ISSN: 0974-4908
http://www.rels.comxa.com

Res. Environ. Life Sci.
2(3) 181-184 (2009)
rel_sci@yahoo.com

Effect of low temperature on flowering response and biochemical changes during the process of floral induction in *Cucumis sativus*

Gopal Tewari*, Sandeep Kaur, Manoj Kumar Soni and Y.K. Sharma
Department of Botany, University of Lucknow, Lucknow - 226 007, India
***e-mail:** tewarigopal@gmail.com

(Received: January 8, 2009; Revised received: July 27, 2009; Accepted: August 03, 2009)

Abstract: Seeds of cucumber, *Cucumis sativus* L., variety Jyoti Green Long, were exposed to low temperature (4°C) for – 0 (as control), 24, 48 and 72 hr and then allowed to germinate and grow in normal conditions for two weeks inside the laboratory. After two weeks five seedlings from each petridish were transplanted in pots and their development patterns up to anthesis were observed using morphological and biochemical (metabolites and enzyme activity) parameters. It was observed that exposure of seeds to low temperature had a profound effect on the flowering response in cucumber. Except the pollen sterility and the total No. of female flowers formed, all the morphological parameters studied *viz*. days to anthesis of male and female flowers; number of node bearing 1st male or female flower, total male flowers formed and ratio of male flowers to female flowers were decreased as compared to control, when low temperature was given for 24 and 48 hr; but different effect was observed on exposure to low temperature for 72 hr. The sucrose and starch content was found to increase with an increase in the duration of low temperature exposure to seeds up to 48 hr, beyond which it decreased significantly at 72 hr exposure, both at pre-flowering and flowering stages while the total protein content showed a different trend though. The activity of sucrose synthase increased significantly with increase in duration of low temperature up to 48 hr beyond which it decreased when compared to control. Similarly the activity of sucrose synthase decreased from pre-flowering to flowering stage in plants raised from seeds that were exposed to low temperature for 24, 48 and 72 hr respectively. Activity of acid invertase showed a trend just opposite to that of sucrose synthase. IAA oxidase showed a definite reduction in the activity with the increased duration of low temperature exposure. While at pre-flowering stage it was highest in control, the activity decreased significantly in plants which were raised from the seeds subjected to low temperature for 24, 48 and 72 hr respectively. The polyphenol oxidase (PPO) activity also increased markedly when plants experienced transition from pre-flowering to flowering stage. A similar trend to that of PPO was observed in case of peroxidase activity.

Key words: Male flowers, Female flowers, Pollen sterility, Sucrose synthase, Acid invertase, IAA-oxidase, Polyphenol oxidase, Peroxidase

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 (Bernier, 1988). Light quality and day length information are not enough for the plant to ensure its developmental adjustment to environment and, for many species; temperature constitutes a major predictable environmental factor regulating flowering time. As light, temperature also shows diurnal and seasonal variation along the year and this variation not only conditions plant growth rate but regulates the timing of many developmental transitions such as germination, bud dormancy and bursting, or flowering initiation. 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 (Ausin *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.

Exposure to low temperature can induce flowering in many temperate monocots and dicots. Experiments involving cooling treatments localized to parts of plants have shown that the shoot apical meristem is itself a site of perception of the low-temperature treatment (Metzger, 1988). 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. 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 glass distilled water (GDW) in 100 mm petridishes, sterilized with 70% alcohol – 20 seeds per petridish; and exposed to low temperature (4°C) for – 0 (as control), 24, 48 and 72 hr and then allowed to germinate and grow in normal conditions (at room temperature) for two weeks inside the laboratory. The whole experiment was performed in triplicate. 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. 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 (Randomized Block Design) 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 (pre-flowering stage) and one week thereafter (flowering stage) to study the changes in the biochemical parameters during the process of floral induction under the influence of low temperature treatments.

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), 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 results are shown in Table 1 to 4. The days to anthesis were 44.80 and 54.00 for male and female flowers respectively in control and it gradually decreased on 24 hr exposure and 48 hr exposure of low temperature. However, days to anthesis increased to 48.00 and 55.76 for male and female flowers respectively on 72 hr exposure. Similarly average nodal positions bearing 1st male or female flower were observed to be greater in 72 hr exposure. Total no. of male flowers formed decreased with increasing duration of low temperature. However, plants raised from seeds which were exposed to 72 hr of low temperature showed least No. of male flowers (12.3). Percentage of pollen sterility, though, increased with the exposure of seeds to low temperature. The percentage of pollen sterility of control plants was 13.55 which increased to 16.13, 21.67 and 23.89 with increased exposure to low temperature. However, different trend was observed in case of no. of female flowers formed. In control the total no. of female flowers formed was 4.1 which increased to 4.7 in plants developed from seeds exposed to low temperature for 24 hr and to significantly higher No. of 5.4 in plants developed from seeds exposed to low temperature for 48 hr. However a longer exposure of seeds to low temperature for 72 hr decreased the total no. of female flowers to 4.0. In coherence to the effect on formation of male and female flowers, exposure to low temperature decreased the ratio of male flowers to female flowers as compared to control. In control plants this ratio was 4.5:1.0 which declined to 3.7:1.0 in plants raised from seeds exposed to low temperature for 24 hr. However the least ratio of 2.4:1.0 was obtained in the plants which were developed from seeds exposed to low temperature for 48 hr. It again slightly increased to 3.1:1.0 when low temperature was given to seeds for longer duration of 72 hr (Table 1).

The sucrose content was found to increase with an increase in the duration of low temperature exposure to seeds up to 48 hr, beyond which it decreased significantly at 72 hr exposure, both at pre-flowering and flowering stages. A similar trend was observed in total starch content which was 0.280 mg g^{-1} fresh weight in control at pre-flowering stage and declined during flowering to 0.244 mg g^{-1} fresh weight. Just like sucrose content, the starch content was also highest in plants which were raised from seeds having exposure to low temperature for 48 hr. Similarly it was the least in plants, at both pre-flowering and flowering stages, which were developed from seeds exposed for 72 hr. The total protein content showed a different trend though, there was a marked increase in the content of total protein when all, control as well as plants grown from low temperature exposed seeds, experienced the transition from pre-flowering stage to flowering stage. While it increased over control with enhanced duration of low temperature up to 48 hr and decreased below control level at 72 hr (Table 2).

The activity of sucrose synthase increased significantly with increase in duration of low temperature up to 48 hr beyond which it decreased when compared to control. However sucrose synthase activity was found to be least in plants grown from seeds having exposure to low temperature for 72 hr. Similarly the activity of sucrose synthase decreased from 173.16 at pre-flowering to 159.22 μmol g^{-1} fresh weight at flowering stage, from 195.65 at pre-flowering to 172.35 μmol g^{-1} fresh weight at flowering stage and from 166.23 at pre-flowering to 147.28 μmol g^{-1} fresh weight at flowering stage in plants raised from seeds that were exposed to low temperature for 24, 48 and 72 hr respectively. Activity of acid invertase showed a trend just opposite to that of sucrose synthase. Thus, acid invertase activity was markedly higher during flowering stage than in pre-flowering stage in all the plants. Similarly, in contrast to sucrose synthase activity, activity of acid invertase was reduced with enhanced duration of low temperature up to 48 hr and increased thereafter in 72 hr as compared to control (Table 3).

IAA oxidase showed a definite reduction in the activity with the increased duration of low temperature exposure. While at pre-flowering stage it was highest in control, the activity decreased significantly to 0.590, 0.580 and 0.560 μg IAA oxidized g^{-1} fresh weight/hr at pre-flowering stage, in plants which were raised from the seeds subjected to low temperature for 24, 48 and 72 hr respectively. The activity of IAA oxidase was found to decline when plants switched to flowering stage from pre-flowering stage. However, the activity of polyphenol oxidase (PPO) showed a trend opposite to that of IAA oxidase. It was found to increase significantly with the increased duration of low temperature at both pre-flowering and flowering stages. The PPO activity also increased markedly when plants experienced transition from pre-flowering to flowering stage. In control it was more than double at flowering stage (0.221 Δ O.D. g^{-1} fresh weight) than at pre-flowering stage (0.103 Δ O.D. g^{-1} fresh weight). The same trend was observed in the plants raised from low temperature treated seeds. A similar trend to that of PPO was observed in case of peroxidase activity. It also showed increase from pre-flowering stage to flowering stage in control as well as in plants raised from low temperature treated seeds. Low temperature exposure to seeds also caused an increase in peroxidase activity at both pre-flowering and flowering stages (Table 4).

Discussion

Results of low temperature exposure to cucumber seeds showed a distinct effect of vernalization on the process of floral

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

Treatments	Male flower (♂)			Female flower ( )			& ratio	Pollen sterility (%)
	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		
Control	44.80±2.13	5.0±0.65	18.6±0.95	54.00±1.58	6.4±0.88	4.1±0.31	4.5:1.0	13.55±1.10
Low temp. (24 hr)	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
Low temp. (48 hr)	38.60±1.77*	3.7±0.37*	13.0±1.07*	45.14±1.06*	5.2±0.52*	5.4±0.38*	2.4:1.0	21.67±0.57
Low temp. (72 hr)	48.00±3.66	5.2±0.68	12.3±0.83*	55.76±1.99	6.5±0.97	4.0±0.17	3.1:1.0	23.89*±0.95

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

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

Treatments	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. (24 hr)	249.2±8.83	227.5±6.78	0.290±0.008	0.274±0.007*	49.76±2.86	59.65±3.68
Low temp. (48 hr)	268.78.52*	249.5±5.43*	0.305±0.006*	0.282±0.004*	54.20±3.45*	63.10±4.15*
Low temp. (72 hr)	215.56.75*	197.5±4.68*	0.268±0.003	0.241±0.005	41.50±3.22	45.75±2.26

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: Effect of low temperature (4°C) treatment to the seeds on enzymes of carbohydrate metabolism (sucrose synthase and acid invertase) during floral induction in cucumber

Treatments	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. (24 hr)	173.16±5.62	159.22±3.68	102.10±2.83	115.18±3.58
Low temp. (48 hr)	195.65±7.56*	172.35±5.52*	78.94±2.52*	99.44±5.43*
Low temp. (72 hr)	166.23±6.83	147.28±5.66	122.45±3.89*	131.29±4.72

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

Table-4: Effect of low temperature (4°C) treatment to the seeds on IAA-oxidase, polyphenol oxidase and peroxidase during floral induction in cucumber

Treatments	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. (24 hr)	0.590±0.010*	0.548±0.027	0.117±0.005	0.224±0.005	23.36±1.88	27.51±1.02
Low temp. (48 hr)	0.580±0.017*	0.536±0.013*	0.129±0.008	0.237±0.009	23.62±1.10	27.94±2.89
Low temp. (72 hr)	0.560±0.008*	0.522±0.022*	0.177±0.008*	0.259±0.010*	24.02±2.25	28.37±2.02

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 O.D. g^{-1} fresh weight

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. 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;Thingnaes *et al.,* 2003; Paltiel *et al.,* 2006).

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. 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). 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.

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.,* 1999). 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. Lastly, it has been observed that many plants in temperate zone require a low temperature stress (vernalization) to induce flowering and fruit production.

References

Agarwal, S.C. and Sharma, C.P.: Pot sand culture techniques for the study of mineral nutrient element deficiency under Indian conditions. *Geophytol.,* **6**: 356-367 (1976).

Artuso, A., Guidi, L., Soldatini, G. F., Pardossi, A. and Tognoni, F.: The influence of chilling on photosynthesis and activities of some enzymes of sucrose metabolism in *Lycopersicon esculentum* Mill. *Acta Physiol. Plantarum,* **22**: 95-101 (2000).

Ausín, I., Alonso-Blanco, C. and Martinez-Zapater, J.: Environmental regulation of flowering. *Int. J. Dev. Biol.,* **49**: 689-705 (2005).

Bernier, G., Corbesier, L., Périlleux, C., Havelange, A. and Lejeune, P.: Physiological analysis of floral transition. *In*: K. Cockshull, D. Gray, G.B. Seymour and B. Thomas (Eds) Genetic and Environmental Manipulation of Horticultural Crops. CAB International, Wallingford, U.K. pp. 103-109 (1998).

Bitonti, M.B., Cozza, R., Chiappetta, A., Giannino, D., Castiglione, M.R., Dewitte, W., Mariotti, D., Van Onckelen, H. and Innocenti, M.A.: Distinct nuclear organization, DNA methylation pattern and cytokinin distribution mark juvenile, juvenile-like and adult vegetative apical meristems in peach (*Prunus persica* (L.) Batsch). *J. Exp. Bot.*, **53**: 1047-1054 (2002).

Burn, J.B., Bagnall, D.J., Metzger, J.D., Dennis, E.S. and Peacock, W.J.: DNA methylation, vernalization, and the initiation of flowering. *Proc. Natl. Acad. Sci. USA,* **90**: 287–291 (1993).

Calvert, A.: Effect of the early environment on development of flowering in tomato. II light and temperature interactions. *J. Hort. Sci.,* **34**: 154-162 (1957).

Chekmin, I.F., Biglov, T.T. and Akhmetov, R.R.: The influence of low temperatures on the RNA of embryos of rye and wheat. *In*: M. Nauka (Ed) Biology of Nucleic Acid Metabolism in Plants. pp. 130-135 (1964).

Chouard, P.: Vernalization and its relations to dormancy. *Ann. Rev. Plant Physiol.,* **11**: 191-238 (1960).

Dejardin, A., Sokolov, L.N. and Kleczkowski, L.A.: Sugar/osmoticum levels modulate differential abscisic acid-independent expression of two stress-responsive sucrose synthase genes in *Arabidopsis*. *Biochemical J*., **2**: 503–509 (1999).

Demeulemeester, M.A.C., Van-Stallen, N. and De Proft, M.P.: Degree of DNA methylation in chicory (*Cichorium intybus* L.): Influence of plant age and vernalization. *Plant Sci*., **142**: 101-108 (1999).

Henderson, I.R., Shindo, C. and Dean, C.: The need for the winter in the switch to flowering. *Ann. Rev. Genet.*, **37**: 371-392 (2003).

Hewitt, E.J.: Essential nutrient elements: Requirements and interactions in plants. *In*: F.C. Steward (Ed) Plant Physiology. Vol. 3, Academic Press, New York. pp.137-360 (1963).

Kandina, G.V.: The effects of treating sprouting cucumber seed with cold temperatures. *Izv. Mold. fil. AN SSR,* **5**: 85-94 (1958).

Kunwar, K.B.L. and Khan, P.A.: Peroxidase and polyphenol oxidase in excised rage (*Eleurine corocona*) cw PR_2O_2 leaves during senescense. *Ind. J. Exp. Biol*., **25**: 412-476 (1982).

Lindsay, D.L.: Cytokinin Induced Gene Expression in *Arabidopsis*. Doctoral Thesis, University of Saskatchewan, Saskatoon, Saskatchewan (2006).

Lowry, O.H., Resebrough, N.J. Farr A.L. and Randall R. J.: Protein determination with folin reagent. *J. Biol. Chem*., **193**: 265 – 275 (1951).

Luck, H.: Peroxidase. *In*: H.U. Bergmeyer (Ed) Methods of Enzymic Analysis. Academic Press, New York. pp. 895 - 897 (1963).

McCready, R.M., Guggolz, J. and Silviera, V.: Determination of starch and amylase in vegetables. *Anal. Chem*., **22**: 1156-1161 (1950).

Metzger, J. D.: Localization of the site of perception of thermoinductive temperatures in *Thalspi arvense* L. *Plant Physiol*., **88**: 424-428 (1988).

Nitsan, J.: Possible role of polysaccharides in floral induction. *Nature*, **198**: 100-101 (1963).

Paltiel, J., Amin, R., Gover, A., Ori, N. and Samach, A.: Novel roles for GIGANTEA revealed under environmental conditions that modify its expression in *Arabidopsis* and *Medicago truncatula*. *Planta*, **224**: 1255-1268 (2006).

Phatak, S.C., Wittwer, S.H. and Teubner, F.G.: Top and root temperature effects on tomato flowering. *Proc. Amer. Soc. Hort. Sci.,* **88**: 527–531 (1966).

Rabin, R.S. and Klein, R.M.: Chlorogenic acid as a competitive inhibitor of indole-acetic acid oxidase. *Arch. Biochem. Biophys.,* **70**: 11-15 (1957).

Samach, A. and Lotan, H.: The transition to flowering in tomato. *Plant Biotechnology*, **24**: 71–82 (2007).

Shukry, W.M.: Effect of industrial effluents polluting the river Nile on growth, metabolism and productivity of *Triticum aestivum* and *Vicia faba* plants. *Pakistan J. Biological Sci.,* **4**: 1153-1159 (2001).

Thingnaes, E., Torre, S., Ernstsen, A.and Moe, R.: Day and night temperature responses in *Arabidopsis*: Effects on gibberellin and auxin content, cell size, morphology and flowering time. *Ann. Bot.,* **92**: 601-612 (2003).

Wang, F., Sanz, A., Brenner, M. and Smith, A.: Sucrose synthase, starch accumulation, and tomato fruit sink strength. *Plant Physiol.,* **101**: 321- 327 (1993).

Wang, F., Sanz, A., Brenner, M. and Smith, A.: Sucrose synthase, starch accumulation, and tomato fruit sink strength. *Plant Physiol*., **101**: 321- 327 (1993).

Wellensiek, S. J.: Dividing cells as the prerequisite for vernalization. *Plant Physiol.*, **39**: 832-835 (1964).

Wittwer, S.H. and Teubner, F.G.: The effects of temperature and nitrogen nutrition on flower formation in the tomato. *Am. J. Bot.*, **44**: 125–129 (1957).