retaining moisture, and the drooping types are difficult to harvest (Sumar et al. 1992).

2. *Seedling vigour*: *Amaranthus* is a rapid grower, but after emergence seedling initially grows very slowly during the first few weeks; thereafter its growth gets momentum. This initial slow growth rate leaves amaranth

Fig. 8.1 Dwarf form of *Amaranthus caudatus* with erect terminal inflorescence

vulnerable to weed competitors. The fragile nature of amaranth seedling makes them susceptible to damage from wind-borne soil. Greater seedling vigour, which could come from larger seed size, might allow amaranth to be planted deeper, which could be desirable with certain soil and weather condition.

3. *Seed weight*: Larger seeds would improve seedling vigour, ease of handling and popping. Wild species are potential source of genes for larger seeds. Artificial polyploids can also be used to increase the seed size. Seed size can be increased by 41–159 % with polyploidy (Pal and Khoshoo 1968; Sun and Yue 1993), with significant changes in the nutritional value (Misra et al. 1971) and improved popping (Pal and Khoshoo 1968). Polyploidy is an established mean to increase seed size.
4. *Grain yield*: Like other crops higher yields are also desirable in amaranths. Increase in grain yield is quite feasible in amaranths. Cultivars occasionally have yielded much higher in replicated research trial (≥3000 kg/ha) (Myers 1996) in comparison with commonly seen farmer yield (≤1000 kg/ha). Heterosis may be a key factor to boost grain yield in amaranths.
5. *Taste and nutritive value*: Few people like the taste of amaranth, while others find it unacceptable. It is blended with other cereal products at such a low percentage that its taste is masked by other grains in the mixture. A milder or widely accepted taste profile would favour the acceptance of amaranths in food marketplace. Research on amaranths in the last few decades has emphasised mainly its good nutritional profile, more precisely its high seed protein level and high-lysine content. Amaranth germplasms show diversity in protein level and other nutritional values. Few cultivars of *A. hypochondriacus* were successfully selected for higher seed protein content in India. Plant breeders can select for nutritionally superior vegetable or forage cultivars. The anti-nutrients like oxalate and nitrate contents vary between genotypes (Devadas et al. 1984). Selection of variant with low oxalate and nitrate content will widen its acceptability as herbage vegetable. Teutonico and Knorr (1985a) developed an improved method to select for low oxalate by screening cultured amaranth cell.
6. *Stress tolerance*: Amaranths can be cultivated on different types of soil even in marginal areas. It is tolerant to high temperature. Cold-tolerant seedlings could be a significant improvement, though there is no clear evidence for the existence of cold tolerance. Germplasm screened for cold tolerance and tolerance to aluminium toxicity in acidic soil showed variability.
7. *Disease and pest*: *Amaranthus tricolor* is the worst sufferer among amaranths from fungal disease, often prematurely killed by a fungus that aggressively colonises the leaves and stems (Bansal 1996). Most of the accessions appear to be very susceptible. Finding of an improved resistant cultivar to the pathogen is desirable. Evaluation of this resistance among diverse amaranth accessions is in progress. Amaranths are susceptible to a number of insects (El-Aydam and Burki 1997). The grain amaranth can recover after insect feeding, but in vegetable amaranths, the damage is severe enough to lower the yield heavily. The insect sucking can damage the developing seeds and reduce the harvest up to 80 %. In worst situation insect can disrupt the vascular supply in inflorescence, destroying the whole branches. Wilson et al. (1989) identified some potential sources of tarnished plant bug resistance evaluating the germplasm for resistance at North Central Regional Plant Introduction Station (NCRPIS), Iowa. In Mexico, the stem borer *Sciara* is a problem, but a resistance was found in the Aztec grain race (Espitia 1994). Resistance to root-knot nematodes is available in some accession of *A. cruentus* and *A. dubius* (Babatola and Awoderu 1986).

Based on the results obtained and problem of amaranth cultivation, the following course of actions was proposed:

1. Detailed genetic studies involving diallel analysis in order to evolve high-yielding genotypes with lower content of anti-nutrient factor and high average nutritive value with late

bolting habit, lodging resistant and non-shattering traits.
2. Standardisation of the agrotechniques to obtain maximum yield, low content of anti-nutrient factor and late bolting habit.
3. Fundamental studies on metabolism of anti-nutrient factors in amaranths.
4. Standardisation of plant protection measures using herbal or safer insecticides with minimum residual toxicity and waiting period.
5. Amaranth is an ideal crop for biocycling of urban waste and for minimising soil, water and environmental pollution. Development of high-lysine lines in grain amaranths is yet another important area of research.

Genetic variability is very high in the populations of wild amaranths and fairly high in cultivated species. It is possible to make use of the unexploited germplasm resources of the wild species for crop improvement and cultivar breeding of this underutilised but promising crop. According to Kauffman (1984), the traits useful for successful agronomic use of amaranths are (1) synchronised drydown of the entire plant at maturity; (2) reduced plant height and reduced branching; (3) non-shattering seeds that facilitate easy harvesting; (4) development of cultivar with light-coloured seeds with high-protein content, fast growth and high water-use efficiency; (5) production of cultivars resistant to fungal or insect diseases; and (6) more branching in vegetable amaranth for multiple cuttings.

In our present agricultural system, both inter- and intraspecific diversities are declining. Only 30 edible plant species have the prime responsibility to feed the world though there is an estimated total of 30,000 (FAO 1996a) to 50,000 (Sánchez-Monge 2002) edible plants known to exist. Out of 30 edible species, only three major crops being maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) performing the major role (FAO 1996a) (Fig. 8.2). The plant breeders involved in amaranth breeding contributed towards utilisation of diversity at the intraspecific level developing novel breeding population, selecting best genotypes, developing genetically homogeneous cultivars and introducing a widely adaptable elite varieties (Fig. 8.3). The decline of genetic variability both at the inter- and intraspecific levels is associated with several risk factors like epidemics of pests and diseases due to greater genetic vulnerability, the lack of adaptability to climate-change-related stresses or other environmental stresses, the lack of genetic variation for specific quality trait and reaching performance plateaus or plateau of stagnation. Therefore a more efficient utilisation of plant genetic diversity is a prerequisite to meet the challenges endangering food security and poverty alleviation (FAO 1996b). General aims and objectives of using Plant Genetic Resources (PGR) in crop improvement are:

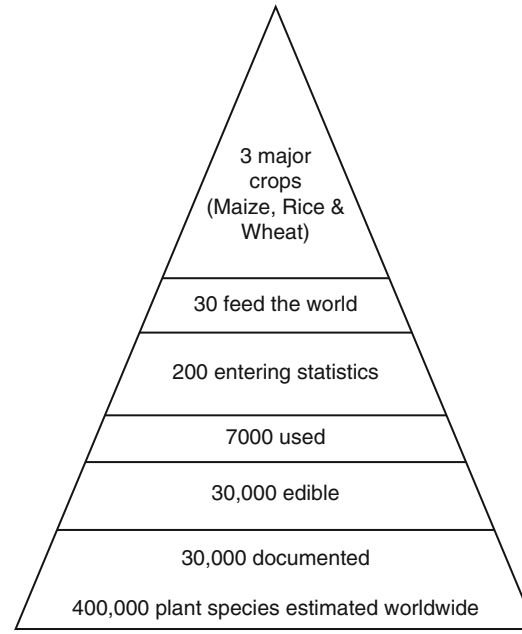

Fig. 8.2 The use of crop species diversity in agriculture (FAO 1996a, b, c)

1. Development of cultivars specifically adapted to abiotic or biotic stresses
2. Assurance of sustained production with increased nutrient and water efficiency in convenient environment through reduced application of agrochemicals
3. Development of alternatives for farmers through development of industrial, energy or pharmaceutical crops

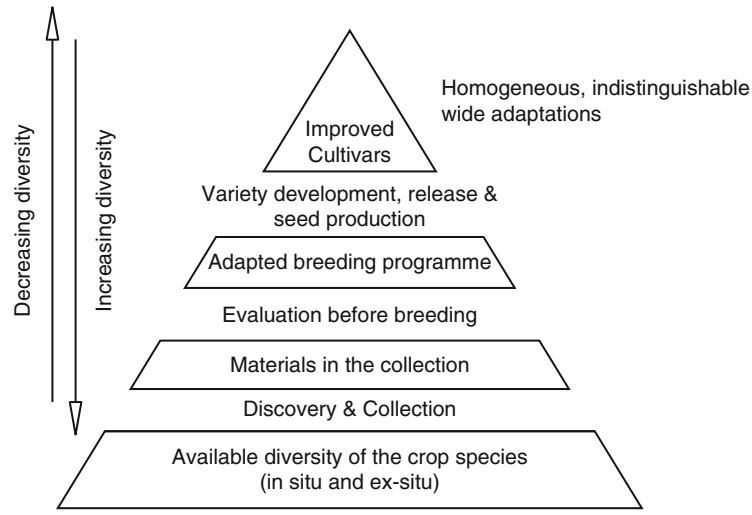

Fig. 8.3 The diversity triangle of plant breeders (FAO 1996a, b, c)

Breeding systems of *Amaranthus* species are complex because they are influenced by both genetic and environmental variations (Hauptli and Jain 1985; Jain et al. 1982). Breeding work on grain amaranth has just began and needs further research for drought resistance, yield improvement and maturation of grain (Brenner et al. 2000; Joshi and Rana 1991; Williams and Brenner 1995). The breeding of new varieties has just begun in Europe in the UK, the Netherlands, Germany, Austria, the Czech Republic and Poland. New breeding lines with potential for high grain and biomass yield should be investigated for performance under different climatic conditions.

8.4 Conventional Breeding by Selection

The work on the genetic improvement of grain amaranths, especially in India, has been achieved through conventional selection methods from local collections of landraces available at different experimental stations. The selection process has been proved to be valuable as it has resulted availability of varieties that are cultivated at present and has provided a foundation from which significant advances in yield can be achieved. Recently ICAR (Indian Council of Agricultural Research) has included crop amaranths under all India-coordinated research project on underutilised and underexploited crop plants for its further improvement involving a large number of research stations in different parts of India. This integrated work has made some progress, and some high-yielding varieties have been identified and became available under commercial cultivation in the rainfed areas.

Field trials of commercial amaranths have been carried out throughout the world, like in China, Iowa, Arizona, India, Kenya, Peru, Montana, Utah, Mexico and Thailand. Through field trials cultivars with desirable traits have been selected. High yield and uniform lines could be selected only after a few years of selection (Yue and Sun 1993). Optimum time of planting, type and application rate of fertiliser and response to fertiliser, planting density, irrigation and control of pest and disease were investigated and standardised in different field conditions. In all experimental works conducted, only a limited selection of diversity of species and cultivars was represented. The research would be more efficient and meaningful if representative collections of most diverse and productive cultivars are selected for evaluation.

A reduction in plant height and branching with high harvest index is of great value in crop improvement. Breeding system in grain amaranths varies from high rates of selfing (over 90 %) to mixed mating with as much as 30 % out-

crossing. Outcrossing rates vary significantly with genotype and environmental parameters. A bidirectional selection based on male/female flower ratio per plant suggested that breeding system is under genetic control and thus can be modified to suit breeding procedure for either inbreeding or outcrossing species (Joshi and Rana 1991). Thirty five genotypes of grain amaranths were examined for their adaptability and yield performance at three different locations (lowland rainfed areas at 300 m above the sea level, upland 1000 m above the sea level and highland more than 1000 m above the sea level) in Chiang Mai, Thailand, from 1982 to 1987 by Senthong (1986). The different types of grain amaranths showed marked variability in yield stability and adaptation. 'African' and 'Mexican' morphological group exhibited higher yield and more stability than other grain amaranth types. The 'South American' group was least well adapted to lower latitude but performed well in highland atmosphere. These observations show probable effect of drought stress in low rainfed regions and by cold atmosphere at highland areas.

Two populations, *A. cruentus* UC 87 and *A. hypochondriacus* UC 99, were subjected to S1 family analysis for agronomic characters (Ayiecho 1986). For mass and recurrent S1 selection for yield, height ratio and harvest index were applied, and selections were advanced to the second-generation mass selection in 1983. In addition, S1 selection for plant height and for days to flower was applied to UC 87 and UC 99, respectively. UC 87 showed high direct and indirect selection gains indicating the presence of additive effect. UC 99 gave the similar result. In both selections, mass selection was more efficient than S selection.

Grain amaranth landrace accessions UCC 192 (*A. cruentus*) and UCH 213 (*A. hypochondriacus*) were used by Vaidya and Jain (1987). In a mass selection experiment, involving both the populations, only 5% of the tallest and highest-yielding plants in both populations were selected for three cycles. Selection gain was highest in the first cycle for both the traits. The results favoured the previous observation about the existence of genetic variability within landrace population. A pure line selection from Koilpatli A 38 gives high yield of succulent leaves and stems and matures in about 8 weeks. A dual-purpose vegetable or cereal selection from Ooty A 50 gives good greens 3 weeks after sowing with medium leaves, and stems developed. A yield of 300 kg/ha of nutritious grain was obtained. A high-yielding variety 'Annapurna' has been developed for cultivation (Joshi 1988) from the germplasm collected from Pauri in Uttrakhand, India, through pure line selection, and it has given an average grain yield of 22 kg/ha under multilocation test. Vivekananda Laboratory, Almora, Uttrakhand, has developed VL-21 a local selection. Likewise NBPGR, New Delhi, has developed a high-grain-yielding selection IC-5564 for cultivation in plain. It would be worthwhile to resort to the most suitable breeding procedure such as biparental mating, recurrent selection or diallel selective mating (Jensen 1970).

Research on amaranth breeding for crop improvement at North Central Regional Plant Introduction Station, Iowa State University, resulted in the release of five improved breeding lines including traits like non-shattering seed cases and large stems with good resistance to lodging for biomass. The non-shattering trait resulted in the better seed retention and subsequent increase in yield and improved ornamental lines for cut-flower use. Accessions have been identified with traits like male sterility, non-shattering seeds, heavy seeds and dark red foliage. Accessions having red foliage pigmentation can be useful for colouring food. Incompatibility in crossing or minimal crossing with weedy species is another useful aspect to maintain pure commercial breeding line. The accessions having minimum crossability with weedy amaranth are to be documented as the next goal.

The knowledge about interrelationship between various agronomic and quality characters along with direct and indirect influence of component characters on yield can be very effective to improve the foliage yield of vegetable amaranths. Shukla et al. (2010) conducted an investigation to explore the interrelationship among various agronomic traits and quality traits and their direct and indirect effect on foliage

yield in 39 distinct cultivars of vegetable amaranth (*A. tricolor*). Among the agronomic traits, plant height and number of inflorescence were positively correlated with foliage yield, while chlorophyll a, chlorophyll b, carotenoid, fibre and ascorbic acid showed positive correlation with foliage yield. Chlorophyll a and chlorophyll b showed significant positive correlation with carotenoid, fibre and ascorbic acid, while ascorbic acid was positively correlated with fibre and carotenoid. Protein content was linked with plant height, branches per plant and 500 seed weight. Foliage yield showed direct positive correlation with chlorophyll a, carotenoid and inflorescence length but revealed negative correlation with branches per plant, leaf size, seed yield, chlorophyll b, moisture content and ascorbic acid. Suitable traits have been marked out to enhance foliage yield in vegetable amaranth.

8.5 Hybridisation and Heterosis in Amaranths

Hybridisation has been proved to be the most efficient breeding procedure to create new variations both under domestication and natural conditions. Inter-varietal and intraspecific crosses are the most successful in the sense that such hybrids are viable and fertile. In contrast hybrids from wide crosses either suffer from nonviability or sterility or from both, as such promptly eliminated by the forces of natural selection. Very often occasional hybridisation in nature between incompletely isolated species (geographical races) leads to the development of hybrid swarms, i.e. highly variable populations consisting of segregating progenies of species hybrid at geographical boundaries that separates distribution regions of species. Such an interspecific hybridisation (that apparently looks like crossing between two habitats) producing partially fertile hybrids is called introgressive hybridisation or just introgression (Anderson and Hubricht 1938). Introgression can be defined as 'the infiltration of germplasm from one species to another by repeated backcrossing' or more precisely 'the transfer of genetic materials across an incompletely developed interspecific barrier, usually via a partially sterile F_1 hybrid, by means of repeated backcrossing and selection of well-adapted types'. Though the success of introgressive hybridisation in overlapping habitats is dependent upon the availability of a suitable ecological niche for the establishment of introgressed types, introgressive hybridisation is supposed to have played a vital role in the origin and diversification of amaranths especially in grain amaranths. Improvement of heritable qualitative traits that are governed by one or a few major genes or gene complexes is one of the prime objectives of introgression. Generally, the conventional backcrossing procedure is applied to introgress traits like resistances or restorer genes from wild relatives into recipient breeding materials (= the recurrent parent).

Hybridisation studies are very significant to establish evolutionary linkage and accessible gene flow for traditional breeding programme. Murray (1940) was one of the first persons to classify interspecific hybridisation within the genus *Amaranthus*. He divided monoecious species according to the arrangement of the male flower in the inflorescence. Two categories of plants were recognised – type I plants having male and female flowers intermingled with each other and type II plants having male flowers arranged at the apical part of inflorescence. He performed quite a few crosses between and among type I monoecious species (e.g. *A. caudatus, A. hybridus, A. retroflexus* and *A. powellii*), type II monoecious species (*A. spinosus*) and dioecious species. Crosses between monoecious species produced hybrids with different ease. Crosses involving type I and type II plant showed most difficulty in hybrid production. Crosses between type I monoecious species and type I dioecious species readily produced hybrids indicating a phylogenetic proximity between these taxa. Crossing involving *A. hybridus* and *A. caudatus* was among the most consistent with the weak prezygotic isolation indicating close affinity. Interestingly, crosses between *A. hybridus* and *A. caudatus* with *A. tuberculatus* were similarly prolific and consistent predicting evolutionary relationship.

Natural hybrids of grain amaranths were detected by characters intermediate between *A. caudatus* or *A. cruentus* and species of *A. hybridus* complex. In some cases morphology of hybrid reflects three-way hybridisation among the species (Tucker and Sauer 1958). The hybrid forms may be found in nature which indicates that first-generation hybrids were fertile enough to advance to more stable subsequent generations. Khoshoo and Pal (1972) involved *A. hypochondriacus* (as male parent) in crosses with *A. hybridus* and *A. caudatus,* all having 32 chromosomes. Hybrids that evolved from these crosses showed the formation of 16 bivalents. Hybrids derived from crosses between *A. hypochondriacus* and *A. hybridus* showed much higher pollen fertility than hybrids produced through crosses between *A. hypochondriacus* and *A. cruentus*. Surprisingly crosses between *A. hybridus* and *A. caudatus* resulted in lethal seedlings. Hybrid fertility in this study consolidated the concept that domestication of grain amaranths has occurred independently and evolutionary *A. hybridus* should be placed much closer to *A. hypochondriacus* and *A. caudatus*.

Grain amaranths and their putative progenitor showed two basic chromosome numbers, i.e. $2n=32$ (*A. hybridus, A. hypochondriacus, A. caudatus, A. quitensis*) and $2n=34$ (*A. cruentus* and *A. powellii*). Pal and Khoshoo (1982) tried to explore the phylogenetic relationship between these two basic numbers applying dibasic cross between *A. hypochondriacus* with $2n=32$ and an African race of *A. hybridus* with $2n=34$ chromosomes. At the metaphase I, most of the meiotic cells of the interspecific F_1 hybrids showed 15 bivalents and one trivalent association of chromosomes. F_2 hybrid progeny showed 1:2:1 segregation pattern for 32, 33 and 34 somatic chromosomes, respectively. This type of meiotic configuration in dibasic hybrids indicated that $n=17$ may have evolved by aneuploidy probably involving reciprocal translocation resulting in a decrease in chromosome number from $n=17$ to $n=16$. Many authors studied the meiotic behaviour in various crop-wild hybrids and provided clues to understand gene pool accessibility and phylogenetic relationship. The meiotic configuration of 13 different crop-wild and wild-wild spontaneous hybrids was studied by Greizerstein and Poggio (1995), and the information was used to formulate the first set of genomic configuration for those species (Brenner et al. 2000). Crop-wild hybrids have been produced to fulfil all major breeding objectives:

1. Increase in grain yield
2. Increased tolerance against pest
3. Increase in protein content
4. Early maturity and improvement in growth
5. Improvement in harvestability
6. Reduction in grain shattering

One of the most widely used grain amaranth varieties in the USA is *A. hypochondriacus* var. Plainsman. The variety was derived from a cross between *A. hypochondriacus* and a Pakistani *A. hybridus* accession (Baltensperger et al. 1992). Biomass heterosis and combining ability with domesticated species have been measured using interspecific crosses between *A. hypochondriacus* and *A. hybridus* (Lehman et al. 1991). Useful traits inherent in wild species have been transferred to crop species through hybridisation, e.g. crop-wild hybrids have been developed to transfer non-dehiscence property of *A. powellii* to *A. cruentus* and *A. hypochondriacus* breeding lines in an effort to reduce grain shattering. Hybridisation with *A. cannabinus*, a wild dioecious species, might be beneficial to get hybrids with greater seed size (Brenner et al. 2000). Introduction of herbicide resistance from wild species to cultivated species is of great implication. Herbicide resistance evolved in *A. hybridus* has been transferred to elite breeding lines of *A. hypochondriacus* and *A. cruentus*. Research related to hybridisation involving wild-crop relatives of amaranths subsequently emphasised the study of gene flow between two problematic weeds, viz. *A. hybridus* and *A. tuberculatus* (Trucco et al. 2006a). This study has attracted breeder's interest because *A. hybridus* is almost unequivocally regarded as the common progenitor of the domesticated grain species. The frequent occurrence of herbicide resistance in amaranth weed suggested that it should be pos-

sible to transfer the disease-resistant trait from weed to crop via hybridisation or alternatively to select that same trait in cultivated crops.

Interspecific hybridisation among most of the *Amaranthus* species has been investigated (Sauer 1950), and some of them have been even treated as 'promiscuous' (Trucco et al. 2005b). Though hybridisation occurs frequently between *Amaranthus tuberculatus* and *A. hybridus,* genetic introgression takes place only in one direction, from *A. hybridus* to *A. tuberculatus* (Trucco et al. 2009). Interspecific hybridisation was experimentally documented under field conditions for these two species (Trucco et al. 2005a,b). Chromosome numbers are variable among weedy *Amaranthus* species. Documents on interspecific hybridisations in *Amaranthus* reveal that equal chromosome number is not a prerequisite for hybridisation to take place, but hybrid progeny appears to be more viable and stabilised when their parental species have the same chromosome number, as in the case of hybrids derived from crosses between *A. hybridus* and *A. tuberculatus* (Trucco et al. 2009). Floral structure may also be an influencing factor in hybridisation, as pollen grains from other species have to face greater pollen competition in self-pollinating species than in dioecious species.

Amaranths are usually wind pollinated with the exception of grain amaranths which are insect pollinated. Species included under section *Blitopsis* are predominantly self-pollinating due to the number of staminate flowers per glomerule and small axillary inflorescence. On the other hand, single staminate flower per glomerule and big inflorescence are responsible for more cross-pollination in sect. *Amaranthus* (the grain amaranths). Interspecific hybrids are formed as a result of cross-pollination of two different species. Eight naturally occurring interspecific hybrids have been reported in vegetable amaranths species with varying degree of sterility. In the case of natural hybridisation involving *A. edulis* and *A. hybridus*, complete chromosome pairing was observed, but the pollen fertility was less than 50 % (Sreelathakumary and Peter 1993). Interspecific hybrids of *A. tricolor* and *A. lividus* showed even higher degree of hybridisation defect (about 90 % pollen sterility). Hybridisation between closely related species like *A. edulis* and *A. caudatus* yields a hybrid showing hybrid vigour, probably as a result of overwhelming effect of minor genetic disharmony by superiority of heterozygous genotypes of the hybrids. Intraspecific hybridisation between different cultivars of *A. hypochondriacus* and *A. cruentus* has shown no heterosis in the hybrids, yet interspecific hybridisation among grain species showed biomass heterosis. Interspecific hybridisation rather than intraspecific hybridisation can increase biomass productivity in the hybrids (Lehman et al. 1991). Interspecific hybridisation between grain and vegetable amaranths often produces hybrids with deformities, high pollen sterility and chromosomal aberrations indicating the presence of a great incompatibility barrier between species of sect. *Amaranthus* and sect. *Blitopsis* (Mohideen and Irulappan 1993). Natural hybrids of *A. spinosus* ($n=17$) and *A. dubius* ($2n=32$) are easily formed in areas where the two species grow side by side. Genome analysis of the resultant triploid suggested that 17 chromosomes homologous to the chromosome complement of *A. spinosus* are present in *A. dubius* (Behera and Patnaik 1982). However study on characteristics of the amphidiploid *A. dubio-spinosus* ($2n=49$) does not support this claim (Sreelathakumary and Peter 1993). From the present knowledge of chromosome number relating to relationship within the genus, it appears that aneuploid condition in all probability has emerged as a result of interspecific hybridisation (Grant 1959a, b, c). Aneuploidy remains a major factor responsible for species variation, speciation and taxonomic ambiguity of the genus. Three species of amaranths (*A. hypochondriacus, A. caudatus and A. edulis*) were rendered tetraploid (Pal and Khoshoo 1977). In comparison with diploid, the raw autotetraploids are shorter, sterile and thus non-lodging.

Eight hybrids derived from interspecific crosses between *A. edulis* and *A. hypochondriacus*, *A. edulis* and *A. caudatus*, *A. edulis* and *A. caudatus* var. *atropurpureus*, *A. caudatus* and *A. hybridus*, *A. edulis* and *A. hybridus*, *A. caudatus*

and *A. hypochondriacus*, *A. hybridus* and *A. hypochondriacus* and *A. powellii* and *A. hypochondriacus* were investigated. The crosses were successful when crossing made between *A. powellii* and *A. hypochondriacus*, *A. edulis* and *A. hypochondriacus*, *A. caudatus* and *A. hypochondriacus*, *A. edulis* and *A. caudatus*, *A. edulis* and *A. caudatus* var. *atropurpureus* and *A. hybridus* and *A. hypochondriacus* (Pal and Khoshoo 1973a, b). Hybrids evolved from crossing between *A. hypochondriacus* (as the male parent) and *A. hybridus*, and *A. caudatus* showed the formation of 16 bivalents. However, hybrids produced from crosses between *A. hypochondriacus* and *A. hybridus* showed much greater pollen fertility than hybrids derived from crosses between *A. hypochondriacus* and *A. caudatus*. Interestingly, in hybrids between *A. hybridus* and *A. caudatus*, seedling was lethal (Trucco and Tranel 2011). Ranade et al. (1997) found that the hybrid of *A. edulis* and *A. caudatus* is associated with *A. caudatus* in a single cluster, while the hybrid of *A. hybridus* and *A. hypochondriacus* is included in the cluster of the latter species. The low genetic distance values between these hybrids and other accessions of *A. caudatus* and *A. hypochondriacus*, respectively, indicated the fact that these are not strongly and completely differentiated genetically. Hybrids produced through crosses with wild species have addressed all major breeding objectives including yield improvement, pest management and grain harvestability (Brenner et al. 2000). The crop-wild hybrids have been produced to transfer non-dehiscence trait from *A. powellii* to *A. cruentus* and *A. hypochondriacus* breeding lines, with an intention to reduce grain shattering and improve grain harvestability.

Herbicide resistance in weed represents an excellent model system to study evolutionary concept. The most common herbicide used to control weed in the world is glyphosate. But simultaneous evolution of resistance against this herbicide is a problem in agriculture. This resistance is due to an overexpression of a novel gene 5′-enolpyruvylshikimate-3-phosphate synthase (EPSPS gene). The resistant plant possesses 40- to more than 100-fold more copies of EPSPS gene than susceptible individual. The resistant species acquire a rare and unique trait of rapid amplification of EPSPS gene that enables them to resist the herbicide glyphosate. This unique trait may be transferred from resistant species to other related species exposed to similar selective process through interspecific hybridisation. According to Heap (2011), glyphosate resistance has been reported in 21 species globally. Amplification of the 5′-enolpyruvylshikimate-3-phosphate synthase gene has recently been reported in *Amaranthus palmeri* which has enabled them to resist glyphosate. This evolved property could be introgressed to other weedy *Amaranthus* species through interspecific hybridisation. Gaines et al. (2012) evaluated the feasibility of interspecific hybridisation as a measure to transfer this trait from *A. palmeri* to other weedy amaranths like *Amaranthus hybridus*, *Amaranthus powellii*, *Amaranthus retroflexus*, *Amaranthus spinosus* and *Amaranthus tuberculatus* via pollen grains of *A. palmeri.* Crossing was conducted under both the field and greenhouse conditions using glyphosate-resistant male *A. palmeri* as pollen donors and the other weed *Amaranthus* species as pollen recipients. Hybridisation between *A. palmeri* and *A. spinosus* occurred with frequencies of <0.01–0.4 % in field crosses and 1.4 % in greenhouse crosses. A majority of the hybrids of *A. spinosus* and *A. palmeri* were monoecious, grown to flowering and produced viable seed. Hybridisation frequency in field condition between *A. palmeri* and *A. tuberculatus* was <0.2 % and between *A. palmeri* and *A. hybridus* <0.01 %. This is considered as the first documentation of hybridisation between *A. palmeri* and both *A. spinosus* and *A. hybridus*.

A key evolutionary concept is the relative importance of acquiring a unique adaptive trait via interspecific gene transfer versus evolution of the same adaptive trait within a species. Interspecific hybridisation can be influential on evolution if hybrid genotypes are more superior to one or both parents (Abbott 1992; Barton 2001). As far as the introgression of herbicide resistance genes are concerned, the hybrid would clearly have higher fitness than the susceptible

parental species when exposed to herbicide selection. Much effort has been devoted to study the transfer of herbicide-resistant genes from crop species to related weed species (e.g. Ellstrand et al. 1999; Legere 2005; Gaines et al. 2008), but information regarding interspecific hybridisation between weed species are still not adequate. Intraspecific hybridisation has been proved to be useful in the transfer of herbicide resistance at the commercial field scale in *Lolium* (Busi et al. 2008) and *Amaranthus* (Sosnoskie et al. 2009).

Glyphosate resistance has evolved in *A. palmeri* due to overexpression of EPSPS (5′-enolpyruvylshikimate-3-phosphate synthase) gene. This trait is transmissible to other weedy *Amaranthus* species, particularly *A. spinosus* via pollen-mediated gene flow. The hybridisation rate was found higher in field condition because such condition represents ideal natural situation where *A. palmeri* coexists with other *Amaranthus* species. Thus, interspecific hybridisation may be anticipated to occur in field conditions and represent a potential route of evolutionary adaptive change for related species currently lacking this novel resistance mechanism, with considerable agronomic relevance for managing *Amaranthus* weeds. Evolved glyphosate resistance mechanism in *A. palmeri* is widespread and is of great potential for the rapid selection and spread of glyphosate resistance in other troublesome *Amaranthus* species via interspecific hybridisation with *A. palmeri*. In the case of hybrids with *A. spinosus*, glyphosate-resistant (GR) hybrid progeny are self-fertile, and their progeny are viable and GR. *Amaranthus spinosus* a common weed is a close relative of *A. palmeri*, a problematic agricultural weed with widespread glyphosate resistance. These two species are known to hybridise facilitating transfer of glyphosate resistance. According to Nandula et al. (2014), glyphosate resistance in *A. spinosus* is caused by the amplification of EPSPS gene. Part of EPSPS amplicon is found to be present in GR *A. palmeri*. This is due to hybridisation between *A. spinosus* and GR *A. palmeri*. Though *A. spinosus* is not of such stature of *A. palmeri* and *A. tuberculatus*, still its ability to germinate over a broad range of temperature, profuse seed production and hybridise with *A. palmeri* may create management problem in agricultural field in the future.

Both *Amaranthus tuberculatus* and *A. hybridus* are phylogenetically linked to the *A. hybridus-A. powellii-A. retroflexus* complex (Franssen et al. 2001b; Trucco et al. 2007) regarding acquisition of glyphosate-resistant trait from *A. palmeri* through interspecific hybridisation, prior to the evolution of glyphosate resistance within those species. Though glyphosate is used intensively on *Amaranthus* sp., only *A. tuberculatus* and *A. palmeri* have evolved glyphosate resistance (Culpepper et al. 2006; Legleiter and Bradley 2008; Norsworthy et al. 2008; Steckel et al. 2008). It indicates the preference of interspecific hybridisation as significant evolutionary avenue for glyphosate-resistant trait to occur in related *Amaranthus* species more rapidly than by independent evolution within each species because interspecific hybridisation occurs more rapidly than independent evolution. This also indicates that the major troublesome *Amaranthus* weed species would not be manageable with glyphosate (Trucco et al. 2009). Depending on the similarity on chromosome number ($2n = 34$), pollen morphology (Franssen et al. 2001a) and genome size (Rayburn et al. 2005), it can be predicted that *A. palmeri* and *A. spinosus* may have evolved from a more recent common ancestor than the other *Amaranthus* species examined. The rapid evolution of herbicide resistance in palmer amaranth due to multiple copies of the EPSPS genes suggests that the genome of it may be rearranged. Epigenetic responses to a changing environment based on alterations in genome architecture rather than changes in the underlying DNA base sequence could explain at least some of its capacity to adapt rapidly (Ward et al. 2013). A large number of non-hybrid progeny appeared from controlled crosses made between dioecious *A. palmer*i (palmer amaranth) and *A. tuberculatus* (common water-hemp). All the non-hybrids were examined for two interspecific crosses, and all plants were female and had DNA content very similar to the female (palmer amaranths). Among the hybrids eight were nonviable (lethal or neuter), and only one hybrid (triploid) continued gene movement (Trucco et al. 2006b). As a dioe-

cious species, palmer amaranth is an obligate outcrossing species. Grant (1959b) observed that heteromorphic sex chromosomes are not present in the karyotype of any dioecious amaranth species. Apparent agamospermy has been reported in female palmer amaranth plant pollinated by common water-hemp (Trucco et al. 2007). This requires further investigations. Wetzel et al. (1999b) reported the transfer of ALS resistance via hybridisation and backcrossing between palmer amaranth and common water-hemp. Gaines et al. (2012) also reported low level (<0.2 %) of interspecific hybridisation between palmer amaranth and common water-hemp but the highest level of fruitful hybridisation between palmer amaranth and spiny amaranth producing viable and fertile F_1 progeny.

Some weedy amaranth species can hybridise with the cultivated grain amaranths (Brenner et al. 2000) because the crossability barrier between grain amaranths and their weedy relatives is very fragile. In the USA the common weed which is cross-compatible with grain amaranths is either dioecious (*A. palmeri*, *A. arenicola*, *A. tuberculatus*) or monoecious (*A. hybridus*, *A. powellii*, *A. retroflexus*) (Wassom and Tranel 2005). Weed-crop hybrids appear as weeds in grain amaranth field and produce off-type brown seeds in place of white seed lots of grain amaranths (Hauptli and Jain 1984). These off-type brown seeds though nutritionally similar to white seeds (Pond et al. 1991) create a cosmetic problem that reduces the marketability (Sooby et al. 1998). Weed-crop hybrids are still a major seed purity problem. The frequency of outcrossing between the cultivated grain amaranths ranges from 0 % (on highly male fertile) to 100 % (on male-sterile plant) and is heritable (Hauptli and Jain 1985). Outcrossing rate in the available landraces of grain amaranths varies between 7.6 % and 41.4 % (Espitia 1994) which is responsible for frequency variability of cultivar-weed outcrossing.

The weed-crop hybrids are phenotypically distinguishable from amaranth cultivars as hybrids typically express the trait of dominant weed (Brenner et al. 2000). Molecular parameters have also been applied to establish the paternity of amaranth hybrids (Wassom and Tranel 2005). The domesticated amaranths function as maternal parent characterised by white seed-coat colour trait which is recessive to brown seed-coat colour. Hybrids derived from crosses with dioecious amaranth species are distinct from those hybrids that have evolved from crosses with monoecious species, since dioecism is considered as a dominant trait (Trucco et al. 2006b). Furthermore F_1 hybrids obtained from crosses between grain amaranth cultivar and monoecious weedy amaranths are often fertile, but hybrids from crosses between monoecious species and dioecious male show reduced fertility but close resemblance to dioecious weedy parent (Murray 1940; Trucco et al. 2006b). Two grain amaranth cultivars, *Amaranthus* 'D136-1' (PI 538327), an interspecific hybrid developed at Rodale Institute (USDA 2013) with pale green inflorescence, and *Amaranthus hypochondriacus* 'Plainsman' (PI 558499) developed at Rodale Institute and University of Nebraska's Panhandle Research and Extension Centre (Baltensperger et al. 1992; USDA, 2013) with maroon inflorescence, were evaluated for crossability with weedy members (Brenner et al. 2013). Outcrossing rates with weed amaranths were assessed at nine locations in the USA. The 'Plainsman' has about ten times more weed hybrids than D136-1. The proportions of hybrid progeny which were either monoecious or dioecious were generally similar between D136-1 and 'Plainsman' variety. Both the accessions had one male flower in each glomerule sampled in contrast to the relationship between varied male frequencies and outcrossing that was observed with another amaranth population (Hauptli and Jain 1985). The biological mechanism underlying the significant differences in outcrossing across various locations could not be explained. The low outcrossing can be helpful to select cultivars that can be maintained more easily for the production of high-value grain with few brown seed contaminants in field. There could be other grain amaranth cultivars having even more extreme differences in outcrossing.

Interspecific and intraspecific factorial matings among grain amaranths, from varied origins and types (vegetable, grain and weed), were tested for heterosis and combining ability. An intraspecific mating within *A. hybridus* exhibited highly significant (P=0.01) accession heterosis for biomass and GCA (general combining ability) and SCA (specific combining ability) effects. No hybrid yielded more biomass than its best parent, and mid-parent heterosis ranged from −36 to 29 %. Date of maturity had little effect on biomass heterosis in *A. hypochondriacus* matings, whereas late flowering of the accessions per se did cause greater biomass. The magnitude of biomass heterosis in intraspecific *A. hypochondriacus* crosses suggests that the Asian accessions may not be useful as parents in biomass or forage breeding programmes. However, among the factorial matings, only the intraspecific *A. hypochondriacus* mating exhibited SCA effects for biomass. This result is consistent with the idea that the 'Hindustani' region is a secondary centre of diversity (Grubben and van Sloten 1981). An intraspecific mating between *A. cruentus* grain and vegetable types showed significant (P–0.05) GCA effects for the grain types when used as male parents, but average heterosis was zero. Mid-parent heterosis ranged from 0 to 57 % for intraspecific *A. cruentus* hybrids. The small variation for flowering and modest biomass heterosis in *A. cruentus* may be due to a narrowed genetic base. Two theories that explain this narrow genetic base are either (1) a post-Cortez introduction of the crop to tropical Africa with subsequent selection or geographic isolation or (2) an evolutionally recent, sexual isolation of *A. cruentus* from the other grain amaranths. An interspecific factorial mating displayed high biomass productivity, with an average of over 60 % more biomass accumulated when *A. hypochondriacus* rather than *A. cruentus* was the male parent. Date of flowering had a major influence on biomass production.

The use of interspecific amaranth hybrids seems to be a promising way to increase biomass productivity of *Amaranthus*. When vegetable types are used as parents, heterosis from interspecific hybrids may double to quadruple the biomass of the parents. As forage, interspecific hybrids could substantially increase biomass yields over open-pollinated varieties. As the grain amaranths possess a photosynthetic pathway with an optimum temperature above 40 °C (El-Sharkawy et al. 1968) and higher water-use efficiencies (Miller et al. 1984), their biomass heterosis might be utilised in hot, arid environments. Before the value of interspecific amaranth hybrids can be understood, their fodder quality, hybrid seed production and cropping niches need assessment. Male sterility sources (Peters and Jain 1987; Gudu and Gupta 1988a) may be helpful in these studies.

Biomass heterosis has long been observed in *Amaranthus*. Interspecific crosses among the grain amaranths species and their weedy relatives yielded sterile hybrids but with increased biomass. The putatively palaeopolyploid nature of amaranths coupled with two gametic numbers in the genus ($n=16$ or 17) is probably responsible for interspecific hybrid sterility (Pal et al. 1982). According to Lehman et al. (1991), interspecific crosses among diverse amaranth germplasms produced biomass yield of 0–57 % above the mid-parent value. Biomass yield can be simply and rapidly estimated in hybrid amaranth (Lehman 1990). Basal stem diameter taken at harvest measured by hand callipers predicts the dry weight when harvest sample size is at least 40 plants and plant populations are high.

Grain protein percent was studied by Pandey and Pal (1985) in the F_1 and F_2 of a diallel cross of six *A. hypochondriacus* genotypes showing additive and nonadditive gene effects. Data from a six-parent diallel without reciprocal studied by Pandey and Pal (1985) indicated that hybrids from six crosses exceeded the mean parental value for protein content of the grain, and hybrids from three of these crosses exceeded the better parent. Heterosis with respect to the six parents was 15 %.

Grain yield heterosis has been estimated in *A. hypochondriacus* (Pandey 1984a). Using six-parent, nonreciprocal, diallel crosses that created 30 hybrid progenies, seven of these hybrids outyielded their better parents by 33–71 %. This result indicated that increased heterosis is obtain-

able by employing genetically diverse parents. High general and specific combining ability in parents suggested that inbreed line development might follow the maize pattern in which distinct lineages are maintained that produce consistent hybrid vigour. Pandey (1984b) further analysed yield-contributing traits in the F_1 and F_2 of *A. hypochondriacus* and determined that both additive and nonadditive genetic variances were important.

Reports on grain yield are lacking in *A. cruentus*, though Kulakow and Jain (1987) found appreciable inbreeding depression in comparison of F_1 and F_2 generation in terms of anthesis, plant height, petiole length, leaf length, leaf width, panicle length and weight. They imposed two cycles of selection and found rapid gains for anthesis time and leaf length, suggesting a large additive terms in the total genetic variance. Overall heterosis for either grain yield or forage yield appears to be promising in *Amaranthus*. Male sterility is available in *A. hypochondriacus* only but not in other species. *A. hypochondriacus* ($n = 16$) is the most likely species to get benefit from initial heterosis with male sterility to enhance breeding and hybrid seed production. A source of male sterility in *Amaranthus cruentus* ($n = 17$) is needed. The latter may cater to enhance interspecific forage crosses and permit reciprocal analysis of mating.

Amaranth is still categorised as an underutilised crop. The main attributes responsible for its limited domestication are the seed shattering before harvest, tiny seeds, lack of public awareness on its food value, lower demand and production than conventional cereals, lack of production research and development programmes, difficulty in maintaining pure stands due to cross-pollination by black-seeded wild species and lack of specialised breeding projects (Rana et al. 2005).

8.6 Male Sterility in Amaranth Breeding

Male sterility is a reproductive deficiency of few plants where male reproductive organs in hermaphrodite flowers are non-functional and produce nonviable pollen grains through microsporogenesis. The entire process of microsporogenesis is delicately balanced under the genetic control of many loci, and mutation at any one locus may upset the entire process and result in the formation of non-functional pollen, i.e. male sterility. As the sterility is a genetic abnormality in a natural population, hence sterile plants are eliminated by selection forces. However if properly maintained under domestication, they can prove to be an asset to plant breeding providing a natural and effective means for genetic emasculation of plants. Therefore male sterility simplifies the hybridisation on commercial scale. It is of more importance in crops where flower structure is an obstacle to artificial emasculation or in monoecious plants. Male sterility can be utilised for developing mass reservoirs, particularly in outbreeders, for the conservation of variability and also as a tester genotype for assessing the combining ability of a large number of stocks, making hybrid products a requirement for certain breeding programmes and genetic studies.

The floral structure in amaranth with an initial staminate flower followed by an indefinite number of pistillate flowers in compact cluster creates a definite problem in pollination studies. Development of a male-sterile line is very helpful to facilitate effective crossing. Most of the basic breeding works on amaranths have been done at the Organic Gardening and Farming Research Centre, PA, USA (Kauffman 1980). The phenomenon of male sterility in higher plant is due to either recessive or dominant *ms* genes or it has cytoplasmic causes. In cross-fertilisation it is of considerable agronomic importance in connection with the utilisation of the heterosis effect. It is not a regular phenomenon in amaranths. Male sterility is available only in *Amaranthus hypochondriacus* (Peters and Jain 1987; Gudu and Gupta 1988a and Brenner 1993) but not in other *Amaranthus* species. Peters and Jain descried the diagnostic features of male sterility and identified some cytoplasmic male sterility type. Brenner (1993) found the unusual off-type black and translucent seeds in 'Plainsman' seed lots that frequently developed male sterility and

other aberrant types. These aberrant types may be the result of hybridisation with wild species and/or seed immaturity (Brenner et al. 2000). The source of male sterility is available through the USDA ARS (1999) – these include selection of Peters and Jain (1987) and Brenner (1993) and other spontaneous male sterility found in landrace germplasm population.

Gudu and Gupta (1988a) identified twenty male-sterile plants in a normal population of Jumla variety (ex Nepal). They could be distinguished from normal plants by the colour and morphology of their inflorescence. The sterility was conditioned by a single recessive nuclear gene, *ms*. Male sterility was discovered by Peters and Jain (1987) in selfed families of several open-pollinated as well as interpopulation hybrid individuals in grain amaranth (*A. hypochondriacus*). Segregation patterns in the F_2 generation of numerous crosses involving male-sterile plants clearly suggested gene-cytoplasmic mode of inheritance. Segregation ratios provided evidence for one or two restorer nuclear genes in different populations. Cytological studies showed male sterility to be associated with abnormal tapetal cell functioning and microsporogenesis failure prior to the first metaphase, leading to abortive anthers. Further work on genetic selection and mode of gene expression is needed to explain the relative deficiency of male sterility in certain segregating families.

A comparative study of microsporogenesis in male-sterile and male-fertile grain amaranth was conducted using electron-microscopy (Fang et al. 1996). The onset of microsporogenesis gets deviated in male sterility at the mononuclear pollen stage following the release of microspores from tetrads. Abnormality in the behaviour of degenerated tapetum was observed, which failed to envelop individual microspores after their release, leading to an abnormal vacuolation in the mononuclear pollen grains. As a result, the normal thickening of pollen wall could not occur, and pollen grains could not engorge, causing male sterility.

8.7 Wild Gene Pool, Landraces and Heritability

There are few problems in amaranth breeding like high degree of heterozygosity, low heritability of some trait and susceptibility to some diseases specially in *A. caudatus* which are to be addressed and solved (Flores et al. 1982). Numerous major genes have been identified that may be useful for mutation breeding in amaranth, such as genes coding flower, embryo and seed pigmentation, leaf characters, type of starch in perisperm, early/late flowering, inflorescence architecture and vegetative architecture (Table 8.1). Goals in improving cultivars of grain amaranth are similar to those in other grain crops – improving and stabilising the yield, increasing pest resistance and improving harvestability (Brenner et al. 2000). Several desired traits were identified, viz. vigorous seedling growth, determination of the plant growth, timing and uniformity of flowering and seed maturation within plants, synchronous drying of plant and inflorescence, reduction of leafiness in the inflorescence area, reduction in seed retention, increasing size of seeds, pale seed pigmentation and enhanced food quality traits (increasing seed proteins).

Grain amaranth cultivars are generally uniform, but due to high phenotypic plasticity, it appears to be heterogamous in field plantings (Guillen et al. 1999). The high phenotypic variability displayed by these cultivars indicates the ability of amaranths to adjust to environmental variations, but it can also make selecting within cultivars unreproductive (Guillen et al. 1999). In contrast to single-plant selections, some grain amaranth landraces have been shown to be genetically variable (Hauptli and Jain 1984) and have responded to selection.

A landrace is a local variety, regional ecotype of a domesticated plant species which has evolved over time largely to adapt in the natural and cultural environment in which it exists. It differs from conventional cultivars which have been selectively bred to conform to a particular stan-

Table 8.1 Several traits with associated hypothecated gene in amaranths

Trait	Hypothetical gene	Source	References
Embryo colour	*Pe/pe* homozygous prevent expression of Pe	*A. caudatus*	Kulakow (1987)
Pink/pale			
Floral feature			
1. Male fertile/male sterile	*Ms1/ms1*	*A. hypochondriacus*	Gudu and Gupta (1988a, b)
2. Cytoplasmic inheritance of male sterility	S1/S2 cytoplasmic genes; Rf1/rf1, Rf2/rf2 and Rf3/rf3 nuclear genes	*A. hypochondriacus*	Peters and Jain (1987)
3. Early flowering (temperate)	*Ea/ea*	*Amaranthus* sp. x *A. hybridus*	Murray (1960)
2. Late flowering (tropical)	*Ea/ea*	*Amaranthus* sp. x A. *hybridus*	Murray (1960)
3. Early flowering/late flowering	*Ea/ea*	*A. retroflexus* x *A. cruentus*	Kulakow and Jain (1985)
Inflorescence			
1. Determinate/indeterminate	*Dt/dt*	*A. caudatus*	Jain et al. (1984), Kulakow (1987)
2. Erect/drooping	*Pd1/pd1, Pd2/pd2* and modifying factors	*A. caudatus*	Kulakow (1987)
Hybridisation barrier abnormal hybrid/normal hybrid	*Ah1/ah1, Ah2/ah2*	*A. caudatus* x *A. hypochondriacus*	Jain et al. (1984)
Herbicide resistance Triazine tolerant/non-tolerant	With chloroplast	*A. caudatus*	Cheung et al. (1988) Jordan (1996)
Leaf and other markings		*A. hypochondriacus*	Jain et al. (1984)
1. Blade 'V' mark/no mark	*Vm1/vm1, Vm2/vm2*	*A. retroflexus*	Kulakow et al. (1985)
2. Blade spot/no spot	*Ls/ls*	*A. hypochondriacus*	Kulakow et al. (1985); Gupta and Gudu (1990)
3. Blade/red, vein/green	*R1/r*	*A. tricolor*	Matsumura (1938)
4. Blade/red, vein/green	*R2/r*	*A. tricolor*	Matsumura (1938)
5. Blade/pale red/green	*R3/r*	*A. tricolor*	Matsumura (1938)
6. Blade of both surfaces red/green	*R4/r*	*A. tricolor*	Matsumura (1938)
7. Blade red/green	*A/a, B/b, C/c*	*A. tricolor*	Deutsch (1977)
8. Stem red/green	*A/a*	*A. tricolor*	Deutsch (1977)
Perispermic starch glutinous/ non-glutinous	*Gl/gl*	*A. caudatus*	Okuno and Sakaguchi (1982)
Pigmentation			
Red/green	*R/r*	Several species	Jain et al. (1984)
Orange/green	*O/o*	*A. tricolor*	Jain et al. (1984)
		A. caudatus	Jain et al. (1984)
		A. cruentus	Kulakow et al. (1985)
Chlorophyll present/absent	Three genes	*A. caudatus* x *A. hypochondriacus*	Jain et al. (1984)

(continued)

Table 8.1 (continued)

Trait	Hypothetical gene	Source	References
Normal/chlorophyll variegated	Three genes	*A. caudatus* x *A. hypochondriacus*	Jain et al. (1984)
Yellow (flavonoid)/nonyellow	*C/c*	*A. tricolor*	Matsumura (1938)
Red seedling/flowering	*Lp/lp*	*A. caudatus A. hypochondriacus*	Jain et al. (1984)
Red whole plant/base of the plant	*Bd/bd*	*A. hypochondriacus A. tricolor* and various other species	Jain et al. (1984)
Red normal/diminished	One gene	*A. caudatus*	Jain et al. (1984)
Red not intense/intense	One gene		Jain et al. (1984)
Seed-coat colour	*P/p*	*A. caudatus*	Kulakow et al. (1985)
Black/pale		*A. hypochondriacus*	Coons 1982
	Y/y	*A. caudatus*	Kulakow et al. (1985)
		A. hypochondriacus	Gupta and Guru (1990)
Brown/pale	*Br/br*	*A. caudatus*	Kulakow et al. (1985)
Black/dark medium light brown/pale	Two genes	*A. cruentus x A. retroflexus*	Jain et al. (1984), Kulakow et al. (1985)
Shattering			
Dehiscent utricle/indehiscent utricle	*Dh/dh*	*A. hypochondriacus*	Jain et al. (1984)
Nonpersistent utricle/persistent utricle	Few genes	Various species	Jain et al. (1984)
Vegetative structure			
Tall/dwarf	*Dw/dw*	*A. hypochondriacus*	Pandy (1982)
		A. caudatus	Jain et al. (1984)
Cotyledon long/short	Multiple genes	*A. caudatus*	Walton (1968)
	Ok/ok	*A. cruentus* x *A. retroflexus*	Jain et al. (1984)
Narrow blade/normal blade	*V/v*	*A. tricolor*	Kihara and Matsumura (1935)

dard of characteristics. Specimens of a landrace tend to be relatively genetically uniform, but are more diverse than members of a standardised or formal breed. Landrace populations often show variability in morphological appearance, but they have a certain genetic similarity and can be identified by their appearance. Landraces have a genetic continuity with improved varieties. The relatively high level of genetic variation of landraces is one of the advantages that these can have over improved varieties. Stability of landraces against adverse conditions is typically high, but yields may not be as high as improved varieties. As a result, some individuals of a landrace population but not all become susceptible to a new pests or diseases. Landraces are quite distinct from both ancestral wild species of modern stock and separate species or subspecies originated from the same ancestor as modern domestic stock. Landraces are not all derived from ancient stock, largely unaltered by human breeding interests. *Amaranthus* has a rich stock of landraces which can be a rich source of wild gene pool that can be utilised in amaranth breeding. The collections of landrace germplasms are the backbone of the varietal improvement programme which aims at raising agronomically acceptable lines using conventional plant breeding and selection meth-

ods. To generate varieties resistant to abiotic stress, certain types of germplasm-landraces, wild relatives or wild progenitors may play a basic role in the success of a breeding programme. Landraces are often capable to produce some yield even in difficult situation where the modern varieties fail or proved less reliable. Landraces of self-pollinated species like *Amaranthus* spp. are mixture of a great number of homozygote genotypes (Ceccarelli et al. 2004). Therefore landraces are the rich sources of readily usable genetic variation. Selection within landraces is one of the easiest, oldest and cheapest methods of plant breeding. Landraces as the source of trait like drought tolerance is well documented in the case of barley in the Syrian Arab Republic. Landraces are capable of yielding yield more than modern cultivars under low input and stress condition. Genetic variation in landraces was studies by Jain (1985) applying qualitative markers and quantitative traits and allozyme variation. The New World collections were found to be varied in the amount of genetic variation from region to region and between species. Most landraces seem to be highly homozygous and carry a significant amount of variation for quantitative traits such as plant height, branching, flowering time, head length and harvesting index. Accessions showed no or little allozymic variation within and among population, though they showed high morphological variability. The heterozygosity and interspecific gene transfer in northern Indian population were found to be higher in comparison with New World landraces. Landrace population from two states of India was studied in greenhouse by Vaidya (1984) for the evaluation of genetic variation of few qualitative and quantitative characters.

Genetic variability in the germplasm is the prerequisite for the success in any crop improvement programme. Several researchers have studied the potentials of improvement of grain amaranth through the study of its genetic resources (Pandey and Singh 2010; Prashantha and Nagaraja 2011). A wide range of variability has been observed in respect of agroeconomic traits in grain amaranths (Pandey and Singh 2010; Prashantha and Nagaraja 2011).

The presence of high variability in this crop offers much scope for its improvement. Cultivated landraces of the three species of grain amaranth have a wide degree of genetic diversity which can be utilised to develop selections of improved lines with suitable characteristics for grain production. Traditional landraces of grain amaranth that are still extant in various agroecosystems may provide the genetic variability needed to diversify the gene pool of improved amaranth varieties. These landraces can therefore be exploited to significantly enhance grain amaranth productivity in various agroecosystems. Systematic survey, collection and evaluation of local amaranth germplasm are of great importance for current and future agronomic and genetic improvement of this crop.

Yield and its component traits are controlled by polygenes, whose expression is greatly influenced by the environment. Sufficient genetic variability available in the germplasm material is a necessary prerequisite for yield improvement through proper selection method (Ali et al. 2008). The genetic variance of any quantitative trait is made up of additive variance (heritable) and non-additive variance which comprise dominance and epitasis (nonallelic interaction). Therefore, it becomes compulsory to divide the observed phenotypic variability into its heritable and non-heritable compartments with suitable parameters such as phenotypic and genotypic coefficient of variation, heritability and genetic advance. To initiate and sustain an effective long-term plant breeding programme, genetic variability which is a heritable difference among genotypes is needed in an appreciable level within a population.

Heritability denotes the proportion of phenotypic variance that is due to genotype which is heritable. Heritability is known to differ to some extent depending on the populations handled. The estimates of heritability serve as a useful guide to the breeder. Selection for this trait would be fairly easy as there would be close correspondence between genotype and phenotype due to a very little contribution of environment to the phenotype. But for a character of low heritability, selection may be considerably difficult or virtually impractical due to the masking effect of environment on

the genotypic effect. The degree of gain in a trait obtained under a given selection pressure is expressed as genetic advance which is another important criterion that guides the breeder to choose a selection programme (Hamdi et al. 2003; Shukla et al. 2004). High heritability and high genetic advance for a given trait are indicative of the fact that it is governed by additive gene action and therefore offers the most effective condition for selection (Tazeen et al. 2009). Germplasm characterisation and evaluation are being conducted at a regular basis at a number of locations around the world. Extensive, well-documented amaranth germplasm characterisation has been conducted by several organisations in India, Peru and Mexico (Joshi 1981a, b; Sumar et al. 1983; Espitia 1986). Phenotypic and genotypic variances, phenotypic and genotypic coefficient of variation, heritability and genetic advance have been used to assess the magnitude of variance in grain amaranth germplasm (Rana et al. 2005; Shukla et al. 2010; Pandey and Singh 2011; Prashantha and Nagaraja 2011).

Genetic parameters for grain yield and its associated traits were studied (Sravanti et al. 2012) by evaluating 40 germplasm lines along with three checks (Co-1, Arikarai and RNA-1) of amaranth (*Amaranthus* spp.) in a randomised block design with three replications at the National Bureau of Plant Genetic Resources, Regional Station Hyderabad, Andhra Pradesh, India, during kharif, 2010. Analysis of variance revealed highly significant differences for genotypes, indicating magnificent variation among the genotypes for all the 19 characters taken into consideration, viz. plant height, stem girth, stem weight, number of branches per plant, leaf area, leaf length, leaf width, petiole length, total leaf weight, number of leaves per plant, days to 50 % flowering, inflorescence length, lateral spikelet length, days to 80 % maturity of seed, leaf/stem ratio, 1000 seed weight, protein content, dry matter content and seed yield per plant. High heritability and genetic advance were observed for all the characters, which suggested that they are controlled by few genes. On the basis of mean performance for grain yield and its components, five germplasm lines, namely, IC 588812, IC 526832, IC 585672, IC 588820 and IC 588811, which were found to be superior over the checks (Co-1, Arikarai and RNA-1), are useful for making selection to fix the desirable characters to be exploited for developing suitable parental materials. The future of amaranth is dependent on the careful recombinant selection of genotypes from the germplasm collections.

The extent of genetic variability among different *Amaranthus* species was investigated by Khurana et al. (2013). The investigation also aimed at the estimation of the heritability values, correlation coefficients and their partitioning into direct and indirect effects by path analysis. Differences among genotypes in respect of all the characters under study were highly significant. Estimates of heritability and coefficients of genotypic and phenotypic variability in both, i.e. summer and rainy season, crops were found to be high for traits, namely, total green yield, number of leaves per plant and leaf area index. Genetic advance expressed in terms of percent of mean was found to be higher in characters like leaf area index and leaf width in both summer and rainy season. Traits like plant height, number of branches per plant, leaf length, leaf width, number of leaves per plant, leaf area index and protein content were significantly and positively correlated with total green yield in both summer and rainy seasons.

Genotypic variability and character association in grain amaranth genotypes for agronomically useful and yield-contributing traits were evaluated (Yadav et al. 2014). The experiment was conducted at the National Bureau of Plant Genetic Resources, Regional Station, Shimla, Himachal Pradesh, during 2013–2014. The materials for study consisted of 27 grain amaranth germplasm accessions. The characters like width of leaf blade, length of lateral spikelet and grain yield per plant showed high genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) values. The differences between PCV and GCV were very less for all the characters taken into consideration. This showed the close proximity between the corresponding estimates of PCV and GCV in almost all the characters except petiole length, which suggested

that environmental parameters have very little influence on the expression of these characters, and variability was caused by genetic constitution only. High degree of heritability associated with high genetic advances was recorded for the characters, like plant height, inflorescence length and days to 80 % maturity, which suggested that these characters can be recognised as favourable features and as an indication of additive gene action. Similar types of results were also reported by Mulugeta et al. (2012). On the basis of evidence, most of the traits appeared to be correlated and associated with grain yield and intercorrelated among themselves. Significant positive correlation was found in few cases at phenotypic level like positive correlation between seed yield per plant and days to 80 % maturity and plant height with days to 50 % flowering. Though the rest of the features showed nonsignificant correlation, plant height, inflorescence length, lateral spikelet length and days to 50 % flowering were recorded with high GCV, PCV, heritability and genetic advance percentage of mean, which suggested that maximum emphasis should be given on the above features during the selection of grain amaranth with higher grain yield. Hybridisation and subsequent phenotype-based selection could be helpful in the transfer of these characters to the progeny. On the other hand, concomitant selection based on high inflorescence length, plant height and high leaf blade width would be an effective selection method for the improvement of grain amaranth.

One hundred accessions (50 from India and 50 from exotic sources) of grain amaranth (*Amaranthus hypochondriacus*) germplasm were grown in complete randomised block design. Data were recorded on six quantitative and two qualitative characters, viz. protein content (determined by the conventional Kjeldahl method), oil content (determined by nondestructive method using New Port NML), plant height, number of leaves, leaf length, leaf width, inflorescence length and seed yield/plant (Rana et al. 2005). Heritability was high for protein content, oil content and seed yield; moderate for inflorescence length and leaf length; and low for plant height, leaf width and number of leaves. Moderate to low estimates of broad-sense heritability indicate that improvement through selection would be limited. Genetic advance expressed as percentage of mean was high for seed yield, leaf length, inflorescence length, number of leaves and was moderate to low for other characters. Relationship of heritability and genetic advance also gives an idea about the type of gene action. It appeared that both the additive and nonadditive gene actions influence all the characters, which suggests that simple selection methods alone would not be effective; rather hybridisation followed by selection would be a better option for amaranth improvement. Other workers have also reported the role of additive and nonadditive gene action and emphasised recombinant breeding for grain amaranth improvement. Direct positive effects of inflorescence length, number of leaves and leaf length and negative direct effects of oil content, plant height and leaf width were observed on seed yield. A character showing positive correlation may not have direct effect on seed yield but may contribute to yield via other characters. Besides seed yield, inflorescence length, number of leaves and plant height were found to be important characters for selecting better yielding genotypes.

Several genes have been identified governing different traits in amaranths like pigmentation, determinant terminal inflorescence, drooping inflorescence, embryo colour, early flowering, male sterility, etc. Amaranths show variability in pigmentation. The red, yellow and orange pigments in amaranths are due to the presence of betacyanin pigments (Cai et al. 1998). Among the grain amaranths, six major genes have been identified that control the pigmentation pattern (Kulakow 1986). Diversity in variegated pattern may be due to quantitative inheritance. Pigmentation markers are useful for varietal identification, estimation of outcrossing, controlled hybridisation and aesthetic value. Wild and vegetable amaranths generally have black or dark brown seeds, while grain amaranths typically have yellow- and white-coloured seeds. Polymorphism in seed-coat colour in controlled progenies has suggested for one or two genes (Kulakow et al. 1985) with indications of multiple

alleles and additional loci for brown seed coat in some crosses (Kulakow and Jain 1985, 1990b).

The genetic control over four developmental characters in *Amaranthus caudatus*, viz. determinance, panicle orientation, dwarfism and embryo colour, and their inheritance patterns were studied (Kulakow 1986, 1987). The grain amaranth species and various landraces show variability in the structure of terminal inflorescence. The determinant inflorescence trait that has been found in both *A. caudatus* and *A. hypochondriacus* is regulated by a single gene having the allele for the common indeterminate inflorescence completely dominant over determinant allele. Drooping orientation of inflorescence typically found in *A. caudatus* landraces is determined by two major genes with erect panicle incompletely dominant over drooping panicle, while other minor genes are modifying panicle orientation. A single recessive gene determines dwarfism. Pleiotropy was responsible for abnormal growth of dwarf plant.

Some genotypes of *A. caudatus* have pink-coloured embryos where pigmentation appears during embryo development. This trait is determined by one gene with the intensity of the pigmentation and is modified by other genes (Kulakow 1987). This is the first trait known in amaranths where seedling genotypes can be identified on the maternal plant. It is useful in detecting hybridisation in some population. Grain amaranth *A. hypochondriacus* reported to have both the glutinous and non-glutinous types of periplasmic starch (Okuno and Sakaguchi 1982). In grain amaranth, starch granules are stored in the perisperm of the diploid cells derived from the nucleus. Therefore the starch property in the perisperm of grain amaranths is maternally inherited. Two types of starch property at least in *A. hypochondriacus* differentiated in Mexico and *A. hypochondriacus* with both glutinous and non-glutinous perispermic starches were propagated from Mexico to Nepal and India. Non-glutinous plant believed to be a natural hybrid between glutinous and non-glutinous plant was selfed by Okuno and Sakaguchi (1982). Segregation ratio among 500 progenies and in the F_3 derived from them indicated that a single major dominant gene determines the non-glutinous starch. Pink-coloured embryo is determined by two complementary epistatic genes with one locus determining the presence or absence of red betacyanin pigment and the other regulating its expression. All these genes are responsible for some of the largest morphological differences in *A. caudatus*. An accession of weedy *A. retroflexus* has rapid flowering under both short-day length (8 h) and long-day length (16 h). Hybrids and backcrosses between *A. cruentus* and *A. retroflexus* showed that the early flowering is regulated by a single gene with the dominant allele determining the earliness (Kulakow and Jain 1985). Isolation of a single gene determining very early flowering may be useful for developing short-season varieties facilitating rapid cycling of crosses (Kulakow and Jain 1985).

8.8 Mutation Breeding

Conventional plant breeding involves three major steps: the screening of relatively large populations, utilisation of genetic variability and selection of the desired genotypes. There are simple and efficient techniques to induce genetic variation, and the use of radiation is one of such techniques. Application of radiation in appropriate dose followed by selection for desired traits has become a common practice in plant breeding. Mutation breeding in amaranths mostly emphasised the use of radiation mutagenesis to enhance quality and quantity of amaranth grain and to evaluate the performance of selected mutants and parent lines. For this purpose the seeds of two genotypes of grain amaranth *Amaranthus cruentus,* genotype 'Ficha' and hybrid K-433 (products of interspecific hybridisation between *A. hypochondriacus* and *A. hybridus*) were irradiated by γ radiation dose 175 Gy (Gajdošová et al. 2008) followed by positive selection performed during eight mutant generations (1998–2008). The morphological observations were recorded and selection on desired traits was done starting from M_2 generation. The plant with negative features like weak seedling growth, undeterminated plant growth, abundant leafiness in the inflorescence area, nonuniform flowering and seed maturation,

low seed size, etc., was removed from the field, and only plants with positive traits were collected. The weight of 1000 seeds (WTS) was determined using seed counter Contador (2 × 500 seeds), recorded and statistically evaluated. Finally, several putative mutant lines of *A. cruentus* and hybrid K-433 were selected and characterised by highly and significantly increased WTS (in comparison with control) showing gradual tendency towards stabilisation of this trait when compared with the samples of the previous generations. All those selected plants are expected to have genetically fixed WTS. Therefore, these plant materials can be considered as valuable mutation lines useful in future amaranth breeding programme.

The genetic variability is a prerequisite for developing new cultivars, and this variability is either induced or is a natural phenomenon that occurs spontaneously. The rate of spontaneous mutation is quite low and can't be utilised for breeding programme; for this reason mutations are artificially induced treating with physical and chemical mutagen. Several useful genetic changes have been induced artificially by mutagen treatment like high yield, flower colour, disease resistance and early maturation and so on in crops, vegetables, medicinal herbs, fruits and ornamental plants. So far, over 3000 mutant varieties of rice, wheat, barley, sorghum, legumes, cotton, edible oil, ornamental plants and fruits (www-mvd.iaea.org) have been officially released over 60 countries. India and China are the two major producers of mutant varieties in the world to feed their ever-growing human population.

Amaranthus tricolor is a nutritious vegetable crop that is used as a subsistence or cash crop in the rural areas in Africa, Asia and especially in India. Its yield and production are severely limited by abiotic stresses such as drought. Mutation technology was previously used as a tool to create genetic variation and to select for lines with improved drought tolerance (Kgang et al. 2008). *A. tricolor* seeds were subjected to different doses of gamma radiation, and to ensure subsequent seed germination, 160 Gy was selected. The evolved mutant lines were screened over several generations under both field and greenhouse conditions, and seven promising drought-tolerant lines were selected. Further physiological and morphological interpretation on two of these mutant *Amaranthus* lines were reported by Kgang et al. (2008)). These mutant lines showed increased recovery after withdrawal of water for 2 weeks and also showed increased protein content per gramme of dry weight in comparison with the wild type. Improvement of the grains and development of new varieties of *A. tricolor* plants were previously achieved at the ARC Vegetable and Ornamental Plant Institute, South Africa, where seeds were treated with different doses of gamma radiation to induce increased drought tolerance. Radiation is known to cause changes to the genome, but plant performance of both the wild-type and mutant *Amaranthus* plants may be similar (Jie et al. 1993). Hence, this study asks for the comparison of the phenotypic performance of irradiated and wild-type *Amaranthus* lines during drought conditions. Two mutant lines were compared to a wild type, and the parameters considered were plant height, relative water content, protein content of the leaves and genomic differences between the two mutants and the wild type applying RAPD analysis.

In order to increase food availability and household incomes of families in the Andean region of Peru, induced mutation techniques were applied to improve barley (*Hordeum vulgare*) and kiwicha (*Amaranthus caudatus*) cultivars (Gomez-Pando et al. 2009). Kiwicha is a native and an ancient crop of the Andean region but has been rediscovered as a promising crop with high-quality seed protein and a drought-tolerant habit. To improve the traditional cultivar Ancash of *Amaranthus caudatus*, physical mutagen gamma rays were utilised. Dried seeds of the traditional cultivar 'Selection Ancash', previously purified in isolated conditions, were treated with gamma rays at doses of 100, 200, 300, 400, 600, 800 and 1,000 Gy. In M_2 generation, two types of mutations were identified from the materials treated with gamma rays at a dose of 400 Gy. In M_3 and M_4 generation, mutants with different yield potentials were selected among the 36 mutant lines. Five mutant

lines appeared to have greater yield potential than the parental cultivar. From these high-yielding lines, Centenario cultivar was selected and released in March 2006 with similar quality, better yield and different plant colours than the parental material. The yield varied between 3500 and 5500 kg/ha at farmer location in the coastal region, but the range was 2500–3700 kg/ha in the highland areas. Few salient features of Centenario cultivar like the better yield potential, tolerance to *Sclerotinia* sp., colour and size of the grains were contributing factors in the preference of Centenario over other commercial cultivar.

Slabbert et al. (2003) investigated the dryland yield and growth, community acceptance and palatability of seven selected amaranth (*Amaranthus tricolor*) and seven cowpea (*Vigna unguiculata*) mutant lines. The study indicated mutant line A19 as the highest yielding and mutant line A5 as the least yielding. The mutant lines showed significant morphological variabilities. Linear regression analysis indicated the amaranth mutant lines A19 and A2 well adapted to environmental/seasonal changes while A6, A550 and A993 poorly adapted. Mutant line A5 appeared to be the most stable in yield during environmental changes, while A554 showed to be the most sensitive to environmental changes. Community members opined that A550 was the tastiest of the amaranth mutant lines.

Mutations are induced by physical mutagens (like gamma radiation, high- and low-energy beams, etc.) and chemical mutagen (like ethyl methane sulphonate or EMS) treatment of both seed and vegetatively propagated crops. Among physical mutagens, gamma radiation has been widely applied to induce mutation for both seed and vegetatively propagated crops. Recently ion energy technology – heavy ion beam (HIB) and low-energy ion beam (LIB) – is being applied to induce mutation in wide range of crops. HIB is frequently used for inducing mutations in plants (Jain 2010). They participate in linear energy transfer (LET) and enhance the induction of higher biological effects. Several *Arabidopsis* mutants have been generated through deletions, insertions and chromosomal translocations by HIB.

Plant tissue culture techniques have made it possible to regenerate plants from all major food and horticultural crops in aseptic culture. Micropropagation via organogenesis is now routinely used for clonal propagation of ornamental and other vegetatively propagated plants. Explants like shoot meristem, adventitious buds and microspores can be directly treated with mutagen, and shoots can also be directly regenerated from the treated explants followed by root formation (Suprasanna et al. 2010). Regenerated plants are maintained in the greenhouse and then put under the selection pressure. Similarly somatic embryos of vegetatively propagated crops like banana, date palm, cassava and others can also be readily induced. The mutagen treatment of embryogenic cell suspension can either eliminate or reduce the chimaeras drastically and enables to get mutant somatic embryos which can be subsequently regenerated into plantlets in appropriate culture media. Embryogenic cells are plated on a filter paper and put on agar solidified medium; cells are treated with gamma radiation followed by the transfer on culture medium and allowed to form somatic embryos. The treated cells can also be put under the selection pressure in order to isolate mutants, e.g. disease-, salt- and drought-tolerant mutants. The selected mutant plants are transferred in the greenhouse and finally to the field evaluation and used for crossing with other varieties. The lethal dose (LD50 dose) for each experimental plant should be determined following the radiosensitive curve to avoid too high or too low dosage. Moreover, plants and even varieties differ in radio sensitivity. Low dose of gamma radiation has promoted growth in *Citrus* depending on cultivar, maintained embryogenic nature of date palm for 2–3 years, promoted growth in orchids, enhanced secondary metabolites in medicinal plants and also improved the shelf life of postharvest products. Collaborative research programme under the Food and Agriculture Organization and International Atomic Energy Agency (FAO/IAEA) has given emphasis on crop improvement by induced mutation using nuclear techniques (Jain 2000) intended to produce strains of cereals

with higher concentrations of micronutrients and improved bioavailability by reduction in the phytic acid concentration.

Several mutant genes have been successfully introduced into commercial crop varieties of maize, barley, soybean and sunflower to enhance the nutritive value. Traditionally, micronutrient and trace element content of crops can be improved by using field fortification strategies, applying enriched fertilisers in the soil. Biotechnological tools have opened up new avenues to improve the amount and availability of nutrients in crop plant. These involve simple selection of varieties with high nutrient concentration in the seeds, followed by crossbreeding to incorporate a desired trait within a plant and ultimately genetic engineering for the manipulation of the nutrient content of the plant (King 2002). Production of 'Golden rice' represents an example of successful biotechnological application in breeding where a gene necessary for the accumulation of carotenoids (vitamin A precursors) in the endosperm that are not available in the rice gene pool has been successfully transferred.

Recent advances in plant genomics have opened new possibilities for application of mutation techniques in crop improvement. Using the reverse genetic strategy called TILLING (Targeting Induced Local Lesion IN Genomes), it is possible to induce a series of alleles in a target locus provided that its sequence is known (McCallum et al. 2000). TILLING strategy was at first developed for model plant and animal species as an approach towards functional genomics, but now it has become a valuable parameter in crop breeding as an alternative to the transgenic approach. The TILLING techniques require a high frequency of mutations induced by chemical mutagen in combination with a thorough screening method for single nucleotide polymorphism (SNPs) in the targeted sequence. The applicability of this technique for generating a series of new alleles in a gene of interest has already been already exemplified in barley, maize and wheat.

FAO/IAEA has initiated a programme on genetic improvement of underutilised and neglected species through a Coordinated Research Project on 'Genetic Improvement of Underutilised and Neglected Crops in LIFDCs through Irradiation and Related Techniques' in 1998. The overall objective was to safeguard food security, enhance nutritional balance and promote sustainable agriculture in LIFDCs (IAEA-TECHDOC.1426, 2004, Jain 2009). The species which were given emphasis for study comprised medicinal and aromatic plants that are important for the West Asia and North Africa [e.g. argel (*Solenostemma argel*), caper (*Capparis* spp.), oregano (*Origanum syriacum*), mint (*Mentha piperita*), liquorice (*Glycyrrhiza glabra*), aloe (*Aloe* spp.), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*) and henna (*Lawsonia inermis*)], Andean grain crops for Latin America [e.g. quinoa (*Chenopodium quinoa*), canihua (*C. pallidicaule*) and amaranth (*Amaranthus caudatus*)] and nutritive millets for Asia [e.g. finger millet (*Eleusine coracana*), Italian millet (*Setaria italica*) and little millet (*Panicum miliare*)] (Jain 2009).

The FAO/IAEA Mutant Variety Database or MVD collects information on plant mutant varieties (cultivars) released officially or commercially worldwide. The information includes data on the mutagen and dose used, the characters improved and agronomic data if available. The purpose of the database is to demonstrate the significance of mutation breeding as an efficient way for safeguarding and promoting global food security, to serve as a platform for breeders to represent their varieties to a global audience and to stimulate germplasm transfer for cultivation, breeding or genomic studies. By 2000, the Mutant Variety Database (MVD) had information on 2252 mutant varieties or cultivars evolved by mutation and officially released in 59 countries worldwide, mostly in Asia (1 142), Europe (847) and North America (160). Most of the desired genetic variations explored in breeding programmes have occurred naturally and are preserved in different germplasm collections. However, when these collections of germplasms fail to offer a source for a particular trait, it is necessary to depend on other sources of variation. In such cases, mutation techniques can be helpful for the rapid emergence of desired traits.

Though the great majority of induced mutations are recessive and deleterious in nature from a breeder's viewpoint, it is possible to detect desired genotypes from adequately large mutated populations using proper selection tools. Due to these unique probabilities, mutation breeding techniques have significantly contributed to plant improvement worldwide, have a profound impact on the productivity of some crops and gained popularity among the plant breeders.

Three mutant varieties of amaranth have been developed and registered in MVD, viz. Centenario in Peru (mutant variety ID No. 2950), New Asutake in Japan (mutant ID No. 2474) and Sterk in Russian Federation (mutant ID No. 226). The mutant variety Centenario was evolved by irradiation of seed with gamma rays (400 Gy) and approved in 2006. The improved feature of the mutant variety was improved seed production trait. The variety New Asutake was officially approved in 2006 and developed by irradiation with gamma rays (500 Gy). Improved trait of the mutant variety was early maturity. The Sterk mutant variety was developed by the treatment of hybrid seeds of *A. paniculatus* and *A. nutans* by watery solution of chemical mutagen. The improved attribute of the mutant variety was resistance to drought and medium resistance to low temperature.

Mutation breeding has some advantages along with few limitations. The advantages include development of new gene alleles that do not exist in collected germplasm (seeds) pools and introduction of that new gene allele for a commercial variety. So new varieties with desired mutated alleles can be directly used as a commercial variety. The limited genetic changes of any single plant of a mutated population mostly of recessive nature enable breeders to develop a new variety in a short breeding cycle. The disadvantage of mutation breeding is its limitation in generating the desired dominant alleles. It is also less effective than crossbreeding for a trait needs a combination of multiple alleles, such as tolerance to abiotic stresses. The low frequency of mutation needs growing and screening of a large population for selection of desired mutants. This becomes very expensive for traits that have to be evaluated through tedious phenotypic analysis.

Genetic analysis of mutation-induced changes is another aspect. The irradiation-induced changes that occurred in the genome of mutant amaranth can be identified at molecular level employing molecular markers. Amaranth accessions consisting of both control genotypes and mutant lines were analysed with microsatellite markers which yielded reproducible polymorphic band profile clearly differentiating both the species-specific band profile and mutant line-specific profile (Razna et al. 2012). There is a possibility to study molecular markers to distinguish γ-radiation-induced changes on the molecular level. *Amaranthus* accessions of control genotypes and mutant lines when analysed by microsatellite markers produced reproducible polymorphic banding patterns with a potential to distinguish both the species specificity and mutant lines specificity, too (Razna et al. 2012).

8.9 Polyploidy in Amaranth Breeding

Polyploidy has a great implication to bring about sudden abrupt changes in the phenotypes. Some of these may be useful to plant breeding. According to Stebbins (1947) 'polyploidy, one of the best known natural forces of evolution, is the most rapid method of producing radically different but vigorous and well adapted genotypes', hence suitable for development as a technique in plant breeding. Polyploids are of wide occurrence, estimated to be around one-third of angiosperm in nature. Among domesticated species, polyploids are frequent. Stebbins (1957) pointed out that many of the cultivated crops have originated through polyploidy. Natural hybridisation has played a key role in it. Artificially induced polyploidy is useful in plant breeding in many ways, with a definite assumption that polyploidy would revolutionise breeding methods and would help sudden or rapid development of seemingly superior genotypes. Proper handling of polyploids has opened new vistas in plant breeding,

like (1) rapid development of inbreed lines from haploid, (2) development of tetraploid to sustain hybrid vigour, (3) rendering of sterile and fertile hybrids through amphidiploidy and (4) production of intrinsically superior polyploids.

Plant breeders have tried to increase the performance through inducing polyploidy in grain amaranths (Pal and Khoshoo 1977; Behera et al. 1974; Sun and Yue 1993) and vegetable amaranths (Madhusoodanan and Pal 1984; Behera and Patnaik 1975). Polyploids are induced treating the seeds with colchicine or by applying it to the growing point according to the method of Murray (1940) and Behera et al. (1974). Murray (1940) used colchicine solution and observed that one application of 0.25 % aqueous solution of colchicine, applied to the growing point of *A. caudatus*, gave positive results. Madhusoodanan and Pal (1984) generated triploid vegetable *A. tricolor* with desirable performance, but they were impractical to propagate vegetatively or by large-scale production of triploid seeds from diploid-tetraploid crosses. Pal and Khoshoo (1968) worked on agronomic disadvantages in grain producing tetraploids and found a 7 % reduction in the number of female flowers that matured into seeds and the appearance of 'stray' predominantly male plants. Polyploids are shorter and thicker stemmed than normal diploid, and seed size increased by 42–159 % with polyploidy (Pal and Khoshoo 1977; Sun and Yue 1993). Tetraploids showed increase in size of various plant organs though there was very little differences in plant size; tetraploids flowered a week later than the diploids. Amphidiploidy was induced in sterile F_1 hybrid between *A. caudatus* ($n = 16$) and *A. retroflexus* ($n = 17$). All amphidiploids were perfectly fertile and phenotypically alike. The amphidiploids were much longer than tetraploid race. The tetraploid of *A. caudatus* showed 50 % increase in the seed weight. Polyploidy was induced in *A. hypochondriacus*, *A. edulis* and *A. caudatus* by Misra et al. (1971) with colchicine treatment. Tetraploids of *A. caudatus* had 60 % more protein, more lysine and threonine than diploids. It is concluded that polyploidy has increased grain size and weight without decreasing the fertility or nutritive value. Pal and Khoshoo (1977) observed some plants were either predominantly male or female in the C_1 generation and suggested that later generations should be studied to evaluate the stability of grain tetraploids. Pal and Pandey (1982) found that in C_{10} generation tetraploid grain amaranths performed similarly to early generations and concluded that polyploids are stable in *Amaranthus*.

8.10 Genetic Engineering and Biotechnological Approach in Genetic Improvement

Genetic engineering is not just an extension of conventional breeding, but it is also a complementary part of it. In conventional breeding method, new plant varieties are developed through the selection process and require the expression of genetic material which is already existing within species. Conventional breeding involves process that occurs in nature. Genetic engineering primarily involves artificial insertion of foreign genetic material followed by selection. Conventional breeding believes in mixing of features from different populations within a species and then selecting plant complemented with different features from a population or natural genetic element. However genetic engineering relies on incorporation of genetic element in random location that can disrupt complex gene interactions. Many of the products of such random insertion may yield unexpected effect.

The amount and availability of nutrient in plant can now be improved applying biotechnological strategies. Those strategies include simple plant selection for varieties with nutrient-rich seeds, crossbreeding to incorporate a desired trait within a plant and genetic engineering to manipulate the nutrient content of the plant. In plant crossbreeding, all genes of the parent plants are combined together, and the progeny has both desirable and undesirable traits. To eliminate undesirable traits, plant breeders used to 'backcross' the new plant varieties with other plants over several generations. Using the genetic engineering techniques, the gene(s)

responsible for a desired trait(s) are introduced in a precise and controlled manner within a relatively short period of time. Golden rice, containing carotenoids, and rice with higher amount of iron are two classical examples of genetically engineered plants enriched with nutrients. However, public concerns regarding safety, appearance and ethics must be overcome before these products can be effectively introduced into the food supply.

Advancement in molecular biology has encouraged the significant development in biotechnology which has dramatically enriched our knowledge to understand plant genomics and manipulate plant gene pool. Marker-assisted selection (MAS) is one of such techniques which has enhanced the efficiency of breeding for specific trait, and genetic engineering has made it possible to transfer genes from almost any organism into a crop species. The practice of plant breeding has been shifted from the public domain, and thus, selection criteria are increasingly vulnerable to being dominated by private profit motives rather than public good motives (Simmonds 1990).

Plant tissue culture is a promising applied branch of plant biotechnology which includes a set of in vitro techniques, methods and strategies. Tissue culture techniques are actually part of a large group of strategies and technologies, ranging from molecular genetics, recombinant DNA technologies, genome characterisation, gene transfer techniques to aseptic growth of cells, tissues, organs and in vitro regeneration of plants. Tissue culture protocols are available for most crop species, though standardisation or optimisation of methodologies for specific purpose is yet to be achieved for many crops, especially cereals and woody plants. Tissue culture methods, in combination with molecular techniques, have been successfully utilised to incorporate specific traits through gene transfer. In vitro culture of isolated protoplasts, anthers, pollen grains, ovules and embryos has been used to create new genetic variation in the breeding lines, often via haploid production. Cell culture has also produced somaclonal and gametoclonal variants with crop improvement potential. The culture of single cells and meristems can be effectively used to eradicate pathogens from planting material and thereby dramatically improve the yield of established cultivars. Large-scale micropropagation laboratories are already established supplying millions of plants for the commercial ornamental market and the clonally propagated crop market.

8.11 Genetic Transformation in Breeding Amaranths

Technological innovation in plant biotechnology is an important catalyst in any crop enrichment programme. Stable *Agrobacterium*-mediated genetic transformation offers advantages in transferring one or few copies of DNA fragments carrying the genes of interest. It can be carried out on the whole plant by applying either tissue infiltration techniques (Tague and Mantis 2006) or floral dip (Yasmeen et al. 2009). This method is advantageous over formal tissue culture-based techniques as it directly results into transformed seed by passing the lengthy tissue culture process and somaclonal variations (Yang et al. 2009).

A protocol for *Agrobacterium*-mediated genetic transformation of *Amaranthus tricolor* was generated through cocultivation of explant with *Agrobacterium rhizogenes* (Swain et al. 2010). Bacteria plant-specific factors which influenced transformation were optimised. Of the two *Agrobacterium* strains used, LBA9402 was found to be more infectious compared to A4. Few significant observations were recorded, like greater response of explants from garden-grown plants to in vitro culture than those from in vitro cultures and better response of stem internodes than leaves. Significantly higher transformation frequency was achieved by immersing pre-pricked explants in bacterial suspension rather than the direct injection method. The infection of internode explants with the LBA9402 strain followed by cocultivation on growth regulator-free MS medium (MS0) for 5 days resulted in the emergence of hairy roots up to a maximum frequency of 97.22 %. Roots were individually cultured in MS0, but fortified with bactericidal antibiotic

(500 μg ml^{-1} cefotaxime). Rhizoclones showing prolific growth were maintained through successive subcultures in MS0. Opine gene was introduced, and expression of transgene was revealed by positive agropine and mannopine synthesis in all selected transformed rhizoclones. Shoot regenerated from root clones showed capability of auxin-independent growth and opine proficiency. Shoot regeneration was stimulated in MS augmented with 2.0 mg l^{-1} zeatin. Pal et al. (2013) have optimised a genetic transformation protocol for major leafy vegetable crop, *Amaranthus tricolor* L., using epicotyl explant cocultivated with *Agrobacterium tumefaciens.* Two disarmed *A. tumefaciens* strains EHA 105 and LBA 4404, both carrying the binary plasmid p35SGUSINT containing the neomycin phosphotransferase II gene (*npt*II) and the β-glucuronidase gene (*gus*), were evaluated as vector. The former exhibited a higher transforming efficiency. Several key factors influencing the transformation events were optimised.

Mature embryos of *Amaranthus hypochondriacus* (amaranth) were involved to develop an in vitro culture system to be utilised for plant regeneration and genetic transformation (Jofre-Garfias et al. 1997). Plants were regenerated from embryo-derived callus cultured on Murashige and Skoog medium supplemented with 10 μM 2,4-dichlorophenoxyacetic acid or 3,6-dichloro-2-methoxybenzoic acid and 10 % coconut liquid endosperm. Transgenic plants were generated by infection of mature embryo explants with a disarmed *Agrobacterium* strain containing the plasmid pGV2260 (pEsc4), harbouring the genes encoding neomycin phosphotransferase type II and *β*-glucuronidase. The presence of transgenes in the genome of transformed amaranth plants and in their progeny was detected by Southern blot hybridisation. A pea chlorophyll a/b-binding protein promoter was used for tissue-specific and light-inducible expression in transgenic amaranth plants and their progeny.

The success in the transformation of *Amaranthus* by floral dip would broaden the possibilities for the exploitation of the available reported method on many other alternative crops. *Agrobacterium*-mediated transformation by floral dip has been evolved and successfully applied in the popular model plant such as *Arabidopsis thaliana* and *Medicago truncatula. Agrobacterium*-mediated transformation through floral dip followed by rapid selection process after transgenic event has become a phenomenal approach as it will help to overcome the difficulties anticipated during lengthy tissue culture procedures and screening transformed progenies. Munusamy et al. (2013) developed three constructs (p5b5 (14,289 bp), p5d9 (15,330 bp) and p5f7 (15,380 bp)) in pDRB6b vector, with hygromycin as a selectable marker gene, which were introduced individually into *Agrobacterium tumefaciens* strain (AGL1). The bacterial cell suspension was applied to *Amaranthus* inflorescence drop by drop and left to produce seeds (T_1). The T_1 seeds were germinated and grown to raise the seedlings under non-sterile condition. Hygromycin selection of seedling on cotyledonary leaves resulted in identification of 12 putative transformants, three from construct p5b5, four from construct p5d9 and five from construct p5f7. All positive putative transformants that were selected at the first stage or primary screening through hygromycin spraying also showed positive result in leaf disc hygromycin assay. A ~750 bp amplified hygromycin gene was further verified through sequencing. The results indicated that *Amaranthus* inflorescences could be transformed easily, and the transformed progenies could be identified through a combination of simple and rapid methods.

The plant metabolite oxalic acid is commonly considered as a food toxin having negative effects on human nutrition. Oxalic acid is present as nutritional stress in many crop plants like amaranth and *Lathyrus*. Oxalic acid has also been reported to be involved in the attacking mechanism of several phytopathogenic fungi. So degradation of oxalic acid in the crop plants through introduction of a specific gene would have twofold advantages: firstly, nutritional stress will be minimised, and secondly resistance to fungal pathogen will be conferred. Oxalic acid is degraded by two major pathways, i.e. decarboxylation and oxidation. Decarboxylation of the oxalic acid takes place either by conversion of oxalic acid to oxalyl CoA by means of oxalyl-

CoA decarboxylase or directly to CO_2 and formic acid by oxalate decarboxylase. In oxidation process oxalic acid is broken down to CO_2 and H_2O_2 that has been detected in plants. Mehta and Datta (1991) earlier reported purification and partial cDNA cloning of oxalate decarboxylase from *Collybia velutipes*. The enzyme has several advantages over other oxalate degrading enzymes. Firstly, OXDC1 is specific for oxalate, and it catabolises oxalic acid to formic acid (nontoxic organic acid) and CO_2 in a single step without requiring a cofactor. Secondly, the enzyme is active at low pH which would be helpful as most of the oxalates are localised in plant cell vacuoles, where pH is low.

Decarboxylative breakdown of oxalic acid is catalysed by oxalate decarboxylase (OXDC) in a substrate-specific reaction, producing formic acid and carbon dioxide. Little success has been achieved in attempts aimed at reducing oxalic acid levels and understanding of the biological significance of OXDC in crop plants. To study the function of OXDC and the metabolic consequences of oxalate downregulation in a heterotrophic, oxalic acid-accumulating fruit, Kesarwani et al. (2000) generated transgenic tobacco and tomato (*Solanum lycopersicum*) plants expressing an OXDC (*FvOXDC*) isolated from the fungus *Flammulina velutipes* specifically in the fruit. These fruits of transgenic plant showed up to a 90 % reduction in oxalate content, with concomitant increases in calcium, iron and citrate. A full-length cDNA for oxalate decarboxylase was isolated by using 5′-rapid amplification of cDNA ends polymerase chain reaction of a partial cDNA as cloned earlier (Mehta and Datta 1991). Genomic library from *Collybia velutipes* was screened with this cDNA as a probe, and a genomic clone was isolated and sequenced. Genomic sequence was analysed and compared with the cDNA sequence which revealed that the cDNA was interrupted with 17 small introns. The cDNA has been successfully expressed in cytosol and vacuole of transgenic tobacco and tomato plants. The transgenic plants showed normal phenotype, transformation was stable and the transferred trait was stably inherited to the next generation. The recombinant enzyme showed partial glycosylation, and the transgene showed oxalate decarboxylase activity both in vitro and in vivo conditions. Transgenic tobacco and tomato plants expressing oxalate decarboxylase activity showed significant resistance to fungus *Sclerotinia sclerotiorum* that uses oxalic acid during infestation. The work also demonstrated a novel approach to develop resistance against fungal infection through transgenesis (Kesarwani et al. 2000; Chakraborty et al. 2013). The transformation procedure using the cDNA of oxalate decarboxylase followed in the case of tomato and tobacco can be considered as standard protocol to transform vegetable amaranths where oxalate content to some extent is lowering its acceptability.

Protein is essential for normal physical growth and development. So deficiency of protein is the most crucial factor regarding increase of morbidity and mortality especially in developing countries. Efforts to improve protein quality and quantity have received limited success. Improvement in nutritive values of crop plants, in terms of amino acid composition and availability of essential amino acids, has been a major long-term objective of plant breeding programmes. Raina and Datta (1992) reported earlier the cloning of a novel seed albumin gene *AmA1* from *Amaranthus hypochondriacus*. The *AmA1* protein was purified to homogeneity. It is nonallergenic in nature and rich in all essential amino acids, and the amino acid composition complements well with standard for optimal human nutrition as proposed by the World Health Organisation. The protein is a 35 kDa monomer with four isoforms that can be separated by chromatography. A full-length cDNA of *AmA1* protein was characterised and isolated. The *AmA1* gene is expressed during early embryogenesis. Germinated seeds did not contain any *AmA1* mRNA; no RNA was detected in leaves, root or other parts of the plant indicating the tissue-specific expression of the gene. The *AmA1*-coding sequence was successfully introduced in potato to improve the nutritive quality of potato, and expression of the gene was tuber-specific and constitutive nature. The experiment showed unique outcome. The transgenic population showed a significant increase in growth and

production of tubers, as well as both qualitative and quantitative improvements of protein. Chakraborty et al. (2000) reported the development of transgenic potatoes with enhanced nutritive value by tuber-specific expression of an *AmA1* seed albumin protein gene, in seven genotypic backgrounds suitable for cultivation in different agro-climatic regions. Transgenic tubers showed up to 60 % increase in total protein content and a significant increase in the concentrations of several essential amino acids which are otherwise limited in potato. Moreover, this genetic manipulation also exhibited enhanced photosynthetic activity, moderate increase in tuber yield and a concomitant increase in total biomass. The comparison of protein profile suggested that the proteome rebalancing might have caused increased protein content in transgenic tubers (Chakraborty et al. 2010). Furthermore, the data on field performance and safety evaluation indicated that the transgenic potatoes are suitable for commercial cultivation.

Transgenic potato plant with engineered *AmA1* seed storage protein gene in tuber might have utilised the free amino acids for the synthesis and accumulation of the storage protein causing a depletion in endogenous amino acid pool. Such depletion of endogenous free amino acid might have triggered photosynthetic activity and increased the photosynthetic rate. It is evidenced that increased photosynthesis is responsible for the increase in protein synthesis and yield. Thus in transgenic potato, the increased rate of photosynthesis increased total protein content. Seed storage proteins are considered to act as the sink to control the movement of photosynthate into developing organs. It is suggested that *AmA1* seed storage protein probably acts as a sink protein in transgenics, thereby regulating the movement of metabolites, specially the amino acids, into the developing tuber where they are utilised to synthesise new proteins and promote growth (Chakraborty et al. 2010). This study can be treated as a major breakthrough in gene expression and translational research in which a genetically engineered plant with a transgene of seed storage protein has led to nutritional improvement with essentially no negative collateral effects on crop quality or yield. The applicability of genetically altered plants on commercial scale depends on several factors like stable integration and expression of the transgene under the different genotypic backgrounds of the host species, wider environmental adaptability and sustainable production, including food safety. In vitro and in vivo studies on experimental animals demonstrated that the transgenic tubers are also safe for human consumption.

The nutritive value of wheat is mainly characterised with low content of essential amino acid like arginine, lysine, hystidine and threonine. During milling process with the removal of aleurone layer and embryo tissue, high amount of essential amino acids is lost. Several attempts have been made to improve the amino acid content by conventional breeding methods. Recently as a pioneering achievement, herbicide-resistant transformed wheat has been developed through microprojectile bombardment of regenerable embryonic callus (Vasil et al. 1992) which has opened up a new avenue to improve agronomic traits of transgenic crops, as well as the grain composition and quality. An attempt was made to express recombinant storage protein with the appropriate amino acid profiles in the wheat endosperm tissue to improve the essential amino acid content in wheat flour (Cecilia 2009). The transgene *AmA1* was stably integrated into the wheat genome by a competent wheat endosperm-specific promoter (1Bx17 HMW-GS), expressed in the wheat endosperm and transmitted to the next three generations. The transformation pattern of the foreign gene showed tissue specificity. The amount of in vivo expression of *AmA1* protein gene showed simultaneous increase in the essential amino acid content of the transgenic flour with no significant change in the total protein content. Nutritive value of several other crops can be enriched by transformation with the novel *AmA1* protein gene.

All the experimental outcome emphasised the fact that expression of novel *AmA1* gene might be a potential strategy for the nutritional improvement of food crops. Transformation of other crops with this unique *AmA1* protein gene is quite feasible.

Unwanted transgene escape occurs in several crops like oilseed, rapeseed, maize, cotton, etc. They are found in the variant cultures and wild types as well as in hybrids. The consequence of the continuous transgene escape in the cultivation field cannot be predicted reliably, because that depends surely on the ability of the transgene to influence the fitness of the plant containing the introgressed gene. It would not be logical to wait until something adverse happens. Transgene flows destroy the gene pool at its centre of origin and domestication. Care must be taken to minimise the transgene escape into the wild as effectively as possible applying all available measures during developing genetically modified (GM) crops.

8.12 Molecular Markers and Genome Research for Using Genetic Resources in Plant Breeding

The utility of molecular markers and genome research regarding the utilisation of Plant Genetic Resources (PGR) for crop improvement include several aspects:

1. Studies on diversity to differentiate genetically similar or distinct accessions and to determine individual degrees of heterozygosity and heterogeneity within PGR populations
2. Genetic mapping to identify markers in close proximity to genetic factors affecting quantitative trait loci (QTLs), followed by marker-assisted selection (MAS) of desired genotypes in segregating populations
3. Exploitation of valuable QTLs from PGR
4. Studies on the allelic variability of PGR collections and identification of those alleles which are beneficial for important agronomic traits

Molecular markers should be selectively neutral, highly polymorphic, co-dominant, well dispersed throughout the genome and cost- and labour-efficient to be utilised efficiently in diversity analysis (Bretting and Widrlechner 1995). Protein markers like isozymes and DNA markers like restriction fragment length polymorphisms (RFLPs), microsatellites, simple sequence repeats (SSRs), etc., satisfy the above parameters. The development of molecular markers like microsatellites and SSRs requires a prior knowledge of DNA sequence. A few dominant and universal molecular markers such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphisms (AFLPs) have also been employed to analyse the variability of in Plant Genetic Resources.

A heterotic clustering of genotypes appropriate for hybrid breeding represents a different approach of diversity analysis at the molecular level with a principle of searching out a positive correlation between genetic distance and heterosis, i.e. the more the distance between two genotypes of a crop species are, the more will be the heterozygosity genetically. Hybrids originating from crosses between them are expected to show heterosis (Melchinger et al. 1999; Reif et al. 2003a, b). Since high degree of heterosis does not necessarily mean high hybrid yield, so the crossing effect on heterosis and hybrid performance needs to be differentiated and evaluated separately. Recent studies have shown that if the molecular markers applied for genetic diversity assessment are linked to performance QTLs, rather than from using neutral markers, the correlation between diversity measures and hybrid performance appears more consolidated and conclusive (Vuylsteke 1999; Vuylsteke et al. 2000; Jordan et al. 2003).

Marker-assisted selection (MAS) can be helpful (i) to select individuals carrying molecular markers which are linked to the trait of interest, instead of exercising extensive phenotypic evaluation (foreground selection), and (ii) to minimise unwanted parts of the donor genome, including the linkage drag (background selection). Foreground selection needs a strong linkage between the trait of interest and its flanking markers for which one is selecting. Background selection requires genotyping with a larger number of markers, covering the whole genome. MAS has been proved efficient for the transfer of simply inherited qualitative traits from germplasm resources into elite breeding lines applying backcross procedures. It is particularly useful for

the traits which are recessive in nature, can be assessed only after flowering or that are very difficult and expensive to assess. Through combined application of both foreground and background selections, the transfer of monogenic trait from a germplasm resource into an elite breeding line may be completed within 3–4 generations in place of usual six generations of conventional laborious backcrossing with the same proportion of the recurrent parent genome (Ragot et al. 1995; Frisch et al. 1999). MAS for multigenic, quantitative traits at first requires the identification of the genomic regions (QTLs) that affect the trait of interest. In classical QTL mapping, a segregating population (e.g. F_2, F_3 or recombinant inbred population) is developed from two inbred lines. This mapping population is evaluated for the trait(s) of interest. Simultaneously, the population is genotyped with a number of markers, and a genetic map is constructed from the marker data. In the final QTL analysis, data is analysed for co-segregation of particular markers with the trait of interest which is ultimately followed by transfer of favourable QTL alleles into elite breeding lines via pure MAS or MAS in combination with phenotypic selection.

8.13 Molecular Breeding in Amaranths

In general, plant molecular breeding depends on the understanding of the genetic control of target traits of interest at the molecular level. Information on molecular genetic is also of great help in framing a proper mutation breeding strategy. Firstly, it is important to assess the feasibility and potentiality to induce a mutation of interest. Secondly, a mutation may have pleiotropic effects if the gene is situated at the upstream or at the middle of a long biosynthetic pathway. Thirdly, knowledge of genes controlling a trait of interest would constitute the very basis of the TILLING (Targeting Induced Limited Lesions IN Genomes) method. Induction of mutation is the starting point in mutation breeding, and a low frequency of mutation has been a severe limiting factor. Equipped with knowledge of the DNA damage and repair, we should be able to design strategies of mutagenic treatment to significantly increase the mutation frequency. When the genes responsible for DNA damage repair are deleted or mutated, these breeding lines could become highly vulnerable and sensitive to mutagenic treatment, and frequency of mutation could be significantly enhanced. Such lines are commonly termed as ‘super mutable genetic lines’. Once mutation of important traits is induced and identified in such ‘super mutable genetic lines’, the transgenes and mutated genes can be separated through self-crossing as they are not linked to one another; hence non-transgenic stable mutant lines could be generated. In conventional mutation breeding, mutations generated through induced mutation are used either directly or indirectly (through crosses with other varieties) to develop a new variety or elite breeding line. It is now possible to tag the mutated genes, accumulate them into a single elite breeding line and follow them up in subsequent breeding programmes.

Improved knowledge on molecular genetics and genomics and better understanding of genotypic variability and rapidly emerging molecular techniques have enabled plant breeders to apply induced mutation techniques in plant breeding to evolve novel varieties more judiciously and precisely than ever before. Plant molecular mutation breeding has emerged as a strong, efficient procedure for plant improvement in a very short period of time in which genomic and molecular information are utilised in framing the breeding strategies, screening, selection and verification of induced mutants and in the subsequent utilisation of mutated genes in the breeding process. It is based on the knowledge of DNA damage, repair and mutagenesis, plant molecular genetics and genomics of important agronomic traits as well as induced mutations.

Responses of different genotypes and plant tissues to different mutagens, usually measured using lethal doses (LD), genetic chimaeras formed due to mutagenic treatment and their effect on transmission of mutated alleles and segregation in the following generation. Such knowledge of genetics is very significant to determine

proper doses and modes of mutagenic treatment, as well as for the methodology of growing M_2 populations and harvesting.

In spite of great potentiality of grain amaranths as a source of nutritious food, little efforts have been devoted to explore its genomics. A partial transcriptome *of A. hypochondriacus* has been reported for the understanding of the mechanism adopted by the species of the family Amaranthaceae in response to the environmental stress (Delano-frier et al. 2011). A limited sequencing of the genome of *A. tuberculatus* has been done to understand the mechanism applied by the species to resist three herbicides. A more systematic and concrete approach towards exploring the amaranth genome includes creation of BAC libraries for all three grain amaranths (Maughan et al. 2008), development of microsatellite markers (Mallory et al. 2008) and construction of linkage map using single nucleotide polymorphism (Maughan et al. 2010). Studies on the genomics of grain amaranths in respect to their unique nutritional properties and C_4 photosynthesis among the edible dicots are limited. Keeping in view these objectives, Sunil et al. (2014) working on genome and transcriptome of *A. hypochondriacus* reported a draft genome as the first of its kind of grain species highlighting the high-lysine phenotypes and C_4 evolution under Caryophyllales. Out of 411 linkages, single nucleotide polymorphisms (SNPs) derived from homozygous region of the grain amaranths, 355 SNPs (86%) were reported to be present in scaffolds and 74% of the 8.6 billion bases of the sequenced transcriptome map to the genomic scaffold. The genome of *A. hypochondriacus* reported codes for at least 24829 proteins and contains 13.76% of repeat elements. They have also placed two workable hypotheses for high-lysine phenotype including gene number polymorphism for AK (aspartate kinase) and eQTL of DHDPS (dihydrodipicolinate synthase). Lysine biosynthesis is mainly regulated by two allosteric enzymes aspartate kinase and dihydrodipicolinate synthase. Any kind of polymorphism within these two gene loci is expected to have a correlation with high-lysine phenotype in amaranths. The high free lysine content in seeds correlates well with expression level of DHDPS gene, suggesting eQTL for high-lysine phenotype, though expression profiles of all other enzymes comprising AK show no correlation with high-lysine content in seeds. Annotations of all the genes in the lysine biosynthetic pathway using comparative genomics and expression analysis offer insight into high-lysine phenotype. The proposed draft genome (Sunil et al. 2014) can be very useful for the advancement of the understanding of diverse phenotypes that are unique to amaranths like rich nutritional profile, rapid growth, drought resistance, wide adaptability and characterisations of genes for betalain pigment.

It has been well documented that DNA is subjected to continuous damage, and the cell has its own machinery to address such injury. There are different ways for the repair of DNA damages caused by different types of mutagen. This knowledge is very important for proper framing of mutagenic experiments, so that an enhanced mutation frequency can be achieved. Cells with damaged DNA will survive only when these damages are repaired either correctly or erroneously. The repair done erroneously will be fixed in the genome as induced mutations. The nature of DNA damage caused by different types of mutagens to a great extent determines the molecular feature of induced mutations.

8.14 Plant Tissue Culture Techniques in Amaranth Breeding

Currently, there are not much published reports on the tissue culture of *Amaranthus*. Probably the first report on in vitro growth and morphogenetic response was published by Flores et al. (1982). He has developed an in vitro culture system for both grain and vegetable amaranths. Leaf discs and hypocotyl segments from 2 to 3 weeks old seedlings of *A. hypochondriacus*, *A. cruentus* and *A. tricolor* were cultured in MS and B5 media supplemented with 2,4-dichlorophenoxyacetic acid, α-naphthyl acetic acid, benzyladenine and zeatin in various combinations. The tissue culture

showed various responses like rapid callus growth, abnormal root formed on the leaf disc and embryo-like structure developed from the surface and veins on the disc with slight modification of media. Viable protoplasts from primary leaf mesophyll tissue were isolated from all the three species enzymatically. Protoplast culture is of great potential for genetic manipulation of plant. Primary result indicated that protoplast from *A. tricolor* can expand and form a new cell wall. This was a pioneering work in amaranth tissue culture forming a base for future biotechnological work like in vitro propagation, germplasm preservation and transfer, and agronomic improvement through genetic manipulation. Shoot tip-derived plantlet of *A. caudatus*, *A. tricolor*, *A. hypochondriacus*, *A. retroflexus* and *A. viridis* flowered in vitro following 8–32 weeks of culture. Shoot tip was cultured on MS medium containing salt and 30 g/l sucrose, 100 mg/l *myo*-inositol, 0.4 mg/l thiamine and 8 g/l agar. Addition of NAA (0.1 mg/l) enhanced inflorescence production but was not necessary for flower production. The fruits produced by *A. gangeticus* and *A. retroflexus* dehisced, and their seeds were dropped on the surface of agar medium, where they germinated immediately. Bennici et al. (1992) studied on several species and varieties of *Amaranthus* and showed its potential with regard to dedifferentiation and morphogenetic responses in vitro. On the background of these studies, Guidea et al. (2012) reported micropropagation of selected genotypes and their subsequent exploitation, rescue of the genetic variation or induction of new variation, phytoremediation studies and use of cell biomass to obtain phytochemicals of practical interest. The study was to evaluate the growth and morphogenetic responses of various types of explants from three varieties of *Amaranthus* species: *Amaranthus cruentus* 'Amont', *Amaranthus hypochondriacus* 'Intense Purple' and *Amaranthus* ssp. 'Plenitude'. The seeds belonging to these three varieties of *Amaranthus* species were germinated in vitro in aseptic conditions on MS basal medium containing half strength as regards the concentration of macro- and microelements, 3 % sucrose, 0.8 % agar noble, pH 5,8, without the addition of hormones. Chemical compositions of the culture media containing inorganic salts and hormones have an important role to play for successful aseptic in vitro culture of explants. Preferential depletion of some elements leads to symptoms of deficiency or toxicity, sometimes with necrosis of the inoculum. The underexploited crops, including amaranth, offer a special challenge for the use of in vitro approaches, because extensive efforts are required from the plant breeders to select and improve this plant material. In vitro systems have important practical applications not only for rapid breeding of this rediscovered crop but also for producing cell biomass to be used as source of phytochemicals of practical interest.

Tissue culture studies in amaranths are just beginning. There are several areas which can be emphasised, like the control of oxalate/nitrate content in cell culture may provide clues regarding regulation of nitrogen assimilation and consequently may lead to the selection of low-oxalate cell/plant. The wide variability available in the germplasm can be used to identify cell lines more tolerant to stress and also to involve in plant regeneration system. Amaranth germplasm represents a potent source of herbicide-resistant trait. This resistance could be introduced in cultivated form (grain and vegetable) via protoplast fusion with their weedy relatives.

In spite of significant potential, the absence of adequate knowledge on basic process governing cell division and differentiation pattern in amaranths is creating obstacles before the application of cell and tissue culture techniques for their improvement. Conditions required for induction of somatic embryogenesis are determined empirically. Definite guideline is yet to be standardised, except for few model systems. Research is slowly gaining momentum towards a primary understanding of the process involved, through the use of mutant cell lines (Sung and Dudits 1981). Though application of tissue cultural methodologies in amaranth is limited, still there are several areas where tissue culture can be of great potential.

Evolution of Sexuality in Amaranths

9

9.1 General

The genus *Amaranthus* is unique in showing a wide range of sexuality from gynomonoecy, monoecy to dioecy and also variability in mating behaviour from self-compatibility to obligate outcrossing, i.e. from monomorphic reproductive system to dimorphic reproductive system. Bisexual flower represents the ancestral condition in angiosperm, and monoecy is considered to have been derived from bisexual condition through intermediate gynomonoecious or andromonoecious forms. Dioecy results in a division of labour between sexes and may ultimately lead to greater reproductive efficiency. A variety of hypotheses have been proposed for evolution of dioecy from hermaphroditism through monoecy, gynodioecy and androdioecy. Monoecy in the grain amaranths are supposed to have originated from the dominant cosexual form having exclusively bisexual flower through sterility mutation followed by subsequent specialisation in flower form and position. Dioecy may have evolved from monoecy through disruptive selection on male and female reproductive allocation, followed by gender specialisation ultimately leading to unisexual plants. In the case of vegetable amaranths, genetic modification in female fertility of hermaphrodite population might have resulted in the formation of bisexual member with non-functional gynoecium. Later male sterility gene might have played a role to give rise to female member. Subsequent inbreeding between these derivatives having sexual compatibility, gynomonoecious member might have originated. Monoecy in amaranths might have transmitted from cosexual species of Chenopodiaceae-Amaranthaceae alliance or Chen-Am alliance. Molecular analysis of the Caryophyllales established Chen-Am alliance as a monophyletic lineage.

9.2 Trend of Sexuality in Plants

Flowers are the most varied structure in the flowering plants. Such variability is a key factor and primarily instrumental to achieve mating success. Selective forces are responsible for origin and maintenance of sexual diversity in flowering plants. Flowering plants are mostly hermaphrodite producing bisexual flowers. Few flowering plants (−10 %) have unisexual flowers with a wide spectrum of gender strategies that involve varied combinations of female, male and hermaphrodite flowers at the plant population level. Though dioecy is widely distributed in flowering plants occurring in nearly half of all families, overall frequency of dioecious species is only 6 % (Renner and Ricklefs 1995) signifying its rare association with successful evolutionary diversification. Dioecy is commonly associated with unspecialised pollination system that involves wind, water or generalised pollinator rather than the more specialised pollinator that commonly drives floral diversification and

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reproductive isolation in many cosexual flowering plants (Johnson et al. 1998). Sexual structure can be spatially separated in a flower or can become functional at different times reducing the likelihood of self-pollination. Gradual sexual diversity has emphasised the mechanism that aimed at promoting outcrossing and reducing likelihood of inbreeding depression. Conventionally floral traits have been considered as anti-selfing mechanism that encourages cross-pollination preventing self-pollination

Strong empirical evidences indicate that bisexual flower represents the ancestral condition in angiosperm (Richard 1997; Doyle 1998; Endress 2001). Monoecy with separate male and female flowers on the same plant is considered to be derived condition from bisexual condition (Mitchell and Diggle 2005). Monoecy has traditionally been considered as originating from gynomonoecy or andromonoecy (Wilson 1979; Bawa and Beach 1981; Bertin 1982). Many research have been directed to explore the evolutionary transition giving rise to dioecious condition, i.e. male and female flowers on separate plants (Renner and Won 2001; Gleiser and Verdu 2005; Case et al. 2008). Dioecy results in a division of labour between sexes and may ultimately lead to greater reproductive efficiency (Lloyd 1982). Male and female reproductive function may be optimised through different selective processes ultimately leading to phenological dimorphism in male and female plant.

Despite seemingly complex pattern of sexual diversity in flowering plants, two broad and fundamentally distinct patterns of gender variation or sexual system have been recognised – monomorphic sexual system and dimorphic sexual system (Bawa and Beach 1981). In the former system, species bears bisexual female and/or male unisexual flowers on the same individual, leading to monoecious, gynomonoecious, andromonoecious and trimonoecious condition. In the latter system, species have dimorphic sexual system, i.e. unisexual individual such as dioecy, gynodioecy and androdioecy.

Despite the simultaneous/common occurrence of hermaphroditism and monoecy, little attention has been paid to the factors favouring evolutionary transition between these two systems. Establishment of monoecy requires specialisation in the shape, size and positioning of the male and female flower (Shmida et al. 2000). But before such specialisation, a mutant with some unisexual flowers needs to be established first. Only after the unisexual mutant is firmly established, subsequent mutation related to specialisation in flower form and position has given rise to monoecy.

Many investigations have been directed towards exploring traits leading to dioecy (Renner and Won 2001; Gleiser and Verdu 2005; Case et al. 2008). A variety of hypotheses have been proposed for evolution of dioecy from hermaphroditism through monoecy, gynodioecy and androdioecy. But not all these pathways have received the same theoretical attention. There are no well-authenticated cases of androdioecy as an intermediate stage in the evolution of dioecy and very limited evidence for the evolution of androdioecy from hermaphroditism. Two fundamental evolutionary pathways for the origin of dioecy through monoecy and gynodioecy are generally recognised. Both involve the transition from gender monomorphism to dimorphism. In the gynodioecy pathway male sterility genes spread in bisexual population, resulting in an intermediate stage that involves females and hermaphrodites. Genetic modifiers of female fertility gradually convert hermaphrodites to male resulting in dioecy. The monoecy pathway is less well investigated, assumed to involve disruptive selection on male and female allocation in monoecious population which gradually increased gender specialisation until unisexual plants originated. Monoecy has originated several times from the dominant cosexual condition in angiosperm population having exclusively hermaphrodite flower. This might have occurred through sterility mutation that produced unisexual flowers.

A lot of investigations have been done on the gynodioecy, but till date little attention has been given to monoecy pathway. It is not clearly resolved whether the transition is determined solely by selection or quantitative genetic variation governing sex allocation or whether major male sterility genes are also having a role. A recent molecular phylogenetic study (Renner and

Won 2001) provides convincing evidence of multiple origins of dioecy from monoecy in the primary Neotropical shrub family Siparunaceae. It is very vital to determine the circumstances under which selfing rate increased resulting in inbreeding depression in ancestral cosexual population. Such condition facilitates the spread of unisexual variants that favour outcrossing. A link between origin of gender dimorphism and conditions that promote inbreeding depression might occur through the action of polyploidy. Chromosome doubling in plants can result in the inhibition of self-incompatibility leading to self-compatibility (Chawla et al. 1997) providing opportunities for self-fertilisation. Molecular phylogeny in North American *Lycium* (Solanaceae) indicates that gender dimorphism has evolved only in species that are polyploid and self-compatible but their close relatives are diploid and self-incompatible. Such pattern also found to have originated independently in South African species of *Lycium.* Polyploidy might have acted as a trigger for the evolution of gender dimorphism (Miller and Venable 2000). Large plant size might be another condition that can potentially lead to gender dimorphism (de Jong and Klinkhamer 1994). In plant species with large stature (like shrubs and trees), a considerable amount of selfing can occur due to the presence of many open flowers on a plant at the same time leading to inbreeding depression. Recent marker gene analysis of the clonal aquatic plant *Sagittaria latifolia* offers evidences that indicate that geitonogamous selfing and strong inbreeding depression have influence in evolutionary transition from monoecy to dioecy (Dorken et al. 2002). Population of both sexual systems occur together in this species making it an excellent model for studying the evolution of combined versus separate sexes.

9.3 Sexuality in Amaranths

The genus *Amaranthus* is unique in mating behaviour ranging from self-compatibility to obligate outcrossing, i.e. from monomorphic reproductive system to dimorphic reproductive system. It shows a wide range of variability in sexuality from gynomonoecy, monoecy to dioecy. Dioecy is the rare breeding system among angiosperm. It may affect the ability of a lineage to avoid extinction or encourage speciation. Monoecy is the predominant phenomena in amaranths. Grain amaranths are exclusively monoecious, while vegetable amaranths are predominantly monoecious with exception. It would be logical to presume that both grain and vegetable amaranths were evolved and domesticated in their respective centre of origin from their weed progenitor having wide range of variability in sexuality ranging from monoecy to dioecy.

Amaranths followed the general evolutionary trend in sexuality, i.e. from monoecy to Dioecy. It can be presumed that monoecy in amaranths might have transmitted from cosexual species of Chenopodiaceae-Amaranthaceae alliance or Chen-Am alliance. The Chen-Am alliance is of worldwide distribution, comprising 2400 species. The alliance is noted for the evolution of C_4 photosynthesis, halophytism, xerophytism and a variety of breeding system. The close relationship of Chenopodiaceae and Amaranthaceae has been recognised based on core floral formula consisting of five tepals, five stamens and 2–3 carpels (Hershkovitz 1989). Molecular analysis of the Caryophyllales (Manhert and Rettig 1994; Downie and Palmer 1994; Downie et. al. 1997; Cuenoud et. al. 2002) established Chen-Am alliance as a monophyletic lineage.

The grain amaranths are grown and consumed both as green vegetable and pseudocereal. Though grain amaranths are crops of America but later they migrated to Asia and Southeast Asia, Europe and North America. Vegetable amaranths specially *Amaranthus tricolor* and *A. dubius* probably originated and domesticated in Asia and Southeast Asia (Grubben and Van Sloten 1981) and later spread throughout tropical and temperate regions of Africa, Central America and Europe by the immigrants (Martin and Telek 1979). *Amaranthus blitum* aggregate included in sect. *Blitopsis* of subgen. *Albersia*, comprising *A. blitum* and *A. emarginatus,* was studied morphometrically (Das and Iamonico 2014). *Amaranthus blitum* with two varieties are supposed to have originated and domesticated in Mediterranean

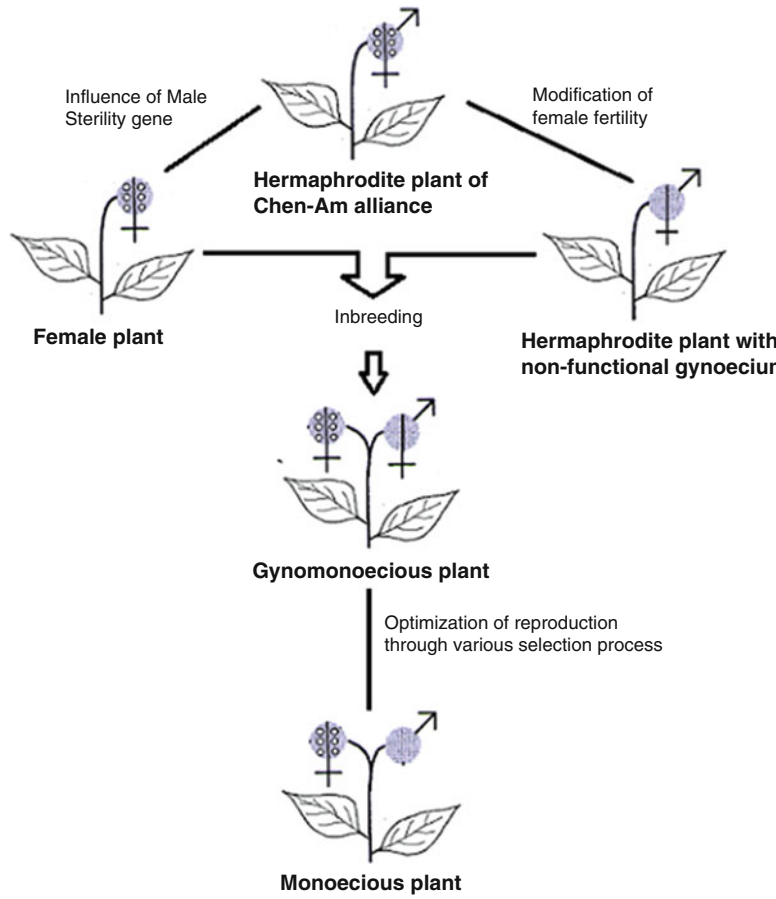

Fig. 9.1 Evolution of sexuality in vegetable amaranths

Basin, Europe and North America and *A. emarginatus* in tropical America. The member of 'blitum complex' later might have migrated to Asia, Southeast Asia and other parts of the world. *Amaranthus bengalense*, a new variant of *A. blitum*, was reported from West Bengal, India (Das and Iamonico 2014). A new gynomonoecious species *Amaranthus parganensis* Saubhik Das was discovered from Lower Gangetic Plain of West Bengal that closely resembles *A. tricolor* of sect. *Pyxidium* subgen. *Albersia* (Das 2015). The new species shows structural gynomonoecy with rudimentary gynoecia in bisexual flowers and provided a clue regarding origin of monoecy through intermediate sexual system. Dioecious species are confined to a small area in North America (Sauer 1957), though sporadic appearance has been reported from time to time (Brenan 1961). Monoecy in amaranths may have evolved from hermaphrodite Chen-Am member through various processes like spread of male sterility genes and gender modifier gene of female fertility in hermaphrodite population followed by optimisation of male and female reproductive function through different selective processes. In case of vegetable amaranths, genetic modification in female fertility of hermaphrodite population might have resulted in the formation of bisexual member with non-functional gynoecium. Later male sterility gene might have played a role in giving rise to female member. All these derivatives originated through genetic modification and have sexual compatibility. Subsequently, through inbreeding process gynomonoecious member might have originated. Monoecy is a derived condition originated from gynomonoecious condition through optimisation of male and female reproduction through different selection processes (Fig. 9.1). Further evolution of

sexuality is not observed in vegetable amaranths. On the basis of available morphological and phytogeographical evidences, Mosyakin (2005) suggested that dioecy would have evolved in plants growing in open habitats such as coastal areas, river valleys, disturbed plant communities, deserts, semi-desert and prairies. It was accompanied by development of many adaptive morphological traits in some groups (perianth reduction for more successful cross-pollination through anemophily, indehiscent utricles for more successful hydro- and anemochory, etc.). Dioecious amaranths probably independently evolved from monoecious ancestor at least twice questioning the monophyly of the subgen. *Acnida* (Mosyakin 2005).

Monoecy in the grain amaranths are supposed to have originated from the dominant cosexual form having exclusively bisexual flower like most of the angiosperms through sterility mutation followed by subsequent specialisation in flower form and position. Two main evolutionary pathways for the origin of dioecy though monoecy and gynodioecy are identified, of which monoecy line might have been followed by grain amaranths. Gynodioecious pathway is not acceptable due to lack of any member with intermediate sexuality. Origin of dioecy from monoecy may have evolved through disruptive selection on male and female reproductive allocation, followed by gender specialisation ultimately leading to unisexual plants.

Increased selfing rates resulted in inbreeding depression in ancestral cosexual population. Such condition facilitates the spread of unisexual variants that favours the outcrossing. Frequent inbreeding in monoecious vegetable amaranths has resulted in a large number of morphotypes. Relatively greater self-pollination in members of sect. *Blitopsis* and members of sect. *Pyxidium* has resulted in breeding depression that favoured the spread of outcrossing. Such outcrossing might have resulted in formation of gynomonoecious member. Grain amaranths show relatively greater outcrossing forming number of variants.

Cultivation of Amaranths

10

10.1 General

Amaranths are a type of crop that can be grown on any type of soil even in marginal areas in the wild as an escape from cultivation; they need no special agricultural attention and can tolerate environmental stress. Collection and preservation of seeds are the primary aspect of cultivation. Seeds should be dried below 12 % moisture content to store for at least 2 years before regeneration. Common amaranth seeds remain dormant when shed; dormancy is lost after 2–3 months of storage in dry condition. Recommended temperature for germination is 20°–30°, while germination is poor below 20° temperature. Temperature above 25° is optimum for growth, but growth ceases at temperature below 18°. Lower temperature and short-day length favours flowering. Seeds of grain amaranths deteriorate significantly after 1 year of storage. Germination in field depends upon age of seed and depth of sowing. Leafy amaranths are warm-season crop adapted to hot and humid climate, whereas grain amaranths are grown in tropical lowland to an altitude of 3500 mt. Amaranths can be grown on various types of well-drained soil. Grain amaranths are drought-tolerant plant, but leafy amaranths require frequent irrigation to keep soil moist. Most of the amaranths are day neutral but differ in their day-length requirement and respond differently to changes in photo- and thermoperiodism. Seeds are broadcasted on farm but well-pulverised seedbed; germination takes place within 4–5 days after sowing. Amaranths do not require high amount of nitrogen like maize but respond well to fertilisers. There are a number of obstacles in the breeding of amaranth – like nonavailability of improved variety, heavy lodging and seed shattering and lack of information about agrotechniques. Few attributes related with higher seed production were evaluated like mixed cropping, spacing and population trials, transplantation versus direct sowing, etc., with positive result. Vegetable amaranths are harvested by uprooting or repeated clipping. In multicut method, the first cutting is done 25–35 days after sowing; subsequent cutting is done with an interval of 7–10 days. Quality and yield get deteriorated after flowering. For greater nutritional value, amaranth leaves are to be harvested at a young stage (20 days after sowing). On average grain amaranths have longer growth period than weedy species. In grain species, terminal inflorescences are cut when the plants are still green, sundried for 6–7 days in thrashing yard and thrashed by biting to collect the grains.

Amaranths are a crop of marginal areas that require no special agricultural requirement. Being a pseudocereal they are adapted to fragile environmental condition and can tolerate difficult condition and environmental stress, specially the vegetable amaranths. They can grow in the wild, also as an escape from cultivation. Vegetable amaranth species are reported to be tolerant to adverse environmental effects (Dieleman et al. 1996; Ghorbani et al. 1999). Amaranths are being

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cultivated from prehistoric period till now in almost all environmental conditions ranging from the true tropics to semiarid regions and also in few highest farms in the world. Ecotypes having the ability to withstand alkaline sandy soils with pH as high as 8.5, as well as the acidic clays of hillside slash-and-burn fields of the tropics, have been evolved. Although traditionally amaranths are cultivated within 30° latitude of the equator, it can be grown in higher latitudes using strains that flower in spite of the longer-day length (photoperiod) than that of the tropics. Most of the grain amaranths have been concentrated in highland valleys, such as the Sierra Madre, Andes and Himalayas. Compared to the cereals, the yield of grain amaranths has been very low, and the difference continues to widen. Factors responsible for the low grain production in the Himalayas are many. The available varieties of these crops in this region are the mixture of landraces, the crop takes a long time to mature, and their growing season overlaps with that of the cereals. High degree of seed shattering and lodging of plants at maturity makes it less productive. Other factors responsible for the poor yields are: (1) crops are generally grown in lands of marginal productivity without applying adequate amount of fertilisers and pesticides, and (2) there is lack of information on scientific cultural practices for this crop to take maximum advantages of its yield potential.

10.2 Seed Dormancy and Seed Viability of *Amaranthus*

Cultivation of any crop plants and preservation of germplasm require a profound knowledge about seed dormancy and viability. The primary objective of storing seeds is to maintain the genetic integrity of the preserved accessions as long as possible. Deterioration of seeds in storage may cause low vigour, reduced number of viable seeds and genetic drift. The precise guideline is required to protect or reduce the decline in viability of conserved germplasms. This would help gene-bank personnels to frame the viability testing and estimate regeneration intervals on stored germplasm and to increase quality and retain the genetic integrity of the germplasm. The viability of seeds in storage condition is a good indicator of seed quality and vigour in many crops. Seeds may be stored for a short period as required for carry-over seeds or for considerably longer period as in the case of germplasm accessions and high-value seed stocks. One can get full benefit from storage system only when the seeds have high-initial quality. Therefore in germplasm preservation, maximum seed quality and vigour are of paramount importance. Amaranth seeds start losing genetic integrity when germination capacity is below 40 %. Under ambient condition storage period should not exceed 3 months for best performance of amaranth seeds. Seed preservation is the most common method of ex situ maintenance of genetic resources of about 70 % of the Earth's plant species. A variety of problems has been encountered to be associated with ex situ conservation strategies, one of which is the problem of genetic changes in storage due to ageing and/or field rejuvenation (Roberts 1988). Chromosomal aberrations occur during seed ageing in storage.

Seed viability equation developed by Ellis and Roberts (1980) can be used as a valuable guideline for estimating deterioration of seeds in storage especially when initial viability levels are high (Mead and Gray 1999). The following equation reflects seed longevity over a range of storage temperature and moisture content:

$$V = K_i - P / 10^{KL} - C_1 m - C_2 t$$

This equation relates probit percentage viability (V) at any time (P), to any combination of moisture content (m) and temperature (t). Once the values of the species' constants K_L, C_1 and C_2 are known, longevity of seeds of that species can be calculated for specific storage condition with certain temperature and seed moisture content. The initial viability of individual seed lot is represented by their K_i value which is dependent on the genotype, the prestorage environment and their interactions (Ellis and Roberts 1980). The equation enables to predict percent viability expected after any given period under different combinations of storage temperature and

moisture content within medium-term gene-bank storage. It has been shown that usefulness of this equation regarding storage behaviour persists as long as initial viability is high. Longevity difference has been found in seeds of various species during storage at comparable temperature and moisture content. It has been suggested that the differences between the seed chemical compositions affect longevity by influencing the water-binding capacity and chemical activity in seeds, hence the rate of deterioration (Walters 1998).

Seed collectors should dry amaranth seeds during prolonged collection mission to avoid probable high rate of deterioration. The seeds should be dried below 12 % moisture content to store for at least 2 years before regeneration. Normal storage by the farmers for over a year is possible for seeds of amaranths without major viability loss, at temperature ≤20 °C in any combination with ≤12 % moisture content. The species constants obtained can be used to give an estimate of anticipated longevity of seeds for the species during storage within short- to medium-term storage condition.

Seeds in the state of rest do not germinate, in spite of potentially favourable conditions for germination, known as seed dormancy. Three types of seed dormancy are distinguished: (1) resulting from the external determinants of germination (2) determined by physical and biochemical factors occurring inside the seeds, but provoked by external factors, and (3) resulting from internal physiological state of the germ, independent on the surrounding conditions. Seed dormancy of most species can be primary (inborn) or secondary. Primary dormancy is associated with natural maturing of seeds. In many species such a state is induced by the germination temperature that is improper for them, or not meeting the light conditions creating favourable conditions for germination. Inborn dormancy may be of physical and mechanical nature, associated mainly with the hardness of the seed cover. Secondary dormancy occurs in nongerminating seeds, in which the stage of absolute dormancy has already been passed and the seeds are able to germinate.

Wild species and few vegetable accessions of *Amaranthus* have seed dormancy. The cultivated and especially white-seeded grain types lack seed dormancy and will germinate in 3–4 days at 21 °C or above (Myers 1996). Kigel (1994) reviewed the seed dormancy; Deno (1993) wrote about cool-moist treatment to overcome seed dormancy of many plant species having the kind of dormancy like amaranths. A month or more of moist stratification at approximately 2–5 °C will be enough to overcome seed dormancy for many accessions. Years of dry storage and many chemical and scarification options can abolish dormancy (Kigel 1994), but the cool-moist treatment is recommended because it is safe and reliable. Germination can be achieved on blotter or sand (Baskin and Baskin 1998). After stratification efficient seed germination will takes place with 20 °C (night) and 30–35 °C (day) temperature. Attempts to explain the reasons for low germinating capacity of amaranth seeds were made based on studies on different species comprising grain amaranths and different vegetable amaranths. To combat the unevenness in germination, a number of methods for presowing seed processing have been employed. The lack of readiness of seeds to germinate leads to unevenness of emergences, and a number of methods for presowing seed processing were developed in order to stimulate them for better germination and emergence of seedlings in the shortest period of time possible under a wide range of environmental conditions (Jisha et al. 2013).

From the biological point of view, the seeds of amaranth are fruits and small nuts, in which the proper seed is surrounded with the hard pericarp. Such structure of fruits favours durability of seeds. In their natural environment, plants sometimes have to wait for a long time for suitable subsoil moisture to germinate. Even when the climatic conditions and habitat are similar to that required by amaranth, still plant emergences sometimes become difficult, although seed germination proceeds very fast – only in a few days. Amaranth seedlings are very small and the soil moisture in the surface layer determines their survival. Almost all the methods known from

agricultural practice were used in experiments to increase the germinating capacity of seeds of amaranths, viz. treatments affecting an increase in permeability of the seed cover and soaking seeds in water (Musa et al. 2014), in the alcohol solution of $CaCl_2$ (Colmenarez de Ruiz and Bressani 1990) and in the sulphuric acid solution (Soomarin et al. 2010), by cooling (Zharare 2012) and by the action of alternating magnetic field to seeds (Dziwulska-Hunek and Kornarzyński 2009). Most of the above-mentioned methods as presowing treatment of amaranth seeds achieved the expected results. The most spectacular effects were given by the use of various treatments to seeds stored for several years, overwintered in soil and exposed to the salt stress, for those which have naturally weakened germination capacity (Moscova 2012). In laboratory conditions, the germination energy and capacity of amaranth seeds of the cultivar Rawa much more depend on the germination temperature than on the kind of preparation in which seeds are soaked. Germination capacity at 25 °C can be regarded as satisfactory, irrespective of the method for seed material treatment. Effective stimulation of amaranth seed germination at 15 °C is induced by soaking them for 8 h in 0.03 % water solution of Pol-Gibrescol, as well as in a mixture of 0.03 % Pol-Gibrescol and 2 % Betokson 025 Super SL.

Although ISTA (1985) recommended germination at either 20 °C or 20/30 °C for *Amaranthus* sp., Grubben (1976) suggested that 20 °C was approaching a germination temperature boundary, and germination was poor at temperatures lower than 20 °C, particularly in the presence of white light. Germination of *Amaranthus* seed is photo inhibited. In the dark, germination is independent of temperature, but in the light, germination at constant temperature increases with increasing temperature. In *A. cruentus* germination was usually greater at 20/30 °C than at 20 °C. Seed dormancy in *Amaranthus* spp. is a type of relative secondary dormancy, a photo dormancy (Kendrick and Frankland 1969) induced by prolonged exposure of seeds to white and far-red light. It can be broken by exposure to red light, but the more conventional seed testing methods of prechilling and KNO_3 (ISTA 1985) proved adequate for *A. cruentus*.

Aufhammer et al. (1998) conducted an experiment in incubator to find out the effects of several factors on the germination of two amaranth cultivars. The factors considered in the experiment were year of harvest, crop type of the mother plant, seed position on the mother plant, stage of maturity, temperature, light and seed dressing. In the experiment percentage germination and germination speed were also recorded. Most effects appeared in interaction with cultivars. Germination percentage was above 80 % when the temperature was higher than 16 °C. The germination speed showed close relationship with temperature, and speed decreased with decrease in temperature. Light or even a short illumination inhibited germination and slowed it down at temperatures below 25 °C, while presoaking of seeds accelerated germination. Seed stored for more than 1 year showed decreased germination percentage. An early harvest of homogenous and dense amaranth crops is recommended for amaranth seed production. More research regarding presowing treatment of amaranth seeds is required.

Seeds of grain amaranths are considered among the most sensitive seeds susceptible to significant deterioration after storage of 1 year. Seeds are required to be preserved in safe storage since they are harvested in the preceding season and generally used for sowing in the next season often after a time gap of 6 months or more. Seeds gradually lose their vigour and germinability and ultimately become less viable during the ageing process in storage (Maity et al. 2000). Losses in seed quality also occur during field weathering, harvesting and storage. Several intrinsic and extrinsic factors influence the viability of seeds during storage. Among intrinsic and extrinsic factors, seed moisture content, relative humidity, temperature of storage, pests and diseases and oxygen availability are more important. Storage of seeds wrapped in polyethylene and aluminium foil was found effective in preventing moisture uptake and maintaining seed viability, while storage of seeds in paper and cloth-made containers were found least effective (Wilson and McDonald

1992). Seed deterioration is an inexorable and an irreversible event. One of the symptoms of seed deterioration is membrane deterioration (Copeland and McDonald 1995). Seed deterioration alters the semi-permeability properties of the membrane and membrane integrity (Berjack and Villiers 1972). Electrical conductivity of seed leachate increased gradually over period of storage. With increase in storage period, the germination capacity, vigour and protein content decrease, while the electrical conductivity increases irrespective of treatments and storage containers. This may be probably due to increased moisture content of the seeds.

Weed species which form persistent seed banks are of concern for future weed management (Egley and Chandler 1983). In the absence of viable seed production, a decline in amaranth seed bank might be due to seed mortality through physiological age and herbivory or microbial decay (Egley and Williams 1990; Buhler and Hartzler 2001). Persistent seed bank of a few *Amaranthus* species has been investigated earlier (Steckel et al. 2007). Palmer amaranth (*A. palmeri* S. Watson) is known as prolific seed producer, a single female plant may produce up to 600,000 seeds (Keeley et al. 1987). Investigation on seed persistence of palmer amaranth is lacking. It shows seasonal seed dormancy and an extended emergence period, resulting in a season-long interference and severe yield reductions in several crop plants. *Amaranthus* species have low persistence in soil seed banks (Steckel et al. 2007). The redroot pigweed (*A. graecizans*) exhibits a sharp decline in the viable seed bank within 2 years of burial, with no viable seeds remaining in the upper 0–15 cm depth of soil after the third year (Egley and Williams 1990). Several studies on natural and artificial seed banks reported that the rapid decline of *Amaranthus* seed bank within a year of burial might be due to physiological death of seeds, fatal germination, seed herbivory or microbial seed deterioration in the soil (Cardina et al. 1996; Kremer 1993).

Generally common amaranth seed remain dormant when shed. The time of emergence and the growing conditions experienced by the parent plant have a definite effect on the level of dormancy. Fresh seed collected from distinct populations of common amaranth may show difference in their germination behaviour, but such differences are reduced during dry storage. The tough seed coat does not rupture easily and, while water uptake is not prevented, seed germination is prohibited by it. In laboratory studies, the minimum temperature for germination was 10 °C (Wiese and Binning 1987; Ghorbani et al. 1999), while maximum germination occurred at 35–40 °C. The ripe seeds soon after harvest showed some germination when incubated at temperature of 40 °C. Nitrogen is suggested to promote germination, but the effect of light on germination is yet to be documented conclusively.

Seed of common amaranth showed 50 % less germination at 15 °C than at 25 °C (Chakraborty 1977). In laboratory experiment, when dry-stored seeds are sown on moist paper or soil in the light at a constant temperature of 18–20 °C, 70 % germination was achieved (Cross 1930–1933). Germination was nearly 90 % when the seeds were incubated at alternating temperatures of 0/30 °C or 8/20/30 °C. In other experiments, little difference was observed between germinations under diffuse light or light filtered through a leaf canopy to reduce the red light ratio compared with the far-red light (Taylorson and Borthwick 1969).

The optimum depth of seedling emergence is a crucial factor that varies from 0.6 to 1.3 cm depending on temperature (Mohler 1993). In the laboratory condition best seed germination was observed between 5 and 30 mm deep in soil (Ghorbani et al. 1999). Germination was found better in clay soil than in sandy soil. Below the depth of 40 mm and on the soil surface, seed germination in both the cases was much less. However, when seeds are sown in trays of field soil subjected to different cultural treatments, the highest germination was achieved from seeds left on the soil surface (Chepil 1946). Seedling emergence decreased with increasing burial depth, and cultivation increases seedling emergence by bringing seeds into the upper soil layers, but fatal germination did not occur and a greater number

of seeds simply remained nongerminated. Seedling emergence was less in untilled soil than in tilled soil even when seeds were maintained at the same depth (Mohler and Galford 1997). Few of the seeds sown in a 7.5 cm layer of soil in open cylinders in the field and stirred periodically seedlings emerged soon after sowing in autumn (Roberts 1986). In the following year, the seedlings emerged from May to August. Germination appeared to require a high temperature. A gradually reducing number of seedlings emerged in subsequent years, but some viable seeds still remained after 5 years.

Common amaranth seeds spread in moist soil, persist and can remain viable but dormant for over 30 years (Crocker 1916). However, other investigations reported just 1 % seed viability after 5.5 years in soil (Egley and Chandler 1983). In an experiment to study the relationship between burial depth and germination percentage, it was found that seed buried at 20, 56 and 107 cm showed 9, 11 and 18 % germination, respectively, after 1 year; 11, 36 and 48 % after 10 years; and none after 16 years (Toole 1946; Goss 1924). After a dry storage at low temperature for 30 months, seeds retained full viability, but after burial in field soil for the same period, viability was less than 13 % (Egley and Chandler 1978). Common amaranth seeds sown in the field and followed by cultivation of wheat in winter and barley in spring over a 5-year period showed an annual decline in germination of around 40 % (Barralis et al. 1988). Emerging seedlings represented only 8 % of the seed bank. The viability of seeds recovered from 10 cm deep in soil declined from 98 % to 90 % in a 12-month time period, while the viability of seeds recovered from the soil surface declined from 93 % to 62 % over the same time period (Omami et al. 1999). Soil solarisation is the main factor for speedy decline in germinability and viability, and common amaranth seeds are very sensitive to soil solarisation. When the seeds were sown in pots of moist soil and treated with worm air for 6 h, germination was reduced by 30 % at 47 °C, 60 % at 52 °C and 80 % at 54 °C (Laude 1957).

If germination is slow, soil surface needs to be lightly stirred; amaranth seeds require some sunlight after a period of darkness for germination. This trait helps them to adapt in disturbed or overturned soil. The weedy amaranths are often called ‘pigweed’ because they would germinate in hordes in an area after pigs had passed and turned the soil, exposing their seeds to the light so they could germinate. Amaranth seeds can be stored safely for up to 3 years at a temperature below 8 °C and at 10 % relative humidity in a tightly closed moisture-resistant container (Hartmann et al. 2011). Ideal containers are airtight such as a sealed glass jars, metal cans or foil envelopes as they maintain seed water content best. Seed in containers should be stored in a cool, shady and dry place to extend seed shelf life (Hartmann et al. 2011).

Seeds from different crops can be stored for different periods of time after harvesting. Seed viability at the end of the storage period is determined by the initial viability at harvest and the rate of deterioration during storage. Deterioration differs with seed species as well as the storage conditions including temperature and relative humidity (Muyonga et al. 2008). Seeds can be classified as either recalcitrant or orthodox based on their genetic potential to tolerate storage. Recalcitrant seed are those that do not tolerate seed moisture below 25 % after seed maturation, while orthodox seeds can tolerate drying from 10 % down to 4 % moisture content after seed development, and these differ in the length of time they can tolerate storage (Barker and Duarte 1998). According to Hartmann et al. (2011), orthodox seeds can be further divided into medium-lived and long-lived categories. Medium-lived seeds can remain viable for periods of 2–5 years provided that seeds are stored at relative low humidity and temperature. Seeds of most vegetables, flowers and grain crops belong to this group. It is important for seed storage to be designed in such a way that it should not create conditions that will negatively affect seed and/or seedling vigour. During seed deterioration the seed first loses vigour or the ability to germinate when environmental conditions are not favourable. Seed deterioration during storage is stimulated by high respiration and other metabolic rates which injure the embryo (Hartmann et al. 2011).

Moisture content in seeds is the most important factor in seed longevity and, therefore, is important to consider during storage (Baskin and Baskin 1998). For example, seed having orthodox characteristics can be best stored at a non-fluctuating low moisture level as they can tolerate low moisture content. Seed moisture content of about 4–6 % is suitable for prolonged storage of seed from many vegetable species. However, many storage problems may arise when seed moisture content is elevated during storage (Baskin and Baskin 1998): (1) at about 8–10 % moisture content, several insects are active and can reproduce; (2) above 12 % seed moisture content, fungi are active and can multiply to produce spores; and (3) at the higher seed moisture content levels, respiration, germination and disease activity are stimulated leading to reduced seed viability (Barker and Duarte 1998). On the other hand, too low water content in some seeds can have a reducing effect on seed viability and germination rate (Abdullah et al. 2011). For this reason, hydration is necessary for seeds stored at a humidity atmosphere below 2 %, to avoid seed injury, as this can influence the moisture content of the stored seed. Conversely, in the case of some species, dry climate increases seed longevity, while high relative humidity (RH) results in shorter seed life (Barker and Duarte 1998). Amaranth seeds can be preserved safely for up to 3 years at a temperature below 8 °C and at 10 % RH in a tightly closed moisture-resistant container (Hartmann et al. 2011). Ideal containers are airtight such as sealed glass jars, metal cans or foil envelopes as they best maintain seed water content. Seed in containers should be stored in a cool, shady and dry place to extend seed shelf life (Hartmann et al. 2011).

As mentioned earlier, germination of all seed is affected by the viability of the seeds, seed dormancy and adequate environmental factors. If one of the three aspects is not sufficiently considered, germination can be severely delayed or inhibited, leading to secondary dormancy (Tucker 1986). Seed viability represents the ability of nondormant seeds to germinate, and viability testing is essential in determining seed quality. Seed dormancy is the condition whereby seeds do not germinate even if they are subjected to favourable conditions that are normally beneficial for germination (Mayer and Mayber 1995). Essentially, dormancy is an adaptation mechanism that prevents seeds from germinating after it has been dispersed by mother plants and that only permits germination when environmental conditions are favourable (Baskin and Baskin 1998). Dormancy in amaranth seed is reported to be high at the time seeds are detached from mother plants, but decline as seed water content decreases (Baskin and Baskin 1998). Generally, dormancy is at its peak within 2–3 months after seed harvesting. In amaranth seed dormancy after ripening is associated with naturally occurring compounds present in the seed at maturity, and oven drying or naturally air-drying can help to reduce amaranth seed dormancy (Hartmann et al. 2011).

10.3 Agroclimatic Condition for Cultivation of Amaranths

10.3.1 Climate

Leafy amaranths or vegetable amaranths are a warm-season crop adapted to hot-humid climatic conditions. It is grown throughout the year in tropics and in autumn, spring and summer seasons in temperate regions. Amaranth can tolerate full sun, drought conditions and high temperatures. Grain amaranths are grown in wide geographic areas ranging from tropical lowlands to 3500 m in the Himalayas. Altitudes above 1000 m in the tropics are considered best for grain amaranth cultivation though they are tolerant to drought condition and low soil fertility. It performs much better under conditions that are considered ideal for maize (corn). It can be intercropped with maize, beans, peppers or squash. In the Andes region, it is often intercropped with quinoa (*Chenopodium quinoa*) and other pseudocereals by the local farmers. For grain amaranth cultivation, altitude is not a severe limiting factor. Amaranths can grow satisfactorily from sea level to above 3000 m; the only exception is *A. caudatus* which is known to grow at an altitude of 3000 m in the Andean region and

Himalayas. Some of the grain species of *Amaranthus* are sensitive to day length. Some accessions of *A. hypochondriacus* will not set flower in summer; however they do mature in the greenhouse during short-day situation in winter. Some strains of *A. cruentus* remain vegetative for a long time in its equatorial house. However, it starts to set seed very early when introduced into the long-day conditions. *A. caudatus* thrives well in high hills and is familiar as a short-day species. It generally flowers and sets seed only when day length is less than 8 h. Amaranth population are easily miniaturised, and their flowering cycle can be accelerated by controlling the environment (Brenner and Widrlechner 1998). *Amaranthus* production guide (Sooby et al. 1998) is a useful reference for field management of amaranth.

10.3.2 Soil

Amaranths can be grown on various types of soil including marginal soil. Amaranth comes up well in well-drained loamy soil rich in organic matter. The ideal pH is 5.5–7.5, but there are types which can come up in soils with pH as high as 10.0. It can be grown on marginal areas with little soil management. It is evident from the field observations that amaranth can grow well on soil containing variable amount of soil nutrients. Initial studies at Rodale Research Centre (RRC) in Pennsylvania showed that young grain amaranth plants grow taller with fertiliser but with little improvement in grain yield. On the other hand, vegetable amaranth needs high soil fertility, particularly potassium and nitrogen. Grain amaranths prefer well-drained cultivation field with neutral or basic soils (pH values above 6). However, this aspect has not been studied carefully considering the wealth of amaranth germplasm variability that exists. It is most likely that types that can tolerate acidic condition can be identified for cultivation in tropical lowlands where acid soils are common. The genus is not known for high salt tolerance, but they have the ability to withstand mild salinity and alkalinity as apparent in some species of amaranth. Moreover, *A. tricolor* has demonstrated (Foy and Campbell 1984) tolerance for soil with high aluminium levels. Cultivation practices differ according to the methods of harvest, duration, growth pattern of variety, etc. Land is prepared to a fine tilth by thorough ploughing and harrowing. Well-decomposed and powdered organic matter at 20–25 t/ha is incorporated with the soil at the time of final ploughing. The poor amaranth stands in field can be attributed to various reasons like soil crusting, low soil moisture, poor seed-to-soil contact, uneven planting depth and wind erosion in some areas. Proper addressing of such primary reasons will result in a good stand of amaranths (Sooby et al. 1998).

10.3.3 Temperature

Temperature is an important parameter for seed germination, growth, emergence of inflorescence, etc. Grain amaranths grow best when the daily high temperature is at least 21 °C. Various accessions of grain amaranths have shown optimal germination at temperature range of 16–35 °C. The speed of emergence is encouraged at the higher end of the temperature range. The grain species *A. hypochondriacus* and *A. cruentus* are not frost hardy and can withstand high temperature. Growth of the plant stops at about 8 °C and below 4 °C the plants get injured. However, *A. caudatus* being native to Andes and high Himalayas is more resistant to chilling than the other two grain species. The optimum germination temperature for amaranth has been reported to be between 20 and 30 °C (ISTA 2010). The best temperature for germination and early seedling growth ranges between 25–30 °C for *A. cruentus* and 25–35 °C for *A. hybridus*, respectively. Amaranth seeds require soil-temperature range of 18–25 °C for germination and an air temperature above 25 °C for optimum growth. The growth ceases at temperatures below 18 °C. Lower temperatures associated with shorter-day length will induce flowering with a subsequent reduction in leaf yield. As the crop grows during summer with the onset of the rains, frost damage should not be a problem. However,

harvesting of the crop may be effected by frost. As amaranth is an annual crop, it does not mature completely in areas having a short growing season. Frost is necessary to terminate the crop's growth.

10.3.4 Rainfall

Grain amaranths are drought-tolerant crops, but vegetable amaranths require frequent irrigation to keep soil moist. Frequency of irrigation depends on soil. Seeds of amaranths require well-moistened soil to germinate and establish roots, but once seedlings are established, grain amaranths perform well with limited water; in fact they grow best under dry, warm conditions. Vegetable amaranths, on the other hand, require moisture throughout the growing season. Grain amaranths have been grown in dry agricultural areas that receive as little as 200 mm of annual precipitation, while vegetable amaranths are routinely grown in areas receiving 3000 mm of annual rainfall.

10.3.5 Day Length

Most of the vegetable amaranths are day neutral in habit but differ in their day-length requirements and respond differently to changes in photo- and thermoperiodism. Grain types, *A. caudatus*, *A. cruentus* and *A. edulis*, are short-day species, while *A. hypochondriacus* is day neutral. Many of the amaranths are sensitive to day length. For example, accessions of *Amaranthus hypochondriacus* from the south of Mexico will not flower in the summer in Pennsylvania. However they will flower in the greenhouse during the short-day conditions in winter. The reverse happens with *Amaranthus cruentus* from Nigeria. It remains vegetative for a long period in its equatorial home. However, it sets seed very early when placed under the long-day conditions in Pennsylvania and can be used to breed for early-maturing traits. *Amaranthus caudatus*, on the other hand, is known to be a short-day species. It usually flowers and sets seed only when day length is less than 8 h. However, some accessions of *Amaranthus caudatus*, such as the ornamental 'love-lies-bleeding', will set seed when the day length is longer (El-Sharkawy et al. 1968).

10.4 Cultivation Practices

10.4.1 Sowing of Seeds

The field must be well levelled and two to three ploughings are sufficient for sowing of amaranth seed. To initiate amaranth cultivation, a well-worked, firm and moist seedbed is required. It is necessary to firm the soil over the seed to make good contact between the seed and the soil. A loose (but firmly packed), friable soil is preferred in seedbed. Amaranth seeds are very small in size, and the weight of 1000 seeds is varying from 0.7 to 0.9 grammes. It has been found that the largest amaranth seeds are only 1/16 in., and some varieties have seed as small as 1/23 in. in diameter. As per the recommendation, 1.2–3.5 kg seed/ha is to be planted to an average depth of 1.3 cm (Webb et al. 1987). According to the authors, planting depth needs to be monitored carefully as the deeper range might delay and decrease seedling emergence. On the other hand, shallower planting depth is also believed to decrease seed-soil contact, exposes the seeds to pests and increases the risk of being washed away by water (Stallknecht and Schulz-Schaeffer 1993). Further, amaranth seed requires a firm moist seedbed with a soil temperature above 15 °C to ensure proper seedling emergence and good plant establishment. Consequently, drying of the top soil during the germination process can reduce seed germination and subsequently seedling establishment. Therefore, amaranth seed germination depends mostly on moisture and temperature, with an optimal temperature range between 16 and 35 °C (Muthomi and Musyimi 2009). As is the case with all crops, amaranth also experiences harsh abiotic and biotic stress conditions when planted in the field. Water-deficit stress and other environmental factors, like stress of unfavourable temperature, have been shown to reduce seed germination and seedling development.

Seeds may be planted in a nursery bed for subsequent transplanting in cultivation field or sown directly in the field. Transplanting ensures a very efficient use of seeds and the growing area to be weed free just before the seedlings are transplanted. If direct seeding is practised, sowing in rows is recommended. Seed sown at a depth of 1.3 cm (0.5 in) or less in soil with temperature of 15 °C will establish a good plant stand. Seed will germinate within 3–4 days with soil temperature of 20 °C (68 °F). Before sowing, application of 50 quintals of farmyard manure per hectare has been found to be good to enhance yield. Plant density, i.e. spacing between plants, has a great impact on yield and harvesting specially in the case of vegetable amaranths. One common practice is to grow plants at a spacing of 5–10 cm (2–4 in) and harvest by uprooting when the plants are 5–7 weeks old. Another common method is to sow seed less densely at a spacing of 15–30 cm (6–12 in) and harvest by cutting the stem tips and tender leaves periodically starting from 4 to 6 weeks when the plants are about 15 cm tall. The crop is generally sown in the first or second week of June just after the first monsoon shower. However, amaranth can be propagated from seed in the early summer (Muyonga et al. 2008). Traditionally, the seeds are broadcast, but better crop stand is achieved if seeding is done in rows. The depth of sowing should be less than 2 cm in view of very small grain size, with 50 cm spacing between rows and between plants. On a large scale, grain amaranth is directly seeded into the field at a seed rate of 1–1/2 kg to 2 kg/ha for good grain yield. Amaranth is harvested by pulling out and by frequent clippings (multicut).

Field studies have shown that amaranth yields remain constant across a range of 0.3–4.5 kg/ha (0.25–4 lbs/a). There are approximately 1000–3000 amaranth seeds per gramme (1,000,000–2,500,000/kg) (Sooby et al. 1998). Plant spacing recommended for grain amaranth varies widely. One recommendation is to maintain a spacing of 23 cm (9 in) between plants and 75 cm (30 in) between rows. This results into a planting density of approximately 38,000 plants per hectare (15,400 per acre). If harvesting is to be done manually, the less dense spacings are advisable. The wider rows gave the highest yields. Amaranth seeds, being small in size, are mixed with fine sand and sown uniformly by broadcasting. The seeds are covered either by raking up soil and by covering with a thin layer of sand or soil. This is followed by a light irrigation. Soil is kept moist by frequent irrigation. Grown-up seedlings are selectively pulled out at 30 days after sowing and marketed in small bundles along with roots.

10.4.2 Seedling Growth and Interculture

Vegetable amaranth species are reported to be tolerant to adverse environmental effects (Dieleman et al. 1996; Ghorbani et al. 1999). They have been growing wild in arid and semi-arid regions, which mean that they could be more tolerant to low-water and high-temperature conditions (Hurro and Cees 1991; Modi 2007). Many African communities believe that because amaranth is found in the wild, there is no need to cultivate these plants (van Rensburg et al. 2007). Seedling growth is highly dependent on both moisture and temperature, with *A. hybridus* being more tolerant to high-temperature and low-moisture conditions than *A. cruentus*.

Seed germinates within 4–5 days after sowing and needs maximum care till it attains a height of about 25–30 cm. In fact this is the most critical stage for obtaining maximum yield potential of the crop. During this phase, it must be properly spaced, made free from weeds and must receive adequate moisture. One more weeding is necessary after 30 days of sowing. At the seedling stage, one spray of some fungicides to check the attack of damping off is necessary. Once the stand is established, then its maintenance is relatively easy. The land, after thorough ploughing and levelling, is made into shallow trenches/basins of 50–60 cm width and convenient length. Well-decomposed farmyard manure is applied in trenches and thoroughly incorporated in soil by digging. Seedlings (20–25 days old) already raised in nursery are transplanted in trenches at 20–25 × 10–15 cm spacing. Seed requirement for transplanted crop is only 500 g/ha. Amaranth is a

short-duration and shallow-rooted crop. A light hoeing is needed to prevent soil crust formation after irrigation and to keep soil loose. Field also should be kept weed free, especially during initial stages.

Direct seeding though requires much less labour, but it invites a greater risk of poor stand due to diseases and predators of young seedlings and poor competition with existing weeds in the crucial initial couple of weeks. If direct seeding is practised, sowing should be done in rows to facilitate cultivation. Because of the shallow depth of plantation, special care must be taken to prevent the soil from drying out or soil solarisation until plants are established. Transplanting or thinning is vital, and it may be done within about 2 weeks after sowing when plants are 5–10 cm tall (2–4 in). However, any delay in transplanting has an adverse effect on yield.

10.4.3 Manuring

Amaranth does not require a high amount of nitrogen fertiliser like maize, but responds well to fertilisation. A leguminous cover crop tilled under prior to seeding is enough to provide sufficient nitrogen for amaranth. Animal manure or chemical fertiliser at a rate of 135 kg per hectare (135 lbs/a) is also sufficient. The requirement of chemical fertilisers is less when the cultivation is preceded by a leguminous crop such as beans or soybeans. Singh and Whitehead (1993) studied the growth responses to three different pH levels 6.4, 5.3 and 4.7. Results indicated that pH 6.4 is the best for growth and growth decreases with the increase in soil acidity. Stallknecht and Schulz-Schaeffer (1993) recommended soils with pH above 6.0 suitable for growth. Fertiliser like NPK showed no significant effect on yield, but increased the seed protein content in the two lowest-yielding ecotypes, while effects on seed fat content were inconsistent. Amaranth is not well adapted to soils markedly deficient in available phosphorus; amaranth seedlings have repeatedly failed to establish. In the same trials, cereals such as corn, sorghum, wheat, triticale and even small-seeded millets established satisfactorily. In these situations, satisfactory stands of amaranth were established applying readily soluble phosphorus fertiliser directly below the seeds. Under hilly condition in India, NPK at the rate of 20:30:20 kg/ha is sufficient to meet the full requirements of the crop.

Amaranths are heavy feeder and high-yielding crop; 20–25 tonnes/ha of FYM (farm yard manure) and 50:25:20 kg NPK/ha are recommended as basal dose. Under pulling-out method, 20 kg nitrogen should be top-dressed twice during subsequent pulling-out of seedlings. For clipping varieties, a still higher dose of 75:25:25 is advisable. Nitrogen should be applied after every clipping or cutting. Foliar spray of 1 % urea or diluted cow urine at every harvest is good for promoting further growth and for high yield.

10.4.4 Obstacles in Productivity in Grain Amaranths

The major productivity constraints of the crop identified are:

1. Nonavailability of improved variety for cultivation till recently
2. The lack of information on agrotechniques for high seed production
3. Heavy lodging and seed shattering
4. Little or no use of inorganic fertilisers
5. Little or non-utilisation of seed for various domestic and agroindustrial products by mass consumers
6. The lack of public (consumer) awareness about the organoleptic taste and quality of both grains and greens for production of various domestic, agroindustrial delicious products.

To identify and standardise the attributes for high seed production in grain amaranths, agronomic trials concerning five attributes were conducted at NBPGR, Regional Station, Shimla, with newly released variety Annapurna during 1985 and 1986. The attributes were (1) spacing and population trial, (2) sowing date trial, (3) mixed cropping with French bean, (4) transplanting vs. direct sowing and (5) leaf picking vs. nonpicking.

Plant-to-plant spacing of 20 cm has been observed to be the optimum spacing for obtaining highest grain yield of 34 q/ha. To standardise the optimum time for sowing of seed in the hills and for obtaining highest seed yield, a randomised block-design trial with four sowing dates with an interval of 15 days was conducted. The highest grain yield of 16.1 q/ha was obtained when sowing was done on first date of sowing (June 2) followed by the second date of sowing (June 17), and the last was recorded on the last date of sowing (July 17). The reasons for low yield levels in this trials were due to heavy lodging and seed shattering. The delayed sowing beyond June highly reduced the plant height, inflorescence length and glomerule number per plant. It also induced tenderness to the plants. Early sowing caused a big stem borer problem as compared to late-sown plants which were completely free from this disease. Highest yield of 21 q/ha was obtained under mixed cropping when French bean (viny type) was grown between two rows of amaranth at 25 cm apart. Mixed cropping with French bean not only provided the nitrogenous fertiliser to the amaranth plants but also held them by twining and preventing lodging which is a serious problem in the hills. Highest grain yield of 24.3 q/ha was obtained in the case of direct sowing, and there was a reduction of 7 q/ha under transplanting. Early maturity by a week time and reduction in plant height, inflorescence length and leaf size were observed in the case of transplanted plants. The farmers in the Himalayas generally pluck the leaves of grain amaranth at vegetative stage when the plant is tender for vegetable use and leave the rest for grain yield. There was a reduction of 2.9 q/ha grain yield in the case of leaf-plucked plant than the normal ones, but there was 20 q/ha additional foliage yield for vegetable use. In the case of leaf picking, plant height is reduced and number of branches at the top and number of leaves increased. The size of inflorescence increased, but the number of glomerules decreased. The leaf-plucked plants became stunted and stout and did not lodge which is the most desirable character. Late maturity by a week was observed in the case of leaf picking than the normal ones. Thus leaf picking at the vegetative stage is recommended for taking maximum advantage of the crop in the hills.

10.4.5 Harvesting

Vegetable amaranths are harvested by uprooting tender plant or by repeated clipping or cutting. Harvesting by repeated clippings, with an interval of 2 or 3 weeks is common through the end of the season (usually the short-day length period of the year). Frequent clipping is helpful to increase both the yield and quality of leaves. At the end of vegetative growth, flowering begins, and subsequently harvest becomes inferior both in quality and quantity. Vegetable amaranth is harvested early in the morning by pulling out or by clipping. In the first method, grown-up plants are pulled out at 30, 45 and 55 days after sowing, along with roots, washed and sent to market in small bundles. In multicut method, first clipping or cutting is done 25–35 days after sowing. Subsequent cuttings are made at 7–10-day intervals. Premature flowering or bolting is a serious problem in cultivation of amaranth. Bolting is usually associated with planting of short-day varieties during November to December, deficiency of nitrogen, extreme high temperature and poor soil erosion. Practices like raising of crop at ideal time depending on locality, frequent application of nitrogen fertilisers and manures and keeping soil loose by light hoeing may be some of the preventive measures. Prolonged flowering is a serious problem in cultivation of amaranth. Quality and yield are deteriorated after flowering. Flowering of *Amaranthus* species usually starts 4–8 weeks after showing (Grubben and Denton 2004). However the growth and development pattern are highly variable between species to species and cultivars to cultivars, depending on photoperiodism, altitude and cultivation practices (Wu et al. 2000).

Wild leafy vegetables are harvested from crop fields by rural people at different stages of plant growth. There may be a specific or preferred plant developmental stage when flavour and palatability are most favourable for human consumption. In the case of leafy vegetable, it is not likely that flavour and palatability are influenced

by environmental condition. Data on changes in nutritional quality of leaf in response to plant age and environmental condition is scanty. Modi (2007) made an attempt to study the effect of growth temperature on amaranth leaf yield and nutritional value at different stages of plant growth involving five species, viz. *A. hybridus*, *A. hybridus* var. *cruentus*, *A. hypochondriacus*, *A. tricolor* and *A. thunbergii* in South Africa. Vegetable amaranths are the most widely occurring leafy vegetable in South Africa and Africa in general (Modi 2007). Nutritional quality of *Amaranthus* leaves is significantly influenced by growth temperature and developmental stages at which harvesting has been done. It was found that cool environmental condition favours the increase in total protein and amino acid content in leaves. However the amount of mineral element like calcium and iron increased in leaves in response to increase in growth temperature. It was recommended that for greater nutritional value, amaranth leaves should be grown under warm condition and leaves should be harvested at young stage (20 days after sowing). Warm condition was found to be associated with high yield and improved germination capacity.

On an average the three grain types have longer growth period than weedy species. Amaranth grains mature much earlier and the plant dries up quite late. If the heads are allowed to remain till the plant dry up, heavy shattering of grains is noticed which leads to heavy grain loss. The terminal inflorescences or heads are cut when the plant is still somewhat green and start weathering and kept for sundrying for 6–7 days in the threshing yard. Threshing is done by beating. The produce is threshed and winnowed like other cereals. Unusually the harvesting is done early in the morning when the plants are somewhat wet due to night dew to avoid grain shattering in cut heads. When the grains mature, the inflorescence heads are cut dried under shade.

10.4.6 Pests and Diseases

In general, soil fungus, damping-off, leaf blight, white rust and mycoplasma and virus-related diseases have been identified as the serious diseases of grain amaranth in India. Amaranths are generally affected with some fungal diseases, specially damping-off disease of seedlings caused by *Pythium*, *Rhizoctonia* and *Aphanomyces* spp. and cankers caused by either *Phoma* or *Rhizoctonia*. Various root and stem rots can occur later in the season when the soils are wet, which contribute to lodging problem. *Alternaria* leaf spot is the most serious foliar disease in amaranth. Amaranth is generally considered tolerant to nematodes and often has been recommended as a rotation crop to reduce nematode populations for subsequent crops. The presence of root-knot nematodes in amaranth roots has been reported. It is important to know whether or not amaranth can be used to control nematodes and/or whether it can be cultivated where nematodes are a problem. A lot of insects are known to feed on amaranth leaves that may account for severe and sustaining yield loss. A few insects may cause substantial damage. Amaranth may succumb to caterpillars, webworms, blister beetles, lygus bugs and stem borers. The lygus bug, coffee bug or tarnished plant bug (*Lygus* spp.) is a sucking insect that attacks flowers and seeds and causes severe damage both by preventing flowers from producing seeds and also by reducing seed weight. Solutions of pyrethrum or synthetic pyrethrins are helpful to control lygus. Other insects that can injure the developing amaranth include fall armyworm (*Spodoptera frugiperda*), corn earworm (*Heliothis zea*) and the cowpea aphid (*Aphis craccivora*). The amaranth weevil (*Conotrachelus seniculus*) can damage roots, resulting in lodging or other root diseases. The potato flea beetle (*Epitrix cucumeris*) can damage seedlings, and the beet leafhopper (*Circulifer tenellus*) can transmit curly top virus, but this has been seen only in areas nearing large areas of sugar beet production.

Hymenia recurvalis and other caterpillars are serious pests of amaranth like *Cletus* sp., *Asparia* sp. and *Lygus lineolarus* bug (cabbage looper), *Trichoplusiani* (European cornborer), *Ostrinia nubilalis* (corn earworm), *Heliothis zea* (cowpea aphid), *Aphis craccivora* (striped blister beetle), *Epicauta vittata*, *a* weevil, spinach flea beetle, *Disonycha xanthomelas*, etc. Lygus bugs, more

specifically the tarnished plant bug, are recently considered to be the most important insect pest of grain amaranth. These insect feedings result in localised wilting and tissue necrosis followed by abscission of fruits, morphologically deformed fruit and seed and altered vegetative growth. Serious damage of amaranths has been caused by spider mites and stem weevil. Stem borer (*Lixus truncatucus*) is another problem in Africa and Asia in early-sown plants which causes high degree of lodging in plants. A considerable damage is done by the leaf rotters during rainy seasons. Seedbeds should be protected against ants and termites. The other pests damaging the crops are *Hypolixus nubilaus* (Egypt), *Rhachi creagra* (Costa Rica), *Chrotogonus* (Pakistan), *Hyphurus* (India), *Geocoris* (California), *Thysanoptera* (Hawaii), *Diabnotica barberi* and *Spodoptera exigua*. The fungal population was higher in the pre-flowering stage of the host. Pandy and Gupta (1985), while studying the leaf surface mycoflora of *Amaranthus paniculatus* grown in Almora hills, observed 24 fungal species on leaves of this crop. McLean and Roy (1988) reported *Colletotrichum domatium* damaging *Amaranthus hybridus* plant in Mississippi, USA. The other species damaging the crop are C. *truncatum* and *C. capsici. Choanephora cucurbitarum*, causing wet rot, is the most troublesome disease of amaranth in Africa. *Albugo bliti*, causing white rust, is a serious disease in Southeast Asia. Reddy et al. (1980) reported *Xanthomonas amaranthicoia* as a causal organism of bacterial leaf spot disease of amaranths in India. Sharma et al. (1981) and Naseema et al. (1983) reported that *Aspergillus flavus*, *A. niger* and *Rhizopus stolonifer* were externally as well as internally seed borne in most of the seeds of *Amaranthus gangeticus* and *A. caudatus* and are the major storage fungi of amaranth crop in India. Sammons and Barnett (1987) reported about tobacco ringspot virus damaging *Amaranthus hybridus* in California, USA. A severe mosaic disease caused by cucumber mosaic virus in *Amaranthus caudatus* was reported in Himachal Pradesh by Sharma and Chawla (1987) damaging the crop. The other viruses affecting the crop are ivy vein clearing virus, alfalfa mosaic virus and beet western yellow virus.

Future Prospects in Amaranth Research

11

Amaranths especially the grain amaranths are considered as the golden crop of future. Though the cultivation of grain amaranths was initiated in the prehistoric period with a promising note, later it lagged behind conventional crop a lot, because a large section of the people rejected that due to its unpalatability. Later, a number of research works on unique nutritive value of both vegetable and grain amaranths rediscovered the plant and projected the group as useful super crop of future keeping in view its minimum agronomic demand and food security of huge world population. The available literatures on amaranth research reveal that this underutilised crop has not received much attention as it deserves. Most of the research works were concentrated on its nutritive value as human and animal feed, utilisation of starch, basic biology, genetics and breeding practices. A little work has been done on taxonomy, phylogeny and germplasm maintenance and screening and biotechnological approach in breeding programme. Much of the attentions was directed towards grain species of amaranths; vegetable group received very little attention. Weed amaranths too are not evaluated for utilisation in breeding programme. Thus, the area of amaranth research is vast and wide. A multidisciplinary research approach is very much needed to project amaranths as golden super crop of future.

Cultivation of amaranth has gradually crept into a number of countries. Research work on re-evaluation of its significance and importance and contribution towards genetic manipulation of other crops has gained a momentum in recent years. Breeding programme related to genetic improvement has escalated a lot. But still there are few areas which are to be given emphasis. While no fundamental obstacles to the crop's future development are apparent, many technical details remain to be explored. Agencies funding agricultural research for developing countries should consider supporting amaranth research and testing. Some of the thrust areas for recommendations and research needs are listed here:

1. *Collection and screening of germplasm*: Amaranth offers more genetic diversity in its present undeveloped state than do many conventional crops. The broad geographic spread of the genus has resulted in the evolution of many landraces in widely separated areas. Several features of their highly variable breeding system and overall reproductive biology provide ample choice of breeding methods. This huge gene pool will be very important to the future development of the crop. Further systematic collections of amaranth germplasm should be made in Latin America, the Caribbean, India, Nepal, China and the Pacific. These collections should be coordinated with the International Board on Plant Genetic Resources (IBPGR), which is starting germplasm collections in Southeast Asia and has recently completed one in Peru. Proper screening of germplasm

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collection is necessary for the future development of the crop.

There is a need to develop an international cooperation network to enrich the germplasm collection with exotic elite lines or cultivars also to test elite lines and germplasms of amaranths in as many locations as possible under various agroclimatic conditions.

2. *Adaptability trials*: Selection and standardisation of uniform high-yielding lines for multilocation yield trials are essential to fish out types with desirable features like wide adaptability in the hill. Studies on inheritance and combining ability, correlation and genetic divergence need due emphasis for varietal improvement. The varieties selected for adaptability trials should include a range of species and morphological types that have agronomic potential. This would be an excellent way to obtain indications of the geographical areas where given types will grow best. It would be the first step towards developing 'zones' for amaranth adaptability, similar to those used for soybeans. Larger trials, involving perhaps 100–500 varieties, should also be undertaken by geneticists and screening nurseries. Demonstration trials under farm conditions are also recommended.
3. *Ethnobotanical aspects of the crop*: Studies of the ways people grow and use grain amaranths in Central and South America and the Himalayas, as well as those methods employed to grow and use vegetable amaranths elsewhere, could be very informative. Data could be collected about the most favourable environments within which the various species can be grown and much useful information could be collected by documenting the rainfall, temperature, day length and soil conditions of these areas. This would enable to target new areas for amaranth cultivation. It would also demonstrate soil requirements, crop ecology and social aspects of amaranth cultivation and use. Such studies will not only benefit those wanting to grow amaranth for the first time but will also assist people who traditionally cultivate the crop.
4. *Role of weedy amaranths*: From agronomic point of view, amaranth, specially the weedy members, is interesting for their drought tolerance, low susceptibility to disease and pest (Barba de la Rosa et al. 2009) and environmental plasticity, i.e. ability to grow in areas where traditional crops fail to adapt (Brenner et al. 2000). Amaranths are leading the modern weed science research and have played as a model system for the study of weedy nature of plants (Basu et al. 2004).

Many amaranth species have developed unique resistance to few herbicides, recommended for the control of annual dicot weed. Heap (2002) reported about six amaranth species among 25 worst resistant weeds in the world. Amaranth weeds provide valuable genomic resources that can be utilised for improving important agronomic crop trait, like potentiality to generate herbicide resistance. Genome analysis of resistant species can be helpful to explore the evolution of herbicide resistance in plants as well as the possibility to transfer the resistant trait from weed to crop.

Seeds of the shattering, weedy amaranth species (e.g. *Amaranthus hybridus*, *Amaranthus palmeri*, *Amaranthus retroflexus* and *Amaranthus spinosus*) should not be distributed for cultivation. Nevertheless, these weeds can be useful to the amaranth breeder. *Amaranthus hybridus* is the wild progenitor species of present-day cultivated *Amaranthus hypochondriacus* and easily exchanges genes with it. For introducing desirable traits like faster maturation, disease resistance and wider adaptability in the cultivated forms, the role of *Amaranthus hybridus* could be invaluable. *Amaranthus spinosus* (a diploid), sometimes a troublesome weed, can be utilised in raising hybrids (F_1 triploids) with *Amaranthus dubius* (a tetraploid) on a commercial scale for forage. This is made possible by the peculiar distribution of male and female flowers in *Amaranthus spinosus*. The hybrids are fast-growing and

sterile and have very soft spines. Feeding trials and nutritional studies are, however, a prerequisite before using such hybrids for forage.

5. *Taxonomic delimitation in amaranths*: Taxonomic disputes in amaranths are still not clearly resolved. The presence of a large number of morphotypes, landraces and overlapping morphological features and frequent misapplication of names and a large number of synonyms have made taxonomic delimitation in amaranths much complicated. Subcategorisation in grain amaranth and its derivation from weed amaranths almost unequivocally resolved applying both morphological and molecular parameters. Weedy and grain amaranths are phylogenetically and historically linked inseparably. Disputes regarding taxonomic segregation in vegetable amaranth are still awaiting proper attention. From Lower Gangetic Plain of West Bengal, India, few new species and varieties of vegetable amaranths have been identified having specific morphological and reproductive identity. The plains of India and other Southeast Asian countries are rich source of vegetable amaranth germplasm with unique diversity. This diversity should be explored to broaden the vegetable list of nutritive potential.

   It is assumed that both the grain and vegetable amaranths have originated from their respective weed progenitors at different locale of the globe. But unlike grain amaranth, we don't have any concrete idea about the putative progenitor of vegetable amaranth and phylogenetic linkage among them as well. Information on genetic diversity and relationship within and among crop species and their wild relatives is essential for the fruitful utilisation of the Plant Genetic Resources. It is of common consensus that the monoecious grain and vegetable amaranths form two distinct groups. The subgenus *Acnida* represents dioecious weedy amaranths. Its relation with grain amaranths is yet to be explored conclusively which is very important for their utilisation in any breeding programme.

6. *Biotechnological approach in genetic improvement of amaranth*: Previously the genetic improvement of amaranths has been done by conventional selection method and hybridisation. But recently biotechnological procedures have been applied and found to be very effective. A novel protein *AmA1* has been isolated from the seeds of *Amaranthus hypochondriacus* and subsequently purified and characterised; its cDNA was cloned, and through transformation, transgenic potato and wheat plant has been raised. This has opened a new avenue to improve the nutritive value of several other crops through introduction of *AmA1* protein gene by *Agrobacterium*-mediated transformation.

   Much of the research activities done on amaranths has focussed on its exceptional nutritive value. The main reason could be the content of protein, fat and active substance with antidiabetic, anti-hyperlipidemic, spermatogenic and anticholesterolemic effects (Sangameswaran and Jayakar 2008; Girija et al. 2011) and antioxidant and antimicrobial activities (Alvarez-Jubete et al. 2010; Tironi and Aron 2010). Additional interest is generated by the oil and carbohydrate profile of amaranth seeds which offers scope for various industrial applications. Nutritive value of vegetable amaranths is somewhat degraded by the presence of some antinutrients like oxalic acid and nitrates. The presence of saponin and phenolic compounds in grains is responsible for its unpalatability. Decarboxylative degradation of oxalic acid is catalysed by enzyme oxalate decarboxylase. A full-length cDNA for oxalate decarboxylase was cloned and characterised, and transgenic tomato plant was raised showing oxalate decarboxylase activity. This approach is very promising for vegetable amaranths to reduce oxalate problem and make it more acceptable. A protocol for *Agrobacterium*-mediated transformation of *Amaranthus tricolor* has already been developed and standardised. This could be utilised to produce transgenic amaranth with oxalate decarboxylase activity.

7. *Some specific agronomic requirements*: From agronomic point of view, though amaranth requires little agronomic attention in comparison with other conventional crops, still few steps need to be taken to improve yield. Amaranth cultivation and harvesting practices require several types of research in the following aspects:
   1. Selection of type best adapted to local conditions
   2. Application of mixed cropping system in amaranth cultivation and crop rotation
   3. To learn about the most extreme rainfall, evaporation and soil characteristics under which amaranths grow and produce a reasonable yield
   4. Knowledge about soil requirements, i.e. fertility, tolerance to salinity, need for organic matter and maximum and minimum moisture
   5. Knowledge about the effects of growth conditions on chemical composition and nutritive value
   6. Determination of best planting dates, plant density and weed and pest management
   7. Development or adaptation of machinery, planting implements, thresher, winnower and grain cleaner
   8. Control of diseases and pests causing significant damage of the crop
   9. Proper strategies of seed storage to sustain seed viability for longer period of time

   Future research should be directed towards achieving few breeding objectives which are yet to be achieved. Presently, breeding objectives of grain amaranths prescribe selection for the following traits:
   1. Desirable growth characteristics such as reduced plant size, reduced sensitivity to photoperiod, synchronous flowering, early maturity, reduced lodging and uniform drydown
   2. Environmental adaptations such as drought tolerance, pest and disease resistance, herbicide tolerance and efficient fertiliser utilisation
   3. Food quality such as white seeds, palatability and high levels of protein and essential amino acids
8. *Processing of grain amaranths*: Research is needed on the physiology of postharvest handling, especially on the effect of moisture on grain quality and storage. Studies are also needed on some other agrotechnical aspects:
   1. Grain cleaning
   2. Removal of sand and weed seeds from the grain
   3. Applicability of existing machineries to handle amaranth
   4. Drying and storage of harvested grains
   5. Processing of the whole grain, such as by extrusion cooking, milling whole and popped seed and toasting, rolling, sprouting and popping, to assess any changes in nutritional value or chemical compounds from such processing
   6. Commercial development requirements, such as dry and wet milling and derivatives
   7. Storage and shelf life of products
   8. Value of the grain and crop residues for ensilage and for direct feeding to livestock
9. *The use of grain amaranths as food*: Regarding the use of grain amaranths, research is needed in the following aspects:
   - Basic characteristics of seed starch, protein, bran, germ and oil
   - Uses in products, including breakfast foods and weaning mixtures, as well as recipe development
   - Nutritional testing in humans
   - Amaranth's value as a wheat extender or a supplement for added nutritional value in traditional foods such as chapattis, tortillas, weaning foods, chicha and arepas
   - Its use in infant foods
   - Nutritional availability of minerals, vitamins, proteins and starch
   - Amaranth's functional characteristics (viscosity, density, freeze-thaw stability, heat stability, emulsifying properties) when used in foods, and how grain types differ from one another in those characteristics.
   - Anti-nutritional constituents

10. *Vegetable amaranths*: Vegetable amaranths have been more thoroughly investigated than the grain amaranths in Asia and more specifically in Southeast Asia. Several important aspects are still left for improvement. Selections have been made by Asian growers for many years; varieties have been identified suitable for widespread culture. Nevertheless, further improvement of the crop could be achieved by studies of the following:
    1. Pest and disease resistance
    2. Nutrient uptake and nutrient content at different stages of harvest or crop growth
    3. Leaf yield
    4. Food quality, including tenderness and storage methods to prolong the life of the harvested produce
    5. The use of amaranth leaves as a remedy for vitamin A deficiency
    6. Anti-nutritional factors and heavy-metal accumulation in response to type and quantity of fertilisers used and type of soil
    7. Production of leaf-nutrient concentrate
    8. Regrowth after harvest
    9. Comparison of yield from clipping versus successive planting
    10. Seed production and farmer-selection techniques
    11. Leaf/stem ratio
    12. Late emergence of inflorescences
    13. Planting and cultural practices for efficient use of land, water and fertiliser
    14. Crop rotation to avoid soil-borne diseases
    15. Proper timing of harvest
    16. Benefits and possible toxic problems of vegetable amaranth as a forage
11. *New uses*: The germs and brans of grain amaranths contain about 20 % oil. There is a need to study and screen the germplasm for edible oil. The amaranth oil is the rich source of squalene (a high-priced material found in amaranth seed but normally obtained from shark livers and used in cosmetics). The industrial application of amaranths is an area which is to be explored in much extensive manner. The nutraceutical activities of amaranths are yet to be explored adequately. There is a need to evaluate the impact of processing and cooking on the nutritional properties of the species. Amaranth can also be a potent source of natural dyes and pharmaceuticals.
12. *Environmental impact*: It is important to study the weediness of the most problematic *Amaranthus* species and the likelihood of their becoming pests. Amaranth pollen and grain may cause allergic reactions in some people, and this needs to be addressed. Both the grain- and vegetable-type amaranths could provide many nutritious foods for the world. The small seed size is a limitation in planting as well as in harvesting, threshing and cleaning the grain. But modern experience in the Northern Indian plains shows that they have a good chance of adapting successfully. They might complement other cereals such as sorghum, millets or barley, thus helping countries that import large amounts of wheat. In addition, they would provide a local source of feed grain for the poultry industries of developing nations. Grain amaranths are the new promising crop for drylands (areas with 600–800 mm of rainfall per year) and for tropical highlands up to extreme elevations (3500 m and above) and as a quick-maturing, dry-season crop for monsoon areas. It would be a daydreaming and too much optimism to expect amaranth to be on dinner plates next year; it took a century for the American people and the farmers to accept the soybean, and it took two centuries for Europeans to give recognition to potato. Comparing with such now-established crops, amaranth has attracted the attention of scientific research or testing though in small scale. With the help of today's communications and technology, the day is not far away when amaranth would find its niche. Within a few years, it seems likely that this ancient grain of the Americas will return to grace in the modem age. Eventually, it may prove to be as a rich leg-

acy of the American Indian as maize and beans.

Despite the growing evidence in amaranth's favour, much research needs to be done before the crop be commercially produced on large scale like conventional cereals and widely accepted. Nevertheless, the researchers are studying the crop's responses to climate, soil conditions, pests and diseases. Also, they are engaged in breeding of short-statured plants of uniform height with sturdy, wind-resistant stalks and high-yielding seedheads that hold their seeds until they are harvested. Much of the amaranth's development has been done in the Rodale Research Centre near Emmaus, Pennsylvania, where more than a thousand different accessions collected from all parts of the world were bred, grown and evaluated. Further collaboration has been initiated with scientists in Africa, Asia and Latin America; as a result, plant lines have been selected to overcome tendencies towards lodging, seed shattering, indeterminate growth, succulence at harvesting time and day-length dependence. This research effort has produced grains with improved baking, milling, popping and taste qualities, as well as machinery adapted to planting, cultivating, harvesting and threshing the crop. Lines of uniform colour and height that bear their seedheads above the leaves, thus making them suitable for mechanical harvest, are now available. The crop can be said to be on the threshold of limited commercial production in the USA. Several companies are testing the grain in their products, and an amaranth-based breakfast cereal is available. Research has mainly emphasised grain amaranths so far, but in 1967 FAO started investigation on vegetable amaranth. The following year it began field experiments in home garden projects in Nigeria and Benin. Later it commissioned germplasm collections. As a result, the vegetable branch of the amaranth family is beginning to attract recognition, and FAO has published a report on these species (Grubben and Van Sloten 1981).

In any country among the crops under cultivation, some may be native, and some may be of non-native origin from other regions. The Plant Genetic Resources for Food and Agriculture (PGRFA) formulated the basis for the establishment of a multilateral system of access and benefit sharing which is applicable to a list of crops under a Standard Material Transfer Agreement (SMTA) for food security and interdependence irrespective of the origin of the crop. Enforcement of the Convention on Biological Diversity (CBD) from 1993 and provisions under trade-related aspects of Intellectual Property Rights (TRIPS) led to the apprehension that exchange of germplasm would get restricted. To increase the food production at global level on sustainable basis, dependence on crop genetic resources that originated from different geographical locations through introduction and exchange is inevitable. This holds good especially in the case of underutilised crop species which have same centre of origin and centre of domestication because domestication process is still in evolutionary phase.

Appendices

Appendix I

Abbreviation

AICRP	All India Coordinated Research Project
ALS	Acetolactate Synthase
AVRDC	Asian Vegetable Research and Development Center
CBD	Convention on Biological Diversity
CGIAR	Consultative Group on International Agricultural Research
CIAT	International Center for Tropical Agriculture
CSIRO	Commonwealth Scientific and Industrial Research Organisation
EPSPS	5'-enolpyruvyl-shikimate-3-phosphate synthase
FAO	Food and Agriculture Organization
GCA	General Combining Ability
IBPGR	International Board of Plant genetic Resources
ICAR	Indian Council of Agricultural Research
ICARDA	International Center for Agricultural Research in the Dry Areas
IPGRI	International Plant Genetic Resources Institute
IITA	International Institute of Tropical Agriculture
IRRI	International Rice Research Institute
ISTA	International Seed Testing Association
LIFDC's	Low-Income Food-Deficit Countries
MAF	Major Allele Frequency
NAS	National Academy of Sciences
NBPGR	National Bureau of Plant Genetic Resources
NCRPIS	North Central Regional Plant Introduction Station
NPU	Net Protein Utilization
PER	Protein Efficiency Ratio
PGR	Plant Genetic Resources
PGRFA	Plant Genetic Resources for Food and Agriculture
PIC	Polymorphic Information Content
PPO	Protoporphyrinogen Oxidase
ROS	Reactive Oxygen Species
RRC-	Rodale Research Center
SCA	Special Combining Ability
SMTA	Standard Material Transfer Agreement
SNPs	Single Nucleotide Polymorphisms
TILLING	Targeting Induced Local Lesion IN Genomes
TRIPS	Trade-Related Aspects of Intellectual Property Rights
UPGMA	Unweighted Pair Group Method with Arithmetic mean
VPKAS	Vivekananda Parvatiya krishi Anusandhn Shala

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USDA-ARS	United States Department of Agriculture-Agricultural Research Service
USDA-GRIN	United States Department of Agriculture-Germplasm Resources Information Network
UUC	Underutilized Crop

Appendix II

Compiled list of *Amaranthus* species (including synonyms) based on Australian Plant Name Index (APNI), International Plant Names Index (IPNI), Index Kewensis (IK) and Gray Card Index (GCI)

Species	Synonyms	References
Amaranthus abyssinicus hort. ex L.H. Bailey		Man. Cult. Pl. 252(1924) in Syn
Amaranthus acanthobracteatus Henrickson		Sida 21(1): 12. 2004
Amaranthus acanthocarpa		Wohlpart & Mabry 1968
Amaranthus acanthochiton J.D. Sauer	= *Acanthochiton wrightii* Torrey in L. Sitgreaves	Madrono 13:44, 1955
Amaranthus acroglochin Spreng		Syst.Veg. (ed 16) [Sprengel] 1: 927.1824
Amaranthus acutilobus Uline & W.L. Bray		Bot. Gaz. 19: 320.1894
Amaranthus adulterinus Thell.		Repert. Spec. Nov. Regni Veg. 24: 301, hybr. 1928
Amaranthus aeneus Besser		Cat. Hort. Volhyn. (1816) 8
Amaranthus affinis Thell.		Repert. Spec. Nov. Regni Veg. 21: 324. 1925
Amaranthus albiformis Moq.		Prodr. [A.P. de Candolle] 13(2): 263.1849
Amaranthus albomarginatus Uline & W.L. Bray.		Bot. Gaz. 19: 318.1894
Amaranthus albus L.	= *A. albus* var. *pubescens* (Uline & Bray) Fernald	Fl. Cap. (Thunberg, ed.2) 215. 1823
	= *A. pubescens* (Uline & Bray) Rydberg	
Amaranthus alius E.H.L.Krause		Beih. Bot. Centralbl., Abt. 2. 33(2): 481, sp. aggreg. 1915
Amaranthus alopecurus Hochst ex A. Br. & C.D. Bouche		Index Seminum [Berlin] 1: 1872. Sauer (1950)
Amaranthus altissimus Riddell	*Acnida altissimus* (Riddell) Moq.	Syn. Fl. West States 41: 1835
Amaranthus amboinicus Buch.-Ham.		Numer. List [Wallich] n. 6987.1832
Amaranthus ambigens Standley		N. Amer. Fl. 21(2) : 106.1917
Amaranthus anacardana Hook.f.		Fl. Brit. India [J.D. Hooker] 4(12): 719. 1885
Amaranthus anardana Buch.-Ham. ex Moq.	= *A. hypochondriacus* L.	Numer. List. [Wallich] n. 6903. 1832
Amaranthus andersonii J.T. Howell	= *Scleropus urceolatus* Andersson	Proc. Calif. Acad. Sci. Ser. 4, 21: 95. 1933
Amaranthus angustifolius Lam.	= *A. graecizans* ssp. *graecizans*	Encyclopedie Methodique Botanique 1: 115. 1783
	= *A. blitum* var. *graecizans*	
	= *A. graecizans* L.	
Amaranthus annectens S.F. Blake		J. Bot. 53: 103, 1915

(continued)

Species	Synonyms	References
Amaranthus aragonensis Sennen		Bull. Geogr. Bot. 1911, xxi.123
Amaranthus arardhanus Sweet		Hort. Brit. [Sweet] ed. 3. 569.1839.
Amaranthus arctioideus Perr. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 332. 1849
Amaranthus arenicola I.M. Johnston		J. Arnold Arbor. 29: 193. 1948
Amaranthus artineanus Muschl.		Man. Fl. Egypt i 311 (1912). hybr.
Amaranthus arvensis E.H.L.Krause		Deutschl. Fl. (Sturm) ed. 2. 5: 137. 1901
Amaranthus ascendens Loiseleur	= *A. ascendens* sensu auct *Japon* = *A. lividus* L.	Not. Fl. France 141. 1810.
	= *Euxolus viridis* var. *ascendens* (Lois) Moq.	
*Amaranthus aschersonianus*Thell.		Graebn. Syn. Mitteleur. Fl. V. 309 (1914)
Amaranthus asplundii Thell.		Repert. Spec. Nov. Regni Veg. 21: 322. 1925
Amaranthus ataco Thell.		Repert. Spec. Nov. Regni Veg. 16: 23. 1919.
Amaranthus atropurpureus Roxb.	= *A. oleraceus*	Fl. Brit. Ind. [J.D. Hooker] 3. 608. 1885
Amaranthus atrosanguineus Hort. Lugd.		(18380 ex Moq. In DC. Prod. Xiii.II 266
Amaranthus aureus Hort. ex Moq.	= *A. hybridus* L.	Prodr. [A.P. de Candolle] 13(2): 259. 1849. Kirpicznikov (1969)
Amaranthus australis (A. Gray) J.D. Sauer	= *Acnida australis* A. Gray	Madrono 13: 15. 1955.
	= *A. alabamensis* Standley	Sauer (1955)
	= *A. cannabina* L. var. *australis*	
	(A. Gray) Uline & W.L. Bray	
	= *A. cuspidata* Bertero ex Sprengel	
Amaranthus bahiensis Mart.		Herb. Fl. Bras.n. 969
Amaranthus batalleri Sennen		Butl. Inst.Catalana Hist. Nat.1932 xxxii.iii. in syn
Amaranthus bellardii Hort. ex Moq.		Prodr. [A.P. deCandolle] 13(2): 259. 1849
Amaranthus bengalensis Saubhik Das & Iamonico		Phytotaxa 181(5): 297 2014
Amaranthus berlandieri (Moq.) Uline & W.L. Bray		Bot. Gaz. 19: 268. 1894
Amaranthus berchtholdi Hort. ex Moq.		Prodr. [A.P.deCandolle] 13(2): 259. 1849
Amaranthus berchtoldii Seidl. ex Opiz.		Boehm. Gen 164. Natural 1. 1823
Amaranthus bicolor Nocca ex Willd.		Sp.Pl. ed. 4. [Willdenow] 4(1): 384. 1805
Amaranthus bernhardi hort ex Moq.		Prodr. [A.P. deCandolle] 13(2): 258. 1849
Amaranthus bigelovii Uline & W.L. Bray		Bot. Gaz. 19: 271. 1894
Amaranthus blitoides S. Watson		Proc. Amer. Acad. Arts. 12: 273. 1877

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Species	Synonyms	References
Amaranthus blitonius St. Lag		Ann. Soc. Bot. Lyon vii: 119 (1880)
Amaranthus blitum L.	= *A. lividus* L.	Sp.Pl. 2: 990. 1753
	= *A. ascendens* Loiseleur	Feine (1981); Robertson (1981); Sreelathakumary & Peter (1993);
	= *A. lividus* ssp. *lividus*	
	= *A. blitum* var. *blitum*	
	= *A. blitum* auct. non L.	
	= *A. graecizans* L.	
Amaranthus blitum var. oleraceus (L.) Hook.F.	= Cultivated form of *A.lividus* L.	Fl. Brit. India 4: 721. 1885
Amaranthus blitum var. *ascendens* (Loisel.) DC		Cat. Pl. Horti Monsp. 4. 1813
Amaranthus bouchonii Thell.	= Aberrant form of *A.powellii*	Le Monde des Plantes 27(160): 4. 1926
	= *Amaranthus bouchonii* Thell.	
	= *A. powellii*	
Amaranthus parganensis Saubhik Das		Novon 23(4): 406. 2015
Amaranthus bengalense Saubhik Das & Iamonico		Phytotaxa 181(5): 297. 2014
Amaranthus brandegeei Standl.		N. Amer. Fl. 2: 109. 1917. Sauer (1978)
Amaranthus brownie m Christoph. & Caum		Bull. Bernice P. Bishop Mus. 81: 25. 1931
Amaranthus buchtienianus Thell.		Repert. Spec. Nov. Regni Veg. 21: 323 91925)
Amaranthus cacciatoi (Aellen ex Cacciato) Iamonico		Willdenowia 43(2): 239 (2013)
Amaranthus californicus (Moquin-Tandon) S. Watson	= *Mengea californica* Moq.	Bot. California [W.h. Brewer] 2: 42.1880
Amaranthus campestris Willd.		Sp. Pl. ed. 4 [Willdenow] 4(1): 382. 1805
		Hooker (1885); Tanaka (1976)
Amaranthus canariensis Besser		Cat. Hort. Volhyn. (1816) 8.
Amaranthus cannabinus (L.) J.D. Sauer	= *Acnida cannabina* L.	Madrono 13: 11.1955
Amaranthus capensis Thell.		Syn. Mitteleur. Fl. [Ascherson & Graebner]. 5(1): 293. 1914
Amaranthus capitatus Cat.		Hort. Turic. (1827–28)
Amaranthus caracam Besser		Cat. Hort. Volhyn (1816)g
Amaranthus caracasanus Kunth		Nov. Gen. Sp. [H.B.K] 2:195 (1818)
Amaranthus caracu Zucc. ex Steud.		Nomencl. Bot. [Steudel] ed. 2. 1:69. 1840.
Amaranthus cararia Besser		Cat. Hort. Volhyn (1816) 8
Amaranthus cararu Jacq ex Zuccagni		Cent. Observ. Bot. [p.46] No. 94. 1806
Amaranthus cardenasianus Hunz.		Bol. Soc. Argent. Bot. Iv. 136 (1951)
Amaranthus carneus Greene		Pittonia 2(8): 105. (1890)
Amaranthus carolinae Kov.		Nauch. Trud. Vissh Selskostop.
		Inst. Plovdiv 23(1): 51. 1978

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Species	Synonyms	References
Amaranthus cathecu Hort. ex Moq.		Prodr. [A.P. deCandolle] 13(2): 259. (1849)
Amaranthus caturus B. Heyne ex Hook.f.		Fl. Brit. India [J.D. Hooker] 4(12): 720. 1885
Amaranthus caudatus L.		Sp. Pl. 2: 990 1753
Amaranthus cauliflorus Link.		Enum. Hort. Berol. Alt. 2:389. 1822
Amaranthus celosioides Kunth		Nov. Gen. Sp [H.B.K.] 2: 194. 1818
Amaranthus centralis J.Palmer & Mowatt		Nuytsia 19(1); 111. (2009)
Amaranthus cernuus Besser		In Cat. Hort. Turic. (1827–8) "An Celosia"?
Amaranthus chihuahensis S. Watson		Proc. Amer. Acad. Arts 21:436. (1886)
Amaranthus chipendalei Kov.		Nauch. Trud. Vissh Selskostop. Inst. Plovdiv 23(1): 52. (1978)
Amaranthus chlorostachys Willd.	= *A. hybridus* ssp. *Hybridus*	Hist. Amaranth. 34 (t.10,fig.19). 1790
	var. *hybridus*	
	= *A. hybridus*	
	= *A. chlorostachys* auct.	
	= *A. powellii*	
	= *A. hybridus* var. *hybridus*	
Amaranthus circinnatus Hort. Parisex Poir.		Encycl. [J. Lamarck & al] Suppl. 1.311. 1810
Amaranthus clementii Domin		Bibliotheca Botanica 89(4) 1928
Amaranthus coesius F. Dietr. Ex Moq.		Prodr. [A.P. de Candolle] 13(2): 266. 1849.
Amaranthus communicates Kerner		Oester. Bot. Z. 25: 194.1875
Amaranthus coracanus Mart.		Hort. Erlang. 197
Amaranthus congestus C.C. Townsend		Kew Bull. 43(1): 103. (1988)
Amaranthus crassipes Schlechtendal	= *A. crassipes* var. *warnockii*	Linnaea 6: 757. (1831)
	(I.M. Johnston) Hendrickson	
	= *A. warnockii* I.M. Johnston	
Amaranthus crispus Terrac.		Atti. Accad. Sc. Napoli Ser. 2 iv. (1890) App. 2
Amaranthus cristatus Noronha		Verh. Batav. Genootsch. Kunst. 5(Art. 4) : 7. 1790
Amaranthus cristulatus Speg.		Comun. Mus. Nac. B. Aires 1: 345. 1901
Amaranthus crocatus Besser		Cat. Hort. Volhyn. (1816) g
Amaranthus cruentus L.	= *A. hybridus* (L.) ssp. *cruentus* L. Thellung	Syst. Nat. Ed. 10. 2:1269. 1759
Amaranthus cruentus L. var. *patulus* (Bertol) Lambinon		Bull. Jard. Bot. Natl. Belg. 47(1–2): 247. 1977.
Amaranthus cruentus L. var. *albus* Saubhik Das		Nordic J. Bot. 30(4): 418. 2012
Amaranthus curvifolius Spreng.		Syst. Veg. ed. 16 [Sprengel] 1: 928. 1824
Amaranthus cuspidifolius Domin		Bibliotheca Botanica Ixxxix 78. 1921

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Species	Synonyms	References
Amaranthus deflexus L.		Mantissa Plantarum Altera 295. 1771
Amaranthus delilei Richter & Loret		Bull. Soc. Bot. France 13: 316. 1868.
Amaranthus desfontanii Kov.		Nauch. Trud. Vissh Selskostop. Inst. Plovdiv. 23(1): 51. 1978
Amaranthus diacanthus Raf.		Fl. Ludov. 31. 1817
Amaranthus diandrus Spreng.		Neue Entdeck. Pflanzenk. 3:20. 1822.
Amaranthus diffusum Dulac		Fl. Hautes-Pyrences 174. 1867
Amaranthus dioicus Michx ex Moq.		Prodr. [A.P. de Candolle] 13(2): 277. 1849
Amaranthus dinteri Schinz		Mem. Herb. Boiss. No. 29. 15
Amaranthus divaricatus Andrz. ex Lindem.		Prod. Fl. Cherson. 185.
Amaranthus dubius Mart ex Thell.	= Tetraploid	Fl. Adv. Montpellier 38: 203. 1912. Thell. in Aschers & Graebn. Syn. Mitteleur Fl. 591): 265. 1914
Amaranthus dussil Sprenger		Bull. Reale Soc. Tosc. Ortic. 21: 178. 1896.
Amaranthus edulis Speg.	= *A. caudatus* L.	Physis (Buenos Aires) 3: 163. 1917
	=*A. caudatus* ssp. *Mantegazzianus*	
Amaranthus edulis var. *spadiceus* Hunz.		Revista Argent. Agron. 10: 330. 1943
Amaranthus edulis var. *typicus* Hunz.		Revista Argent. Agron. 10: 330. 1943
Amaranthus emarginatus Salzm. ex Uline & W.L. Bray		Bot. Gaz. 19: 319. 1894
Amaranthus enervis (F. Muell.) Kov.		Nauch. Trud. Vissh Selskostop Inst. Plovdiv 23(1): 50. 1978
Amaranthus esculentus Besser		Cat. Hort. Volhyn. (1816) g. Ex Moq. in DC. Prod. Xiii. II.266
Amaranthus eugenii Sennen		Butl. Inst. Catalana Hist. Nat. Xxxii. 111. 1932.
Amaranthus farinaceous Roxb. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 266. 1849; Sauer (1950)
Amaranthus fimbriatus (Torr.) Bentham ex S. Watson	= *A. fimbriatus* var. *denticulatus*	Bot. California [W.H. Brewer] 2:42. 1880
	(Torrey) Uline & W.L. Bray	
Amaranthus flavus L.	= *A. hypochondriacus* L.	Syst. Nat. Ed. 10. 2: 1269. 1759; Feine (1981)
Amaranthus floridanus (S. Watson) J.D. Sauer	= *Acnida floridana* S. Watson	Madrono 13: 25. 1955; Sauer (1955)
Amaranthus frumentaceous Buch.-Ham.	= *A. paniculatus*	Buch.-Ham. in Roxb. Fl. Ind. Iii. 699
	var. *frumentaceous*	
	= *A. hypochondriacus* L.	
	= *A. hybridus* ssp. *hybridus*	
Amaranthus filicaulis Sennen		Cavanillesia ii 34. 1929. Hybr. ?
Amaranthus flexuosus Ambrosi		Fl. Tirolo Mer. 2: 187. 1857
Amaranthus floridus Benth.		Bot. Voy. Sulphur [Bentham] 158, t. 51. 1846

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Species	Synonyms	References
Amaranthus frutescens Hort. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 348. 1849
Amaranthus furcatus J.T. Howell		Proc. Calif. Acad. Sci. Ser. 4, 21: 94. 1933
Amaranthus galii Sennen & Gonzalo		Cavanillesia ii. 34. (1929) hybr.
Amaranthus gangeticus L.		Syst. Nat. Ed. 10. 2: 1268. 1759
Amaranthus giganteus Besser		Cat. Hort. Volhyn. (1816) 91 F. G. Dietr. Lexik. Gaertn. i. 313.
Amaranthus glaucus Biv.		Nuove Piante II, footnote, 1838.
Amaranthus glomeratus Posp.		Fl. Oesterr. Kustent. i. 375. 1897
Amaranthus gracilis Desf.	= *A. viridis* L.	Tabl. Ecole Bot. 43. 1804
Amaranthus graecizans L.	= *A. albus* L.	Sp. Pl. 2: 990. 1753
	= *A. graecizans* var. *graecizans*	
Amaranthus grandiflorus (J.M. Black) J.M. Black		Transactions and Proceedings of the Royal Society of South Australia 60. 1936
Amaranthus greggii S. Watson		Proc. Amer. Acad. Arts 12: 274.1877
Amaranthus guadeloupensis Hort. ex Moq.		Prodr. [A.P. de candolle] 13(2): 257. 1849
Amaranthus hierichuntinus Vis.		In Att. Ist. Ven. Sc. Ser. III, iv. (1858–59) 139
Amaranthus haughtii Standl.		Publ. Field Mus. Nat. Hist. Bot. Ser. 11: 149. 1936
Amaranthus hungaricus Soo.		Repert. Spec. Nov. Regni Veg. 22: 318, hybr.1926
Amaranthus hunzikeri N. Bayon		Novon 17(3): 294. 2007
Amaranthus huttonii Hort. Veitch.		Gard. Chron. 215 (1872)
Amaranthus hybridus L.		Sp. Pl. 2: 990. 1753
A. hybridus var. *bouchonii* (Thell.) lambinon		Candollea 52(2): 273. 1997
A. hybridus L. var. *chlorostachya* Thell.	= *A. chlorostachya* Willd.	Fl. Advent. Montpellier 205. 1912
A. hybridus L. var. *densus* Farw	= *A. paniculata* L. var. *densus* Regel.	Rep. (Annual) Michigan Acad. Sci. 20; 175. 1918
A. hybridus L. var. *erythrostachys* Moq.		Prodr. [A.P. de Candolle] 13(2): 259. 1849
A. hybridus L. var. *hecticus* (Willd.) Moq.	= *A. hecticus* Willd.	Prodr. [A.P. de Candolle] 13(2): 260. 1849
A. hybridus L. var. *hypochondriacus* (L.) *B.L. Rob.*	= *A. hypochondriacus*	Rhodera 10: 32. 1908
A. hybridus L. var. *paniculatus* Uline & W.L. Bray	= *A. paniculatus*	Mem. Torry Bot. Club 5: 145. 1894
A. hybridus L. var. *batalieri* (Sennen) Carretero		Collect. Bot. (Barcelona) 11: 129.1979
A. hybridus L. var. *bellardii* Moq.		Prodr. [A. P.de Candolle] 13(2): 259. 1849
A. hybridus L. var. *laetus* (Willd.) Moq.	= *A. laetus* Willd.	Prodr. [A. P.de Candolle] 13(2): 259. 1849

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Species	Synonyms	References
A. hybridus L. var. *leucocarpus* (S. Watson) Hunz.	= *A. leucocarpus*	Revista Argent. Agron. 10: 340.1943
A. hybridus L. var. *patulus* Thell.	= *A. patulus* Bertol	Fl. Advent. Montpellier 206. 1912
A. hybridus L. var. pergaminensis Covas		Darwiniana 5: 336. 1941
A. hybridus L. var. *prostrate* Moq.		Prodr. [A. P.de Candolle] 13(2): 260. 1849
A. hybridus L. var. *pseudoretroflexus* (Thell.) Carretero		Collect. Bot. (Barcelona) 11: 125. 1979
A. hybridus L. var. *quitensis* (Kunth) Covas	= *A. quitensis* Kunth	Darwiniana 5: 336. 1941
A. hybridus L. var. *rubricaulis* Moq.		Prodr. [A. P.de Candolle] 13(2): 259. 1849
A. hybridus L. var. *sanguineus* Farw.	= *A. paniculatus* L. var. *sanguineus* Regel.	Rep (Annual) Michigan Acad. Sci. 20; 175. 1918
Amaranthus hypochondriacus L.	= *A. hybridus* ssp. *hybridus* var. *erythrostachys*	Sp. Pl. 2: 991. 1753
	= *A. hybridus* var. *erythrostachys*	
Amaranthus hypochondriacus L. var. *macrostachys* Moq.		Prodr. [A. P.de Candolle] 13(2): 256. 1849
Amaranthus hypochondriacus L. var. monstrosus Moq.		Prodr. [A. P.de Candolle] 13(2): 256. 1849
Amaranthus hypochondriacus L. var. *powellii* (S. Watson) Pedersen	= *A. powellii* S. Watson	Monogr. Syst. Bot. Missouri Bot. Gard. 74(2): 1245. 1999
Amaranthus hypochondriacus L. var. *racemosus* Moq.		Prodr. [A. P.de Candolle] 13(2): 256. 1849
Amaranthus hypochondriacus L. var. *tortuosus* Moq.		Prodr. [A. P.de Candolle] 13(2): 256. 1849
Amaranthus inamoenus Willd.	= *A. mangostanus*	Sp. Pl. Ed. 4 [Willdenow] 491): 386. 1805
Amaranthus incarnates Hort. Ex Moq.		Prodr. [A. P.de Candolle] 13(2): 257. 1849
Amaranthus incomptus Willd.		Enum. Hort. Berol. 64
Amaranthus incurvatus Gren. & Godr.		Prosp. Fl. Fr. 8
Amaranthus induratus C.A. Gardner ex Palmer & Mowatt		Nuytsia 19(1):117. 2009
Amaranthus intermedius Guss. ex Moq.		Prodr. [A. P.de Candolle] 13(2): 259. 1849
Amaranthus interruptus R. Br.		Prodr. Fl. Nov. Holland. 414.1810
Amaranthus lecocarpus S. Watson	= *A.leucospermum S. Watson*.	Proc. Amer. Acad. Arts 10: 347.1875
	= *A. hybridus* L.	
	= *A. hypochondriacus*	
	= *A. paniculatus*	
Amaranthus jansen-wachterianus Thell.		In Graebn Syn. Mitteleur. Fl. v. 347 (1914)
Amaranthus japonicas Hoult. ex Willd.		Sp. Pl. Ed. 4 [Willdenow] 4(1); 386. In syn. 1805
Amaranthus johnstonii Kov.		Nauch. Trud. Vissh Selskostop. Inst. Plovdiv 23(1): 50. 1978. n*om.nov*

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Species	Synonyms	References
Amaranthus jonesii (Uline & W.L. Bray) Kov.		Nauch. Trud. Vissh. Selskostop. Inst. Plovdiv 23(1); 49.1978
Amaranthus kloosianus Hunz.		Bol. Soc. Argent. Bot. 4:138.1951
Amaranthus laetus Willd.		Amaranth 28; Sp. Pl. Iv. 391 (1805)
Amaranthus lanatus Dum. Cours.		Bot. Cult. 1: 640. 1802
Amaranthus lancifolius Roxb.		Hort. Bengal 67
Amaranthus lancifolius Del. Ex Moq.		Prodr. [A.P. de Candolle] 13(2): 276. 1849
Amaranthus lanceolatus Roxb.		Fl. Ind. III. 607
Amaranthus laxiflorus Comell ex Poll.		Fl. Veron. Iii. 114. 1824
Amaranthus lecocarpus S. Watson	= *A. leucospermus* S. Watson	Proc. Amer. Acad. Arts 10: 347. 1875
Amaranthus lineatus R. Br.		Prodr. Fl. Nov. Holland. 414.1810
Amaranthus littoralis Bernh.		In Hort. Tur. (1813) ex Moq. in DC. Prod. Xiii. II.274
Amaranthus lividus L.		Sp. Pl. 2: 990.1753
Amaranthus lividus L. var. *ascendens* (Loisel.) Hayw. & Druce		Adent. Fl. Tweedside 177.1919
Amaranthus lividus L. var. *polygonoides* (Moq.) Thell. ex Druce	= *Euxolus viridis* var. *polygonoides* Moq.	Rep. Bot. Soc. Exch. Club Brit. Isles 5: 574. 1920
Amaranthus lombardoi Hunz.		Bol. Soc. Argent. Bot.4:141.1951
Amaranthus looseri Suess.		Littoa 4:128. 1939
Amaranthus macrocarpus Benth.		Fl. Austral. 5: 216. 1870
Amaranthus macrocaulos Poir.		Encycl. [J. Lamarck & al.] Suppl. 1: 314. 1810
Amaranthus macrostachyus Merat *ex* Moq.		Prodr. [A.P. de Candolle] 13(2): 256. 1849
Amaranthus major Salzm.ex Moq.		Prodr. [A.P. de Candolle] 13(2): 274. 1849
Amaranthus mangostanus L.	= *A. tricolor* L.	Cent. Pl. I. 32. 1755
Amaranthus mantegazzianus Passer.	=*A. caudatus* (differs significantly in DNA content as in *A. caudatus*)	In Hort. Parm. 4. 1864; Greizerstein & Poggio (1994)
	= *A. caudatus* var. *edulis*	
	= *A. caudatus* ssp. *edulis*	
Amaranthus margaritae Dam.		Wiener III. Gart.-Zeitung (1887) 433-35
Amaranthus melancholicus L.	= *A. tricolor* L.	Sp. Pl. 2: 989. 1753
Amaranthus miamiensis Riddell		Syn. Fl. West St. 41. 1835
Amaranthus microphyllus Shinners		Sida 1: 248. 1964
Amaranthus miniatus Hort. Avign. Ex Hook.f.		Fl. Brit. India [J.D. Hooker] 4(12): 721. 1885
Amaranthus minimus Standl.		N. Amer. Fl. 21(2): 119. 1917
Amaranthus minor Gray.		Nat. Arr. Brit. Pl. 2: 289. 1821
Amaranthus mitchellii Benth.		Fl. Austral. 5: 214. 1870
Amaranthus monstrosus Cal.		Hort. Div. (1837); Hort. Tonelle ex Moq. in DC Prod.

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Species	Synonyms	References
Amaranthus moquinii Sennen.		Cavanillesia ii. 34. 1929
Amaranthus morosus Rchb.		Fl. Germ. Excurs 585
Amaranthus mucronatus Poir.		Encycl. [J. Lamarck & al.] Suppl. 1. 311. 1810.
Amaranthus mucronatus Hort. Petrop. ex Hook.f.		Fl. Brit. India [J.D. Hooker] 4(2): 720. 1885.
Amaranthus muelleri (Uline & W.L. Bray) Kov.		Nauch. Trud. Vissh. Selskostop Inst. Plovdiv 23(1): 50. 1978
*Amaranthus muricat*us Gillies ex Moq.	= *Euloxux muricatus* Gillies ex Moq.	Prodr. [A.P. de Candolle] 13(2): 276. 1849.
Amaranthus myrianthus Standl.		Bull. Torry Bot. Club 41: 506.1914.
Amaranthus necticus Willd.		
Amaranthus neglectus Hort. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 259. 1849.
Amaranthus nepalensis Cal.		Hort. Lugd. (1836); ex Moq. in DC Prod. Xii. II. 259
Amaranthus nettii Kov.		Nauch. Trud. Vissh. Selskostop Inst. Plovdiv 23(1): 50. 1978
Amaranthus obcordatus Standl.		N. Amer. Fl. 21(2): 107. 1917
Amaranthus obovatus S. Watson		Proc. Amer. Acad. Arts xii. 275. 1877
Amaranthus oleraceus L.	= *A. blitum* var. *oleraceus*	Sp.Pl. ed. 2. 2: 1403. 1763
	= *A. lividus* ssp. *lividus*	
Amaranthus obtusiflorus (Mart.) Kov.		Nauch. Trud. Vissh. Selskostop Inst. Plovdiv 23(1): 50. 1978
Amaranthus officinalis Gromov ex Trautv.	= *A. blitum* subsp. *oleraceus* (L.) Costea	Trudy Imp. S.-Peterburgsk. Bot. Sada 9. 139. 1884.
Amaranthus olitorius Besser		Cat. Hort. Volhyn. (1816) g
Amaranthus pachystachys Rchb. ex Moq.		Prodr.[A.P. de Candolle] 13(2): 265. 1849.
Amaranthus pallidiflorus F. Muell		Fragm. (Mueller) 1(5): 140. 1859
Amaranthus pallidus M. Bieb.		Fl. Taur.–Caucas. 2: 399.1808
Amaranthus palmeri S. Watson		Proc. Amer. Acad. Arts 12: 274.1877.
Amaranthus paniculatus L.	= *A. hybridus* L. var. *paniculatus* (L.) Thell.	Sp. Pl. ed. 2. 2: 1406. 1763
Amaranthus paniculatus L. var. *cruentus* (L.) Moq.	= *A. cruentus* L.	Prodr. [A.P. de Candolle] 13(2): 257. 1849.
Amaranthus paniculatus L. var. *purpurascens* Seub.		Fl. Bras. (Maritius) 5(1): 238.1875
Amaranthus paniculatus L. var. *sanguineus* (L.) Moq.		Prodr. [A.P. de Candolle] 13(2): 238. 1849.
Amaranthus paniculatus L. var. *speciosus* L.H. Bailey	= *A. speciosus* Sims.	Stand. Cycl. Hort. 1: 270. 1914
Amaranthus paolii Chiov.		Nuovo Giorn. Bot. Ital. Ser. 2, 34: 845. 1927
Amaranthus parganensis Saubhik Das		Novon 23(4): 406. 2015
Amaranthus paraguayensis D. Parodi		Anales Soc. Cl. Argent. V. 273. 1878

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Species	Synonyms	References
Amaranthus parisiensis Schkuhr.		Bot. Handb. [C. Schkuhr] 3: 249. 1802
Amaranthus parodii Standl.		Publ. Field Mus. Nat. Hist., Bot. Ser. 17: 240. 1937
Amaranthus parvulus Peter		Repert. Spec. Nov. Regni Veg. Beih. 40: 25. 1932
Amaranthus patulus Bertol	= *A. hybridus* L.	Comment Itin. Neapol. 171,t. 12. 1837
	= *A. patulus* auct. non L.	
	= *A. cruentus* L.	
Amaranthus pedersenianus N. Bayon & C. Pelaez		Novon 22(2): 133. 2012
Amaranthus pendulus Hort. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 255. 1849.
Amaranthus perennis Bellardi		Ex Colla, Herb. Pedem. Iv. 578. 1835
Amaranthus persicarioides Hort. ex Poir.		Encycl. [J. Lamarck & al] Suppl. 1. 311. 1810
Amaranthus persimilis Hunz.		Bol. Soc. Argent. Bot. Iv. 133. 1951
Amaranthus peruvianus (Schauer) Standl.		Publ. Field Mus. Nat. Hist., Bot. Ser. 13(2): 487. 1937
Amaranthus polychroa Raeusch.		Nomencl. Bot. [Raeusch] ed. 3. 275. 1797
Amaranthus polyflagellus Spreng.		In Hort. Argent. (1834). ex Moq. in DC. Prod. Xiii. II. 266
Amaranthus polygamus L.	= *A. tricolor* L.	Cent. Pl. I. 32. 1755
Amaranthus polygonoides L.	= *A. lividus* L.	Pl. Jamaic. Pug. 27. 1759
	= *A. berlandieri* (Moquin-Tandon)	
	Uline & W.L. Bray	
Amaranthus polystachyus Willd.	= *A gracilis* L.	Sp. Pl. ed. 4 [Willdenow] 4(1): 385. 1805
Amaranthus powellii S. Watson	= *A. hypochondriacus* L. var. *powellii* (S. Watson) Pedersen	Proc. Amer. Acad. Arts 10: 347. 1875
	= *A. chlorostachys* Willd. var. *powellii* (S. Watson) Priszter	
	= *A. bracteotus* Uline & W.L. Bray	
	= *A. retroflexus* L. var. *powellii*	
	(S. Watson) B. Boivin	
Amaranthus powellii S. Watson subsp. bouchonii (Thell.) Costea & Carretero		Sida 19(4); 964. 2001
Amaranthus powellii S. Watson subsp. cacciatoi (Aellen ex Cacciato) Iamonico	= *A. bouchonii* Thell.	Nordic J Bot. 30(1): 13. 2012
	= *A. bouchonii* Thell. var. *cacciatoi*	
Amaranthus praetermissus Brenan		J.S. African Bot. 47(3): 478. 1981
Amaranthus pringlei S. Watson		Proc. Amer. Acad. Arts 22: 476. 1887
Amaranthus probstii Thell.		Repert. Spec. Nov. Regni Veg. 23: 271. 1926

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Species	Synonyms	References
Amaranthus prostratus T. Bastard	= *A. blitum* L.	Essai Fl. Maine-et-Loire 344. 1809
Amaranthus pseudogracilis (Thell.) G.H. Loos.		Jahrb. Bochum. Bot. Vereins 1: 117. 2010
Amaranthus pubescens Rydb.		Bull. Torry Bot. Club 1912 xxxix. 313
Amaranthus pumilus Rafinesque		Med. Repos. New York v. 360. 1808
Amaranthus purgans Hort. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 257. 1849
Amaranthus pycnostachys St.-Lag.		Etude Fl. Ed. 8 [A. Cariot] 2: 697. 1889
Amaranthus pyramidalis Noronha		Verh. Batav. Genootsch. Kunst. 5 (Art. 4): 7. 1790
Amaranthus quitensis Kunth		Nov. Gen. Sp. [H.B.K.] 2: 194. 1818
Amaranthus recurvatus Desf.		Tabl. Ecole. Bot. Ed. 3. (Cat. Pl. Horti Paris) 39. 1829
Amaranthus retroflexus L.	= *A. powellii* S. Watson	Sp. Pl. 2: 991. 1753
	= *A. retroflexus* var. *salicifolius*	
	I.M. Johnston	
	= *A. bulgaricus* Kov.	
Amaranthus retroflexus var. *powellii* (S. Watson) B. Boivin		Naturaliste Carad. 93: 641. 1966
Amaranthus retroflexus var. *pseudoretroflexus* (Thell.) Boivin	= *A. chlorostachys* Willd. var. *pseudoretroflexus*	Phytologia 17: 70. 1968
Amaranthus retroflexus var. rubricaulis Thell.	= *A. retroflexus* L. f. *rubricaulis* Thell. ex Probst.	In Asch & Graebn-Syn. Mitteleur. Fl. [Ascherson & Graebner] 5, Abt. 1: 260. 1914
A. retroflexus var. *salicifolius* I.M. Johnston		J. Arnold Arbor. 25:157. 1944
Amaranthus reverchonii (Uline & W.L. Bray) Kov.	= *A. blitoides* S. Watson	Nauchni Trudove Selskost Inst. "Vasil Kolarov" 23: 49. 1978
Amaranthus rhombeus R. Br.		Prodr. Fl. Nov. Holland. 414. 1810
Amaranthus rigidus Schult. ex Steud.		Nomencl. Bot. [Steudel] ed.2.1: 70. 1840
Amaranthus rosengurttii Hunz.		Kurtziana iii. 201. 1966
Amaranthus rotundifolius Herb. Par. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 261. 1849
Amaranthus roxburghianus Nevski		Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 4: 311. 1937
Amaranthus roxburghianus var. *angustifolius (Moq.)* N.C. Nair		J Bombay Nat. Hist. Soc. 73(1): 61. 1976
Amaranthus roxburghianus var. *aschersonianus* (Thell.) N.C. Nair		J Bombay Nat. Hist. Soc. 73(1): 61. 1976
Amaranthus ruber E.H.L.Krause		Beih. Bot. Centralbl., Abt. 2. 33(2): 479. 1915
Amaranthus rubescens Hort. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 257. 1849

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Species	Synonyms	References
Amaranthus rubricaulis Page & Hort. Angl.ex Moq.		Prodr. [A.P. de Candolle] 13(2): 267. nomen. 1849
Amaranthus ruderalis Koch ex moq.		Prodr. [A.P. de Candolle] 13(2): 274. 1849
Amaranthus rudis J.D. Sauer		Madrono 21(6): 1972
Amaranthus ruebelii Thell.		Repert. Spec. Nov. Regni Veg. 24: 299, hybr. 1928
Amaranthus salicifolius Hort. Veitch		Sauer (1950); Kirpicznikov (1969); Wohlport & Mabry (1968)
Amaranthus sanguineus L.		Sp. Pl. ed. 2. 2: 1407. 1763
Amaranthus sanguinolentus Schrad. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 267. 1849
Amaranthus scariosus Benth.		Bot. Voy. Sulphur [Bentham] 158, t.51. 1846
Amaranthus scandens L.f.		Suppl. Pl. 419. 1782
Amaranthus scleropoides Uline & W.L. Bray		Bot. Gaz. 19: 316. 1894
Amaranthus schinzianus Thell.		Vierteljahrsschr. Naturf. Ges. Zurich Ivii. 535. 1912
Amaranthus sclerantoides (Anderson) Anderson		Kongl. Svenska Freg. Eugenix Resa, Bot.2: 59. 1861
Amaranthus sylvestris Vill.		Cat. Jard. Pl. Strasbourg iii. 1807
Amaranthus sparganiocephalum Thell.		In Graebn. Syn. Mitteleur. Fl. V. 312 (1914), in obs.
Amaranthus spathulatus Desf. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 276. 1849
Amaranthus speciosus Sims		Sauer (1950)
Amaranthus spinosus L.		Sp. Pl. 2: 991. 1753
Amaranthus spiratus Zipp. ex Span.		Linnaea 15: 345. Nomen. 1841
Amaranthus splendens Hort.		Vilm. Blumengartn., ed. 3. 1: 868, in Syn. 1895
Amaranthus squamulatus B.L. Rob.		Proc. Amer. Acad. Arts xiiii. 32 (1907)
Amaranthus squarrulosus Uline & W.L. Bray	= *Amblogyna squarrulosa* A. Gray	Bot. Gaz. 19: 270. 1894
Amaranthus standleyanus Parodi ex Covas		Darwiniana v. 339. 1941
Amaranthus strictus Ten.		Syll. Pl. Fl. Neapol. 127. 1831
Amaranthus sylvestris Desf.	= *A. graecizans* ssp. *sylvestris*	Tabl. Ecole. Bot. 44, nom. Nud. 1804
	= *A. blitum* var. *sylvestris*	
	= *A. sylvestris* Vill. = *A. graecizans*	
Amaranthus tamaulipensis Henrickson		Sida 18(3): 808. 1999
Amaranthus taishanensis F. Z. Li. & C.K. Ni		Acta Phytotax. Sin. 19(1): 116. 1981
Amaranthus tamariscinus Nutt.	= *A. rudis* J.D. Sauer	Trans. Amer. Philos. Soc. Ser. 2, 5: 165. 1835
Amaranthus tarraconensis Sennen & Pau		Bull. Geogr. Bot. xxi. 124. 1911,

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Species	Synonyms	References
Amaranthus tenuiflorus Fisch. Hort. Hal. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 267. 1849
Amaranthus tenuifolius Willd.	= *Mengea tenuifolia*	Sp. Pl. ed. 4 [Willdenow] 4(1): 381. 1805
Amaranthus tenuis Benth.		Fl. Austral. 5: 216. 1870
Amaranthus thellungianus Nevski ex Vassilcz	= *A. graecizans* ssp. *thellungianus*/	Fl. USSR, ed. Komarov vi. 365 1936; Townsend (1980)
	= *A. polygamus*/*A. blitum* var.*polygonoides* /*A. polygonoides*	
Amaranthus thevenoei Degen & Thell.		In Graebn. Syn. Mitteleur. Fl. V. 346. 1914. hybr.
Amaranthus thunbergii Moq.		Prodr. [A.P. de Candolle] 13(2): 262. 1849
Amaranthus timeroyi Jord. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 259. 1849
Amaranthus torreyi Benth. ex S. Watson		Bot. California [W.H. Brewer] ii. 42. 1880
Amaranthus tortuosus Hornem.		Hort. Hafn. Suppl. 107
Amaranthus tricolor L.		Sp. Pl. 2: 989. 1753
A. tricolor L. var. *acutus* Saubhik Das		Phytotaxa 88(2): 27.2013
A. tricolor L.var. *tristis* (Willd.) Mehrotra, Aswal & B.S. Bisht		Companion to Chopra's Gloss. Indian Medicin. Pl. 9. 1987
Amaranthus tricolor splendens		Kirpicznikov (1969)
Amaranthus tristis L.	= *A. tricolor* L.	Sp. Pl. 2: 989. 1753
	= *A. dubius* Mart ex Thell	
	= *A. tristis* Moq. = *A. dubius*	
Amaranthus trivialis Rota.		Giorn. Bot. Ital. Ii. II. 287. 1846
Amaranthus tuberculatus (Moq.) J.D. Sauer	= *Acnida tuberculatus* Moquin-	Madrono 13; 18. 1955
	Tandon	
	= *A. rudis* J.D. Sauer	
	= *A. tamariscinus* var. *tuberculate*	
	(Moquin-Tandon) Uline & W.L. Bray	
Amaranthus tucsonensis Henrickson		Sida 18(3): 804. 1999
Amaranthus turcomanicus Gand.		Bull. Soc. Bot. France 66: 222. 1919
Amaranthus undulatus R. Br.		Prodromus Florae Novae Hollandiae. 414. 1810
Amaranthus urceolatus Benth.		Bot. Voy. Sulph. 158
Amaranthus ulinei Kov.	= *A. bigelovii* Uline & W.L. Bray var. *emarginatus*	Nauch. Trud. Vissh Selskostop. Inst. Plovdiv 23(1): 49. 1978
Amaranthus velutina Spruce ex K. Schum		Proc. Amer. Acad. Arts 22: 366. 188
Amaranthus venulosus S. Watson		Proc. Amer. Acad. Arts 17: 376. 1882
Amaranthus vernus Opiz ex Moq.		Prodr. [A.P. de Candolle] 13(2): 267. 1849
Amaranthus verticillatus Sesse & Moc.		Fl. Mexic. ed. 2. 217. 1894
Amaranthus violaceus Hort. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 270. 1849

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Species	Synonyms	References
Amaranthus viridis L.	= *A. gracilis* Desfontaines ex Poiret	Sp. Pl. ed. 2. 2: 1405. 1763
Amaranthus viscidulus Greene	= *A. bracteosus*	Pittonia 3(19): 344. 1898 ******
Amaranthus vulgatissimus Speg.	= *A. vulgatissimus* auct.	Anales Soc. Ci. Argent. 53: 281. 1902
	= *A. standleyanus*	
Amaranthus warnockii I.M. Johnst.		J. Arnold Arbor. 25: 153. 1944
Amaranthus watsonii Standley	= *A. torreyi* var. *suffruticosum* Uline & W.L. Bray	Bull. Torrey Bot. Club 41: 505.1914
Amaranthus wrightii S.Watson		Proc. Amer. Acad, Arts 12: 275. 1877
Amaranthus zanensis Horn. ex Moq.		Prodr. [A.P. de Candolle] 13(2): 267. 1849

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