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Flowering, Seed Production, and the Genesis of Garlic Breeding

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I. INTRODUCTION

Garlic is one of the oldest horticultural crops. There are Egyptian and Indian references to garlic 5000 years ago, clear evidence of Babylonian usage 4500 years ago, and usage in China 2000 years ago, although some

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writings suggest that it was grown there as long as 4000 years ago (Hahn 1996). In spite of its long history, little is known about early garlic production or plant types used for cultivation.

Garlic production today relies completely upon asexual propagation of the crop and, while flowering in garlic has long been known, no record of true seed production in garlic is known before 1950. While domestication implies selection for improved adaptation and production in cultivation by humans, it could be argued that despite its long cultivation, garlic still remains undomesticated. This statement presumes that domestication only proceeds when humans can tap the broad range of heritable variation resulting from sexual reproduction. However, it seems likely that sexual reproduction and selection among products of meiosis and syngamy may have been realized in cultivation from time to time during garlic's long history. Furthermore, clonal selection has clearly generated new cultivars of non-sexual, clonally propagated crops such as seedless bananas, sweet potatoes, *Caladium*, *Colocasia*, potatoes, apples (Sharma 1956), and also garlic (Burba 1997). Nevertheless, flowering and seed production research in garlic over the last 50 years sets the scene for utilizing a system of classical plant breeding in this important crop that has been used as the mainstay of plant breeding today. True seed production in garlic provides a foundation for dramatically changing the production and reproduction technologies used by garlic producers in the future. Etoh (1985) reviewed the early research on garlic sterility and seed production, while Etoh and Simon (2002) recently reviewed garlic diversity, fertility, and seed production.

Darlington (1939) maintained that, in nature, asexual reproduction provides only limited genetic options for continued adaptation, saying: "With loss of sexual recombination, the apomict . . . is cut off from ultimate survival. Apomixis is an escape from sterility but it is an escape into a blind alley of evolution." Human intervention has apparently sustained and even favored asexual reproduction throughout the agricultural history of garlic. The prospects for true seed production in garlic now hold out the possibility of capturing the broader diversity that allogamy and recombination can bring.

II. GARLIC PRODUCTION TRENDS

Garlic is widely grown, with current worldwide production at nearly 10 million tonnes on nearly a million hectares, and it is used as a spice, condiment, and vegetable (Table 5.1). China and India are the largest producers of garlic, collectively accounting for 60% of the production area and 69% of the world yield. World garlic production area has more than doubled since 1970, and its availability has almost doubled in that

Table 5.1. World garlic production and availability, 1961–2000. Source: FAO Yearbook Production Statistics; average of 3 years for 1970, 1980, 1990, and 2000 (i.e., 1970 = average of 1969–1971).

Continent	Year	Area (% world)	Yield (t/ha)	Availability (kg/capita)
Africa	1961	1	10.5	0.2
	1970	1	23.2	0.4
	1980	2	16.3	0.4
	1990	3	12.7	0.4
	2000	3	11.4	0.4
Asia	1961	86	5.7	2.2
	1970	74	7.4	1.2
	1980	74	7.3	1.3
	1990	77	9.0	1.7
	2000	76	10.7	2.2
Europe	1961	8	5.2	0.8
	1970	16	5.3	0.8
	1980	15	5.2	0.9
	1990	13	5.4	1.0
	2000	12	6.1	0.7
North and Central America	1961	1	4.3	0.1
	1970	2	6.8	0.2
	1980	2	9.7	0.4
	1990	3	13.6	0.6
	2000	3	15.2	0.8
Oceania	1961	<1	1.0	<0.1
	1970	<1	1.0	<0.1
	1980	<1	7.0	<0.1
	1990	<1	5.9	<0.1
	2000	<1	6.0	<0.1
South America	1961	4	3.1	0.6
	1970	6	3.7	0.6
	1980	6	4.5	0.6
	1990	5	5.1	0.6
	2000	6	7.4	0.9
World	1961	771	5.6	1.4
	1970	464	6.9	0.9
	1980	627	7.0	1.0
	1990	771	8.4	1.2
	2000	985	10.0	1.6

period to a current annual consumption of 1.64 kg/capita. Asia is by far the major garlic production region, but increased area and availability have kept up with population in the rest of the world, with especially sharp rises between 1990 and 2000 in the Americas.

In spite of the wide geographic range of garlic production today, little is known about the similarities and differences among major cultivars grown in different growing regions. In this paper, we use the term "clone" to indicate garlic cultivars, strains, or genotypes that are asexually propagated.

The major distinction among garlic clones is the tendency to produce a flower stalk, or not. Those producing a flower stalk are referred to as "bolting," "stalking," or "hard neck" types, whereas those without a flower stalk are "non-bolting," "non-stalking," or "soft neck." Sometimes a third category, "incomplete or partial bolting," is used for clones in which the inflorescence begins to develop but stem elongation is incomplete and mature flowers do not develop (Takagi 1990). There is a presumption that this character is stable across environments but this is unproven. Likewise, it is assumed that, like onion, garlic bulb formation is a response to photoperiod and some reference is made to "short-day" cultivars (which produce bulbs as photoperiods lengthen slightly in the spring in regions near the Equator), and "long-day" cultivars (which produce bulbs as photoperiods lengthen dramatically in the spring in regions farther from the Equator). This has not been substantiated either, although both bolting and non-bolting garlic clones are produced in nearly all production regions.

Grower and consumer preferences for garlic vary among different geographic regions and end uses. Intact or processed garlic bulbs constitute the usual form of the crop traded in commerce, but in parts of Asia and North Africa leaves are marketed. Preferred garlic cultivars for fresh bulb sale include those with red or pink cloves surrounded by white bulb scales and those with white cloves and bulb scales. Processors of prepared fresh garlic (e.g., minced, chopped, crushed, or sliced) use both types. Dehydrators require the latter type with little pigment and high dry matter content. The inclination of consumers toward unusual and pigmented vegetables has resulted in specialty markets for novel and colored garlic. The development of garlic for the nutraceutical industry has resulted in another specialty market with high levels of sulfur compounds such as allicin, that are often correlated with strong flavor. True seed production of garlic will likely provide a more diverse array of variation from which users can select.

The strictly asexual propagation of garlic brings with it several viruses, nematodes, and other pests, which lower garlic yield. Consequently, an extensive research effort and very significant production resources are dedicated to reducing or eliminating these pathogens from "seed" garlic, plants used for crop production rather than consumption (van Dijk 1994; Verbeek et al. 1995; Salomon 2002). The prospects of true seed production in garlic could dramatically reduce the resources dedicated to this aspect of garlic production, especially if genetic resistance can be identified and incorporated into new cultivars.

III. GARLIC TAXONOMY AND GENETIC VARIATION

Plant systematists have struggled with the taxonomy of garlic, *Allium sativum* L. It is a diploid species ($2n = 2x = 16$) in the subgenus *Allium* of the Alliaceae (formerly in the Liliaeceae, and then the Amaryllidaceae). The other cultivated plants in this subgenus are leek, usually tetraploid, or elephant garlic, usually hexaploid (both *A. ampeloprasum* L.). Leek and garlic have flat, folded leaves. Elephant garlic and garlic form a bulb, but leek does not. Elephant garlic bulbs consist of 2 to 6 large cloves and several small cloves, while garlic bulbs usually have more cloves of a relatively consistent size, especially for bolting types. Bolting garlic, leek, and elephant garlic all have a solid scape, unlike the hollow scape of the most economically important *Allium*, onion (*A. cepa* L.). Garlic flower color ranges from white to pink to purple and inflorescences almost always include not only flowers but also bulbils (small, undivided bulbs occurring in the inflorescence; also referred to as “topsets” or “air bulbs”), whereas *A. ampeloprasum* inflorescences form bulbils only sporadically.

Early classifications considered *A. sativum* to be a species known only in cultivation, with *A. longicuspis* Rgl. as a closely related wild relative originating in Central Asia (Vvedensky 1935; Jones and Mann 1963). The diversity of Central Asian garlic supports the idea that this region is the primary center of origin of garlic, although this region was likely much larger in past history (reviewed by Engeland 1991; Etoh and Simon 2002). The distinctiveness of *A. longicuspis* as a separate species, primarily differing from garlic in having exserted anthers, has been brought into question. Several botanical varieties of garlic are described in the literature, including *A. sativum* var. *sativum* L., which rarely or never flowers; *A. sativum* var. *ophioscorodon* (Link) Doll (the varietal name meaning “serpent garlic”), which regularly flowers; and *A. sativum* var. *pekinense* (Prokh.) Makino, which rarely flowers, like *A. sativum* var. *sativum*, but has wider leaves (Helm 1956; Jones and Mann 1963). Several other garlic subspecies names have been used in the literature. For example Kuznetsov (1954) and Kommisarov (1964, 1965) referred to ssp. *vulgare* and ssp. *sagittatum*, while Kazakova (1971, 1978) referred to ssp. *sativum* or *mediterraneum* and ssp. *asia-mediae* Kaz., but the contrast of bolting versus non-bolting has been the primary distinction made among subspecies. Most recent considerations of *A. sativum* taxonomy include either two or three groups. Hanelt (1990) included a “common garlic group” (consisting of *A. sativum* var. *sativum*, *A. sativum* var. *typicum* Rgl., and *A. pekinense* Prokh.) and an “ophioscorodon group” (consisting of *A. sativum* var. *ophioscorodon* (Link) Doll, *A. ophioscorodon* Link, and *A. sativum* var. *controversum* (Schrad.)

Moore jr.), while Fritsch and Friesen (2002) added a third "*longicuspis* group" (consisting of *A. longicuspis* Rgl.). More recently, *A. tuncelianum* has been proposed as the wild progenitor of garlic (Mathew 1996). This suggestion has generated discussion (Etoh and Simon 2002; Fritsch and Friesen 2002), including the point that garlic is regarded as a Central Asian species, while *A. tuncelianum* is currently found in Turkey.

A wide range of morphological diversity has been observed in garlic, including flowering ability (and scape length of flowering clones), leaf traits (color and attitude), bulb traits (shape, outer leaf scale color, clove color, number, size, and organization of cloves), plant maturity, bulbing response to temperature and photoperiod, cold-hardiness, bulbil traits (number, size, color, and ease of removal), and flower traits (timing, number, size, color, petal closure, and stigma position) (McCollum 1976; Hwang 1993; Messian et al. 1993; Lallemand et al. 1997; Hong et al. 2000a; Senula and Keller 2000; Jenderek and Hannan 2002). Detailed classification of garlic germplasm collections based solely on morphological diversity has been considered difficult due to the subjective nature of categorization for several of these traits, although Hwang (1993) and Senula and Keller (2000) were able to develop detailed comparisons based upon principal component analysis of 10 traits and cluster analysis of 16 traits.

Concomitant with this morphological variation, evaluations of molecular variation have noted significant levels of variation in isozymes, RAPDs, and AFLPs (Etoh and Ogura 1981; Siqueria et al. 1985; Etoh 1985; Tsuneyoshi et al. 1992; Pooler and Simon 1993a; Maaß and Klaas 1995; Bradley et al. 1996; Al-Zahim et al. 1997; Lallemand et al. 1997; Hong et al. 1997; Etoh et al. 2001; Garcia Lampasona et al. 2002; Ipek et al. 2003). In earlier studies utilizing isozymes, relatively few polymorphisms were detected and consequently unique "fingerprints" were unable to be determined for even clearly morphologically different clones, whereas with the utilization of RAPDs, and more recently AFLP molecular markers, hundreds of polymorphic bands can be detected to allow for clear demarcations of differences in diverse garlic germplasm collections. Generally the groupings ascertained by morphological variation are positively correlated with molecular variation, and clustering of clones in the *ophioscorodon* and "common" (*sativum*) botanical varieties is evident. Maaß and Klaas (1995) included subtropical and *pekinense* clones in their study, and suggested that subtropical clones were "clearly separated from all other types," while the *pekinense* subgroup was relatively similar to the stalking types. Interestingly, most molecular "fingerprinting" studies which included *A. longicuspis* clones produced no basis for distinction between *A. sativum* and *A. longicuspis* as separate species. Field evaluation of a genetically—and geographically—diverse collection of all *A. sativum* subspecies, *A. longicuspis*, and *A. tuncelianum* in very diverse climates, combined with molecular fingerprinting, may help further resolve garlic taxonomy. A combination of field evaluation and molecular fingerprinting will also be helpful in characterizing world garlic cultivars. Cultivars are clones, so any garlic production plot is expected to be genetically invariable. Molecular fingerprinting studies have demonstrated wide DNA variation concomitant with wide phenotypic variation observed among cultivars in a growing area. However, it is not known how cultivars from one region compare to those from another, since cultivar names are rarely constant over diverse growing areas. Knowledge of these relationships may shed light on crop history and adaptation.

Variation among chromosomes in the garlic karyotype has also been noted for centromere location, chromosome length, and the number of chromosome satellites (Takenaka 1931; Krivenko 1938; Mensinkai 1939; Battaglia 1963; Gohil and Koul 1971; Konvicka and Levan 1972; Verma and Mittal 1976; Etoh and Ogura 1978; Etoh 1979; Etoh 1983b; Etoh 1985; Hong et al. 2000a). The extensive karyotypic evaluation by Hong et al. (2000a) demonstrated a preponderance of the “basic” karyotype (2 sets of chromosomes) in bolting Central Asian clones proven to produce seed, while a collection of partial-bolting clones from the Iberian Peninsula usually had “non-basic” karyotypes, including heteromorphic pairs and unusual chromosome variation in terms of centromere location and/or satellites.

Assessment of morphological variation in garlic can be complicated by what is perceived as a gradual response by garlic to the environment. For example, an accurate assessment of yield, maturity, and even flowering ability for a garlic clone is often reserved until 3–5 years of propagation after arrival at a new location (Engeland 1991; Pooler 1991), especially if large changes in photoperiod or climate are involved. With this, garlic clones adapted to shorter summer photoperiods (i.e., at lower latitudes) will usually grow well the first year or two after being moved to a long summer photoperiod environment, but in subsequent years smaller bulbs are produced and eventually no bulbing occurs before senescence, and the clone is lost. This is thought to be a photoperiod response, but it is unproven.

One variable that has a significant negative effect on garlic performance and a confounding effect on germplasm evaluation is virus infection. The potyviruses onion yellow dwarf virus and leek yellow stripe virus are most economically important. Because virus infection is so common in garlic, and resistance has not been identified, there has been extensive research and effort devoted to generating virus-free garlic for production areas around the world (van Dijk 1994; Verbeek et al. 1995;

Salomon 2002). It is not uncommon for the virus-freeing process to increase garlic yield from 5 to 20% with a delay of plant senescence of up to 5 weeks.

IV. GARLIC GROWTH AND REPRODUCTIVE BIOLOGY

A. Morphology and Growth

Factors influencing garlic morphology and growth have been discussed in many reports, including several comprehensive studies (Mann 1952; Mann and Minges 1958; Takagi 1990). The garlic bulb of commerce typically weighs 60 to 120 g, and consists of cloves surrounded by the dried basal sheaths of the foliage leaves. Bolting garlic bulbs usually consist of 4–12 cloves that tend to be relatively similar in size, while nonbolting garlic usually have more cloves that can vary greatly in size. Cloves are sessile lateral bulbs, that originate from axillary buds of inner (younger) foliage leaves. An outer, thin protective leaf and an inner, thickened, bladeless storage leaf are the most predominant parts of a garlic clove, with the storage leaf accounting for most of the volume and weight of the clove. The storage leaf subtends a central vegetative bud over a flattened basal plate, which is a modified very short stem. The central vegetative bud includes a predominant sprout leaf and several foliage leaf primordia that surround the apical meristem.

The growth of a garlic plant typically begins from an individual clove that has been exposed to cool temperatures (15°C or less) and depleted or "broken" dormancy (Rahim and Fordham 1988; Takagi 1990). Depending upon how long the mature bulb was stored, the sprout leaf and foliage leaves may still be contained within the storage leaf, or may have elongated (sprouted) through the top of that leaf to protrude above it. Roots grow quickly around the perimeter of the basal plate and precede further leaf development. Leaves arise in an opposite and alternate orientation. Since a given leaf blade emanates from a broad meristematic arch, the bases of consecutive leaves are extensively overlapped. This overlap of leaf bases results in the production of a "pseudostem" that consists of closely appressed leaf sheaths surrounding young leaves. Above the pseudostem, leaf blades extend without overlap. The root system and flat leaves usually develop before clove initiation (bulbing) ensues from inner (younger) leaf axillary buds. Many variables, including storage, temperature, growing temperature, planting time, and photoperiod, interact with clove size and the genetic predisposition of a garlic clone to determine the number and size of leaves and cloves realized in a production cycle. Plant maturation and senescence usually ensues after garlic bulb production. As the main stem of the bulb dies, intact and living basal plates remain on each clove to carry on future growth.

Studies investigating broad ranges in photoperiod, air and soil temperature, and light intensity during production, and bulb storage time and temperature have noted relatively small effects on garlic leaf size and number, bulb and clove size, and bulb dormancy, compared to the effects of those environmental variables in other Alliaceae (Takagi 1990; Pooler and Simon 1993b), although extended cold storage increased the incidence of flowering plants for some clones, shading slowed maturity and reduced yield, and "short day" cultivars often produced small, undivided bulbs ("rounds") in long photoperiods.

B. Reproductive Biology

Most reports of garlic morphology and growth focus on the plant and bulb, although several include evaluations of floral initiation and development (Vanin 1947; Aoba 1966; Takagi 1990; Pooler and Simon 1993b). Like other alliums, garlic flowers are perfect, with 6 petals, 6 anthers, and 3 locules consisting of 2 ovules each (Fig. 5.1). Garlic flowers are smaller

Fig. 5.1. Garlic flowers and a bulbil from a male fertile clone.

than onion. The number of flowers in an umbel varies from less than 10 to over 300, but the typical range is from 150 to 200. The inception of routine seed production in garlic has provided a wealth of information about its reproductive biology (Etoh 1985; Pooler and Simon 1994; Jenderek 1998). Garlic flowers are protandrous with 2 to 4 days between pollen shedding and stigma receptivity. The stigma is receptive for 1 to 2 days and styles are usually longer than petals. Across a garlic inflorescence, anthesis occurs over 5 to 20 days. The opposing petals of most mature garlic flowers form an angle of approximately 45° although some seed-derived plants have nearly closed petals. Petal color in bolting garlic clones is usually light purple, with or without darker coloration of pedicel and petal tip. In seed-derived plants, lilac and white petals also occur. Anthers of typical clonally-propagated garlic are yellow or purple, while in seed-derived plants light gray and lilac anthers also occur.

Development of the floral apical meristem in bolting garlic requires exposure to low temperatures (< 5°C) before onset of the primary growth period. The details of garlic vernalization requirements are not well known. Most garlic is fall planted and if winter temperatures are lower than 5°C, the bolting phenotype is presumed to be induced. However, a systematic study of cold-treated non-bolting garlic clones typically grown in warmer climates could reveal bolting clones. A very significant genetic predisposition contributes to flowering in garlic, as some clones almost never flower with inductive conditions (non-bolting clones), while others flower readily (bolting clones) (Takagi 1990; Pooler and Simon 1993b). No treatment to routinely induce flowering in non-bolting garlic has yet been devised.

As with other alliums, garlic clones that tend to flower must attain a minimum size or physiological maturity to realize that potential. Therefore, spring planting, which reduces bulb yield and does not allow enough time for plants to reach a certain maturity level, can significantly reduce or eliminate flowering. Low and, especially, high temperatures during inflorescence and scape elongation can also result in incomplete bolting (Takagi 1990).

The garlic seed stalk develops from the tip of the main stem, precluding further vegetative growth (Mann 1952). Seed stalk formation represents the only significant internode elongation in the life of a garlic plant. Early scape elongation precedes any visible indication in the apical meristem that floral development is commencing. During scape elongation the meristematic region at its tip begins to differentiate floral initials and subdivides into identifiable flowers interspersed with bulbils (Kothari and Shah 1974a,b; Etoh and Ogura 1977; Etoh 1985; Qu-Ying et al. 1994; Kamenetsky and Rabinowitch 2001, 2002).

For incomplete bolting clones, the arrested scape development often results in a very short scape with flower and bulbil formation ranging from slightly above the cloves within the bulb, to a more elevated position in the pseudostem, to a short stem protruding slightly above the pseudostem. Complete bolting in garlic involves floral induction, scape elongation, inflorescence development, and floral maturation (Takagi 1990; Kamenetsky and Rabinowitch 2001, 2002). Clove development tends to be initiated somewhat earlier in the development of non-bolting plants than in incomplete or complete bolting plants, suggesting that there is a balance or competition between bulb and inflorescence development, with bulb development taking precedence and floral development being optional. The extent to which a plant initiates floral development is strongly influenced by genotype and contingent upon exposure to low temperatures.

During the growth of flowering garlic, there is a balance or competition between storage organs and flowers in terms of relative development and resource allocation (Pooler 1991; Pooler and Simon 1993b, 1994). The interplay between clove development in the bulb (bulb formation) and clove development in the inflorescence (bulbil formation) at the whole plant level seems to have a counterpart in the interplay between flowers and bulbils within the inflorescence. Bulbing without floral initiation and development is common, as seen in non-bolting garlic, but floral development without bulbing is not reported except for rare instances during in vitro cultivation. In most bolting cultivated garlic, bulbil formation takes precedence over floral development, although usually not to the complete exclusion of at least some floral development. Thus we can represent the relative strength of these three competing resource sinks as follows:

1. Bulb development excludes bulbil and flower development in non-bolting or unvernalized bolting plants;
2. Bulb development predominates over bulbil development and flower development in most cultivated bolting garlic plants; or
3. Bulb development, bulbil development, and flower development occur in some bolting cultivars, wild garlic, and some seedlings.

Once the environmental conditions to stimulate floral development have been met, the rate and degree of floral and bulbil development varies widely among flowering garlic clones (Fig. 5.2). Without the development of mature flowers, the production of viable gametes and seed is not possible. Garlic fertility is primarily determined by genetic background, and fertile clones usually have numerous healthy flowers and small bulbils. Infertile, barren flowering clones vary widely in floral and bulbil development. Large bulbils usually indicate little potential for seed production. For some barren clones, mature healthy-appearing flowers develop, but many barren clones develop flowers that senesce early in the bud stage before buds even reach meiosis so mature flowers never

Fig. 5.2. Inflorescences from six garlic clones with bulbils and senesced flowers late in the season.

develop (Weber 1929; Gvaladze 1965; Etoh 1985; Pooler and Simon 1994). The causes of this form of sterility have not been studied. Examination of developing flowers during scape elongation also noted abortion of some flowers in fertile clones (Qu-Ying et al. 1994).

Floral development is no guarantee of viable gamete production. Studies of 154 diverse primarily Eastern garlic clones (Etoh 1985) and of 210 Central and Western clones (Pooler and Simon 1994) yielded complete bolting in 46% and 65% of the clones, respectively, but in the latter study about 1/2 to 2/3 of these clones failed to develop fully mature flowers, since buds withered and died. This premature senescence takes several forms. In some clones, sporogenous tissue failed to enter meiosis, while in others microsporogenesis was complete, but then microspores degenerated at, or beyond, the tetrad stage. In the former study, one flowering clone developed fertile pollen, while in the latter study 27 male fertile clones were recorded, and in both studies these were Central Asian clones. Numerous other clones from the region of garlic's center of diversity have also been collected and found to be male fertile (Etoh 1986; Etoh et al. 1992; Pooler and Simon 1994; Hong and Etoh 1996; Hong et al. 2000a).

The causes of garlic floral abortion and sterility are not well understood. An accumulation of pathogens, chromosome aberrations, postmeiotic tapetal degeneration, and unsuccessful competition of floral development with bulbils have been implicated as contributing to the failure of flowers to develop (Etoh 1985). Konvicka and co-workers noted leaf necrosis in flowering plants and microscopic inclusions resembling rickettsia-like organisms, both of which became less apparent with antibiotic treatments (tetracycline and tylosine) of cut stems (Konvicka 1973, 1984; Konvicka et al. 1978). Some clones with complete synapsis, bivalent formation, and normal microsporogenesis are male sterile. In these clones, tapetal hypertrophy and other disturbances inhibit further microspore development (Novak 1972; Etoh 1982). These observations were interpreted by Konvicka to support a pathogenic cause of garlic floral abortion and sterility. However, Novak and Havranek (1975) and Pooler (1991) observed ambiguous results with antibiotic treatments, while Etoh (1980) observed phytotoxicity and arrested development of pollen with antibiotic treatment of cut scapes. Furthermore, the extensive production of virus-free garlic has not resulted in increased fertility of bolting clones, although it could be argued that the methods to produce virus-free garlic may not be expected to also remove rickettsia-like organisms. Until further substantiation is provided, a pathogenic basis for floral abortion and male sterility in garlic seems unlikely.

While some karyotypic variation was noted in mitosis of diverse garlic clones, evaluations of meiosis indicate an interesting general difference between clones collected in Central Asia (including those from the wild), east of that region, or west of it (which we refer to as Central, Eastern, and Western clones, respectively) that relate to fertility and perhaps floral abortion (Table 5.2). Several authors have observed quadrivalents, hexavalents, octavalents, and decavalents in microsporogenesis of numerous diverse bolting Eastern garlic clones that were all male sterile, while only a small number of Eastern clones, notably those from India, had all bivalents in the first meiotic division. These Eastern clones with bivalents may represent the subtropical group noted by Maaß and Klaas (1995) to differ significantly from other Eastern garlic based on

Table 5.2. Reports of garlic flowering, meiosis, and male fertility.

Reference	No. clones evaluated^z	No. clones flowering	Meiotic^y configuration	No. male-fertile clones	Remarks
Takenaka 1931	1 E	1	II and multivalents	0	
Katayama 1936	3 E	3	8 II (1 clone); 1 VI & 5 II (2 clones)	0	
Krivenko 1938	1 C	1	8 II	1	Climate limits seed production
Kononkov 1953	1 C	1	—	1	Seed produced
Takenaka 1953	1 E	1	IV, VI, VIII + II	0	
Shimada and Shozaki 1954	1 E	1	—	0	Better flower retention with bulbil removal
Gvaladze 1961	1 C	1	variable	Partial	Better flower retention with bulbil removal; suggested aposporic origins of seed
Gvaladze 1965	—	—	—	50–60% fertile pollen; 1–3% normal ovules	
Koul and Gohil 1970	3 E? (not specified)	3	8 II	0	Normal meiosis, but microspores degenerate
Gohil and Koul 1971	1 E (India)	1	Desynapsis (I)	0	
Konvicka and Levan 1972	4 W	3	8 II in most meiocytes; IV in one clone	0	
Konvicka 1972	1 W	1	8 II	0	
Novak 1972	1 W + 1 *A. longicuspis*	2	8 II	0	Post-meiotic tapetal degeneration

Konvicka 1973	14 W	14	8 II predominate	0 (with no antibiotics) to 14 (with antibiotics)	Postmeiotic disturbances alleviated with application of tetracycline
Novak and Havranek 1975	2 W+ 1 *A. longicuspis*	3	8 II	0	Binucleate pollen not viable; better flower retention with bulbil removal and cut stems
Konvicka et al. 1978	3 W	3	8 II	0 (with no antibiotics) to 3 (with antibiotics)	Postmeiotic disturbances; better flower retention with bulbil removal
Etoh and Ogura 1978	1 E	1	VIII + 4 II usually; never 8 II	0	
Koul et al. 1979	5 E (India)	2 or more	8 II	0	2, 4 - D and gamma-irradiation do not improve flower retention; better flower retention with bulbil removal
Etoh 1979	43 (39 E + 4W)	43	VI, VIII, or X + II	0	
Cheng 1982	1	1	II	0	Bulbils compete with flowers
Etoh 1983a	28 (25 E + 2 W + 1 C)	28	8 II in 2 clones (1 W + 1 C); VI or VIII + II in 25 sterile	1 C	Male fertile clone from Moscow; fertile flowers have violet anthers
Etoh 1983b	153	72	8 II in fertile clones; VI, VIII, or X + II in sterile clones	1 C	Seed produced
Etoh 1985	154	71 complete 57 partial	8 II in fertile clones; VI, VIII, or X + II in sterile clones	1 C	Bulbil number correlated with flower number; partial bolting clones failed to retain flowers to meiosis in 50 of 57 clones

(*continued*)

Table 5.2. *Continued*

Reference	No. clones evaluated[z]	No. clones flowering	Meiotic[y] configuration	No. male-fertile clones	Remarks
Etoh 1986	31C	31	8 II	14	Fertile flowers have purple anthers; several male sterile, female fertile clones
Bozzini 1991	1	1	—	1	Tetraploid, not garlic?
Etoh et al. 1992	several			several	
Pooler and Simon 1994	210 W and C	137	8 II in 29 examined	27	
Hong and Etoh 1996	42 C	42	8 II in 39/42	31/35	Fertile flowers have purple anthers
Hong et al. 2000a	30 W + 30 C	0 W (all partial bolting) + 29 C	Not reported	9 (all C)	
Etoh et al. 2001	38 (33C + 4W + 1 E)	38	8 II in 5 of 5	15 (14 C + 1 E)	

[z] Clones collected in Central Asia (C), East of center (E), and west of center (W).

[y] I, II, III, IV, VI, VIII, and X refer to univalents, bivalents, trivalents and associations of 4, 6, 8, and 10 chromosomes at meiosis I, respectively.

molecular "fingerprinting." The multivalent associations in Eastern garlic north of India indicate a clear basis for their male sterility, since gametes that result will have unbalanced chromosome sets. In contrast, Central and Western garlic clones usually form only bivalents, with multivalents rarely noted, although desynapsis has been noted in some clones. Yet Western garlic clones are almost always male sterile, while Central clones are often fertile. Post-meiotic tapetal degeneration is observed in those Western clones closely analyzed (Novak 1972; Gori and Ferri 1982; Gori 1983), as well as in sterile Eastern clones (Etoh 1982), but the cause of degeneration is not known. Genetic and cytoplasmic male sterility in seed-propagated crops is only beginning to be understood after much effort, and a similar mechanism accounting for male sterility in Western garlic may be uncovered.

A fourth contributor to impaired garlic floral development in some studies points to unsuccessful floral competition with bulbils. Katarzhin and Katarzhin (1982) and Etoh (1983b, 1997) produced garlic seed without bulbil removal, but Konvicka (1984), Pooler and Simon (1994), and Jenderek (1998) removed bulbils during floral development to improve development of flowers and seed, although it should be noted that this was only successful for clones with some male fertility without this procedure. It is reasoned that developing flowers are relatively poor competitors with bulbils for photosynthate and, at least in some cases, this competition results in floral abortion. Verification of this hypothesis has not yet been forthcoming.

Konvicka (1984) and Pooler and Simon (1994) carried this notion a step further and decapitated garlic plants just above the pseudostem around the time when the scapes were nearly fully elongated. These cut stems were then placed in containers of water (with and without antibiotics, respectively) for pollination and seed development, with good success. The rationale for this treatment is that developing flowers also compete poorly with the developing bulb. Experiments demonstrating a clear, positive effect from this treatment (versus seed production on intact plants) have not been reported.

V. GARLIC SEED PRODUCTION

A. Importance of Garlic Germplasm for Seed Production

One recurring observation made in all recent studies seeking garlic flowering and seed formation is the need to include a diverse range of garlic germplasm with an emphasis on Central Asian cultivated clones and *Allium longicuspis* (reviewed by Etoh and Simon 2002) (Table 5.3). The

Table 5.3. Reports of garlic seed production.

Reference	No. seeds produced	Germination rate (%)	No. Clones			Remarks
			Evaluated	Flowering	Producing seed	
Kononkov 1953	Several	+	1	1	1	Bulbil removal
Gvaladze 1961	Several	+	1	1	1	
Katarzhin and Katarzhin 1978	120	+	>1	—	—	Two generations produced in field conditions
Katarzhin and Katarzhin 1982	Several	—	4	—	—	The first seed generation was without bulbil removal; two generations of seeds were produced
Etoh 1983b	27	25.9	153	72	1	
Konvicka 1984	41	41.5	7	7	7	Bulbil removal
Etoh 1985	200 harvested, 85 mature	36.8	2	2	2	Seed production from cross-pollination
Etoh et al. 1988	22,850	3 to 83	17	17	17	
Pooler and Simon 1994	655	9.6	210	137	11	
Inaba et al. 1995	52,508	80	—	—	—	Four cycles of selection, up to 248 seeds/plant
Hong and Etoh 1996	346	0.01	42	39	17	All seed from self-pollination
Etoh 1997	3481 (1984)	12.3	17	17	17	Multiple pollination
	>7000 (1985)	0 to 100	10	10	10	
Jenderek 1998	>2M	up to 93	421	—	64	Up to 656 seeds/plant
Hong et al. 2000c	>3500	0.02	49 original + 124 previously derived from seed	—	—	Inclement weather limited seed production

first three reports of garlic seed production came from research in the Soviet Union apparently using germplasm from Central Asia and neighboring regions (Kononkov 1953; Gvaladze 1961; Katarzhin and Katarzhin 1978, 1982). Etoh and co-workers (Etoh 1983b, 1985, 1997; Etoh et al. 1988; Inaba et al. 1995; Hong and Etoh 1996; Hong et al. 2000c) included Central Asian germplasm in their materials, which resulted in successful production of seed. Konvicka (1984) used a diverse germplasm collection in his successful production of garlic seed. Pooler and Simon (1994) found especially good seed production with recently collected wild garlic from Central Asia. Jenderek (1998) also utilized a very diverse germplasm collection of garlic originating from 41 countries, including many from the former USSR, for highly successful seed production. As Central Asia is the center of garlic germplasm diversity and only area where wild garlic or *Allium longicuspis* still occurs, it seems likely that the broad genetic variation observed in visible characters as well as molecular assessments likely contributed significantly to successful seed production. In fact, we may infer that sexual reproduction in wild, and perhaps cultivated, Central Asian garlic has served as the reservoir of recombination to generate most of the new genetic diversity of this crop. As migrating hunters and gatherers, and more recently travelers followed major migration and trade routes through Central Asia, it is easy to envision them collecting and carrying wild garlic for cultivation far from that region. This process likely continues today.

Based on molecular fingerprinting, it does not appear that newly collected garlic from Central Asia represents a different basic gene pool from clones long propagated in cultivation and collected around the world. Therefore, we may speculate that long-term cultivation and even selection has brought an accumulation of genetic changes that limit the ability of garlic to flower and produce seeds. Long-term asexual propagation is well known to accumulate structural and numerical chromosome aberrations (Sharma 1956), which appears to be the likely mechanism for male sterility in Eastern garlic. But other genomic changes that affect gene expression, such as altered patterns of DNA methylation, may account for male sterility in Western garlic. Experiments comparing clonally propagated garlic with true seedlings may lead to a better understanding of the mechanisms contributing to the success or failure of garlic flowering, fertility, and seed production.

B. Processes and Procedures for Garlic Seed Production

With the discovery of several sources of male fertile garlic, the prospects for seed production and breeding seemed likely. Yet while the development of fertile flowers in garlic is necessary for seed production and breeding, it is not sufficient. Once fertile clones are identified in diverse germplasm, flowers must be pollinated and seed produced; seed must be harvested, stored and germinated; and seedlings must be grown to plants to move forward in a breeding program. The extensive research contributions of T. Etoh in seeking male fertility and successful seed production in garlic are particularly notable in their breadth and depth.

1. Pollination and Seed Production. Optimal plant growth conditions contribute to successful garlic seed production. Fall planting in a climate that induces bolting is essential. Once bolting plants produce mature flowers in the spring or summer, they must be prepared for pollination.

In more optimal climates, garlic seed production is most straightforward on plants growing in the field since this is where optimal growth occurs. This requires situating plants at planting time to accommodate pollinating cages, if they will be placed over flowering plants the following spring. Alternatively, in less optimal climates, field-grown plants can be used as a source of cut stems to perform seed production at a different site. This practice allows bulbil removal and pollination to be performed outside the field, for example, in a laboratory or air-conditioned greenhouse. Garlic cut stems are prepared by severing the scape just above the pseudostem when the scape is fully, or almost, straightened and usually after bulbil removal. Cutting the stem too early can impede full development of the inflorescence. Adequate photosynthate is apparently provided by the scape, as seed production capacity does not appear to be limited by this procedure. Production of garlic seed on cut stems is more labor-intensive than seed production on plants in the field, and consequently would not be practical for commercial large-scale production of seed, but the flexibility it provides will likely recommend its continued use in small-scale breeding programs, or in making initial crosses in larger programs.

Bulbil removal is very conducive to prevention of early senescence of flowers (Fig. 5.3), but it is not required for all clones, especially in the second and subsequent generations of seed production. Bulbil removal is a time-intensive and tedious exercise that involves opening the spathe of inflorescences while bulbils are no larger than 2 to 5 mm in diameter, followed by extirpation of all visible bulbils. Care is required in finding all bulbils while not damaging the developing flowers, which are smaller than the bulbils and much more fragile. After the initial removal of bulbils, a second removal 3 to 7 days later is useful to extirpate bulbils that typically remain.

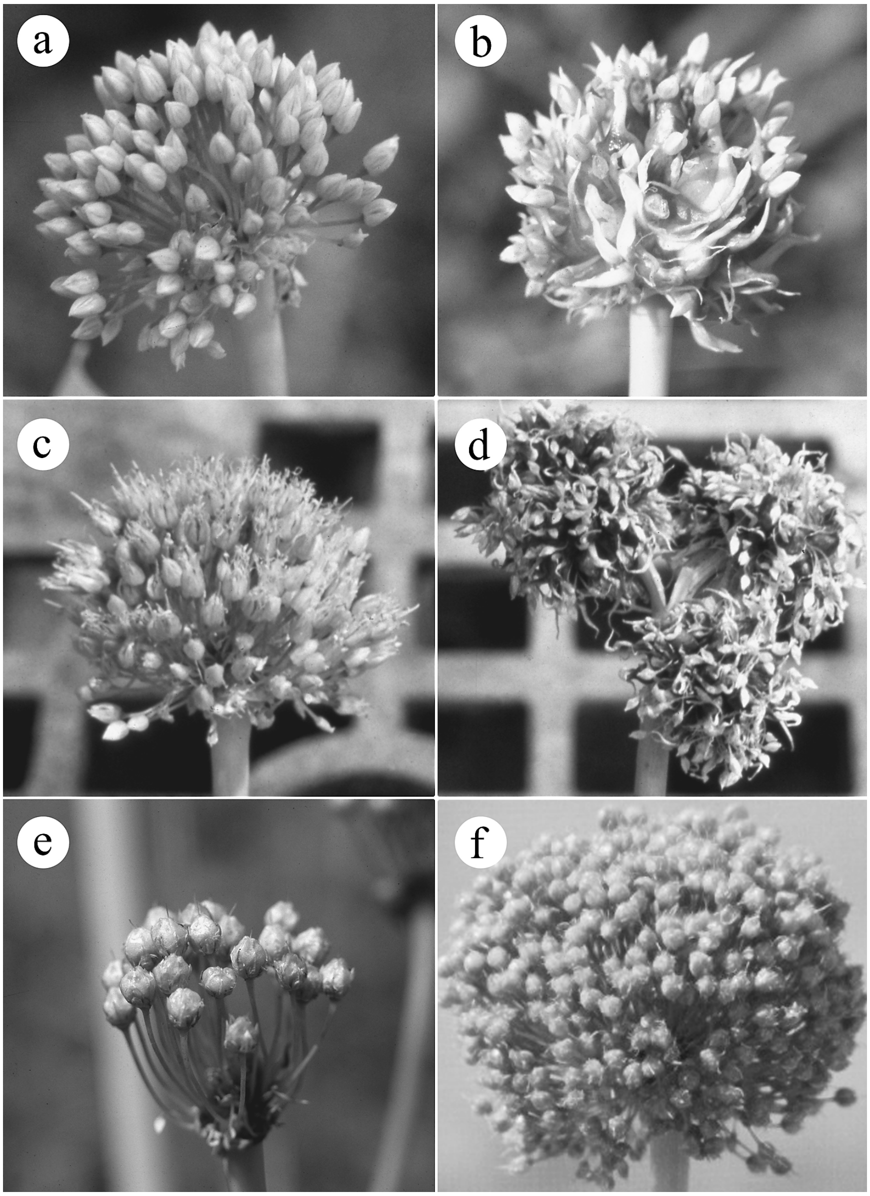

Fig. 5.3. Garlic flower development. Early (a and b) and late (c and d) flowering in the same clone where bulbils have been removed (a and c) or retained (b and d). Fertilized ovules swelling late in the season for clones with few (e) and many (f) flowers.

The most promising flowers for successful seed production usually have pigmented (purple or red) anthers and are male fertile (Etoh 1983a; Konvicka 1984; Etoh 1986; Etoh et al. 1988; Pooler and Simon 1994; Hong and Etoh 1996), although some male fertile plants with yellow anthers do occur and such plants produce seeds (Pooler and Simon 1994; Jenderek 1998). Furthermore, male sterile plants can still be female fertile and set seed. This fact creates an opportunity to produce F_1 hybrid garlic seed by isolating a male sterile/female fertile clone with a fully fertile clone during pollination.

Pollen stainability and germination varied widely among those garlic clones first used for seed production. For example, Hong and Etoh (1996) and Jenderek and Hannan (2000) observed pollen stainability from 0% up to 98.3%. Pollen germination rate is generally lower than stainability. Pooler and Simon (1994) found from 0% to 10.5% germination, while 0.7% to 49.7% was reported by Hong and Etoh (1996) and Jenderek and Hannan (2000). Although male fertility is associated mainly with purple anthers, stainable pollen in yellow anthers was as high as 68.6% with pollen germination rates of 30.1% in the latter study.

To ensure garlic seed production, several authors have used multiple pollination (repeated hand pollination of the same flowers over several days) (Etoh 1983b; Pooler and Simon 1994; Etoh 1997). It is not clear that this practice always improves the seed set.

Several pollination vectors have been used to produce garlic seed. Collection of anthers or pollen with a forceps, on a glass slide, or small brush has been used on a small scale, usually with repeated pollen application to the same flowers over several days. Emasculation has been used to eliminate self-pollination, but it is a tedious exercise. Honey bees (*Apis mellifera*), houseflies (*Musca domestica*), leaf cutter bees (*Megachile rotundata*), and bluebottle flies (*Protophormia terraenovae*) have been used successfully as pollinators in isolation cages. As the style of garlic flowers is small and delicate (Fig. 5.1), seed production in some clones suffers from rough handling and honeybee pollination. Success with bluebottle fly pollination has been very good, and this is the main pollination vector currently used in much garlic true seed production.

Diseases, pests, and weather can take a significant toll on garlic pollination and seed formation. In particular, floral pests such as thrips and mites can quickly decimate recently pollinated flowers and lead to crop failure. Inclement weather such as heavy wind and rain can also interfere with pollination and damage plants in the field (e.g., Etoh et al. 1988). A serious threat to true seed production occurs with hot weather (over 30°C) persisting for several days during anthesis when pollen viability declines rapidly, flowers wither, and flower stalks senesce rapidly. Heat sensitivity varies widely among diverse clones.

2. Seed Harvest, Storage, and Germination. Garlic seed harvest is similar to onion seed harvest. Dried inflorescences are crushed to release seeds, and seeds are separated from chaff. Small seeds are frequently inviable and often occur with great regularity, especially in early generation materials. All seeds are planted in hopes of germination, but as selection for greater viability and seedling vigor proceeds in advanced generations, procedures such as seed separation in a wind column are used to remove chaff and light seed and leave heavier, more vigorous seed.

The number of seeds produced by an inflorescence depends on several variables, including genotype and growing conditions. Clonal planting material produced up to 50 seeds/umbel (Etoh et al. 1988; Jenderek and Hannan 2002), whereas maternal plants derived from true seeds produced 656 seeds/umbel (Etoh et al. 1988; Jenderek 1998).

Garlic seeds are approximately half the size of onion seeds, resembling them in shape and color (Fig. 5.4). The size of true seeds in garlic depends

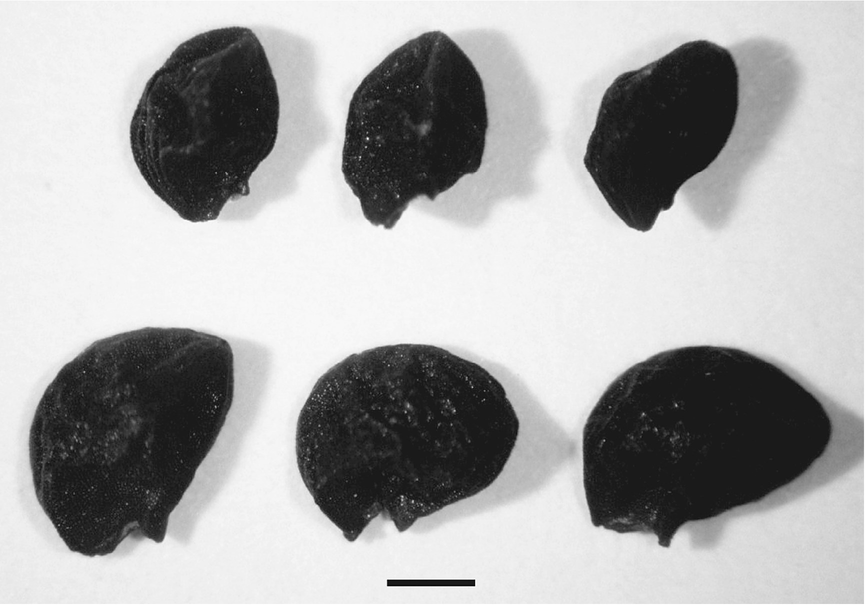

Fig. 5.4. Garlic (above) and onion seeds. Bar = 1 mm.

on the genotype of parental lines. Early publications documenting the ability of certain garlic clones to set a limited number of seeds inspired a series of experiments evaluating conditions and treatments that stimulate seed germination, including hormone treatments, stratification, scarification, and moist chilling. Hormone treatments proved ineffective (Etoh 1983b; Etoh et al. 1988; Inaba et al. 1995). In vitro seed germination allows for easy evaluation of germinating seeds, but requires high labor input with no apparent improvement in germination rate (Pooler and Simon 1994). Like many *Allium* species, garlic has some seed dormancy, which is reduced by cold treatments. Germination rates in clones that had been long propagated asexually ranged from 10% to 35% with some seeds still beginning to germinate 12 months after onset of germination conditions. Breeding efforts have increased germination rates to at least 65%, and up to 100%.

3. Growing Seedlings to Plants. Vigorous growth of garlic seedlings is difficult to achieve in early generations of seed production. Seedling growth is often weak and slow, so optimal growing medium, moisture, and freedom from pests and diseases is essential. Like onion and leek, garlic seed germinates epigeally with the bent cotyledon first to emerge. As with plant development from cloves or bulbils, leaves arise from the apical meristem to generate 5 to 15 leaves, depending on genotype, temperature, and photoperiod. The time from germination to 3 true leaves can take as long as 4 months. Pseudostem development is not very prominent in small seedlings. If seedlings grow large enough, flowering can occur in the first year in optimal growth conditions with the best genetic selections. Usually only one scape and inflorescence forms in flowering garlic, and that in the second year, however 2–3 stalks per plant may develop. A broad range of phenotypic variation is evident in bulbs that develop from true seed (Fig. 5.5).

Garlic seedling vigor and consequently survival usually increases with progressing seed cycles. Populations of all early generation seed derived plants carry, to various degrees, unfavorable characteristics, such as stunted or aberrant growth, or chlorophyll deficiencies. In fact, plants in these early generations can manifest unfavorable growth even in more mature plants. These can include deformed leaves, stunted roots, and limited bulb production (although the latter trait could be a photoperiod response). The appearance of these traits is more frequent in progenies derived from self-pollination (Jenderek 2002), but surviving seedlings and subsequent generations are usually more vigorous in growth, and often they surpass the vigor of plants grown from clonal material.

Fig. 5.5. Garlic bulbs from true seed.

VI. PROGRESS IN GARLIC BREEDING AND FUTURE PROSPECTS

Before the development of techniques to produce garlic seed in relatively large quantities, garlic breeding was not a realistic possibility. In fact, there is no evidence indicating that sexual reproduction and selection were ever utilized by garlic growers throughout history so that, while garlic has been one of the longest cultivated horticultural crops, breeding the crop has just begun. Clonal selection has been successful in altering some traits in garlic such as clove number and earliness (Burba 1997) and routine treatments to reduce or eliminate viruses clearly improve production (van Dijk 1994; Verbeek et al. 1995; Salomon 2002), but without sexual reproduction, desired traits found in different clones cannot be combined. With the possibility of seed production, garlic breeding can commence.

Information on the current status of true seed derived plants is scarce, as most of the large-scale seed production has been performed by private industry. The United States Patent and Trade Office lists three seed derived cultivars to be propagated by cloves. The area in cultivation utilizing those cultivars is not reported.

A. Selection for Improved Floral Characteristics and Fertility

Although the asexually propagated bolting garlic clones used worldwide typically produce inflorescences with predominant bulbils and completely or nearly sterile flowers, selection for improved floral characteristics and fertility has generally been successful. Reduced numbers of small, easy-to-remove bulbils is typically very rapid, so that after two or three generations of seed propagation, bulbil removal is not necessary in some breeding stocks, although they usually are still present. It may, in fact, be possible to breed for bulbil-free strains in some genetic backgrounds.

Selection progress for improved male fertility is also rapid, with high levels of pollen stainability common in the first generation progeny of sexual reproduction. It is presumed that the cleansing effect of meiosis to generate balanced gametes immediately results in meiotic stability and female fertility, although published data are lacking. There is cytological evidence that demonstrates that translocations and inversions occur in many Eastern garlic clones, so one can speculate that some fertile seed progeny of these clones may harbor homozygous translocations or inversions that have no effect upon selfing or intercrossing with similar strains. However, upon crossing with strains lacking these aberrations, sterile translocation or inversion heterozygotes would result. No observations of this type have been published to date. However, several molecular markers have been identified to assist in selection for male fertility so that fertility may be predicted long before a plant flowers (Etoh 1985; Hong et al. 1997, 2000b).

B. Selection for Improved Seed Size and Vigor

Selection for improved seed vigor and size can be dramatic after two or three cycles. Sizable variation in seed size has been noted in early generation seed progeny, and breeding efforts to date have succeeded in selecting for seed size ranging from 339 to 496 seeds/gram (Jenderek 1998). After two to three generations of selection for improved seed germination, germination rates of 65% to 93% have been routinely observed (Inaba et al. 1995; Jenderek 1998), a dramatic improvement over the low rates of 10% to 35% observed in first generation seed. Like any plant breeding effort, selection is not successful in all genetic stocks.

Selection efforts to date have proved that the yield of true seeds could be improved significantly. The possible number of seeds produced by one inflorescence can be up to 1200 seeds in umbels with 200 flowers, based on the assumption that one flower has a potential of developing six seeds.

C. Garlic Breeding Goals

A very wide range of phenotypic variation has been observed in true garlic seed progenies, including variation in all of the characters discussed above in describing germplasm variation. Perhaps most interesting is the observation of non-bolting seedlings, since both parents were obviously bolting plants. These are unusual and no reports have described these segregants beyond first- and second-year field observations. In fact, general descriptions of genetic segregation for any trait in garlic are lacking. Most of the variation described has been for some of the typical bulb and flower traits, including outer and inner bulb scale color, clove number and color, time of bulb maturity, time of flowering, stalk length, and flower petal color, pedicel color, number, and ovary wall color. Given the range of flowering habits observed among diverse garlic clones, a better understanding of flowering genetics will be of particular interest. Of most interest to garlic growers are yield and bulb storage parameters, but no reports to date have included data detailing these attributes. A search for genetic resistance to viruses and other diseases ranks high among garlic breeding goals.

Another very critical trait, likely associated closely with yield, is seedling vigor. This was a crucial variable contributing to the initial successes in obtaining garlic seed, and it will also be critical for success in developing seed-propagated garlic. Since clonally propagated garlic typically develops from a clove several grams in size, and a seedling has a weight equivalent to only a small fraction of a clove, early seedling vigor will be essential for crop propagation from seed. Perhaps other technologies such as transplanting of seedlings, rather than direct seeding, will also be used to make seed-propagated garlic a viable economic possibility, although this would add back another production cost and hence reduce the economic benefits of a seed-propagated crop. Field trails to evaluate the possibilities for direct seeding are underway and they will apply intense selection pressure to test the feasibility of seed-propagated garlic.

D. Garlic Breeding Methods

Initial efforts to produce garlic seed included only a very few fertile clones that were interpollinated and then progeny were either interpollinated again or self-pollinated. As garlic breeding proceeds, there are two distinct directions that can be taken: development of new clones for asexual propagation, or development of seed-propagated garlic cultivars.

The development of new garlic clones for asexual propagation will follow the same process of evaluation and utilization as is currently being used to evaluate new asexually propagated clones that have been acquired from abroad and are being tested for performance. In this system, individual seedlings will be selected based upon superior performance to be used as new clonally propagated cultivars using the same production techniques as have been used until now. The process of adequately testing a new seedling will take several years, since the increase of a single plant to produce an adequate supply of bulbs is necessary to perform replicated field trials, storage trials, and evaluation of added-value characteristics such as phytonutrient content or soluble solids. New cultivars developed this way must fit all existing parameters to meet grower and processor needs, but also exhibit enough superior traits or unique new combinations of traits to warrant considering replacing existing cultivars with them.

New clonally propagated cultivars have likely been gradually replacing older ones in Central Asia throughout history, although this process has never been documented. An important question, which has not yet been answered, is whether new clones from seedlings can out-compete existing clonally propagated materials. Preliminary observations seem to indicate this will occur, but the superior traits offered by new clones selected from seedlings, and the number of existing cultivars that will be replaced is yet unknown. Likely, the large-scale production and processing industry will utilize new clonally propagated cultivars, but smaller-scale growers can benefit to the same extent in improving their operations. The new complication that garlic clones derived from seedlings bring to growers is a potentially huge influx of new clones to be tested. Increase of materials and proper testing over several years for large numbers of seedlings is a monumental task, so new clones from seedlings will likely require striking improvements over existing cultivars to warrant such an effort.

The development of seed-propagated garlic cultivars is only in its very initial stages and no clear indication of its likelihood of success is possible for several years. The possibility that seed-propagated garlic will have low or no virus contamination is a strong incentive for true seed propagation. The combination of tissue culture to remove viruses, plus “seed” garlic field production costs exceed $2500/ha. If the costs of $300/ha for hybrid onion seed are an indication of how expensive garlic seed may someday be, then the economic incentive for true garlic seed is apparent. Inventory management, storage, and transportation costs also favor garlic seed over clonal propagation.

Garlic breeding methods to be used include the usual recurrent phenotypic selection from (at least initially) a broad genetic base of materials. As self-pollination has been demonstrated to be possible and to result in reasonably vigorous and more uniform families, it seems likely that cultivar development strategies for seed-propagated garlic will include an emphasis on inbred development for hybrid cultivars, like onions. Since male sterile/female fertile clones have been well documented, these clones are an obvious choice for seed parents. As for other outcrossing crop species, hybrid cultivars not only can take advantage of hybrid vigor (which has been widely observed in garlic but not reported), but they also encourage growers to return to seed producers for seed in subsequent production years. With the large investment involved in developing seed-propagated garlic, there is little incentive for large-scale seed producers to develop open-pollinated cultivars that could be seed propagated by the grower, although public sector programs may develop open-pollinated seed-propagated garlic cultivars.

VII. CONCLUSIONS

Garlic is a widely recognized and appreciated crop with a long history of asexual propagation. Several inherent aspects of garlic growth and development combined with artifacts of its long asexual reproduction have resulted in a crop in which many clones do not flower, those flowering are nearly or completely sterile, bulbils usually suppress flower maturation, and first-generation seedlings are weak with a high incidence of abnormalities limiting normal growth and development. In spite of these facts, observations and experiments of the last 50 years, and especially the efforts of T. Etoh in the last 20 years, made it apparent that the production of true garlic seed is possible. Access to a diverse range of germplasm, particularly that from near its center of origin, combined with careful application of procedures to enhance seed production and growth, such as bulbil removal and careful seedling husbandry, has set the stage for true garlic seed production of the crop. Thus, the advantages that sexual reproduction brings in generating a balanced genome and combining traits from two unrelated parents could be captured. Utilizing these materials, methods, and meiosis, a small level of success in garlic seed production was realized. Taking advantage of the benefits of these breakthrough efforts, millions of garlic seeds have been generated in the last decade, and garlic breeding is underway. The potential for combining traits of diverse materials to develop new genotypes is only in its infancy, but much genetic variation is apparent and field testing of a seed-produced garlic crop is underway.

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