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Combinatorial effect of low temperature and IAA on flowering responses, metabolites and enzymes in *Cucumis sativus*

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Abstract: The combined effect of IAA and low temperature exposure at the seed germination stage, on the process of floral induction in cucumber was analyzed in this experiment. The low temperature exposure to the seeds decreased the days to anthesis of both male and female flowers to 43.25 and 52.25 respectively. Similarly, the average nodal position bearing first male as well as female flower also decreased as compared to control on providing low temperature and/or IAA, alone or in combination at seed germination stage, the reduction being more noteworthy when IAA and low temperature were given in combination. The total No. of female flowers formed, however, increased with the application of low temperature and IAA and, consequently, the ratio of male flowers to female flowers decreased with the increasing concentration of IAA, the reduction being more when low temperature was provided with IAA. The sucrose content was 240.0 µmol/gm fresh weight in control which declined to 237.5, 235.0 and 233.7 µmol g^{-1} fresh weight in plants which were treated with 25, 50 and 100 mg L^{-1} IAA respectively at seed germination stage. Exposure to low temperature, however, increased the sucrose content. The application of IAA, on the other hand, resulted in the increase in the starch content during both pre-flowering stage as well as flowering stage. The application of IAA also resulted in a gradual increase in the protein content, and this increase was more when IAA and low temperature were given in combination. However, in contrast to starch, the protein contents were found to be increased when plants experienced a shift from pre-flowering to flowering stage. Application of IAA at seed germination stage, however, decreased the activity of sucrose synthase, the decline being proportional to the concentration of IAA applied. Activity of acid invertase, though, showed a tendency just reverse to that of sucrose synthase. At both, the pre-flowering and flowering stages, low temperature exposure of seeds resulted in a decline in the activity of acid invertase while application of IAA at seed germination stage caused an increase in the activity of the enzyme in a concentration dependent manner. The activity of IAA oxidase at pre-flowering stage was significantly lowered in all the treatments as compared to control with the reduction in the activity being proportional to the concentration of IAA applied. A reduction in the activity of IAA oxidase was observed in all the plants when they experienced a shift from pre-flowering to flowering stage. Polyphenol oxidase, though, showed a trend just opposite to that shown by IAA oxidase. At flowering stage, again just opposite to the IAA oxidase, the activity of polyphenol oxidase increased from that in pre-flowering stage in all the plants. The highest activity during flowering stage was recorded in plants that were raised from seeds treated with 100 mg L^{-1} IAA at low temperature (0.252 ΔOD g^{-1} fresh weight). There was, however, not significant alteration in the peroxidase activity in all the treatments over control; though the peroxidase activity increased slightly on the application of IAA and low temperature as compared to control at both pre-flowering and flowering stages.

Key words: Vernalization, Low temperature, IAA, Acid invertase, IAA oxidase, Pre-flowering, Protein, Cucumis sativus, Male and female flowers, Anthesis

Introduction

Flowers represent specialized structures of a reproductive phase of shoot meristem activity. The conversion of the meristem from a vegetative to reproductive function and the development of flowers involve direction by environmental factors, sequential expression of integrated classes of genes, and numerous hormones (Lindsay, 2006). The competence of plants to respond to floral inducing signals is a major determinant in flowering time control. Induction of flowering and flower development are regulated by a large number of genes that may be grouped according to their roles in flower timing, floral meristem identity, and floral organ identity; however, redundancy, overlap, synergy, and interplay between many of these genes have been noted (Irish, 1999; Jack, 2004; Weigel, 1995).Most plants exhibit an early phase of vegetative growth during which flowering cannot occur, irrespective of the environmental conditions, called the juvenile phase (Thomas, 1993). Environmental signals trigger flowering in plants only after they have acquired the competence to flower. Both ambient temperature and long time exposure to low winter temperatures have been shown to have an important effect on flowering time (Henderson *et al*., 2003). In many species from temperate climates a requirement of low winter temperatures ensures spring flowering. The process of exposing such plants to low temperatures to bring about flower production is known as vernalization, and has been extensively studied (Ausín *et al*., 2005). Vernalization effects on flowering are quantitative, so longer cold exposures promotes flowering more than shorter ones till the response is saturated. However, after vernalization, plants do not necessarily initiate flowering but acquire the competence to do so (Chouard, 1960). Usually vernalization exhibits a broad temperature optimum - often from 1 to 7°C. The degree of vernalization reaches an optimum with time, and after this optimum time, extent of vernalization may decrease (over-vernalization). Vernalization may be reversed by high temperatures (devernalization), but only within a few days after cold treatment. To date several lines of evidence indicate that various chemical compounds, including plant growth hormones, sugars, phenolics, oligosaccharins, polyamines and their hydroxycinnamic acid conjugates, can affect flowering. Other external factors such as temperature and water availability are perceived by the shoot apices and root system respectively which may also produce and send some kinds of chemical compounds that affect transition of SAM into reproductive one (Kinet *et al.,* 1993).

Auxins, primarily indole-3-acetic acid (IAA) promote both cell division and cell elongation, and maintain apical dominance. Auxins also stimulate secondary growth in the vascular cambium, induce the formation of adventitious roots and promote fruit growth. The most common naturally occurring auxin is indole-3-acetic acid (IAA). However, other synthetic auxins, including indole-3-butyric acid (IBA); naphthalene acetic acid (NAA); 2,4-dichlorophenoxy acetic acid (2,4-D); and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) *etc*. are known. While these are recognized as synthetic auxins, it should be acknowledged that IBA does naturally occur in plant tissues. Auxin applied to short day plants ordinarily inhibits floral induction. This inhibition has the kinetic characteristics of a saturation phenomenon, approaching a maximum with increase in concentration. This inhibitory effect of auxin is specifically upon floral in-duction and not upon growth of the plants. Under certain conditions the concentrations of auxin effective in the inhibition of flowering may be very low, ap-proaching so called physiological concentration. There are instances, however, in which applied auxin promotes flowering, as with the pineapple, the litchi, some long-day plants, and with various plants to which auxin is applied in low concentrations and at cold temperatures. The exogenous application of auxin replaces the requirement for the presence of active buds after and just before the induction.

Keeping these facts into consideration, the principal problem of research in this investigation has been to attempt to better understand the effects of low temperature on the process of flowering. An attempt has also been made to understand the biochemical and molecular mechanisms involved in the process.

Materials and Methods

Seeds of cucumber (*Cucumis sativus* L.), variety Jyoti Green Long, were surface sterilized with 0.1% $HgCl_2$ and soaked in different concentrations of IAA (0 -as control, 25 , 50 and 100 mg L^{-1}) in petridishes, sterilized with 70% alcohol, 20 seeds per petridish. One set of petridishes was placed at low temperature (4^0C) while one set was placed at normal temperature for 24 hours. Thereafter seeds were allowed to germinate and grow in normal conditions (at room temperature in GDW) for two weeks inside the laboratory. After giving the requisite treatment each petridish was supplied with 5 ml GDW every day and nutrient solution (as described by Hewitt, 1963 and modified for Indian conditions by Agarwala and Sharma ,1976) every fifth day. The whole experiment was performed in triplicates. After two weeks five seedlings from each petridish were transplanted in pots, containing field soil with farmyard manure in standard amount, and placed under normal conditions in wire house in RBD pattern and their development patterns up to anthesis were observed using morphological (days to anthesis of male and female flower, number of node bearing 1st male and female flower, total flowers formed *i.e*. male and female, ratio of male flowers to female flowers and % of pollen sterility) and biochemical such as metabolites (sucrose, starch and total protein) and enzyme activity (sucrose synthase, acid invertase, IAA-oxidase, polyphenol oxidase and peroxidase). Biochemical analyses were done in the leaf nearest to apex just before the appearance of first floral bud (preflowering stage) and one week thereafter (flowering stage) to study the changes in the biochemical parameters during the process of floral induction.

The sucrose, starch and total protein content were determined by the method described by Shukry (2001), McCready *et al*. (1950) and Lowry *et al*. (1951) respectively. The activity of acid invertase, sucrose synthase, IAA-oxidase, polyphenoloxidase (PPO) and peroxidase were determined by the method of Wang *et al*. (1993), Rabin and Klein (1957), Kunwar and Khan (1982) and Luck (1963) respectively. The data observed in the experiment were averaged from three replicates and statistically analyzed for the calculation of standard error (S.E.). Student 't' test was administered for testing the hypothesis with the help of computer software Sigma Stat 2.0. The data shown are the averages of three replicates ± S.E. and significant (*) at $p<0.05$ level.

Results

The combined effect of IAA and low temperature exposure at the seed germination stage, on the process of floral induction in cucumber was analyzed in this experiment. The results obtained in the experiment are shown in Tables 1 to 4.

The Table 1 clearly shows the effect of IAA and low temperature on the morphological aspects of flowering in cucumber. The low temperature exposure to the seeds decreased the days to anthesis of both male and female flowers to 43.25 and 52.25 respectively. Days to anthesis were 44.00 for male flowers and 53.30 for female flowers when 25 mgL^{-1} IAA was provided to seeds at normal temperature but it decreased to 43.10 and 51.70 when low temperature was also provided along with 25 mg L^{-1} IAA. Similarly, the average nodal position bearing first male as well as female flower also decreased as compared to control on providing low temperature and/or IAA, alone or in combination at seed germination stage, the reduction being more noteworthy when IAA and low temperature were given in combination. The similar concentrations of IAA, when applied together with low temperature, the average nodal position of first male flower further reduced to 3.6, 2.9 and 2.2 respectively (the last two values being significantly different). Same tendency was observed in case of female flower as well where the nodal position of first flower was lowered down to 6.1 when low temperature was provided, from 6.4 in control and the significantly different, albeit, minimum No. being recorded in plants that were given 100 mg L^{-1} IAA and low temperature together during seed germination (3.9). The total No. of male flowers formed was 18.6 in control, which declined to 17.4 in plants that were exposed to low temperature during seed germination. The decrease was more discernible when IAA was applied along with low temperature, the diminution being proportional to the concentration of IAA applied. The total numbers of female flowers formed, however, increased with the application of low temperature and IAA and, consequently, the ratio of male flowers to female flowers decreased with the increasing concentration of IAA, the reduction being more when low temperature was provided with IAA. The pollen sterility was lowest in control (13.55) which increased with the application of low temperature and/or IAA alone or in combination. Moreover, the increase in the percentage of pollen sterility was proportional to the concentration of applied IAA.

Exposure to low temperature along with IAA application caused significantly more increase in the pollen sterility which increased to 19.60, 21.14 and 24.61, when same concentration of IAA (25, 50 and 100 mg L^{-1} respectively) was applied along with low temperature to the seeds.

The Table 2 shows the effect of IAA and low temperature on the concentration of important metabolites *viz*. sucrose, starch and protein during floral induction in cucumber. The sucrose content was 240.0 µmol/gm fresh weight in control which declined to 237.5, 235.0 and 233.7 µmol g^{-1} fresh weight in plants which were treated with 25, 50 and 100 mg L^{-1} IAA respectively at seed germination stage. Exposure to low temperature, however, increased the sucrose content. Sucrose content, however, declined when plants shifted from pre-flowering stage to flowering stage. The application of IAA, on the other hand, resulted in the increase in the starch content during both pre-flowering stage as well as flowering stage. When low temperature was given with IAA, this increase in starch content was even more pronounced. Thus it significantly increased to 0.304, 0.319 and 0.329 mg g^{-1} fresh weight when same concentration of IAA (25, 50 and 100 mg L^{-1}) was applied at low temperature. The onset of flowering resulted in a decline in the starch content in all the plants from their corresponding values at pre-flowering stage. The application of IAA also resulted in a gradual increase in the protein content, and this increase was more when IAA and low temperature were given in combination. At pre-flowering stage, the protein content in control plants was 44.12 µg g^{-1} fresh weight while highest content was found in plants developed from seeds to which 100 mg L^{-1} IAA was applied with low temperature (55.98 µg g^{-1} fresh weight). However, in contrast to starch, the protein contents were found to be increased when plants experienced a shift from pre-flowering to flowering stage. It increased to 53.07 µg g^{-1} fresh weight in control while again the highest value was recorded in plants which were treated with 100 mg L^{-1} IAA with low temperature (70.14 µg g^{-1} fresh weight) at seed germination stage.

The changes in the activities of sucrose synthase and acid invertase during the process of floral induction, under the combined effect of IAA and low temperature given to the seeds, are illustrated in Table 3. At pre-flowering stage the activity of sucrose synthase was 172.35 µmol/gm fresh weight which increased slightly to 173.16 µmol/gm fresh weight in plants raised from seeds exposed to low temperature. Application of IAA at seed germination stage, however, decreased the activity of sucrose synthase, the decline being proportional to the concentration of IAA applied. But this decline was some what less when IAA was provided with low temperature than that when IAA was applied at normal temperature. The effect of low temperature and IAA application was, however, same as seen during the pre-flowering stage. Activity of acid invertase, though, showed a tendency just reverse to that of sucrose synthase. At both, the pre-flowering and flowering stages, low temperature exposure of seeds resulted in a decline in the activity of acid invertase while application of IAA at seed germination stage caused an increase in the activity of the enzyme in a concentration dependent manner. This increase was even more pronounced when IAA was applied together with low temperature than when it was applied at normal temperature. Thus at pre-flowering stage the maximum activity of acid invertase was observed when 100 mg L^{-1} IAA was applied along with low temperature (120.14 µmol g^{-1} fresh weight) which was significantly higher than that in control (104.24 µmol g^{-1} fresh weight).

The Table 4 clearly exhibits the combined effect of IAA and low temperature on IAA oxidase, polyphenol oxidase and peroxidase during floral induction in cucumber. The activity of IAA oxidase at pre-flowering stage was significantly lowered in all the treatments as compared to control with the reduction in the activity being proportional to the concentration of IAA applied. Moreover, the exposure to low temperature further reduced the activity of the enzyme. Thus, in confirmation with the trend, the least activity of IAA oxidase at pre-flowering stage was recorded in plants which were provided with 100 mg L^{-1} IAA along with low temperature at seed germination stage (0.561 µg IAA oxidized g^{-1} fresh weight hr^{-1}). A reduction in the activity of IAA oxidase was observed in all the plants when they experienced a shift from pre-flowering to flowering stage. The effect of IAA and low temperature, though, was same as that observed during the pre-flowering stage. Therefore, again the minimum activity of IAA oxidase was recorded in the plants which were grown from seeds treated with 100 mg L^{-1} IAA together with low temperature (0.518 µg IAA oxidized g^{-1} fresh weight hr^{-1}), a significantly lesser activity than control. Polyphenol oxidase, though, showed a trend just opposite to that shown by IAA oxidase *i.e.* the application of IAA and exposure to low temperature at seed germination stage, both enhanced the activity of polyphenol oxidase in a concentration dependent manner at pre-flowering as well as at flowering stage. Thus, at pre-flowering stage a significant difference was observed between the activity of polyphenol oxidase in control (0.103 ΔOD g^{-1} fresh weight) and plants developed from seeds that were provided with 50 and 100 mg L^{-1} IAA with low temperature (0.150 and 0.159 ΔOD g^{-1} fresh weight respectively). At flowering stage, again just opposite to the IAA oxidase, the activity of polyphenol oxidase increased from that in pre-flowering stage in all the plants. The highest activity during flowering stage was recorded in plants that were raised from seeds treated with 100 mg L^{-1} IAA at low temperature (0.252 ΔOD g^{-1} fresh weight). This activity was significantly higher than that in control (0.221 ΔOD g^{-1} fresh weight). There was, however, not significant alteration in the peroxidase activity in all the treatments over control; though the peroxidase activity increased slightly on the application of IAA and low temperature as compared to control at both pre-flowering and flowering stages. The maximum activity of the enzyme at pre-flowering stage was observed in plants grown from seeds to which 100 mg L^{-1} IAA was given together with low temperature exposure (24.96 ΔOD g^{-1} fresh weight). In control it was 21.96 ΔOD g^{-1} fresh weight. At flowering stage the activity of peroxidase too increased over the corresponding values during pre-flowering stage in all the plants. The effect of IAA application and low temperature exposure to the seeds on the peroxidase activity during flowering stage was similar to that observed during pre-flowering stage with not much significant difference between control value (25.12 ΔOD g^{-1} fresh weight) and the maximum value recorded in plants which were given 100 mg L^{-1} IAA and low temperature (27.94 ΔOD g^{-1} fresh weight) at seed germination stage.

Table - 1: Combined effect of IAA and low temperature (4°C) treatment to the seeds on flowering response in cucumber

Treatments	Male flower (♂)			Female flower ( )				
	Days to anthesis (Days after sowing)	Average node position bearing 1st flower	Total flowers formed	Days to anthesis (Days after sowing)	Average node position bearing 1st flower	Total flowers formed	& ratio	Pollen sterility (%)
Control	44.80±2.13	5.0±0.65	18.6±0.95	54.00±1.58	6.4±0.88	4.1±0.35	4.5:1.0	13.55±1.10
Low temp. (LT)	43.25±1.98	4.7±0.44	17.4±1.30	52.25±1.23	6.1±0.72	4.7±0.22	3.7:1.0	16.13±0.32
25 mg L^{-1} IAA	44.00±2.02	4.0±0.51	18.5±1.30	53.30±1.53	5.8±0.72	4.3±0.13	4.3:1.0	15.23±0.53
25 mg L^{-1} IAA + LT	43.10±1.99	3.6±0.38	17.1±0.58	51.70±1.49	5.4±0.63	4.9±0.47	3.8:1.0	19.60±0.98
50 mg L^{-1} IAA	43.00±1.98	3.2±0.37	16.0±1.12	46.20±1.41	5.0±0.43	4.6±0.11	3.5:1.0	16.53±1.00
50 mg L^{-1} IAA+ LT	42.45±1.57	2.9±0.30*	13.3±0.37*	45.14±1.46*	4.8±0.51	5.3±0.66*	2.5:1.0	21.14±0.77*
100 mg L^{-1} IAA	41.57±1.43	2.8±0.21*	12.5±0.58*	45.00±2.44*	4.2±0.48*	4.9±0.14	2.6:1.0	17.82±0.56
100 mg L^{-1} IAA+ LT	40.12±1.14	2.2±0.25*	10.6±0.44*	43.62±1.42*	3.9±0.42*	5.9±0.21*	1.8:1.0	24.61±0.40*

Values are mean of three replicate ± SE and (*) statistically significant at $p < 0.05$ level

Table - 2: Combined effect of IAA and low temperature (4°C) treatment to the seeds on metabolites (sucrose, starch and total protein) during floral Induction in cucumber

	Sucrose[$]		Starch[†]		Total protein[#]	
	Pre-flowering	Flowering	Pre-flowering	Flowering	Pre-flowering	Flowering
Control	240.0±5.95	225.5±5.65	0.280±0.005	0.244±0.003	44.12±2.63	53.07±3.42
Low temp. (LT)	249.2±8.83	227.5±6.78	0.290±0.008	0.274±0.007*	49.76±2.86	59.65±3.68
25 mg L^{-1} IAA	237.5±6.32	221.0±6.02	0.295±0.005	0.251±0.005	45.10±2.89	55.45±4.15
25 mg L^{-1} IAA + LT	239.9±5.78	219.8±6.44	0.304±0.009	0.274±0.008*	50.24±3.95	59.13±3.83
50 mg L^{-1} IAA	235.0±6.02	216.7±6.53	0.310±0.003	0.259±0.005	45.95±2.69	56.24±3.95
50 mg L^{-1} IAA + LT	238.2±6.23	210.5±6.40*	0.319±0.008*	0.276±0.007*	52.02±4.02*	65.86±3.97*
100 mg L^{-1} IAA	233.7±6.35	212.2±7.49	0.315±0.004*	0.263±0.004	46.40±3.76	57.82±4.95
100 mg L^{-1} IAA + LT	240.9±6.67	204.3±5.95*	0.329±0.013*	0.280±0.009*	55.98±3.98*	70.14±4.99*

Values are mean of three replicate ± SE and (*) statistically significant at $p < 0.05$ level; [$] Sucrose content expressed in µmol g^{-1} fresh weight; [†] Starch content expressed in mg g^{-1} fresh weight; [#] Protein content expressed in µg g^{-1} fresh weight

Table - 3: Combined effect of IAA and low temperature (4°C) treatment to the seeds on enzymes of carbohydrate metabolism (sucrose synthase and acid invertase) during floral induction in cucumber

	Sucrose Synthase[$]		Acid invertase[$]	
	Pre-flowering	Flowering	Pre-flowering	Flowering
Control	172.35±6.23	154.48±4.45	104.24±3.22	119.44±4.46
Low temp. (L.T.)	173.16±5.62	159.22±3.68	102.10±2.83	115.18±3.58
25 mg L^{-1} IAA	159.63±6.70	143.05±4.12	116.25±4.72	129.14±4.87
25 mg L^{-1} IAA + L.T.	160.05±5.80	144.16±3.46	117.40±3.46	130.35±3.47
50 mg L^{-1} IAA	153.65±4.39	139.55±5.53	116.75±3.75	129.62±6.10
50 mg L^{-1} IAA + L.T.	155.24±4.36*	141.03±5.12	118.95±5.44*	133.00±5.22*
100 mg L^{-1} IAA	143.34±4.15*	129.16±5.36*	117.00±4.33	130.07±6.18
100 mg L^{-1} IAA + L.T.	144.27±3.26*	130.85±4.63*	120.14±3.86*	134.82±5.38*

Values are mean of three replicate ± SE and (*) statistically significant, at $p < 0.05$ level; [$]Enzyme activity expressed as µmol g^{-1} fresh weight

Discussion

Results of low temperature exposure to cucumber seeds showed a distinct effect of vernalization on the process of floral induction in cucumber. Morphological as well as biochemical changes were visible. The days to anthesis of both male and female flowers as well as the nodal position of first flower was lowered in plants raised from low temperature exposed seeds, indicating a preference towards early flowering (Tewari *et al.*, 2009a,b). This observation is corroborated by Samach and Lotan (2007) who reported that mild ambient temperatures during seedling growth do cause earlier flowering. The early flowering response (reduction in leaf number till first flowering) to low temperatures seems to be limited to the first nine days after cotyledon expansion, termed the 'sensitive phase' (Calvert, 1957; Wittwer and Teubner, 1957). The responsive tissue seems to be the aerial part of the plant (Phatak *et al.,* 1966). Since the number is reduced by low temperature, and the rate is increased by high temperature, faster flowering can be achieved by combining a period of low temperature followed by a period of high temperature (Wittwer and Teubner, 1957).

Table - 4: Combined effect of IAA and low temp. (4^0C) treatment to the seeds on IAA oxidase, polyphenol oxidase and peroxidase during floral induction in cucumber

	IAA oxidase†		Polyphenol oxidase#		Peroxidase#	
	Pre-flowering	Flowering	Pre-flowering	Flowering	Pre-flowering	Flowering
Control	0.652±0.012	0.594±0.014	0.103±0.008	0.221±0.006	21.96±1.02	25.12±2.75
Low temp. (LT)	0.590±0.010*	0.548±0.027	0.117±0.005	0.224±0.005	23.36±1.88	27.51±1.02
25 mg L^{-1} IAA	0.590±0.016*	0.563±0.013	0.137±0.008	0.226±0.009	22.32±1.86	26.34±3.12
25 mg L^{-1} IAA + LT	0.582±0.018*	0.543±0.011	0.141±0.007	0.231±0.008	23.60±1.23	27.04±2.11
50 mg L^{-1} IAA	0.585±0.018*	0.540±0.013*	0.144±0.003	0.234±0.008	22.64±2.01	26.78±3.04
50 mg L^{-1} IAA+ LT	0.569±0.013*	0.532±0.016*	0.150±0.005*	0.241±0.006	24.00±1.76	27.18±2.12
100 mg L^{-1} IAA	0.580±0.017*	0.527±0.018*	0.154±0.006*	0.246±0.014	22.84±1.89	27.06±2.24
100 mg L^{-1} IAA+ LT	0.561±0.018*	0.518±0.013*	0.159±0.007*	0.252±0.009*	24.96±1.99	27.94±2.63

Values are mean of three replicate ± SE and (*) statistically significant at $p < 0.05$ level; †Enzyme activity expressed as µg IAA oxidized g^{-1} fresh weight hr^{-1}; # Enzyme activity expressed as Δ OD g^{-1} fresh weight

In some species there is evidence for the relationship between time-of-day-sensitivity to temperature. For example, in *Arabidopsis thaliana*, warm night temperature caused a more significant reduction in flowering time as compared to warm day temperature (Thingnaes *et al.,* 2003; Paltiel *et al.,* 2006).

Low temperature exposure to seeds also decreased the No. of male flowers and favoured the formation of female flowers, thus, leading to a reduction in the ratio of male flowers to female flowers. This finding or previous workers showed that in many monoecious and dioecious plants, including cucumber, at low temperature female flowers are developed at sites that in plants grown at higher temperature would be occupied by male flowers (Chailakhyan and Khiranin, 1987; Tewari *et al*., 2009a). According to Chekmin *et al.* (1964) the mode of action of the temperature factor on plant sex expression is probably related in some way to changes in metabolism. Kandina (1958) had reported that low temperature treatments of cucumber seeds before sowing change the pathway of physiological and biochemical processes. The contents of reducing sugars and ascorbic acid are increased and this results in an overall increase in the reducing power of the cells of the seedling, which in turn favours female sex expression. Nitsan (1963) showed that the vernalized seedlings of rye contained larger amounts of polysaccharides as compared with unvernalized seedlings.

Together with carbohydrates, activity of sucrose synthase and acid invertase was also altered on exposure of seeds to low temperature. The relatively high activity of acid invertase in plants exposed to low temperature for 72 hr may be due to the effect of longer than threshold exposure to low temperature. Such increase in leaf invertase activity was also reported by Artuso *et al.* (2000) who investigated the effects of low temperature on leaf photosynthesis and sucrose metabolism in tomato plants (*Lycopersicon esculentum* Mill. cultivar Marmande). The results of the determination of starch and soluble sugar content could show that such treatment impaired sucrose translocation. The activity of leaf invertase increased significantly in low temperature exposed plants. Low temperature exposure also led to an increase in the activity of sucrose synthase. The observation is in confirmation with the findings of Dejardin *et al*. (1999) who found two sucrose synthase genes from *Arabidopsis thaliana* to be differentially up-regulated in leaves exposed to cold stress. The differential stress-responsive regulation of these genes in leaves might represent part of a general cellular response to the allocation of carbohydrates during acclimation processes (Dejardin *et al*., 1999). Thus, it is postulated that changes in the metabolites and enzyme patterns due to low temperature exposure may be a result of variable gene expression. Bitonti *et al.* (2002) analyzed the chromatin organization, nuclear DNA methylation and endogenous zeatin localization in shoot apical meristems (SAM) during juvenile and adult phases of peach (*Prunus persica* L. Batsch). They report that nuclear chromatin exhibited chromocentres that were peripherally distributed in SAMs of juvenile and juvenile-like shoots, but were diffusely spread in those of adult shoots. These patterns coincided with a peripheral labelling of DNA methylation in juvenile and juvenile-like meristem nuclei versus a diffuse labelling pattern in adult meristem nuclei. During vegetative growth the level of nuclear DNA methylation was higher in adult meristems than in juvenile and juvenile-like ones.

It has been shown that many plants in temperate zone require a low temperature stress (vernalization) to induce flowering and fruit production. In some plants such as *Arabidopsis thaliana* and *Cichorium intybus* the vernalization response has been linked to changes in DNA methylation (Burn *et al.,* 1993; Demeulemeester *et al.,* 1998), while the seedlings of other plants like *Oryzia sativa* if treated with 5-azacytidine (5-azaC) result into dwarfism and a high tillering number due to hypomethylation of the cytosine nucleotide (Sano *et al.,* 1990; Cherdshewasart *et al.,* 1998). Anuntalabhochai *et al.* (2009) applied the HAT-RAPD DNA amplification methodology to test for the presence of methylation dependent floral induction in jasmine rice (*Oryza sativa indica* KDML 105), petunia (*Petunia hybrida*), spinach (*Spinacea oleracea*) and longan (*Dimocarpus longan*) by inducing with $KClO_3$, 5-azaC and low temperature (10°C) treatments. They found that rice, petunia and spinach showed methylation dependent DNA changes correlated with the vernalization response, potassium chlorate and 5-azaC treatment, suggesting that similar biochemical responses may be involved in the flowering response. Burn *et al.* (1993) using two different plant species in which low temperature induces flowering, have shown that 5-azaC treatment partially substitutes for cold treatment in the promotion of flowering. They have shown that 5-azaC treatment parallels vernalization in a

number of key properties. Both treatments are meristem specific; they are cell division dependent and cell lineage propagative, and their effects are not inherited through successive sexual generations. They further opine that effectiveness of vernalization increases with the length of the cold treatment which may be related to the number of cell division cycles needed to dilute out the initial methylated DNA strands in the dividing aggregate of cells in the inflorescence lineage. Wellensiek(1964) provided evidence that cell division during vernalization is necessary for thermoinduction in *Lunaria annua* and that flowering structures are ultimately derived from the mitotically active cells that were subjected to vernalizing temperatures. He concluded that thermoinduction is a cell-autonomous process that is mitotically propagated. The observation that tissues other than the shoot apical meristem also have the capacity to support thermoinductive responses to vernalization is consistent with the concept of mitotic transmission. Shoots regenerated from leaf cuttings of both *L. annua* (Wellensiek, 1964) and *Thlaspi arvense* (Metzger, 1988) exhibited a developmental state identical to that of plants from which they were obtained: flowering shoots developed from leaves of cold-treated plants, whereas only vegetative rosettes developed from the leaves of nonvernalized plants. Hence classical physiological experiments suggested that vernalization acts in the meristem to promote flowering (Michaels and Amasino, 2000). Initial observations were based on exposing only the leaves or only the apices of celery plants to vernalization treatments, and demonstrating that vernalization of the meristem was sufficient to induce flowering. The vernalization pathway is therefore likely to act in the meristem to reduce FLC expression and thereby induce flowering, and this may also be true for the autonomous pathway in *Arabidopsis thaliana* (Corbesier and Coupland, 2005). Consistent with vernalization acting in the meristem, FLC is expressed specifically in the shoot and root meristems in young seedlings of *Arabidopsis*, although in older plants it is also expressed in expanded leaves (Michaels and Amasino, 2000; Sheldon *et al.,* 2002; Noh and Amasino, 2003; Bastow *et al.,* 2004).

It was observed that low temperature when provided with IAA, somewhat lowered the effect of IAA treatment on sucrose and activity of sucrose synthase. This may be due to the devernalizing potential of exogenous auxin as reported by Tompsett and Schwabe (1974). They made simultaneous quantitative analyses of the endogenous levels of auxin and gibberellin like substances, growth inhibitors, and auxin-oxidizing enzyme activity in the cold-requiring *Chrysanthemum morifolium* cv. Sunbeam and have reported that while little hormone or enzyme activity was found in extracts from unvernalized plants, a striking rise in auxin-oxidizing enzyme activity occurred rapidly after the end of cold treatment. Increased auxin activity was also recorded shortly after vernalization. Exogenous auxin application reduced gibberellin like substance levels and caused devernalization (Tompsett and Schwabe, 1974). Experimental results showed that treatment with IAA also led to early flowering in cucumber which was evident in reduction in the number of days to anthesis, nodal position of first flower of both male and female flowers. These results are similar to many classical physiological studies which show that auxin application causes earlier production of flowers (Chailakhyan and Khiranin, 1987; Pavlová and Krekule, 1990). It has been shown by Yuvadee and Chanrat (2006) that NAA promoted flower bud initiation and along with high phosphorus and boron helped flower development in white kwao krua (*Pueraria candollei* Grah. var. Mirifica). IAA application exhibited a preference for female flower production with concomitant reduction in the ratio of male flowers to female flowers in a concentration and duration dependent manner. Indeed relatively high levels of endogenous auxins are correlated with female sex expression in most of the plants studied. Female plants/flowers of various species show greater amounts of endogenous auxin than the male plants/ flowers (Heslop-Harrison, 1972; Frankel and Galun, 1977). Galun *et al.* (1965) demonstrated that the quantity of auxin in hermaphrodite and andromonoecious cucumber plants differed significantly, the hermaphrodite ones being richer. Chailakhyan and Khiranin (1978) have shown that application, through the root system, of 15 mg L^{-1} IAA to hemp (*Cannabis sativa*) plants having 2–3 pairs of visible leaves caused pronounced shifts of sex expression in the adult individuals resulting in all plants being either female (pistillate flowers) or intersexes (bisexual flowers). Application of IAA, however, caused a rise in the sterility of pollen in a concentration and dose dependent manner. It has been reported by Kalidasu *et al.* (2009) that 2,4-D at 50 and 100 ppm showed 6.2 and 23.2% of pollen sterility, respectively, in coriander.

Activity of acid invertase also increased in the cucumber both at pre-flowering and flowering stages due to application of IAA in a dose dependent manner. It has been reported that the activity of extracellular invertase is stimulated by auxin (Weil and Rauch, 1990). Experimental manipulation of tissue expansion growth by hormones seems to be mediated by invertases. Auxin appears to play a key role in this regulatory mechanism. IAA promotes both growth and invertase activity in segments of young *Phaseolus vulgaris* internodes. The sensitivity to auxin is developmentally regulated and requires mRNA and protein synthesis. It has been reported by Senthil *et al.* (2005) that treatment of both IAA and GA_3 significantly enhanced the peroxidase activity in leaves.

Changes in the levels of IAA, phenolic compounds, peroxidase, polyphenol oxidase, and IAA oxidase activities in the corm and the apical bud of *Crocus sativus* during bud growth and development, with special emphasis on the flowering stage, were also examined by Ebrahimzadeh and Abrishamchi (2001). In the bud, flower formation was accompanied by enhanced activities of peroxidase, polyphenol oxidase, IAA oxidase, and higher contents of phenolic compounds as well as lower levels of IAA. In the corm, during the flower formation, these enzymes showed an opposite behaviour. This also supports the results of experiments 2 and 3, in which a decrease in the IAA oxidase activity while increase in PPO and peroxidase activities were observed as plant shifted into flowering stage from pre-flowering stage. Gaspar *et al.* (1985) also observed that flower initiation takes place during a rise of peroxidase activity following a peak of minimum activity which marked the completion of the flowering inductive phase. Since basic isoperoxidases underwent an inverse variation of activity in the course of successive inductive and initiative phases, it

was hypothesized that the induction of flowering led to a temporary peak of maximum auxin level in the leaves. Thus, it may be concluded that the transition of these plants to flowering is correlated with peroxidase, polyphenol oxidase and IAA oxidase. Furthermore, these enzymes might exert their roles in the regulation of flowering through their participation in IAA catabolism (Ebrahimzadeh and Abrishamchi, 2001).

Sugar production through photosynthesis is the most fundamental activity in plant life. The processes of sugar production, transport, consumption, and storage are dynamic and tightly linked to cellular physiology, organ identity, environmental inputs, and developmental stages. Sugars such as sucrose, glucose, and fructose have an essential function in plant metabolism. These sugars are important for intermediary and respiratory metabolism and are the substrate for synthesizing complex carbohydrates such as starch and cellulose. Sugars also provide the building blocks for amino acid and fatty acid biosynthesis and essentially all other compounds present in plants, thereby affecting the growth and development of the plant including that of SAM and its transition from vegetative to reproductive state. The phase change of SAM from vegetative to floral may occur in response to the attainment of a threshold source strength / carbohydrate production (Tsai *et al.,* 1997). The relation of light intensity and CO_2 content of the air to carbohydrate produc-tion is also thought to be very important. A number of studies have concurred in these conclusions, and propose that a high carbon/nitrogen ratio (organic C:N ratio) favours flower-ing, while plants remain vegetative when this ratio is low (Corbesier *et al.,* 2002). Sugars can also act as signal molecules that may signal alterations in gene expression similar to the concepts developed for hormones. The ability of a plant to monitor and respond to sugar levels could serve as a control mechanism to integrate external environmental conditions including light, other nutrients, and biotic and abiotic stresses, with intrinsic developmental programs directed by multiple plant hormones. In plants, sugars have conventionally been viewed as resources for respiration and metabolic intermediates, as well as structural or storage components. The widely observed effects of sugar on gene expression and on plant growth and development have often been attributed to sugar metabolism and energy production. The previous exclusion of sugars as plant signaling molecules stems from the observation that higher concentrations are needed for sugar activity than for the classically defined plant hormone effects. Recent compelling evidences, however, support the concept that sensing and signaling can be performed even at a millimolar range of signaling molecules using sugar binding enzymes, proteins, or transporters. Carefully designed experiments now reveal the uncoupling of sugar sensing and signaling from sugar metabolism. Despite the anticipated complication of sugar sensing and signaling in photosynthetic plants, exciting progress has been made in the past few years. Many excellent reviews and perspectives on sugar regulated gene expression and sugar sensing and signaling have appeared in the recent years. Sugars as signaling compounds have profound effects in all stages of the plant's life cycle from germination and vegetative growth to reproductive development and seed formation. In principle, any neutral sugar or glycolytic intermediate could have a signaling function but so far this has only been shown for hexoses and sucrose (Smeekens, 2000). Treatments that induce flowering can also lead to increased transport of carbohydrates from leaves to SAM. Sucrose is reported to be the major sugar in both leaf and apical exudates; its level increasing very early and transiently by photo-induction, accumulating very early in the apical meristem of induced plants and has been suggested to have a message like role. This early extra sucrose at the apical meristem does not arise from increased photosynthesis but from mobilization of reserve carbohydrates, presumably starch, stored both in the leaves and the stem (Lejeune *et al.,* 1991,1993).

Recent studies indicate that, in a manner similar to classical plant hormones, sugars can act as signaling molecules that control gene expression and developmental processes in plants. Crucial evidence includes uncoupling glucose signaling from its metabolism, identification of glucose sensors, and isolation and characterization of mutants and other regulatory components in plant sugar signal transduction pathways. The emerging scenario points to the existence of a complex signaling network that interconnects transduction pathways from sugars and other hormone and nutrient signals.

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