Vegetable Science (2015) 42 (1) : 44-48

True seed production of garlic (*Allium Sativum* L.) in sub-tropical plains of India

RK Dhall

Received: August, 2014 / Accepted: April, 2015

Abstract

Garlic (*Allium sativum* L.) genotypes differ considerably in flowering characteristics, pollen viability and seed production ability. In all the ten genotypes studied, removal of developing bulbils resulted in seed production. The maximum seed production was observed in the genotypes 5366 and 5381 which produced more than twenty seeds per umbel. The success of more than 196 total seeds from 20 umbels of all the ten genotypes after bulbils removal confirms that garlic seed setting percentage is highly genotype specific and bulbils removal as critical for seed production success. All the genotypes had yellowish green flowers with light purple tinge. The small size bulbils were usually purple cream or cream purple but with maturity its upper tips become brown. All the genotypes had pollen viability within range of 0-10.5% but on seeding no seed was germinated. Due to short days conditions in Punjab, garlic plants do not have sufficient day length for viable true seed production but if in long day conditions the methodology used in the study is followed, then the chances of viable true seed production of garlic is very high. The production of garlic seed by bulbils removal is labour intensive and consequently would not be practical for commercial large scale seed production but would be used in breeding programme as it generates genetic variation which can be exploited for improvements of yield, tolerance to biotic and abiotic stresses and quality.

Keywords: Garlic, Bulbils, Fertility, Flowering, Pollen viability, Seed

Introduction

Garlic cloves are sometimes refers to as "garlic seed," but seed produced after sexual reproduction is actually a true seed. It is considered to be completely sterile plant and is, therefore, commercially propagated only by cloves. The cause of this sterility has been variously attributed to several possible mechanisms: garlic could be a sterile hybrid resulting from the cross of two fertile ancestral species (Etoh 1985); competition for nutrients between flowers and vegetative buds or topsets or bulbils in the developing inflorescence (Koul and Gohil 1970; Etoh and Simon 2002); the tapetum may degenerate before pollen mitosis (Novok 1990); a series of "degenerative-like-diseases" induced by organisms such as rickettsia, mycoplama and/or viruses which may interfere with sexual reproduction (Konvicka 1973, Konvicka *et al.* 1978); or observed floral morphological abnormalities.

Since garlic has been propagated asexually by cloves for many generations, an accumulation of chromosome aberrations such as aneuploidy and translocations and/or inversions could also significantly reduce the incidence of balanced gametes. Marked variation among garlic genotypes with respect to flowering ability and the ratio of flowers and topsets (bulbils) in the umbel suggested that garlic is undergoing a process of transition from sexual to asexual reproduction (Etoh 1985). According to this hypothesis, ancestral garlic had normal meiosis, was fertile, and developed numerous flowers and bulbils in the long-scaped umbel. Compared with modern sterile cultivars, this type probably had greater tolerance to cold and heat, larger numbers of differentiated foliage leaves, late maturing and sterile. Domestication and subsequent cultivation of garlic would probably have accelerated selection for larger bulbs which promoted sterility, as production of scapes reduces bulb yield, and garlic growers have often eliminated flowering plants. Therefore, variation in garlic occurs only through random or induced mutation (Burba 1993) and/or somaclonal variation (Novak 1990) and new cultivars are bred by clonal selection, induced mutations, somaclonal variation or genetic engineering (Jones and Mann 1963, Rubatzky and Yamaguchi 1997, Robinson 2007). The lack of sexuality in garlic limits the variability that is useful for breeding for economically important

Department of Vegetable Science
Punjab Agricultural University, Ludhiana-141004, Punjab
Email: dhallrk2007@rediffmail.com

traits such as tolerance to biotic and abiotic stress, earliness, yield and quality (Kamenetsky 2007).

Restoring fertility in this crop would provide new genetic combination for breeding purpose or genetic studies. Hence, fertility restoration in garlic has become a major goal of garlic researchers, and attention has been directed towards the morphological and physiological processes during florogenesis (Etoh and Simon 2002, Kamenetsky and Rabinowitch 2001, Pooler and Simon 1994, Simon and Jenderek 2004). Fertility restoration in garlic has been attempted by many researchers (Etoh 1985, Pooler and Simon 1994, Etoh *et al.* 1988, Kamenetsky *et al.* 2004, Konvicka 1984) and it has been suggested that the presence of vegetative bulbils is the major cause of sterility. By removing the bulbils and applying antibiotics to the floral stems, Konvicka (1984) obtained 17 viable seeds. Later Etoh *et al.* (1988) discovered some fertile garlic clones. By manually removing bulbils from the inflorescence, he obtained many viable seeds from fertile and semi-fertile clones. Consequently, studies of floral developmental physiology and sexual hybridization have been possible. Pooler and Simon (1994) improved seed set by scape decapitation and removal of bulbils, but they found that seed germination rates were low, ranging between 10 and 12%. Later, Inaba *et al.* (1995) and Jenderek (1998) obtained 50,000 and 1.2 million garlic seeds, respectively. In the later work, 27 clones were classified as highly fertile, producing over 400 seeds per umbel, with seed germination of 67-93%. The removal of bulbils was necessary only in the early generations, as the strong selection pressure for blooming and seed production resulted in improved seed set. The remarkable effect of selection on the improvement of garlic seed production indicates the significance of the genetic control of this trait. Recently, fertile accessions were identified in the USDA garlic collections (Jenderek and Hannan 2000, Jenderek and Hannan 2004). Sexual reproduction in garlic has the practical significance as it generates genetic variation which can be exploited in garlic breeding for improvements of yield, tolerance to biotic and abiotic stresses, and quality. Seed propagation of garlic would be more economical method to eliminate viruses and nematodes than the labour intensive meristem culture used today. Because of small size of garlic seed, it is easier to handle, store and transport as compared to cloves which ultimately reduce its production cost. Besides economic benefits of true seed production, sexual reproduction in garlic would provide a means for evaluating the extent of variation in garlic genome. Seed propagation of garlic on a massive scale may become a feasible option in the future(Etoh and Simon 2002, Simon and Jenderek 2004).

In spite of the above studies conducted by various researchers, fertile and seed producing germplasm in garlic is still not readily available for garlic breeders and seed producers. The objective of the study was to evaluate fertile garlic genotypes collected from USA for seed producing ability (after bulbil removal from the inflorescence) and fertility under Punjab conditions.

Material and Methods

Plant material, bulbil removal and seed production

For the present study, 10 garlic genotypes from USA collection were used. Five cloves of each genotype were planted at the Vegetable Research Farm of Department of Vegetable Science, Punjab Agricultural University, Ludhiana during October 2012. Ludhiana is situated at 30°54' North latitude and 70°45' east longitude with mean height of 247 m above mean sea level.

Spathe of inflorescences (umbels) of all the genotypes was opened manually when bulbils along with flowers filled the spathe completely. After opening the spathe, bulbils were removed from the inflorescence with fine forceps. Bulbil removal is a combination of plucking them out with tweezers and rocking them out to dislodge them. Care was taken while bulbils removal that no damage occurred to the developing flowers in the inflorescence. After the initial removal of bulbils, a second removal was done 7 days later to extirpate bulbils that typically remain on the inflorescence during the initial bulbils removal process. The senescence of garlic plants was delayed be giving ample water.

The swelled ovaries are indication of seeds development process. Thereafter, the scapes were cut at the level where pseudostem starts but after successful pollination and fertilization of umbels (easily identified with swollen ovaries) and thereafter these scapes were kept in water bucket and placed in netted shade nets till the maturity of seed (Figure 1). The scapes lost colour or became slimy toward their base during the lengthy seed maturation process. Therefore, the brownish or slimy portion of the scape was trimmed and shortened as necessary so that a viable part of the scape remains in water and can continue to sustain the umbel. Harvesting was done when the seeds were completely dried and seed ovaries splits. Dried umbels were threshed to release seeds. Garlic seeds are approximately half the size of onion seeds, resembling them in shape and color.

Pollen grains viability studies

Three flower buds from each genotype were collected before anthesis, when pollen was mature but anthers non-dehiscent, fixed in fixative (1 part 100% ethyl

Figure 1. Different stages of true seed production in garlic (**a**) Stage-I: Spathe opening; (**b**) Stage II: Fully opened umbel having flower buds and bulbils; (**c**) Stage III: Umbel after bulbils removal (**d**) Stage IV: Mature swelled ovaries indicating seed development initiation; (**e**) Stage V: Umbel containing mature true seeds; (**f**) Stage VI: True seeds of garlic

alcohol: 1 part glacial acetic acid) and kept at room temperature. Thereafter the straining solution was prepared as per the method suggested by Peterson *et al.* (2010). After 24 hours of fixation, the buds were placed on the filter paper to absorb fixative's liquid. Anthers were removed from the flower buds and placed on the microscope slide; thereafter anthers were squashed to release pollen. Apply 2-3 drops of strain solution on microscopic slide having dissected anthers. Once the sample was in the strain, slowly heat the slide over the alcohol burner in a fume hood for approximately 30 seconds. A moderate rate of heating (moving the slide in and out of flame) was done as it gave better penetration of dye into the cellulose and protoplasm of the pollen. Place the cover-slip over the samples and sealed them with transparent nail polish. Slides were examined using high power microscope mounted with camera. The fertile pollen grains stained magenta-red and sterile pollen grains stained blue-green.

Results and Discussion

The garlic inflorescence can be described as an umbel like flower arrangement, the branches (flower clusters) of which arise from apical common meristem (Kamenetsky and Rabinowitch, 2001). In all the genotypes studied, true seeds were produced but their number varied. Our observations suggests that if growing bulbils were removed from the inflorescence, then young flowers flourished in all the garlic genotypes studied, the removal of developing bulbils resulted in development of seeds (Table 1). This may be due to reduced or no competition between developing flowers and bulbils after bulbils removal. This is in agreement with results reported by Etoh *et al.* (1988); Jenderek (1998); Konvicka (1984); Koul and Gohil (1970); Pooler and Simon (1994). The maximum seed production was observed in the genotypes 5366 and 5381 which produced more than twenty seeds per umbel. The success of more than 196 total seeds from 20 umbels of all the ten genotypes after bulbils removal confirms our hypothesis that garlic seed setting percentage is highly genotype specific and bulbils removal as critical for seed production success. The less production of seeds in genotypes 5337, 5476 and 5491 may be due to generation of flower before spathe breaking, possibly because of shortage of energy and nutrients due to completion with the sink in the developing underground bulb. Similar results were corroborated by Pooler and Simon (1994), Brewster (1994), Kamenetsky and Robinowitch (2002). The production of garlic seed by bulbil removal is labour intensive 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 programmes or making initial crosses in large programmes.

Table 1. True seed production and pollen viability of garlic genotypes

Sr No	Genotype	Average number of seeds per umbel after removal of bulbils	Pollen viability (%)
1	5351	12	9.6
2	5476	1	0
3	7261	7	6.6
4	5366	24	10.5
5	5381	21	9.2
6	5391	13	7.2
7	5337	1	0
8	5491	2	1.7
9	5338	14	7.5
10	7107	3	2.5

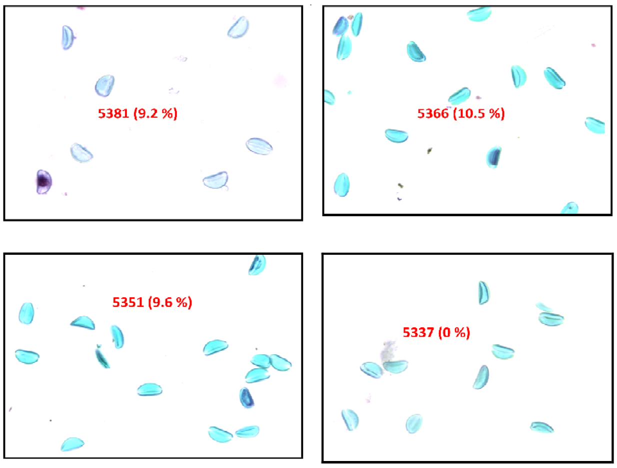

Figure 2. Pollen viability test of four genotypes using Modified Alexander's Stain (fertile pollens are magenta-red while sterile are blue-green)

Pollen viability among all the seed producing genotypes varied from 0-10.5 per cent (Table 1). It was clearly observed from the figure 2 that fertile pollens were strained magenta red while sterile were blue green. The highest percentage of viable pollen was observed in plants of 5366 (10.5%), followed by 5351 (9.6%) and 5381 (9.2%) i.e. the seed production of these genotypes were high among other genotypes (approximately 20 seeds per umbel). The results of pollen viability percentage were in agreement with the seed production rate. Our results are in agreement with those of Etoh (1983), Konvicka (1984).

In addition to these, wide variation in flower abnormalities was observed (figure 3). The different types of flower abnormalities were observed in umbel of different genotypes which includes: the development of thick green leaflets in place of floral buds, the growth of second flower along with primary flower resulting compound umbel, umbel bearing large size bulbils and few floral buds or umbels having thick leaflets, bulbils and few degenerative floral buds. It was also observed that sometime little delay in process of bulbils removal from umbel encourage the development of these flower abnormalities.

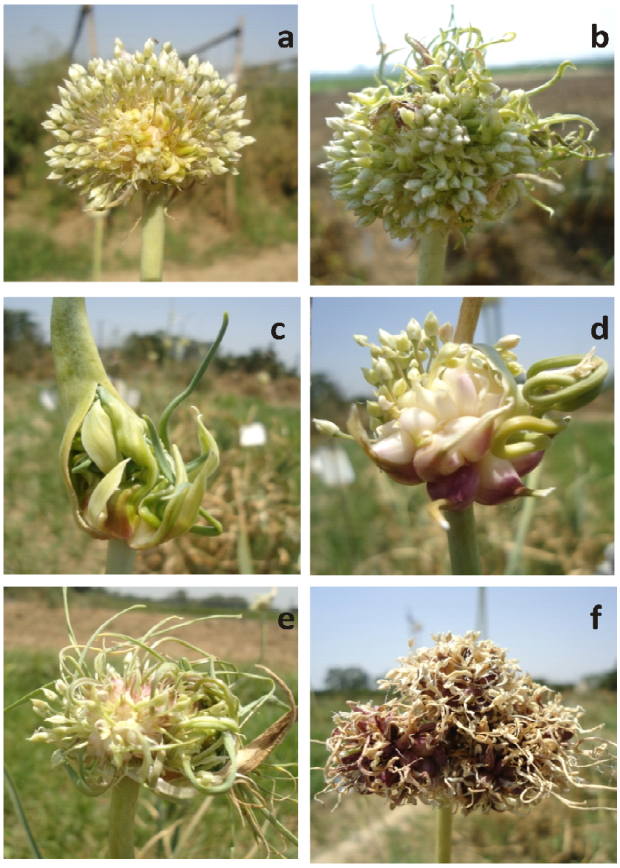

Figure 3. Variation of phenotypic expression of inflorescence in garlic plants propagated from cloves (**a**) Inflorescence bearing small size bulbils and numerous floral buds (**b**) Inflorescence bearing numerous floral buds, bulbils and modified leaflets (**c**) Inflorescence having leaflets only (**d**) Inflorescence bearing too large bulbils and few floral buds (**e**) Inflorescence bearing medium size bulbils, leaflets and few degenerative floral buds (**f**) Inflorescence having compound umbel bearing bulbils and numerous floral buds

सारांश

लहसुन के प्रभेदों में पुष्पन, गुणों, परागकण जमाव एवं बीज उत्पादन क्षमता में महत्वपूर्ण विविधता होती है। अध्ययन में सम्मिलित 10 जननद्रव्यों में विकसित हो रहे प्रकन्दों (बलबिल्स) का निकाल देने से बीज उत्पादन हुआ। सबसे अधिक बीज उत्पादन प्रभेद 5366 व 5381 हुआ जो प्रति अम्बेल 20 बीज से ज्यादा था। सभी 10 जननद्रव्यों के प्रकन्दों (बलबिल्स) को निकाल देने से 20 अम्बेल बने जिनसे कुल 196 बीज प्राप्त हुए, इससे स्पष्ट हुआ कि लहसुन में बीज धारण पूर्णतया उच्च अनुवांशिक विशिष्ट है तथा बीज उत्पादन सफलता के लिए बलबिल्स निकालना क्रान्तिक हैं। सभी जनन द्रव्यों के फूलों में पीले हरे के साथ बैंगनी रंग विकसित हुए। छोटे आकार के बलबिल्स सामान्यतः बैंगनी दुधिया अथवा मक्खन जैसे बेगनी थे लेकिन पकने के साथ पुष्प का उपरी भाग भूरा हो जाता है। सभी प्रभेदों में परागकण जमाव क्षमता 0–10.5 प्रतिशत के बीच था लेकिन बुआई के उपरान्त कोई बीज नहीं जमा। पंजाब की कम दिन मान दशा के कारण लहसुन को पर्याप्त दिन का समय बीज उत्पादन के लिए नहीं मिलता हैं अगर लम्बे दिन मान की दशा में बताई गयी विधि अपनाने से लहसुन से जमाव वाले सत्य बीज प्राप्त करने की सम्भावना बहुत अधिक होती है। लहसुन में बलबिल्स निकाल कर बीज उत्पादन करने के लिए ज्यादा श्रम शक्ति की आवश्यकता होती है और व्यवसायिक स्तर पर ज्यादा बीज उत्पादन करना तर्क संकत व प्रायोगिक नहीं है लेकिन लहसुन में अनुवांशिक विविधता उत्पन्न करने के लिए प्रयोग किया जा सकता है जो प्रजनन कार्यक्रम में प्रयोग हो सकता है। इससे उपज, जैविक व अजैविक प्रतिबलों तथा गुणवत्ता में सुधार लाया जा सकता है।

References

Brewster JL (1994) Onions and other vegetable *Alliums*. CAB International, Wallingford, UK.

Burba JL (1993) Producción de “Semilla” de Ajo. Asociación Cooperadora EEA, La Consulta, Argentina.

Etoh T (1983) Germination of seeds obtained from a clone of garlic, *Allium sativum* L. In: Proceedings of Japan Academy 59: 83–87.

Etoh T (1985) Studies on the sterility on garlic, *Allium sativum* L. Mem. Fac. Agr. Kagoshima Univ. 21: 77–132.

Etoh T, Simon PW (2002) Diversity, fertility, and seed production of garlic. In: Allium Crop Science: Recent Advances (eds. Rabinowitch, H. and Currah. L.), CABI Publication, New York, pp. 101–117.

Etoh T, Noma Y, Nishitarumizu Y, Wakamoto T (1988) Seed productivity and germinability of various garlic clones collected in Soviet Central Asia. Mem. Fac. Agr. Kagoshima Univ. 24: 129–139.

Inaba A, Ujiie T, Etoh T (1995) Seed productivity and germinability of garlic. Breed Sci 45 (Suppl. 2): 310.

Jenderek MM (1998) Generative reproduction of garlic (*Allium sativum* L.) (in Polish). Zeszyty Naukowe Akademii Rolniczejim. H. Kollataja w Krakowie 57: 141–145.

Jenderek MM, Hannan RM (2004) Genetic diversity among U.S. garlic clones as detected using AFLP methods. Hort Sci 39: 485-488.

Jenderek MM, Hannan RM (2000) Seed producing ability of garlic (*Allium sativum* L.) clones from two public U.S. collections. In: Proceedings of the 3rd International Symposium on Edible Alliaceae, Athens, GA, pp. 73–75.

Jones HA, Mann L K (1963) Onions and their allies. Leonard Hill Books, London, UK.

Kamenetsky R (2007) Garlic: Botany and Horticulture. Hort Rev 33: 123-171.

Kamenetsky R, Rabinowitch DH (2001) Floral development in bolting garlic. Sexual Plant Reprod.13: 235–241.

Kamenetsky R, Rabinowitch DH (2002) Florigenesis. In: Allium Crop Science: Recent Advances (eds. Rabinowitch, H. and Currah, L.), CABI Publications, New York, pp. 31–57.

Kamenetsky R, London Shafir I, Zemah H, Barzilay A, Rabinowitch HD (2004) Environmental control of garlic growth and florogenesis. J Amer Soc Hort Sci 129: 144-151.

Konvicka O (1973) Die Ursachen der Sterilitat von *Allium sativum* L. Biol Plant 15:144–149.

Konvicka O (1984) Generative Reproduktion von Knoblauch (*Allium sativum*). Allium Newsletter 1: 28–37.

Konvicka O, Nienhaus F, Fischbeck G (1978) Untersuchungen uber die Ursachen der Pollensterilitat bei *Allium sativum* L. Z. Pflanzenzucht 80: 265–276.

Koul AK, Gohil RN (1970) Causes averting sexual reproduction in *Allium sativum* L. Cytologia 35: 197–202.

Novak FJ (1990) *Allium* Tissue Culture. In *Onions and Allied Crops* Vol. II (eds. Rabinowitch, H. D. and Brewster, J. L.), CRC Press, Boca Raton, Florida, USA, pp. 233-250.

Peterson R, Slovin JP, Chen C (2010) A simplified method for differential staining of aborted and non-aborted pollen grains. International J Plant Biology 1:e13: 66-68.

Pooler MR, Simon PW (1994) True seed production in garlic. *Sexual Plant Reprod*. 7: 282- 286.

Robinson RA (2007) Self-Organizing Agro-ecosystems, Sharebooks Publishing, ISBN 698-0- 9783634-1-3, Available: Sharebooks e-book.

Rubatzky VE, Yamaguchi M (1997) World Vegetables: Principles, Production and Nutritive Values, 2nd ed., Chapman and Hall, New York.

Simon PW, Jenderek MM (2004) Flowering, seed production and the genesis of garlic breeding. Plant Breed Rev 23: 211-244.