Indian Journal of Agricultural Sciences **87** (11): 1554–8, November 2017/Article
https://doi.org/10.56093/ijas.v87i11.75791

Effect of vernalization on flowering and true seed production behaviour of garlic (*Allium sativum*) under North Indian Plains

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Received: 04 May 2017; Accepted: 25 July 2017

ABSTRACT

The effect of vernalization on reproductive potential of garlic (*Allium sativum* L.) especially on flowering and its true seed production behaviour which includes bolting behaviour, scape colour, flower colour, bulbil colour, number of bulbils per umbel, scape length and number of seeds per umbel were studied under North Indian Plain conditions. The thirteen 'seed producing' garlic genotypes which produce true seed under USA (long day) conditions were used in the present study and were given vernalization treatment at 4ºC for two months before sowing. In control treatment, garlic bulbs were kept at room temperature before sowing. The vernalization of garlic bulbs at 4ºC (for two months) resulted in bolting, umbel formation and true seed production in nine genotypes, whereas non-vernalized did not result into bolting, i.e. no true seed production was observed. Out of nine true seed producing garlic genotypes, the maximum seed setting was observed in genotypes 5366, 5351 and 5477 having values of 20.0, 18.0 and 13.0 seeds per umbel. The seed received from these genotypes were further sown but they did not germinated. These genotypes can be used in breeding programme, if these are sown under long day conditions. The highly significant and positive correlation was observed between number of seeds per umbel and scape length (r= 0.99); significant and positive correlation for number of seeds per umbel and number of bulbils per umbel (r=0.58); significant and positive correlation for number of bulbils per umbel and scape length (r=0.61). It was and also observed that when vernalization treatment was given to the 13 different genotypes of the garlic; out of them, nine complete genotypes produce flowers and after bulbil removal process resulted in true seed production. True seed production in garlic has the practical significance as it generates genetic variation which can be exploited in garlic breeding.

Key words: Bulbils, Garlic, True seed, Umbel, Vernalization

Garlic is predominantly reproduced by asexual means which includes cloves and aerial bulbils (Batchvarov 1993). Therefore, each garlic type is generally called as 'clone' rather than 'cultivar'. The bulb of garlic consists of cloves arranged on the basal plate enclosed by several outer foliage leaves (Rubatzky and Yamaguchi 1997). Their number, colour and size are quite variable among different garlic forms and under different environmental conditions (Rubatzky and Yamaguchi 1997). Each clove consists of a modified storage and protective mature leaves. The thickened fleshy storage leaf is the part of the clove which is consumed and within this fleshy storage leaf, there is a central vegetative bud from which new plants sprout. The thin protective leaf surrounds the storage leaf. The central vegetative bud of each clove forms about 5-9 leaves. These leaves surround a round, solid and un-branched floral stalk in flowering garlic types (Hahn 1996) but not in non-flowering garlic types. Sprouting occurs if garlic bulbs are exposed to cold temperature (15^{0}C or less) (Takagi 1990).

Garlic is considered to be completely sterile plant and is therefore propagated through vegetative means. The cause of sterility is due 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 inflorescence (Koul and Gohil 1970, Etoh and Simon 2002); the tapetum may degenerate before pollen mitosis (Novak 1972); 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). 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 of spontaneous mutants and through introduction to various growing environments (Jones and Mann 1963, Rubatzky and Yamaguchi 1997). 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.

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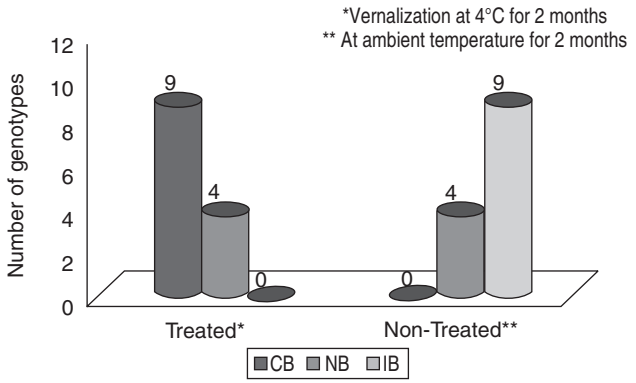

Fig 1 Effect of vernalization on bolting behaviour of garlic

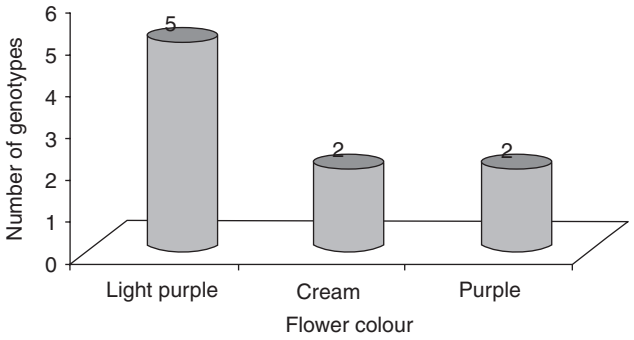

Fig 2 Effect of vernalization on flower colour of garlic

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 reduces its production cost.

Recently, seed-setting garlic clones with fertile flowers have been discovered in central Asia, which has been proposed as the center of origin of garlic (Vavilov 1951, Etoh 1986, Hong and Etoh 1996). Inaba *et al.* (1995) and Jenderek (1998) obtained 50.000, and 1.2 million garlic seeds, respectively. In the latter work, 27 clones were classified as highly fertile producing over 400 seeds per umbel, and seed germination ranging from 67% to 93%. The removal of bulbils was necessary only in the early generations, as the strong selection pressure for flowering and seed production resulted in improved fertility. The remarkable effect of selection on the improvement of garlic seed production indicates the significance of the genetic control of this trait. Recently, 36 fertile accessions were identified in two USA public garlic collections (Jenderek and Hannan 2000). Among seed bearing clones, the number of seeds produced in the first propagation cycle ranged from 0 to 85 per umbel, and a total of about 14000 seeds were harvested. Seed propagation of garlic on a massive scale may become a feasible option in the future (Etoh and Simon 2002, Simon and Jenderek 2003).

Breeding in garlic is less exploited due to lack of fertile restoration and true seed producing germplasm. Considering the true seed production potential of fertile garlic reported by various scientists, the present study was conducted to evaluate the flowering and seed production behaviour of seed producing accessions of USA garlic under North Indian Plain conditions so that genetic base of the garlic can be broadened and new genetic combination are available for breeding purpose or genetic studies.

MATERIALS AND METHODS

The plant material comprised of thirteen genotypes of garlic which included 5337, 5366, 5476, 7261, 5351, 5477, 5491, 7107, 5381, 7187, 5303, 7200 and 7274. All these genotypes were introduced from University of Wisconsin, USA.

Garlic bulbs were stored at 4°C (vernalized) and at room temperature (control) for 2 months before sowing in

Table 1 Mean performance (3 replications) of garlic genotypes for morphological characters after vernalization treatment.

Genotype	Bolting behaviour	Scape colour	Flower colour	Bulbil colour	No. of bulbils/ umbel	Scape length (cm)	No. of seeds/ umbel
5337	CB	G	LP	P	79.33	78.33	0.7
5366	CB	P	C	PB	73.33	76.66	20.0
5476	CB	G	LP	P	68.33	77.33	0.7
7261	CB	G	C	P	66.33	74.33	6.0
5351	CB	G	P	PB	79.33	75.33	18.0
5477	CB	G	P	PB	66.66	73.66	13.0
5491	CB	G	LP	B	73.33	75.00	2.0
7107	CB	G	LP	PB	65.00	76.00	3.3
5381	CB	G	LP	B	62.33	75.00	6.0
7187	NB						
5303	NB						
7200	NB						
7274	NB						

NB = Non-bolters, CB = Complete bolters, G = Green, P = Purple LP = Light purple, P = Purple, C = Cream, PB = Purple brown, B = Brown

the field. In group A, bulbs of all the thirteen genotypes were given vernalization at 4°C for two months and considered as treated. In group B, bulbs of all the thirteen genotypes were kept at ambient temperature and considered as non-treated or control.

The thirteen genotypes of group A and B were sown at Vegetable Research Farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana (30°55' N & 75°54' E) in a Randomized Block Design (RBD) with three replications. Each genotype was planted at a distance of 15 cm between rows and 7.5 cm between plants and standard agronomic practices as recommended in package of practices were followed to raise the uniform crop (Anonymous 2016).

The observations of characters related to reproductive ability of garlic, viz. bolting behaviour (Complete bolters, Incomplete bolters, Non-bolters), scape colour (Green, Purple), flower colour (Light Purple, Cream, Purple), bulbil colour (Purple, Purple Brown, Brown), number of bulbils per umbel, scape length, number of seeds per plant were recorded.

Spathe of inflorescence (umbels) of all the genotypes was opened manually when bulbils along with flowers filled the spathe completely (Dhall 2015). After opening the spathe, bulbils were removed from the inflorescence with fine forceps. Bulbil removal is combination of plucking them out with the tweezers and rocking them out to dislodge. Care was taken while bulbils removal so that no damage occurs to developing flowers in the inflorescence (umbel). 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 by giving ample water. The swelled ovaries are indication of seed 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 the seed. The scapes lost colour and become slimy toward their base during the lengthy seed maturation process. Therefore, the brownish or slimy portion of scape were trimmed and shortened as necessary so that a viable part of the scape remains in water and can continue to sustain the umbel. Seed extraction was done when the seeds were completely dried and seeds ovary splits. Dried umbels were threshed to release seeds. Garlic seeds were approximately half the size of onion seeds, resembling them in shape and colour.

The data of thirteen plants for each replication was statistically analysed and subjected to analysis of variance in Randomized Block Design (RBD) using software CPCS-1 (Singh *et al.* 1991).

RESULTS AND DISCUSSION

In vernalized treatment (4°C for 2 months), nine genotypes, viz. 5337, 5366, 5476, 7261, 5351, 5477, 5491, 7107 and 5381 were identified as complete bolters (plants produced long, thick flower stalk with flowers and topsets); whereas four genotypes, viz. 7187, 5303, 7200, 7274 were identified as non-bolters (plants do not normally form a visible flower stalk) (Table 1, fig 1). In control or untreated group, genotypes, viz. 5337, 5366, 5476, 7261, 5351, 5477, 5491, 7107 were identified as incomplete bolters (plants produced a thin, short flower stalk with only few bulbils and without flowers); whereas four genotypes, viz. 7187, 5303, 7200, 7274 were identified as non-bolters (Table 2, fig 2). In vernalized treated genotypes, nine out of thirteen were identified as complete bolters, i.e. produce flowers and from these nine genotypes, five genotypes, viz. 5337, 5476, 5491, 7107, and 5381 showed light purple colour; two genotypes, viz. 5366 and 7261 showed cream colour and two genotypes, viz. 5351 and 5477 showed purple colour

Table 2 Mean performance (3 replications) of garlic genotypes for morphological characters without vernalization treatment (control)

Genotype	Bolting behaviour	Scape colour	Flower colour	Bulbil colour	No. of bulbils/ umbel	Scape length (cm)	No. of seeds/ umbel
5337	IB	G		P	21.66	51.66	
5366	IB	P		PB	16.66	50.00	
5476	IB	G		P	17.66	57.66	
7261	IB	G		P	14.00	53.33	
5351	IB	G		PB	17.33	57.66	
5477	IB	G		PB	14.00	51.66	
5491	IB	G		B	14.00	59.66	
7107	IB	G		PB	19.00	51.66	
5381	IB	G		B	13.33	52.66	
7187	NB	-					
5303	NB	-					
7200	NB	-					
7274	NB	-					

NB = Non-bolters, IB = Incomplete bolters, G = Green, P = Purple, LP = Light purple, P = Purple, C = Cream, PB = Purple, B = Brown

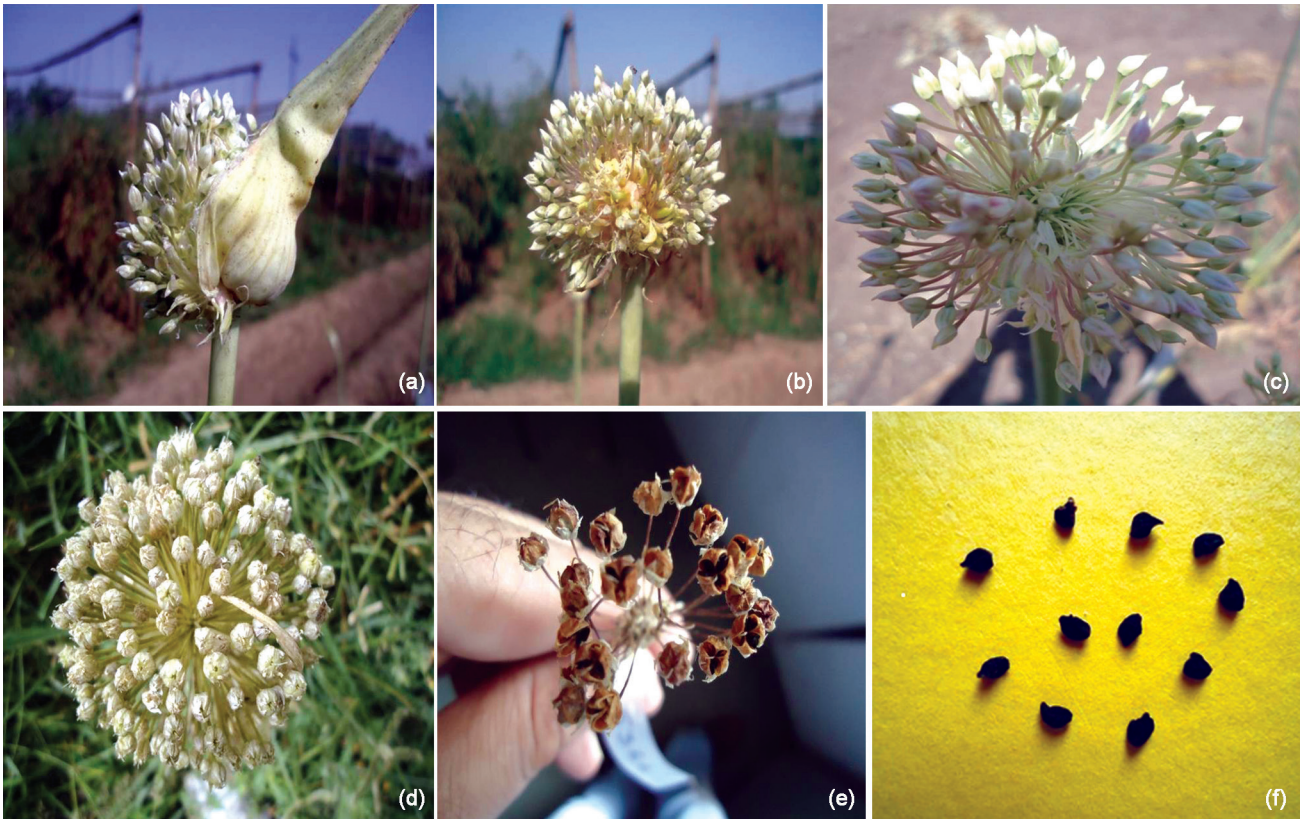

Fig 3 Different stages of true seed production in garlic

(Table 1). In control treatment, all the thirteen genotypes do not flower and were observed to be incomplete and non-bolters (Table 2). In both vernalized and control treatment, eight genotypes out of thirteen, viz. 5337, 5476, 7261, 5351, 5477, 5491,7107 and 5381 showed green colour while one genotype '5366' showed purple colour. It clearly showed that there was no effect of vernalization on scape colour development (Tables 1 and 2). In both vernalized and control treated groups of garlic genotypes, three genotypes, viz. 5337, 5476 and 7261 produced purple coloured bulbils while four genotypes, viz. 5366, 5351, 5477 and 7107 produced purple brown coloured bulbils and two genotypes, viz. 5491 and 5381 produced brown coloured bulbils. The highest number of bulbils per umbel (79.33) was observed in genotypes 5337 and 5351 of vernalized treated group and in genotype 5337 (21.66) of control treatment (Tables 1 and 2). The results were not in agreement with results of Pooler and Simon (1993). The effect of vernalization was observed on number of bulbils per umbel as the vernalized bulbs on sowing resulted into more bulbils of smaller size as compared to the control treatment which produced less bulbils of larger size. The maximum scape length was observed in genotype 5337 (78.33) of vernalized treatment and in genotype 5491 (59.66) of control treatment (Tables 1 and 2). The effect of vernalization was observed on the scape length as the vernalized bulbs on sowing resulted into longer scape length as compared to the control treatment. The maximum number of seeds per umbel were observed in vernalized treated group of genotypes and maximum was observed in 5366 (20.0) followed by 5351 (18.0) and 5477 (13.0) (Tables 1 and 2). No seed formation was observed in control group of genotypes as these genotypes showed incomplete and non-bolting behaviour. The collected true seed of each genotype were sown in pots but none of the seed was able to germinate. Similar results were also reported by Dhall (2015). In the present study, highly significant and positive correlation (r=0.99) was observed between number of seeds per umbel and scape length in garlic genotypes. The significant and positive correlation (r=0.58) was observed between number of seeds per umbel and number of bulbils per umbel. The significant and positive correlation (r=0.61) was also observed between number of bulbils per umbel and scape length (Table 3).

The effect of vernalization (low storage) treatment was observed in nine out of thirteen genotypes used in the present study. This may be due to completion of low temperature requirement for bolting in these nine genotypes, i.e. it resulted into flower development. The production of true seed in these genotypes may be due to transition

Table 3 Correlation between scape length, number of bulbils/umbel and number of seeds/umbel of garlic

Character	Number of bulbils per umbel	Scape length
Scape length	0.99**	1
Number of seeds	0.58*	0.61*

* and ** significant at 5 and 1% respectively

from the vegetative to the reproductive stage followed by differentiation of flower primordia in the apical meristem, which thereafter developed into normal flowers (fig 3). These developed true seeds on sowing in pots do not germinate. This may be due to non-fulfilment of the requirement of long day conditions under north Indian plains. In vernalized treatment, the non-bolting behaviour observed in four genotypes may be due to the reason that these genotypes require more duration of low temperature or further lower temperature for bolting and flower induction. In control or untreated group of garlic genotypes which were kept at room temperature, incomplete bolters were observed in nine genotypes as topsets or bulbils were formed in the inflorescences but does not produce flowers as ambient temperature was not sufficient for induction of flowers in this group and these genotypes does not produce any seed. The clear cut effect of vernalization on scape length was observed and bulbs vernalized before sowing resulted into larger scape as compared to the bulbs which were kept at room temperature before sowing. This may be due to fulfilment of low temperature requirement to overcome dormancy which further resulted into faster growth as compared to control treatment.

Garlic is mainly propagated asexually and its flower is nearly, or completely, sterile with bulbils usually suppressing flower maturation but production of true garlic seed is possible. Availability of fertile clones combined with vernalization and careful removal of bulbils has set the stage for true garlic seed production. The wide scale commercial production of garlic from true seed currently seems difficult but small scale initiatives of F_1 development will definitely generate variability and new better cultivars which can be further multiplied through clonal propagation.

ACKNOWLEDGEMENT

The authors are thankful to Dr P W Simon, Professor, Department of Horticulture, University of Wisconsin, USA for providing the germplasm of fertile garlic.

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