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Full Research Article

Vernalization Responses in Onion (*Allium cepa*) Pre-flowering and Reproductive Phases

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Abstract

The present study was aimed at determining the effect of augmented vernalization on the seed yield and seed yield contributing characters of onion seed crop. The experiment consisted of *in vitro* augmented vernalization at three different temperatures 0±0.5 °C, 5 ± 0.5 °C and 10±0.5 °C for durations 10, 20, 30 or 40 days and two levels of GA_3 at 500 PPM and 1000 PPM in two short day onion varieties- Bhima Kiran and Bhima Super. Bulbs stored at 10 °C for 10 days and 0 °C for 30 days pre-planting produced plants that flowered 11.64 days and 6.44 days earlier than non-vernalized plants in variety Bhima Kiran and Bhima Super respectively. Augmented vernalization resulted in increase of number of scape $plant^{-1}$. *Kharif* harvested variety Bhima Super had highest increase in flowering (56%) where as *rabi* harvested variety Bhima Kiran variety reported 30 % increase in flowering over control. Bulb treatment with GA_3 did not increase the flowers nor yield, majority of the plants appeared showed stunted growth. Indicating the non suitability of GA_3 as a bulb treating hormone. Bulbs stored at 10 °C for 10 days and 0 °C for 30 days gave the highest seed yield in variety Bhima Kiran and Bhima Super respectively. Seed quality parameters except for 1000 seed weight were not affected by the vernalization treatments. From the present study it is concluded that short duration cold treatment of 10 °C for 10 days enhances the seed yield in onion.

Keywords: Onion, vernalization, GA_3, seed yield, seed quality

1. Introduction

Onion (*Allium cepa* L.) is a major *Allium* next to tomato in terms of total production (FAO, 2015). Native of Central Asia widely cultivated for its culinary uses. It is a biannual crop that in subtropical and tropical India is grown in three seasons- summer, winter and rainy reason. After a juvenile stage the summer and winter grown crops are stored for 5-6 months whereas the rainy season crop after curing is usually planted within 15-20 days of harvest for seed production. Seed production is taken up only during winter season in the Indian sub-continent during which the low, vernalizing, winter temperatures prevail with no rains during anthesis marked by warm temperature during seed filling. During which the bulbs undergo vernalization. The exposure to low temperatures either in natural conditions or in artificial cold treatment that causes induction of flowering is called vernalization (Lee et al*.,* 2013; Thomas and Vince-Prue, 1997) where in differentiation of vegetative bud to floral bud takes place, if the prevailing cold period and temperature is not potent enough there will be poor seed yield due to incomplete vernalization (Voss et al*.,* 2013). The differentiation of vegetative meristem to floral meristems commences with flowering and seed set.

Gibberlic acid has been reported to induce early flowering and substitute vernalization as well as photoperiodic requirement for induction of flowering (Geetharani et al., 2008; Zeevaart, 1983). In onion GA_3 application has been reported to enhance the flowering and seed yield by 40 % (Geetharani et al., 2008; Naamni et al*.,* 1980; Loper and Waller, 1982).

Vernalization in onion is of research interest manly to solve the enigma of bolting in bulb crop and to reduce the breeding cycle to shortest period possible (Brewster, 1987). Lack of cold period during seed production is the major problem in onion seed crop in tropical and sub-tropical countries. The onion seed development from bulb to seed can be divided in to (i) juvenile phase (ii) thermo phase (iii) competition phase (iv) completion phase (Kampen, J.V., 1970). Based on the available literature in onion and other alliums it is established that vernalization is obligatory during phase two (Rabinowitch, 1990) and the optimum temperature during thermo phase range between 2-17 °C (Brewster, 1994). The optimum flowering temperature for tropical and subtropical onion is not known (Kamenetsky and Rabinowitch, 2002). Since the effect of vernalization and GA_3 applications has not been studied in seed production of short-day onion cultivars under subtropical Indian conditions, this study was initiated. Specifically, the study aims to determine the optimum temperature, length and concentration of GA_3 application that would augment

the natural vernalization.

2. Materials and Methods

The experiment was conducted in research fields of Directorate of Onion and Garlic Research, Rajgurunagar, Pune, India, during *rabi*/winter 2012–13. The experiment was laid out in randomized block design. The randomization was done using design resource server (Parsad et al., 2011). The seed bulbs were planted in ridges and furrows method at a spacing of 30×45 cm^2 on drip. The plots were fertilized with 150:50:50 kg (N: P: K) and 10 t of FYM $hectare^{-1}$, with full basal dose of P and K. Half of the nitrogen was applied as basal dose and remaining dose was applied in two equal dose at 25 and 50 days after planting. The plots were irrigated regularly throughout the crop cycle to avoid water stress. Fungicide and insecticide were sprayed to prevent disease and pest as required. Pre-emergent herbicide Oxyflurofen (Goal) 0.2% was applied after planting and weeds at later stage were controlled by hand weeding. Rajgurunagar is located in Western Maharashtra Plain Zone the average variation of temperature and RH during the experiment period was 13.55–37.76 °C and 75–33% respectively. The study consists of 12 treatment combination of three Vernalization temperatures-0±0.5 °C, 5±0.5 °C, and 10±0.5 °C for 10, 20, 30 or 40 days and bulb treatment for 15 minutes with two levels of GA_3 at 500 PPM and 1000 PPM on non-vernalized bulbs in onion varieties Bhima Kiran and Bhima Super to study its effect on reproductive and vegetative growth. The variety Bhima Kiran is recommended for *rabi*/winter and the bulbs are harvested during May and stored till November-December and planted in winter thus the bulbs get sufficient rest/after ripening where as Bhima super is recommended for cultivation in *Kharif*/rainy season and the bulbs are harvested during October-November and planted within one month thus the bulbs do not get sufficient rest/ after ripening period.

For observations ten plants were tagged after bulb germination. The plants were monitored regularly from planting of bulbs till scape emergence. The plant height, number of tillers, number of scape, scape length, plant dry weight were recorded after 45 days after planting. The days to first scape emergence were recorded in 10 randomly selected plants regularly. The seeds heads or umbels were harvested, when seed became mature. The umbels were considered to be ready for harvest, when about 20– 30% of the seeds in the umbel turned black. The harvested umbels were sundried, threshed manually and the seed yield 10 $plants^{-1}$ was recorded. Seed germination was conducted by taking 50 seeds in three replication. Seeds were placed on top of moist blotter paper in the 12.5 cm petri dishes at 25 °C±1 in germinator. Water requirements was checked daily and topped-up according to necessity. The first count and final count was taken on 6^{th} and 12^{th} day. The germination % was calculated on normal seedling as per ISTA (1999) protocol. Seedling vigour was calculated by randomly selecting ten normal seedlings at the final count during germination test. The vigour was calculated as per modified vigour index of Abdul Baki Anderson (1973).

Seedling vigour index- I= Germination % X average seedling length of 10 seedlings in mm.Seedling vigour index- II= Germination % X average weight of 10 seedlings in mg.

Statistical analysis was performed by usingstatistical analysis system (SAS) version 9.3. The data collected were subjected to analysis of variance and means were separated by least significant difference test (at P=0.05). Percentages were arcsine transformed prior to analysis.

3. Results and discussion

The effect of augmented vernalization and GA_3 application on onion seed production under subtropical conditions was explored. The vernalization treatment resulted in early scape initiation. In variety Bhima Kiran days to first scape emergence was lowest (34.83 days) in treatment T9 (10 ^{0}C for 10 days) which is 11.64 days earlier over control and in case of variety Bhima Super days to first scape emergence was lowest (37.94 days) in treatment T_1 (0 °C for 30 days) which is 6.44 days earlier over control (Tables 1, 2). This indicates that the vernalization treatment shortened the life cycle of the onion seed crop. Previous studies by Muthamia (1994), Khokhar (2009) also indicate that the cold treatment accelerated the scape emergence. Lee et al*.,* 2013 reported that during vernalization gene AcFT2 is upregulated and the augmented vernalization may have increased the upregulation of AcFT2 gene.

Onion bulb is a modified shoot and has many adventitious buds at the basal plate. The differentiation and emergence of scapes depends on temperature (Zemah, 2001). The vernalization treatment resulted in increase of number of scapes $plant^{-1}$. Which could be due stimulation of production of more number of differentiated adventitious buds in the bulbs. In variety Bhima Kiran highest number of scapes $plant^{-1}$ (4) in treatment T9 (10 °C for 10 days) (Figure 1) which was 0.93more than control and in case of variety Bhima Super highest number of scapes $plant^{-1}$ (5.74) (Figure 1) in treatment

Figure 1: Effect of vernalization treatment on flowering and plant growth

T_7 (0 °C for 10 days) which was 2.14 more than control.

The vegetative traits like plant height and dry weight were significantly higher in control and lowest in vernalized plants (Figure 1, Table 1 and 2) except for variety Bhima Super where plant height did not differ significantly between treatments. Indicating that vernalization treatment possible alters the source sink relation in favor of reproductive growth (Linwattana et al*.,* 1997). The untreated plants accumulated more vegetative biomass as compared to the treated plants.

The result of GA_3 treatment differed from the other researchers. Geetharani et al., 2008 reported that 100 ppm GA_3 spray enhanced the flowering by 47%. Looper and Waller (1982); Naamni et al*.* (1980) reported 30–40% increase in seed yield due to GA_3 application. The concentration of GA_3 used may be higher and cannot be excluded though the concentration of GA_3 used was as per available literature. Majority of the plants with GA_3 treatment in the present experiment exhibited stunted growth and were chlorotic in appearance indicating the toxic effect possibly due to high dosage. The reason for deviations in the result obtained could be. Firstly, varietals difference and growing conditions. The above studies have been done in long day onion cultivars under temperate conditions. Secondly the time and stage of application of GA_3 in the present experiment was onion seed bulb prior to planting where as in the previous studies GA_3 was sprayed at the time of floral initiation. It may be concluded that the GA_3 has beneficial effect on reproductive traits only in long day cultivars when applied at floral initiation stage.

Increasing in seed yield with vernalization treatment was observed in the present study (Table 3 and 4) highest seed yield 10 plants^{-1} (59.30 g) was in treatment T_9 (10 °C for 10 days) which was 57% higher than control. In variety Bhima Super highest seed yield 10 plants^{-1} (55.05 g) was in treatment

Table 1: Effect of vernalization treatment on vegetative and reproductive character of variety Bhima Kiran

Treatments	NSP	SL	PH	DWP	NTP	DFSE
T_1	3.93	38.90	48.07	25.10	4.80	35.73
T_2	3.47	34.89	47.21	27.33	4.47	42.70
T_3	2.60	31.55	41.07	29.70	4.27	46.10
T_4	3.27	33.09	41.25	28.47	3.67	39.19
T_5	3.67	34.58	42.61	29.90	4.53	42.68
T_6	3.87	35.06	41.32	28.37	4.40	37.51
T_7	3.80	36.46	48.93	28.83	4.53	45.15
T_8	3.07	44.37	35.14	32.93	3.60	42.42
T_9	4.00	32.45	41.56	31.43	4.60	34.83
T_{10}	3.33	39.96	45.67	25.60	4.20	42.99
T_{11}	3.07	34.39	44.63	26.53	4.07	42.48
Control	3.07	35.86	50.67	36.23	4.87	47.20
CD (p=0.05)	0.84	NS	7.62	5.79	NS	7.65

T_1: 0 °C for 40 days; T_2: 5 °C for 40 days; T_3: 10 °C for 40 days; T_4: 0 °C for 20 days; T_5: 5 °C for 20 days; T_6: 10 °C for 20 days; T_7: 0 °C for 10 days; T_8: 5 °C for 10 days; T_9: 10 °C for 10 days ; T_{10}: GA_3 500 ppm; T_{11}: GA_3 1000 ppm; NSP: No. of scapes plant^{-1}; SL: Scape length (cm); PH: Plant height (cm); DWP: Dry weight plant^{-1} (g); NTP: No. of tillers plant^{-1}; DFSE: Days to first scape emergence (days)

Table 2: Effect of vernalization treatment on vegetative and reproductive character of variety Bhima Super

Treatments	NSP	SL	PH	DWP	NTP	DFSE
T_1	5.20	38.73	44.13	31.75	5.87	37.94
T_2	4.13	38.21	44.37	33.70	4.53	39.61
T_3	3.60	35.44	45.11	27.52	4.07	38.78
T_4	4.33	43.24	46.23	36.42	4.53	38.44
T_5	4.87	40.61	44.37	37.33	5.20	40.33
T_6	5.07	41.41	47.33	32.93	5.33	38.83
T_7	5.47	39.25	44.01	32.42	6.07	39.33
T_8	4.60	38.64	41.47	34.40	5.80	38.17
T_9	4.07	39.22	42.13	37.21	4.73	39.39
T_{10}	4.13	40.13	40.53	30.98	4.73	44.94
T_{11}	4.07	39.67	42.67	26.63	4.40	41.72
Control	3.33	36.42	42.35	40.02	4.33	44.39
CD (p=0.05)	1.25	NS	NS	7.61	NS	3.6

Table 3: Effect of vernalization treatment on seed yield and seed quality in variety Bhima Kiran

Treatments	SY	SW	Ger*	SV-I	SV-II
T_1	50.47	3.417	96 (78.86)	645.80	149.98
T_2	34.57	3.137	96 (78.49)	467.14	140.17
T_3	30.39	3.216	93 (75.58)	481.11	141.56
T_4	34.81	3.284	95 (77.17)	572.11	139.28
T_5	44.11	3.185	95 (76.78)	475.66	123.73
T_6	46.90	3.422	96 (79.67)	583.81	153.89
T_7	40.70	2.940	95 (77.58)	549.89	132.47
T_8	44.03	3.280	95 (76.66)	591.30	143.50
T_9	59.30	3.231	96 (79.18)	552.82	144.96
T_{10}	43.95	3.085	95 (76.76)	441.52	132.07
T_{11}	35.66	3.081	85 (69.28)	411.24	118.47
Control	37.83	3.049	97 (79.89)	471.27	138.93
CD (p=0.05)	15.00	0.24	NS	NS	NS

SY: Seed yield 10 plants^{-1} (g); SW: 1000 seed weight (g); Ger*: Germination (%)*; SV-I: Seedling vigour index-I; SV-II: Seedling vigour index-I; * Value in the parenthesis are arc signed transformed values

Table 4: Effect of vernalization treatment on seed yield and seed quality in variety Bhima Super

Treatments	SY	SW	Ger*	SV-I	SV-II
T_1	55.05	3.614	95 (77.14)	531.38	188.80
T_2	35.10	3.533	95 (77.24)	616.69	188.33
T_3	36.62	3.546	96 (78.42)	458.72	171.12
T_4	39.24	3.398	95 (77.51)	422.51	184.29
T_5	36.52	3.455	97 (79.63)	419.73	169.36
T_6	37.24	3.630	93 (74.89)	1003.47	170.50
T_7	36.71	3.545	96 (78.57)	415.10	176.53
T_8	37.19	3.435	97 (79.48)	730.21	173.68
T_9	46.43	3.436	96 (78.70)	958.89	170.74
T_{10}	34.00	3.353	95 (77.25)	655.76	176.02
T_{11}	28.48	3.381	94 (75.49)	799.79	182.14
Control	38.00	3.253	95 (76.81)	474.96	167.16
CD (p=0.05)	12.12	0.21	NS	NS	NS

*Value in the parenthesis are arc signed transformed values

T_1 (0 °C for 30 days) which was 45 % higher than control. The increase in yield could be attributed to the more number of flowers in vernalized plants. The results are in agreement with the previous findings of Msika et al. (1997); Khokhar (2009). Onion, being a biennial crop needs vernalization of bulbs before flowering.

Seed quality parameters except for 1000 seed weight did not differ significantly with vernalization treatments. Highest 1000 seed weight in variety Bhima Kiran was in treatment T6 (10 °C for 20 days) where as in variety Bhima Super it was treatment T1 (0 °C for 30 days). According to (Brewster, 1994) during thermo phase the onion seed crop apart from temperature, high concentration of soluble carbohydrates is an important factor promoting flowering in onion and other bulbing *Alliums*. Benkeblia et al. (2004) found the higher activity of soluble invertase, sucrose, fructose and glucose levels in onion bulbs stored at 10 °C than those stored at 20 °C. It may be concluded that the vernalization helped in conversion of complex sugars in the bulbs to simple sugars which were readily available for transition to the reproductive growth and thus resulted in increase in number of flowers and subsequent increase seed yield. The difference in vernalization responses obtained between the cultivars in the present study could be explained to the fact that different cultivars have different length and time to pass through the growth phase (Brewster, 1994) and the sensitivity to cold induction increases with the age of plant as well as bulb (Kamenetsky and Rabinowitch, 2002). In the present experiment the bulbs of Bhima Kiran were stored for 6 months prior to planting where as bulbs of Bhima Super was planted within 30 days of harvesting.

4. Conclusion

Seed yield of onion can be enhanced in short day onion cultivars under subtropical Indian conditions buy augmented vernalization. Vernalization treatment of 10 ^{0}C for 10 days is effective in enhancing the seed yield in onion.

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