

## Chapter 2

# Amaranthus

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### 2.1 Basic Botany of the Species

The *Amaranthus* genus (Magnoliophyta: Caryophyllidae) comprises 70 species grouped into three subgenera (Mosyakin and Robertson 2003). The most economically important is the subgenus *Amaranthus* proper, which includes the three species domesticated for grain production: *Amaranthus hypochondriacus*, *Amaranthus cruentus*, and *Amaranthus caudatus*. Other species of amaranths have been domesticated as leaf-vegetables, for fodder, as potherbs, or as ornamentals; among these species, *A. tricolor*, from South Asia, is probably the most important (Sauer 1967). This chapter, however, will focus on the wild relatives of the grain crops, particularly on species of the *Amaranthus hybridus* aggregate (*A. hybridus* proper, *A. retroflexus*, and *A. powellii*), from which the “pseudo-cereals” are believed to be domesticated. Part of the discussion however – especially that dealing with the development of genomic resources and hybridization with potential for future breeding programs – will refer to wild species of the subgenus *Acnida*, where increasingly studied dioecious (unisexual) weeds are enlisted.

#### 2.1.1 Subgenus *Amaranthus*

The subgenus *Amaranthus* consists of 20 species of annual herbs that are monoecious (Mosyakin and

Robertson 2003), that is, have separate male and female flowers. The species are native to the Americas, with the exception of only one species of possible European origin (Mosyakin and Robertson 2003). Monoecious amaranths are primarily self-pollinated, as female and male flowers are arranged in close proximity (Murray 1940). Stems are usually erect and both axillary and terminal inflorescences are arranged in cylindrical spikes or panicles (Mosyakin and Robertson 2003). Much of the difficulty in taxonomic discrimination of species within the group can be attributed to attempts at recognizing taxa based on pigmentation or growth forms, which are extremely variable within amaranths (Sauer 1967). However, examination of floral parts can result in constant characters from which discontinuities can be used to define well-established taxa. In this sense, tepal (petals and sepals are combined in a single floral whorl) number and morphology are commonly used in taxonomic keys.

*A. hybridus* is a basal species in the crop subgenus and conforms an interbreeding complex with two other *Amaranthus* weeds: *A. retroflexus* and *A. powellii*. As presented 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 European records of the species date back approximately 300 years, with spread in Europe taking place primarily in the Mediterranean region. Spread of *A. hybridus* has been slower than that of other *Amaranthus* weeds, especially when compared to *A. retroflexus*. Presence of the species in western North America, eastern Asia, Australia, and South Africa has been reported as of early to mid 1900s. Today, *A. hybridus* is a worldwide distributed weed of agricultural fields and other

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disturbed habitats, and it ranks among the 18 most serious weeds in the world (Holm et al. 1991).

*A. retroflexus*, like *A. hybridus* and many other amaranths, is a riverbank pioneer. Its earliest distribution expanded from the central-eastern United States to adjacent Canada and Mexico. Sauer (1967) mentions that Linnaeus is blamed for introducing the weed to Europe, where the species quickly spread. By early 1800s, it became a common weed in the temperate regions of the Old World, reaching the Near East and northern Africa soon after. Today, the species is introduced or naturalized nearly worldwide, ranking among the most widely distributed weeds of the world (Holm et al. 1997).

*A. powellii*'s initial distribution included 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 European record of this species is found in German herbarium specimens from the late 1800s, and later introductions can be interpreted from samples of southern India and South Africa. Expansion of *A. powellii* to eastern North America occurred only during the last century.

Partially fertile hybrid swarms between these species can be found in the United States, in areas where their distributions overlap, and in Europe, where all three species are recent immigrants. The amaranth grain crop is derived of ancient domestications of these species or their hybrids, or from their South American close relative, *Amaranthus quitensis*.

*A. hypochondriacus*, one of the three grain amaranths, is cultivated as an alternative crop in North America and Asia. Although initially thought to have Asian origin, it is believed that this distribution is secondary and that the species derives from an *A. powellii* domestication in North America. Hybridization has had 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 Reyes (Michoacan), a cultivar grown to make special “dark” tamales was a putative hybrid between *A. hypochondriacus* and *A. hybridus*. Likewise, a Wariho Indian crop from Rancho Trigo (Chihuahua) was clas-

sified as a hybrid between *A. hybridus* and *A. powellii*. Another putative hybrid between *A. cruentus* and *A. hypochondriacus* is cultivated in the region of Oaxaca (southern Mexico) and is the same crop found in small gardens in Madras, India.

*A. caudatus*, the grain amaranth of South America, is thought to originate from a domestication of *A. quitensis* in the Andean region (Sauer 1967). *A. quitensis* is a weedy member of the *A. hybridus* aggregate, with original distribution as a riverbank pioneer of South America, in mountains in the northwest and at lower elevations in the temperate south. Cultivation of *A. quitensis* forms with incipient domestication is observed from Ecuador to northern Argentina, mainly for the production of pigments needed for coloring of chicha and other maize dishes. Although some cultivated forms of *A. caudatus*–*A. quitensis* are suspected to be the result of interbreeding with *A. cruentus*, the South American amaranths are not thought to readily hybridize with the North American members of this cluster.

### 2.1.2 Subgenus *Acnida*

The subgenus *Acnida* includes nine dioecious species – that is, taxa with separate male and female plants – which are native to North America and have no immediate evolutionary relationship with the amaranth crop. However, recent studies (Trucco et al. 2005a) show that gene exchange may occur between *Amaranthus tuberculatus*, an infamous member of *Acnida*, and *A. hybridus* – as discussed previously, a crop progenitor with residual compatibility with some domesticated forms. In fact, studies of *A. tuberculatus* and *A. hybridus* gene exchange reveal interesting insights as to how the genetic diversity of the dioecious taxon may be available for crop improvement. In addition, as *A. tuberculatus* is increasingly accepted as a model organism for the study of weeds (Tranel and Trucco 2009), a wealth of genomic resources are being developed that may be of use for programs dedicated to the crops. Since these matters will be discussed in more detail in later sections of this chapter, we feel it is pertinent to introduce the basic botany of the species herein.

*A. tuberculatus* is an annual herb flowering during the summer or fall. It has erect stems, which are

usually branched and have terminal inflorescences in the forms of linear spikes to panicles. Female flowers usually have no tepals, although one or two rudimentary tepals may be observed at times. Sauer (1972) separated *A. rudis* (formerly *A. tamariscina*) as distinct from *A. tuberculatus*, primarily based on utricle dehiscence and absence of female tepals.

Sauer's *A. rudis* was first described in Oklahoma in the 1830s and since has shown continuous northward and eastward accretion into midwestern states, overlapping with *A. tuberculatus*, of static range, in 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 record of the former was on average 40 years prior to that of the latter. Many of the samples collected in these areas were classified as putative *A. tuberculatus* by *A. rudis* hybrids, with a higher ratio of hybrids to non-hybrids in artificial habitats compared to natural settings. In Sauer's assessment of dioecious amaranths (1957), *A. tuberculatus* by *A. rudis* hybrids are the most abundant hybrid combination. The author also notes that actual hybridization 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 using molecular and morphological markers suggested both species to be one and the same (Pratt and Clark 2001), and a single polymorphic species, *A. tuberculatus*, is presently recognized (Mosyakin and Robertson 2003). Costea and Tardif (2003), however, encouraged recognition of the two entities at the variety level: *A. tuberculatus* var. *rudis* having more weedy tendencies than *A. tuberculatus* var. *tuberculatus*.

Over the last 20 years, *A. tuberculatus* has gone from virtual anonymity to becoming the most significant weed problem in the midwestern United States (Steckel 2007), one of the world's premier agricultural regions. Success as a weed is attributed, among other things, to its remarkable ability to evolve resistance to herbicides. Herbicide resistance studies with *A. tuberculatus* are discussed in detail in a later section. Although of great concern from a weed management perspective, the ability of this species to respond to selection and the diversity of adaptations identified thus far may be of potential profit to less orthodox crop-breeding initiatives.

## 2.2 Conservation Initiatives

Wild species of amaranths, particularly those closely related to the grain crops, are successful agricultural weeds and found abundantly in areas from which they are native. In situ conservation initiatives of wild *Amaranthus* species are not known, though genetic erosion problems are of concern (Grubben and van Sloten 1981), and materials of interest are actively collected for ex situ conservation. Ex situ conservation of *Amaranthus* germplasm is not very laborious, since seeds are small and long-lived, and efficient protocols for seed regeneration and conservation of genetic diversity exist (Brenner and Widrechner 1998).

The most significant efforts at ex situ conservation of amaranth germplasm were initiated during the late 1970s, mainly as a result of the amaranth breeding initiative by scientists at the Rodale Research Center in Pennsylvania (Kauffman 1992). At its peak, the Rodale collection contained approximately 1,400 accessions, which were donated in 1990 to the North Central Regional Plant Introduction Station, a part of the USDA National Plant Germplasm System (Brenner et al. 2000). The USDA collection is by far the most comprehensive, including 3,200 accessions, with close to 80% of the accessions representing domesticated species. The *A. hybridus* aggregate (including *A. quitensis*) is represented by less than 300 entries, and 51 entries are listed for *A. tuberculatus*.

Other germplasm collections are held by at least 60 different groups or institutions, although most of these tend to have fewer than 100 entries (Brenner et al. 2000). In general, non-domesticated germplasm is poorly represented in these collections. However, at the University of Illinois, for instance, seed collected from several weedy populations of the midwestern USA are conserved for herbicide resistance research and genetic diversity studies.

## 2.3 Studies Using Molecular Markers

As discussed previously, grain amaranths are associated to three putative progenitors: *A. powellii*, *A. hybridus*, and *A. quitensis*. Evolutionary associations are based on morphology, distribution, and some degree of sexual compatibility among species. More recently, molecular marker analyses have contributed

to the elucidation of origin and evolution of cultivated amaranths, and allied wild species have been critical in these analyses. Hauptli and Jain (1984) were among the first to use molecular markers to address evolutionary relationships among the grain amaranths. They observed that with the exception of the *A. caudatus* – *A. quitensis* pair, grain amaranths are more closely related to each other than either is to their putative wild progenitor. This work was based on isozyme polymorphisms and several authors have since expanded molecular diversity studies in the genus.

In a study including both isozyme and random amplified polymorphic DNA (RAPD) markers, Chan and Sun (1997) generated molecular phylogenies of cultivated and wild amaranths. These authors evaluated 23 different species, including the three cultivated for grain as well as accessions of all species in the *A. hybridus* aggregate. For the crop species, they obtained 15 and 240 polymorphic isozymes and RAPD markers, respectively. The level of polymorphic markers increased slightly when considering accessions from putative wild progenitors. Up to 70% of all evaluated RAPD markers (600 in total) were polymorphic when all 23 species were included in the analysis. Both RAPD and isozyme data sets supported a monophyletic origin for grain amaranths, with *A. hybridus* as the common ancestor.

However, molecular studies do not show unanimous consensus regarding the evolutionary origin and proximity of crop–wild allies. Classical studies dealing with hybrid fertility and chromosome numbers tend to support the hypothesis of independent domestication, with *A. hypochondriacus* and *A. caudatus* as the most related crop species (Pal and Khoshoo 1972, 1973). Some molecular data supported a similar conclusion (Transue et al. 1994; Kirkpatrick 1995). However, studies based on restriction site variations in nuclear and cytoplasmic DNA found that *A. caudatus* and *A. cruentus* are more closely related to each other and to their supposed progenitors than either is to *A. hypochondriacus* (Lanoue et al. 1996). Isozyme and RAPD markers were used by other authors and findings tended to agree with the different evolutionary hypothesis presented herein (Ranade et al. 1997; Zheleznov et al. 1997).

The assembly of different phylogenies with different evolutionary implications may result from the intraspecific genetic variation found across amaranth populations, from the residual cross breeding among

sympatric species, and from the choice of accessions selected by researchers for each experiment. For instance, Brenner et al. (2000) note that the number of accessions surveyed by Chan and Sun (1997) was limited, with approximately five accessions representing each crop species and fewer for most wild taxa. Additionally, wild taxa surveyed were not from the area of origin of domesticated material, so they could not represent adequately the diversity within the putative progenitors. Other factors contributing to the ambiguity of molecular phylogenies may be related to the DNA-marker system employed. Many of the early molecular studies used RAPD markers, which are known to provide inconsistent results. Current studies are applying microsatellite markers and genomic sequencing to address evolutionary questions, and these are discussed in more detail in Sect. 2.5.

Some molecular studies have been strictly dedicated to weed species. Wetzel et al. (1999) generated ribosomal ITS restriction-site-based PCR markers to identify common amaranth weeds, which are difficult to identify based on morphological evaluations with a casual eye. Pratt and Clark (2001) used isozymes to address whether *A. rudis* and *A. tuberculatus* should be considered a single species or two. And Wassom and Tranel (2005) used amplified fragment length polymorphism (AFLP)-based markers to assemble a phylogeny of both dioecious and monoecious *Amaranthus* weeds. In this last study, eight weedy species were considered, represented by 141 individuals from 98 different accessions. Interestingly, the dioecious weeds *A. palmeri* and *A. tuberculatus* did not group together, perhaps indicating independent evolutionary paths.

## 2.4 Interspecies Hybridization Studies

Hybridization studies have been very important in establishing evolutionary relations and gene pools accessible for conventional breeding programs. Merritt Murray (1940) was one of the first to systematically assess interspecies hybridization within the genus, in a study to elucidate the mechanisms involved with sex determination in Amaranthaceae.

Murray classified monoecious species according to the arrangement pattern shown by male flowers in inflorescences. He identified two types of species,

with type I plants having male flowers interspersed with female flowers, whereas type II plants have male flowers clustered at the terminal ends of inflorescences. Murray performed a number of different crosses between and among type I monoecious species (including *A. caudatus*, *A. hybridus*, *A. retroflexus*, and *A. powellii*), type II monoecious species (*A. spinosus*), and dioecious taxa. Crosses between monoecious species produced hybrids with different ease, with type I by type II crosses showing the most difficulty at hybrid production. Hybrids were readily obtained among species of the type I floral arrangement and between type I species and dioecious taxa, suggesting evolutionary proximity between these species. *A. hybridus* by *A. caudatus* crosses were among the most prolific, consistent with the weak pre-zygotic isolation expected of closely related taxa. Interestingly, similarly prolific were crosses between *A. hybridus* and *A. caudatus* with *A. tuberculatus* (referred to as *Acnida tamariscina* in Murray's work), insinuating an evolutionary relationship that is closer than is morphologically apparent.

### 2.4.1 Hybridization Within Subgenus *Amaranthus*

Grant (1959) has reviewed reports by different authors on the occurrence of spontaneous *Amaranthus* hybrids, validating in nature part of Murray's greenhouse results. In the studies cited by Grant, natural hybrids were identified by character intermediacy between *A. caudatus* or *A. cruentus* and species of the *A. hybridus* complex. In some instances, hybrid morphology suggested three-way hybridizations among these species (Tucker and Sauer 1958), and the cytogenetic data produced by Grant were consistent with this notion. The fact that hybrid forms may be observed in nature indicates that first generation hybrids are fertile enough to advance to more stable generations. In this sense, speculations regarding the possible hybrid origin of some domesticated forms appear reasonable – see discussion from Sect. 2.1.1.

A somatic chromosome number of 32 or 34 was observed for all 30 species analyzed by Grant, with the exception of the polyploid *A. dubius*, with 64 chromosomes. Khoshoo and Pal (1972) used *A. hypochondriacus* as the male parent in crosses with

*A. hybridus* and *A. caudatus*, all with 32 chromosomes. Hybrids from these crosses showed the formation of 16 bivalent chromosomal associations. However, hybrids produced with *A. hybridus* showed much greater pollen fertility than hybrids produced with *A. caudatus*. Interestingly, *A. hybridus* by *A. caudatus* hybrids here were seedling lethal, a phenomenon not reported previously by Murray for this cross. Hybrid fertility in this study is in consonance with the notion that amaranth domestication occurred independently in the crop species and that *A. hybridus* may be evolutionary closer to *A. hypochondriacus* than to *A. caudatus*.

The two basic chromosome numbers are observed among the grain crops and their putative progenitors. While *A. caudatus*, *A. hypochondriacus*, *A. hybridus* and *A. quitensis* have 32 chromosomes ( $n = 16$ ), *A. cruentus* and *A. powellii* both have 34 ( $n = 17$ ) (Grant 1959). Pal et al. (1982) have explored the evolutionary relationship between the two basic numbers in the grain group by performing a dibasic cross between *A. hypochondriacus* and an African race of *A. hybridus* with 34 chromosomes. At metaphase I, the majority of meiotic cells from the interspecific  $F_1$  showed 15 bivalent and 1 trivalent chromosomal associations. Hybrid progeny ( $F_2$ ) showed 1:2:1 segregation for 32, 33 and 34 somatic chromosomes, respectively. The observance of this meiotic configuration in the dibasic hybrid suggested that  $n = 17$  arose through aneuploidy, perhaps involving a reciprocal translocation resulting in a decrease in chromosome number from  $n = 17$  to  $n = 16$ . Other authors also have analyzed meiotic behavior in crop-wild hybrids, and much of what we know about gene pool accessibility and phylogenetic relations is derived from these studies. Greizerstein and Poggio (1995) studied the meiotic configuration of 13 different crop-wild and wild-wild spontaneous hybrids and this information was used to configure the first set of genomic formulae for these species (Brenner et al. 2000).

Hybridization studies have been of great value for breeders interested in crop improvement through traditional means. Hybrids with wild species have been produced to address all major breeding objectives, including raising yield, improving pest tolerance, and improving grain harvestability. For instance, one of the most widely utilized grain varieties in the USA, *A. hypochondriacus* var. Plainsman, is derived from a cross with a Pakistani *A. hybridus* accession used as a source for earliness (Baltensperger et al. 1992).



Interspecific crosses with *A. hybridus* have been used to measure biomass heterosis and combining ability with domesticated species, in a first step to exploit heterosis in the development of cultivars improved for forage, energy feedstock, or as vegetables (Lehmann et al. 1991).

Brenner et al. (2000) report that crop–wild hybrids have been produced to transfer *A. powellii* non-dehiscence to *A. cruentus* and *A. hypochondriacus* breeding lines, in efforts to reduce grain shattering. The authors also propose that hybridization with *A. cannabinus*, a wild dioecious species, may be useful to obtain germplasm with greater seed size. In an ongoing breeding program, weedy *A. hybridus* with evolved herbicide resistance is being used to introduce herbicide selectivity to *A. hypochondriacus* and *A. cruentus* elite breeding lines (Federico Trucco unpublished data). The introgression of herbicide resistance from wild species and the potential implications of herbicide resistant cultivars is discussed in more detail in Sect. 2.6.

#### **2.4.2 Hybridization Between *A. hybridus* and *A. tuberculatus*, A Species from Subgenus *Acnida***

A recent aspect of research regarding hybridization among wild crop relatives focused in the study of gene flow between two problematic weeds that have been already introduced, namely *A. hybridus* and *A. tuberculatus* (Trucco et al. 2005b). Although this research has been conducted from a weed science perspective, the fact that *A. hybridus* is a common progenitor to the domesticated species makes the findings of these studies of value for breeders interested in exploiting the diversity of the dioecious taxon. Previous experiments indicated that hybrids ( $F_1$ s) between *A. tuberculatus* and *A. hybridus* could be produced but failed to quantify the extent to which this could occur (Murray 1940). Also, subsequent introgression was thought to be compromised by severe sterility in the  $F_1$  (Sauer 1957), and the only viable  $BC_1$  progeny were thought to be those derived from unreduced gametes from the hybrid parent (Murray 1940), resulting in triploidy. First generation backcross progeny would have a full complement of the recurrent species'

genome and only a haploid complement of the non-recurrent parent, and exhibit sterility due to abnormal chromosome pairing. These observations suggested little if any chance for homoploid gene exchange between *A. tuberculatus* and *A. hybridus* – that is, gene exchange without changes in ploidy.

Tranel et al. (2002) were able to transfer a herbicide-resistance allele of acetolactate synthase (ALS) from *A. hybridus* to an advanced hybrid population ( $BC_2$ ) – where *A. tuberculatus* was recurrently used paternally – and with the use of DNA content data suggested that introgression could occur in a homoploid background. However, these authors did not address directly the fertility and genome structure of introgressants. Were heterozygous  $BC_2$ s more fertile than heterozygous  $BC_1$ s, or  $F_1$ s? Was the genomic constitution of these introgressants recombinant (on average 12.5% *A. hybridus* and 87.5% *A. tuberculatus*, or a reconstitution of the  $F_1$ )? What about introgression in the reciprocal direction? From a crop-breeding perspective, all these questions need to be addressed in order to establish the potential for conventional (sex-mediated) gene pool exploitation.

Later experiments showed that hybrids can be produced at relatively high frequencies under field conditions (Trucco et al. 2005a, b). In the case where the monoecious parent was used maternally, the maximum hybridization frequency obtained accounted for close to 50% of the believed intraspecific outcrossing potential of the species. In the reciprocal case, more than 200,000 hybrids could be obtained from a single *A. tuberculatus* plant. These data indicated that little if any gametic incompatibility exists between the studied species and that  $F_1$  production is unlikely to constitute a significant bottleneck for gene introgression.

Although hybrid sterility was well documented by Murray and Sauer, to the extent hybrids were quoted to “run into a blind alley of sterility” (Sauer 1957), a detailed assessment of hybrid fertility showed that as many as 800 seeds could be recovered from a single  $F_1$  (Trucco et al. 2006b). This number is substantial enough to allow expectations of successful gene introgression. However, successful introgression is dependent on recombination between the donor and recipient genomes and this could be unveiled adequately with the use of cytogenetic and molecular markers. Using these tools to profile hybrid progeny from backcrosses to the “pure” species, the following observations were made by Trucco et al. (2005c):

- Most BC<sub>1</sub>s (98%) were homoploid ( $2n = 32$ ), and triploidy was not necessarily the product of unreduced hybrid gametes. This is in agreement with Tranel et al. (2002) and in contrast to Murray (1940). Production of unreduced gametes may vary among populations and this may explain the discrepancy observed in triploidy occurrence in Murray's work (100%) versus Trucco et al. (2%).
- Fertility restitution was not a strict function of reconstitution of the parental species' genomes; in fact, hybrid sterility could be explained by as few as five independently assorting loci. In which case, advantageous alleles unlinked to these loci may be introgressed quickly. The introgression of linked alleles (genes linked to post-zygotic reproductive barriers) may depend on the selection coefficient and population size.

These authors also examined introgression of a herbicide resistance allele of *ALS* from *A. tuberculatus* to *A. hybridus* (a reciprocal of that evaluated by Tranel et al. 2002), and they observed that the *A. tuberculatus* allele could not be introduced into *A. hybridus* monoecious background. Allele introgression was limited to a small number of non-monoecious individuals exhibiting high sterility. The main speculation then was that lack of introgression resulted from linkage of *ALS* to a hybrid sterility locus associated with sex determination, a taxonomically discriminating character for these species.

In a subsequent study by the same authors (Trucco et al. 2009), 192 homoploid BC<sub>1</sub>s were evaluated at 197 AFLP loci, as well as at *ALS* and *PPO* (the gene for protoporphyrinogen oxidase, the enzyme targeted by a second family of herbicides). The parental populations used were polymorphic at the herbicide target genes, and just as observed in the prior study, *A. tuberculatus*' *ALS* and *PPO* alleles could not be transferred to *A. hybridus* monoecious background. This indicated that gene exchange likely is limited by a phenomenon beyond circumstantial linkage.

Indeed, Trucco et al. (2009) were unable to transfer most of 133 AFLP markers from *A. tuberculatus* to *A. hybridus*, with the exception of introgression in a smaller group of non-monoecious BC<sub>1</sub>s, characterized by anomalous phenotypes and high sterility. This observation is striking as the authors uncovered a very different scenario in the reciprocal exchange.

They were able to transfer from *A. hybridus* to *A. tuberculatus* not only *ALS* and *PPO* alleles but also most of the *A. hybridus*-specific AFLP markers. Although introgression at some loci appeared to be disfavored (i.e., showed negative segregation distortion or a fecundity penalty), *ALS* and *PPO* alleles as well as many of the AFLP-markers showed Mendelian segregation in backcross progeny with *A. tuberculatus* and no association with BC<sub>1</sub> reproductive output (measured as pollen viability or seed production). This was not observed in progeny from reciprocal backcrosses, where almost all *A. hybridus* markers showed strong negative distortion and increased introgression was associated with reduced reproductive viability.

Taking monoecism and dioecism as the taxonomic distinguishing characters for *A. hybridus* and *A. tuberculatus*, respectively, we may say that gene exchange between these species is unidirectional. Even if we considered non-monoecious *A. hybridus* backcross progeny as being more "*A. hybridus*" than "*A. tuberculatus*," the use of these individuals in crop-breeding programs seems restricted by the dramatic fitness disadvantage at which they stand compared to their reciprocals. In fact, there is no association between the level of introgression measured in *A. tuberculatus* BC<sub>1</sub>s and their relative seed output.

The data produced by these experiments indicate that *A. tuberculatus* adaptations, some of which may be of great value to the *Amaranthus* crops, may not be transferred to *A. hybridus*, a species from which the crops have evolved. Yet, crop adaptations may be equally transferable to *A. tuberculatus* as those from *A. hybridus*, and this may have alternative implications for crop breeders. First, crop traits such as disease resistance or herbicide tolerance may end up in a highly problematic weed, although crop erosion with *A. tuberculatus* alleles should be of little concern. Secondly, given that *Amaranthus* breeding is at an early stage, the development of an *A. tuberculatus* crop by transferring crop adaptations to this wild relative may be a bold but profitable proposition. Considering that the most distinctive crop adaptations show simple inheritance (Brenner et al. 2000) and that gene transfer may be accomplished readily, the development of a dioecious crop seems within reach. Such possibility could provide a unique opportunity for the exploitation of heterosis in amaranths.

## 2.5 Genomics Resources Developed for *Amaranthus*

Within the last 2 years, genomics resources have been developed for *Amaranthus* species. Two research groups independently developed microsatellite markers from *A. hypochondriacus* (Lee et al. 2008; Mallory et al. 2008). In both cases, the markers were demonstrated to be transferable to other cultivated as well as weedy *Amaranthus* species. 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 *Amaranthus* microsatellite markers were obtained recently from *A. tuberculatus* (Lee et al. 2009). Collectively, these microsatellite markers will be valuable for more detailed phylogenetic studies, as well as for various genetic studies and breeding efforts (e.g., population genetics, construction of genetic maps, map based cloning, and marker-assisted selection).

A second genomics resource for *Amaranthus* is a bacterial artificial chromosome (BAC) library from *A. hypochondriacus* (Maughan et al. 2008). This library contains over 35,000 clones averaging 147 kb, or about a 10-fold coverage of the genome. Utility of the library was demonstrated by using it to obtain the full-length sequences of the *ALS* and *PPO* genes, both of which encode important herbicide target sites (Maughan et al. 2008). In addition to serving as a source for candidate gene isolation and sequencing, this BAC library could be used further to develop a physical map of the *Amaranthus* genome, and could serve as a scaffold for whole-genome sequencing.

A third *Amaranthus* genomics resource recently obtained is shotgun sequence data from *A. tuberculatus* (Lee et al. 2009). Using next-generation sequencing technology, over 40 Mbp of sequence was obtained from *A. tuberculatus*. Included in the dataset was a nearly complete sequence of the chloroplast genome and partial sequences of most currently known herbicide target-site genes. The dataset also provided leads for microsatellite markers, mentioned above. Although the dataset contains only partial sequences for nuclear genes, such information serves as a starting point for candidate gene isolation insofar as designing primers for PCR-based approaches. The same technology has been used to sequence the

*A. tuberculatus* transcriptome (P. Tranel unpublished data). Both the genomic and transcriptomic datasets are being made publicly available via the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>).

Finally, a forth resource in development is a collection of recombinant inbred lines (RILs) derived from an initial crop–wild cross between *A. hypochondriacus* and *A. hybridus* (P. Tranel unpublished data). The initial F<sub>1</sub> plant was selected based on herbicide resistance (which was present in the *A. hybridus* parent, used paternally in the cross) and over 200 lines derived from selfing the F<sub>1</sub> are being propagated via single-seed descent. Given the expected high genetic diversity among the RILs, they should provide an ideal population for development of an *Amaranthus* genetic map.

A current need is the development of a facile genetic transformation system for *Amaranthus*. Only modest success has been reported in regenerating plants from *Amaranthus* callus tissue (Brenner et al. 2000). There is, however, one report of successful transformation of *A. hypochondriacus* by inoculation of mature embryo explants with *Agrobacterium* (Jofre-Garfias et al. 1997). It is surprising that there are not more reports of *Amaranthus* transformation, and we do not know if this is due to lack of effort or technical challenges. Possibly the weedy species are more amenable to genetic transformation and could serve as model systems for developing and optimizing protocols that could then be adapted to the cultivated species.

## 2.6 Herbicide Resistances in *Amaranthus* Weeds

A primary characteristic contributing to the infamy of *Amaranthus* species as weeds of modern agriculture is their demonstrated ability to evolve herbicide resistance. *Amaranthus* weeds comprise over 5% of worldwide cases of herbicide-resistant weeds and have evolved resistances to diverse herbicide modes of action (Heap 2010; Tranel and Trucco 2009). For example, *A. tuberculatus* has evolved resistance to herbicides that inhibit photosystem II (PSII), ALS, PPO, and 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) (Patzoldt et al. 2005; Legleiter and Bradley 2008). In some cases, resistances to more than one of these herbicide groups is present within a



single population (or even within a single plant), making control of *A. tuberculatus* a significant practical problem. In the southeastern US, resistance to glyphosate (which inhibits EPSPS) has become widespread in *A. palmeri* in recent years and is posing a very significant weed management challenge (Culpepper et al. 2006, 2008; Norsworthy et al. 2008).

The frequent occurrence of herbicide resistance in *Amaranthus* weeds suggests it should be possible to select the same traits in cultivated *Amaranthus* crops. Alternatively, it should be possible to transfer the resistance traits from the weeds to the crops via hybridization (although see Sect. 2.4.2). For example, it should be straightforward to cross grain amaranth with *A. hybridus* containing resistance to ALS inhibitors (Trucco et al. 2006a), and then obtain the herbicide-resistant crop by recurrent backcrossing along with selection for the resistance. Unfortunately, however, that these herbicide resistances are widespread in many of the *Amaranthus* weeds would limit their utility in the crop. Nevertheless, the only *Amaranthus* species thus far to have evolved resistance to PPO inhibitors is *A. tuberculatus*, and to EPSPS inhibitors are *A. tuberculatus* and *A. palmeri*. Thus, resistance to one or both of these herbicides in cultivated amaranth may have value, particularly in regions where these two weeds are not present.

The mechanism conferring resistance to PPO inhibitors in *A. tuberculatus* was determined to be a deletion of a glycine residue in a conserved region of the *PPX2* gene (Patzoldt et al. 2006). The gene was predicted to encode both mitochondria- and chloroplast-targeted PPO, thereby resulting in herbicide-insensitive enzymes in both organelles. Through genetic transformation, one could insert the *A. tuberculatus* herbicide resistant *PPX2* into the crop species. The homologous gene was obtained from *A. hypochondriacus* and also shown to contain the dual-targeting signal sequences (Maughan et al. 2008). Site-directed mutagenesis of the native *A. hypochondriacus* *PPX2* to obtain the glycine codon deletion followed by transformation would be another route to obtain resistance to PPO inhibitors. This latter approach might be met with greater public acceptance since the crop would not be carrying a gene from a weed species (although the encoded proteins from the two genes are over 97% identical; Tranel and Trucco 2009).

A major challenge beyond the development of a herbicide-resistant amaranth crop would be maintain-

ing the utility of the trait by preventing its escape into coexisting *Amaranthus* weeds. In this regard, the body of work on interspecific hybridization (reviewed previously herein) should provide the framework for the development of adequate protocols for technology stewardship.

## 2.7 Recommendations for Future Actions

Amaranths have been a staple crop of pre-Columbian cultures, and they have received interest in the last two to three decades as an alternative crop. Much of the recent interest in amaranths is based on the exceptional nutritional profile of the grain proteins, which are rich in amino acids that are usually deficient in other crops (Bejosano and Corke 1998). Additional interest is generated by the oil and carbohydrate profiles of amaranth seeds, which present opportunities for different industrial applications, from the use of amaranth squalene as a cosmetic oil (Budin et al. 1996) to that of micro-sized starch in the formulation of foods (Uriyapongson and Rayas-Duarte 1994). Numerous studies have been conducted to develop and optimize technologies aimed at exploiting these amaranth properties (see works in Paredes-López 1994 for greater detail).

From an agronomic perspective, drought tolerance and environmental plasticity are attractive traits promoting amaranth adoption in areas where traditional crops face greater challenges (Brenner et al. 2000). Yet, modern amaranth cultivars still face several difficulties, which have been overcome in most major crops. Recent breeding efforts to try to solve some of these difficulties have been modest, and very few cultivars have been registered over the last decade. In an unusual contrast, *Amaranthus* weeds have been the subject of leading weed science research over the same timeframe (see Tranel and Trucco 2009 for a revision on the subject). In fact, weedy amaranths have been proposed as a model system for the study of plant weediness (Basu et al. 2004), and valuable genomic resources are being generated with these species as discussed in Sect. 2.5.

This chapter attempts to bridge the research conducted by the weed science and the crop-breeding communities, realizing that perhaps the path to

improving important crop traits may be realized through the judicious exploitation of the wealth found in weedy amaranth resources. The discussions regarding the patterns of gene exchange among the different taxonomic groups constitute a first and incomplete attempt at drafting a roadmap for the exchange of adaptations among species. Similarly, the discussion dealing with herbicide resistance covers a number of possibilities yet to be explored by amaranth breeders. It is important to note that the great success of amaranths as weeds is not found in any one adaptation but in their ability to adapt quickly to changing weed management practices. Infamy due to the evolution of numerous herbicide resistant populations is a reflection of their adaptability, or from a different perspective a reflection of their ability to successfully respond to selection. Interestingly, what constitutes a threat to farm economies at one level may be the most valuable asset for the development of competitive cultivars at another. It is up to us to transform this serious challenge into a beneficial force.

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