Formulation of Flowering Index, Morphological Relationships, and Yield Prediction System in True Garlic Aerial Seed Bulbil Production

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Additional index words. *Allium sativum*, bolting, regression, photoperiod, vegetative propagation

Abstract. The phenomenon of flowering and aerial bulbil production in Asiatic garlic was observed under long photoperiodic conditions of Ladakh, India. Flowers were sterile and the bulbils produced on the umbel were true to type. Observations on a large number of flowering and nonflowering plants have led to the formulation of a precise flowering index (FI) in garlic. Plants with a minimum leaf number of 7, height 25 cm, collar width 0.6 cm, bulb diameter 3.7 cm, bulb weight 22.5 g, and functional leaf area of 182.4 cm² had only shown the flowering. The flowering index formulated was a product of leaf number, plant height, functional leaf area, and bulb weight. For flowering, FI should be more than 788, and availability of a minimum photoperiod of 4020 hours during a growth period of 11 months was another prerequisite. Nonfulfillment of any one of the factors of flowering, although FI and photoperiod were satisfactory, led to nonflowering. Garlic aerial bulbil yield was positively correlated with leaf number, plant height, bulb weight, bulb diameter, length of flower stalk, 100 seed weight, and head diameter. Following the multiple regression model $y = -11.9 - (0.00031 \times \text{number of bulbils}) + (0.147 \times 100 \text{ bulbil weight}) + (4.95 \times \text{head diameter}) + (0.0460 \times \text{length of flower stalk})$, aerial bulbil yield prediction was possible at a mean accuracy of 87%.

Apart from being an important condiment (Alekseeva, 1960), garlic is well known for its blood purification, anticholesterolic, and antibacterial properties since time immemorial (Tokin, 1951), and emphasis on these is growing with man's health consciousness. *Allium sativum* L. var. *sagittatum* Kuz. includes Asian, Caucasian, and Seaside ecotypes of bolting but sterile garlic (Kuznetsov, 1948). Though few seed-producing lines are isolated (Etoh, 1983; Etoh and Nakamura, 1988; Etoh et al., 1988), sterility remains to be the major constraint in its multiplication, propagation, and cultivation. The most common propagation followed in this crop continues to be vegetative using cloves, which restricts the multiplication ratio to about 10. Nonseed formation in garlic is mainly attributed to lesser reserve material for development of fertilized ovules (Etoh et al., 1988) and competition for nutrition by simultaneously developing bulbils (Koul and Gohil, 1970). A successful strategy to restore the fertility in cultivated garlic by managing the photoperiodicity as well as temperatures during development is also formulated (Kamenetsky et al., 2004).

Ladakh, at an altitude of 9000 to 18500 ft above mean sea level in the trans-Himalayan zone of India, is agriculturally unique with its extreme cold arid desert conditions (Table 1). Burgeoning demand had led to the introduction

of garlic into Ladakh in 2001. A unique phenomenon of bolting (Fig. 1) followed by setting of aerial true to type bulbils (Fig. 2) was observed under the long (14 to 16 h) photoperiods (Defence Research and Development Organisation, 2004) of Ladakh, which is highly significant in the fact that the multiplication ratio is the most important factor directing the success of any new crop, especially for nonseed-producing and low-multiplying garlic. Within first 2 years, garlic propagation through true to type aerial bulbils offers 45 times higher multiplication rate over conventional clove method. Albeit flowering being the main factor in species allocation in this genus (Kuznetsov, 1948), studies on climatic as well as morphological requirements for the same did not receive enough attention so far. Plant and environmental factors deciding the bolting and morphological association with bulbil production and techniques for predicting the bulbil yield in Asiatic garlic are discussed.

Table 1. Meteorological observations on temperature and relative humidity in Ladakh, India (2003–04).

Month	Avg temp (°C)		Temp range (°C)	
	Max	Min	Max	Min
September	26.3	13.1	28.0	0.5
October	15.2	-2.9	18.4	-7.0
November	11.1	-8.0	20.0	-15.3
December	2.4	-10.8	7.0	-17.5
January	2.4	-12.2	8.5	-17.7
February	3.0	-9.0	9.2	-15.3
March	6.1	-5.5	16.2	-17.6
April	14.5	0.8	20.9	-5.4
May	15.1	1.5	26.2	-3.4
June	23.9	10.3	30.2	5.5
July	26.9	14.5	33.1	10.5
August	26.1	13.0	31.5	9.3

Fig. 1. Flowering in Asiatic garlic showing the typical large head and sterile flowers emerging from a semitransparent covering sheath ending in a long projection

Fig. 2. Garlic flower head bearing pea-sized vegetative true-to-type aerial bulbils at complete maturity stage.

Material and Methods

Garlic cloves weighing 5 to 8 g were planted on raised beds under open field conditions in the second week of September; standard cultural practices (Singh et al., 2003) were followed, and crops were harvested after 10 months during the second week of July. Crops

Received for publication 30 May 2005. Accepted for publication 1 Aug. 2005.

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remained dormant within soil under open field conditions, without any mortality during the extreme winter and snow fall from November to February, and resprouted during the last week of March. Initiation of flower heads was observed in the last week of May and the crop at this stage until bulbil maturity was used in this study. Observations on bolting behavior, plant height (cm), bulb weight (g), leaves per plant, functional leaf area (cm²), bulb diameter (cm), and collar width (cm) were recorded at the time of 50% flowering in the population over many varieties at different altitudes in the trans-Himalayan zone. During bulbil maturity, observations on cloves per bulb, bulb diameter (cm), bulb weight (g), head diameter (cm), number of leaves, hundred bulbil weight (g), length of flower stalk (cm), plant height (cm), and bulbil yield (g) were recorded following standard morphological descriptors (Mahajan et al., 2000). For comparative photoperiodic studies, a crop was simultaneously raised under open as well as greenhouse conditions, and the photoperiod was recorded using sunshine recorder.

Observations on morphological characteristics of 600 plants, each flowering and nonflowering at 50% flowering stage, were evaluated to arrive at the minimal requisite for flowering. The level of significance of these parameters on flower induction was elucidated through correlation and path coefficient (Wright, 1921) analyses by assigning 1 for flowering and 0 for nonflowering. Parameters showing a significant positive relationship at 1% were used in the formulation of flowering index. Correlation as well as regression analyses of the data at bulbil maturity stage were used in understanding the relationship between morphological characters and bulbil yield. Bulbil yield prediction systems as a function of contributing factors were formulated following multiple regression analysis (Falconer, 1989) and were further compared at field level to choose the most efficient one.

Results and Discussion

Detailed studies on observations at 50% flowering stage have revealed the minimal morphological prerequisites for flowering in garlic. Flowering occurred only in plants with a minimum of 7 leaves. Other prerequisites for flowering in Asiatic garlic included a minimum plant height of 25.0 cm, collar width of 0.6 cm, bulb diameter of 3.7 cm, bulb weight of 22.5 g, and functional leaf area of 182.4 cm². These predisposing factors could be assumed to be stable (Batchvarov, 1993), since, in the present-day cultivars, asexual reproduction system is evolved from an ancestral sexual reproduction system (Etoh, 1985) and characteristics such as plant height, number and size of the leaves, and bulb weight show a high level inheritance and genetic advance and are governed by polygenes (Bhojward, 1980; Sum and Park, 1986).

It was observed that plants grown under a fiber reinforced polyethylene greenhouse (FRP-gh) did not flower. Under a FRP-gh, plants were exposed for an average of 11.2

Fig. 3. Regression plot on head diameter with bulbil yield.

Fig. 4. Regression plot on length of flower stalk with bulbil yield.

Fig. 5. Regression plot on plant height with bulbil yield.

$\text{h}\cdot\text{d}^{-1}$; whereas, in the field, daylight duration averaged 14.9 h during April to July. A cumulative photoperiod of 4020 h during the vegetative period was needed for flower induction in Asiatic garlic in Ladakh. The long photoperiodic requirement of garlic was again evident from the nonflowering of same garlic varieties fulfilling the specified morphological requirements under northern plains of India, where the mean photoperiod was 13.3 h during April to July.

Character association analysis has proved the high level significant positive association of leaf number (0.612^{**}), plant height (0.821^{**}), functional leaf area (0.643^{**}), and bulb weight (0.476^{**}) with flower induction. Collar width had positive significant (0.375^{*}) and bulb diameter had positive nonsignificant (0.059) association with flower induction. However, path coefficient analysis had shown these characteristics to be direct functions of bulb weight and their prerequisites were fulfilled with the same. Thus, no plant had shown nonflowering because the collar width was <0.6 cm or bulb diameter was <3.7 cm, but were automatically met in flowering plants. Based on these results, flowering index (FI) was formulated as a product of leaf number, plant height, functional leaf area, and bulb weight. For flowering in Asiatic garlic, FI should be >788 . Satisfying FI along with the specified morphological requirements and photoperiod had lead to flowering in garlic. Nonfulfillment of any one factor, even though FI is satisfactory, resulted in nonflowering.

The association of characters such as bulb weight, number of cloves per bulb, plant height, length of flower stalk, number of bulbils per head, 100 bulbil weight, leaf number, head diameter and bulb diameter with aerial bulbil yield was revealed from correlation and regression studies at complete bulbil maturity. Head diameter had shown maximum positive significant correlation ($r = 0.938^{**}$) with bulbil yield (Fig. 3). Length of flower stalk (0.796^{**}), plant height (0.769^{**}), and number of leaves per plant (0.735^{**}) also had shown high level significant positive correlation with bulbil yield (Figs. 4, 5, and 6, respectively). Bulb weight (0.619^{**}), 100 seed weight (0.494^{*}) and bulb diameter (0.492^{*}) also had a positive significant relation with bulbil yield (Figs. 7, 8, and 9, respectively). Plant height was the only factor which has significantly (0.455^{**}) increased the individual bulbil weight.

Prediction of character expression using the regression and correlation analysis of characters with positive and highly significant correlation is well demonstrated (Falconer, 1989). Garlic aerial bulbil yield was successfully predicted from morphological observations at complete bulbil maturity. Among the various multiple-regression models compared at field level, $y = -11.9 - 0.00031 C_1 + 0.147 C_2 + 4.95 C_3 + 0.0460 C_4$, where, y – bulbil yield/head (g), C_1 – number of bulbils/head, C_2 – hundred bulbil weight (g), C_3 – head diameter (cm), and C_4 – length of flower stalk (cm), was found to be the most efficient (87%).

Reproduction through aerial bulbils, although limited to *Allium sativum* var. *sagittatum*

Kuz., is very good alternative in mass clonal multiplication, and bulbils act as substitutes for true garlic seeds (Batchvarov, 1966). In garlic, toward the end of the vegetative period, the last two leaves turn fertile; i.e., at the base of the leaves, cloves and flower stem are formed (Batchvarov, 1994). Under Ladakh conditions, it was found that bulbils of pea size planted in September, before the onset of severe winter, formed normal plants with one or two cloved bulbs weighing a maximum of 28.6 g, which in turn produced normal plants bearing many cloves and bulbils in the second year of culture. Since a single normal clove of bolting garlic will multiply into about 10 cloves and 400 bulbils in a year and by the end of the second year, 4500 plants, including 500 normal cloves (400 bulbils of first year + 10 cloves of first year multiplied to 100) and 4000 bulbils (400 each from 10 cloves of first year). Thus, the multiplication ratio over nonbolting cultivars is about 45 times. The multiplication rate of bolting garlic projected for 11 years of continuous culture was $y = -3.4e^{13} + 37.3160X$

where, y = the number of plants obtained after a specific generation and X = the number of plants (including cloves and bulbils) used for planting. It was also observed that bolting garlic multiply by a factor of 10.73 during even years and 37.29 during odd years, since during even years bulbils develop into cloves and during odd years both multiply through bulbils as well as cloves. From an economic perspective, after 2 years of culture, yield realized through bulbils was 3 times higher than conventional system. However, propagation through vegetative means only is not advisable in any crop, since this results in a higher level of heterogeneity within a homozygous system (Chaudhary and Mehla, 1997), which is not desirable in terms of abiotic and biotic stress resistance (Knights, 1991) and manifestation of hybrid vigour (Swaroop, 1886). Temperature, relative humidity, daylength, light intensity, and their combinations can induce frequent changes in the genome of garlic (Batchvarov, 1993). Subzero temperatures, prevalent under Ladakh conditions and under which aerial bulbils are

Fig. 6. Regression plot on leaves/plant with bulbil yield.

Fig. 7. Regression plot on bulb weight with bulbil yield.

Fig. 8. Regression plot on thousand bulbil weight with bulbil yield.

Fig. 9. Regression plot on bulb diameter with bulbil yield.

more exposed than underground bulbs, open a new area of selection breeding.

Another advantage of propagation using true-to-type aerial bulbils is that the bulbils are biologically younger and free from nematode infection and other diseases—mosaic virus in particular (Etoh and Nakamura, 1988). In garlic vegetative propagation of any kind, regardless of the site from which the propagule is selected, all the features of the parents are transferred to the next generation and no deviation from the karyotype has been found among the plantlets (Chang and Park, 1980). In this context, information on morphological

requirements for flower induction as well as prediction techniques on true-to-type bulbil yield are important for clonal multiplication and improvement of garlic.

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