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FLORAL ABSCISSION IN CAPSICUM UNDER HIGH TEMPERATURE: ROLE OF ENDOGENOUS HORMONES AND POLYAMINES

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SUMMARY

High temperature during reproductive stage limits the productivity in capsicum by inducing abscission of floral buds and flowers. In order to understand the mechanism of high temperature induced floral abscission, investigations were carried out on ethylene biosynthesis, indole acetic acid (IAA), abscisic acid (ABA), cytokinins and polyamines in two capsicum cvs, Arka Mohini (susceptible) and Arka Gaurav (relatively tolerant) subjected to temperatures, 35/25°C and 25/20°C (day/night) during reproductive stage. Under high temperature, susceptible cv. Arka Mohini showed 72.4 and 52.4% abscission in floral buds and flowers in contrast to tolerant cv. Arka Gaurav, which recorded 41.1 and 25.1% abscission, respectively. At 35/25°C, ethylene production, ACC (1-aminocyclopropane carboxylic acid)-oxidase activity, and ACC and ABA contents increased, and auxins (IAA) and cytokinins [zeatin riboside (ZR) and dihydrozeatin riboside (DHZR)] contents declined in the floral buds and flowers; the effect was pronounced in the susceptible cultivar Arka Mohini. Free polyamines, putrescine, spermidine and spermine contents under high temperature increased significantly in the reproductive parts, with pattern of polyamine changes being differential in the cultivars. While putrescine increase was greater under high temperature in both the floral organs of the susceptible cv. Arka Mohini, the increase in spermidine and spermine was dominant in the tolerant cv. Arka Gaurav. These results implicated that the floral abscission in capsicum under high temperature is associated with increased biosynthesis of ethylene, and ABA and putrescine accumulation concomitant with decline in IAA and cytokinins.

***Key words*: Abscission, capsicum, high temperature, hormones, polyamines**

INTRODUCTION

The sensitivity of flower retention and fruit set to environmental stresses is major limiting factor for achieving optimum fruit yield in solanaceous vegetable crops (Doorn and Stead 1997). In capsicum (*Capsicum annum* L.), high temperature coinciding reproductive phase is most detrimental to its yield and productivity because of increased abscission of floral organs (Erickson and Markhart 2002). Although mechanism by which leaves abscise under stress has been investigated in great details (Sexton 1995); the abscission of reproductive organs is relatively less studied. The most important factors attributed to abscission in various crops include hormonal imbalance, besides photosynthate levels and partitioning, altered mineral nutrition, changes in proteins and nucleic acids, and impaired sugar metabolism in the source and sink tissues. Many workers opined that the abscission is primarily ascribed to the imbalance of auxin and ethylene (Woodward and Bartel 2005, Goren 2010). Based on exogenous applications and analyses of endogenous levels, it has been demonstrated that the declining levels of auxin in abscising tissue or abscission zone concomitant with increase in ethylene

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are vital for the abscission of plant parts. Additionally, evidences supported by their external applications and also the fact that ethylene and polyamines compete for common intermediate S-adenosine methionine (SAM) for the biosynthesis (Kuznetsov and Shevyakova 2007) also point towards the polyamine role in abscission. High temperature has been reported to increase ethylene production and abscisic acid accumulation and alter the levels of other important hormones including polyamines (Huberman *et al.* 1997, Banowetz *et al.* 1999, Klueva *et al.* 2001, Kuznetsov *et al.* 2006, Wahid *et al.* 2007, Toh *et al.* 2008, Cheng *et al.* 2009). In the present investigation, attempts have been made to study the changes in endogenous hormonal levels and polyamines in floral organs of two capsicum cultivars differing in sensitivity to high temperature with the objective to decipher information on factors linked to the floral abscission induced under high temperature conditions.

MATERIALS AND METHODS

Plant material and stress treatments: Based on growth and yield responses of capsicum (*Capsicum annum* L.) cultivars under high temperature, cvs Arka Gaurav and Arka Mohini were selected for the study. Seeds were germinated under natural light in 12" height pots filled with growing media of garden soil, farm yard manure and sand (2:1:0.5 v/v/v) at the experimental farm of Indian Institute of Horticultural Research, Hessaraghatta. At 4 leaf stage, the seedlings were thinned one plant per pot. All the pots were watered once a day daily with 1.5 litres of water. When potted plants attained reproductive stage (65 days after sowing), 12 uniformly grown healthy plants of different cultivars were shifted to two growth chambers, each maintained at day/night temperatures 25/20°C and 35/25°C with 16 hr photoperiod at 400 µmol $m^{-2}s^{-1}$ PPFD as measured at the top of plant canopy. Irradiance source consisted of the combination of 60% of total wattage from cool white florescent lamp and 40% of total wattage from 100 W incandescent lamps. Relative humidity in the chambers was 70±7%. When floral buds were visible, these were labeled in 10 plants under both the treatments to record abscission of floral buds and flowers. The contents of indole acetic acid (IAA), abscisic acid (ABA), cytokinins-zeatin riboside (ZR) and dihydrozeatin riboside (DHZR) and polyamines, and changes in ethylene biosynthesis (ethylene concentration, 1-aminocyclopropane carboxylic acid (ACC) and ACC oxidase activity) were measured in the intact and abscising flower buds and open flowers.

Abscission measurements: The abscission of floral organs was measured at two temperatures by recording the number of floral buds and open flowers abscised every day from the time of temperature treatments, and percent floral buds or flowers abscised were calculated from the number of floral buds labeled.

Hormonal contents: 1.0 g floral buds or flowers were extracted in chilled 80% methanol containing 0.05 % butylated hydroxytoluene. The extract was centrifuged at 10000 rpm for 20 minutes, supernatant vacuum dried and residue was suspended in 1.0 ml of 20 mM tris buffer containing 5 mM $MgCl_2$ and 20 mM NaN_3 for quantifying IAA (Weiler *et al.* 1981), ABA (Weiler 1982) and cytokinins-ZR and DHZR (Barthe and Steward 1985) by ELISA employing laboratory raised and purified polyclonal antibodies against respective hormones. ELISA protocols followed for IAA and ABA, and cytokinins estimations were as described by Upreti and Murti (2004) and Bhatt *et al.* (2009), respectively.

Ethylene concentration: About 8-10 numbers of floral buds and flowers were sampled in 5.0 ml pre weighed glass vial fitted with rubber septa, and the vial weight was taken immediately after sealing the tubes to record fresh weight. The tubes were incubated for 2 hr at 37°C and ethylene concentration was determined on Gas Chromatograph (GC) (Auto System XL, Perkin Elmer, USA) equipped with Porapak-N column (2.0 m length, 80x100 mesh) and flame ionization detector (FID) according to Galliard and Grey (1969). The column, injection port and detector temperatures used were 60, 110 and 150 °C, respectively and carrier gas (N_2) flow rate was maintained at 40 ml min^{-1}. Standard ethylene (100 µl l^{-1} in nitrogen) was used as external standard to quantify ethylene.

1-aminocyclopropane carboxylic acid (ACC) content: ACC content was determined according to Lizada and Yang (1979). Floral buds and open flowers (250 mg lyophilized powder) was extracted in 2.0 ml of cold 5.0%

sulphosalicyclic acid and centrifuged at 5000 rpm for 15 min at 4°C. The supernatant containing ACC was lyophilized, dissolved in 250 µl of water and further purified over Dowex 50 [H^+] syringe column. The ACC eluted with 2.0 M of ammonium hydroxide was dried under vacuum and dissolved in 250 µl of water. The ACC was estimated by adding 15 µl mercuric chloride to ACC sample in 1.5 ml glass vial kept in an ice bath and fitted with rubber septa. A 50 µl of NaOCl and saturated NaOH (2:1, v/v) solution was injected into the vial, mixture vortexed vigorously and further cold incubated for 5 min at 4°C. The ethylene produced was quantified by GC. The conversion efficiency of ACC to ethylene as analysed by standard ACC was 63.2%.

ACC-oxidase activity: The lyophilized powder of floral buds or flowers (250 mg) was kept in 1.5 ml tubes containing 500 µl of ACC (5.0 µmol). The tubes sealed tightly with rubber septa were incubated for 1 hr at 37°C in an orbital shaker at 90 rpm. The conversion of ACC to ethylene was measured by assaying ethylene produced employing GC.

Polyamines analysis: Free polyamine levels in the floral parts were estimated following the HPLC procedure of Flores and Galston (1982). Samples of floral organs (500 mg) were extracted with 1.0 ml of chilled 5% (v/v) perchloric acid and the supernatant was benzoylated by adding 100 µl of 2.0 N NaOH and 25 µl of benzoyl chloride to 500 µl of supernatant. After adding 250 µl of saturated NaCl, the benzoylated polyamines were extracted with chilled diethyl ether. The ether phase was dried under nitrogen. The residue was suspended in 100 µl methanol and polyamines were analysed by HPLC (Shimadzu, Japan, Model LC-10A) employing µBondapak C_{18} column and UV/Visible detector (Model: SPD-10A, Shimadzu, Japan) adjusted to 282 nm. An isocratic solvent system of methanol (62%, v/v) containing 1% acetic acid at 1.0 ml min^{-1} flow rate was used to resolve benzoylated polyamines. The quantification of free polyamines, putrescine, spermidine and spermine was performed using these as external standards (Sigma, USA).

RESULTS AND DISCUSSION

Under 25/20°C, cv. Arka Mohini experienced 28.9% and 17.7%, and cv. Arka Gaurav 17.7 and 14.2% abscission of floral buds and flowers, respectively. The high temperature (35/25°C) treatment led to substantial increase in the abscission of floral buds and flowers, and response elicited was cultivar dependent. The cultivar differences in the abscission of reproductive organs in capsicum under high temperature have been reported earlier (Wein *et al.* 1989). Under high temperature, the abscission in floral buds and flowers was 72.4 and 52.4% in cv. Arka Mohini and 41.1 and 25.1% in the cv. Arka Gaurav, respectively (Fig. 1). This indicated that cv. Arka Mohini is abscission susceptible and cv. Arka Gaurav is relatively abscission tolerant to the conditions of high temperature, and the flowers are more susceptible than the floral buds.

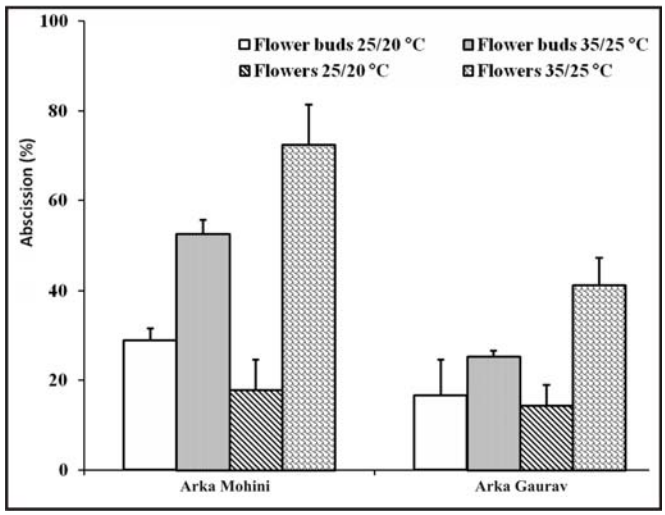

Fig. 1. Floral bud and flower abscission in capsicum cultivars at two temperatures (data represents mean ± SE, n=4)

Ethylene production and ACC-oxidase activity were high in the abscission susceptible cv. Arka Mohini and ACC content in the abscission tolerant cv. Arka Gaurav at 25/20°C, and the flowers, in general, produced higher ethylene than the floral buds (Table 1). Exposure to high temperature (35/25°C) led to significant increase in ethylene (50.7-131.2%) concomitant with increased ACC (35.3-149.1%) accumulation and ACC oxidase activity (56.3-137.9%) in the floral organs, and the changes were predominant in abscission susceptible cv. Arka Mohini. Amongst the floral buds and flowers, magnitude of increases in ethylene levels, ACC content and ACC oxidase activity under high temperature were greater in floral buds (67.5-131.2%, 35.3-149.1% and 59.6-137.9%,

Table 1. Ethylene concentration, ACC content and ACC-oxidase activity in the floral buds and flowers of capsicum cultivars at two temperatures (data represents mean±SE, n=4)

Parameters	Floral parts	Arka Mohini		Arka Gaurav	
		25/20°C	35/25°C	25/20 °C	35/25°C
Ethylene (nl g^{-1} hr^{-1})	Buds	4.26±0.672	9.85±0.811	3.26±0.421	5.46±0.504
	Flowers	6.63±0.861	13.11±1.094	4.99±0.568	7.52±0.837
ACC (mmol g^{-1})	Buds	2.89±0.314	7.20±0.685	3.99±0.341	5.40±0.627
	Flowers	4.35±0.602	8.92±0.769	6.86±0.472	11.41±1.286
ACC oxidase activity	Buds	10.62±1.125	25.26±3.041	9.51±0.724	15.18±1.825
(nl mg^{-1} min^{-1})	Flowers	19.98±2.324	31.22±2.372	12.11±1.428	20.41±2.367

respectively) than the flowers (50.7-97.7%, 66.3-105.1% and 56.3-68.5%, respectively), with flowers of high temperature exposed plants exhibiting high levels. As the ethylene is well characterized for its role in abscission (Abeles *et al.* 1992), the higher ethylene production under high temperature could be of significance for the high abscission of floral organs in the cv. Arka Mohini. However, it is worthy to mention that the ethylene analyses were made by destructive sampling, thus stress induced changes in organ physiology by the tissue excision cannot be ignored. This could be the reason for no relationships between abscission and ethylene in some of the studies (Brown 1997). However, parallel increase in ethylene levels and its precursor, ACC as evident in the present study supported the positive role of ethylene in the high temperature induced abscission by the capsicum cultivars. Higher ethylene production in the abscising flowers has also been reported in Dendrobium by Rungruchkanont *et al.* (2007). Study of Wein and Zhang (1991) concluding that silver thiosulphate, an ethylene action inhibitor, reduced abscission of reproductive organs in pepper also supported our results. ACC oxidase is associated with the conversion of ACC to ethylene in the final step of ethylene biosynthetic pathway (Yang and Oetiker 1998). The high induction in ACC-oxidase activity along with increased ACC availability in the high abscising cv. Arka Mohini under high temperature provide an evidence for high ethylene production, and the cultivar and floral organs susceptibility to abscission. The increased ethylene production has been shown to act directly in the abscission zone to induce the activities of hydrolytic enzymes for faster onset of abscission (Campillo and Bennet 1996, Brown 1997). Wein *et al.* (1989) also reported low carbohydrate availability in the floral organs as the possible cause for abscission in capsicum. Iglesias *et al.* (2006) reported inverse relationship between ethylene production and sugar content in relation to abscission of citrus fruitlets through girdling experiments, which supported the positive role of ethylene in the induction of floral abscission in capsicum under high temperature.

High temperature significantly influenced ABA, IAA and cytokinins, ZR and DHZR contents of floral buds and flowers. While ABA (45.3-170.5%) content significantly increased, the IAA (32.2-35.8%) and cytokinin - ZR (36.1-46.9%) and DHZR (14.8-33.7%) contents in contrast declined in the floral organs following high temperature, and the changes were high in the abscission susceptible cv. Arka Mohini. The ABA increase and IAA decline under high temperature was greater in the abscising flowers than the floral buds. Auxins have been implicated as prominent regulator of abscission, and a continuous supply of auxins is vital for a check on abscission. Ketsa and Rungruchkanont (2007) in Dendrobium reported that auxin decline coupled with inhibition in its auxin transport promoted floral abscission. A decline in auxin promotes abscission by inductions in ethylene biosynthesis and also makes abscission zone sensitive to ethylene (Rahman *et al.* 2001). Bhatt *et al.* (2009) reported decline in IAA and increase in ethylene in the flowers under water stress responsible for floral abscission in tomato. Sakata *et al.* (2010) in barley reported that the decline in auxin also up-regulated certain repressed proteins under high temperature condition which contributed in abscission. Further, there are reports that high ethylene exerts

negative influence on auxin production and auxin transport in abscission zone as in case of cotton (Gruinn and Brummett 1988). Thus decline in auxin and enhanced ethylene production in floral organs under high temperature is expected to activate the gene associated with enzymes of cell wall degradation and promote abscission. High temperature has been reported to promote ABA accumulation (Toh *et al.* 2008), which is expected to trigger abscission as ABA is well characterized abscission promoting signaling molecule. Aneja *et al.* (1999) reported that increase in ABA levels prior to flower abscission in cocoa. The possible mechanisms by which ABA induced abscission are related to its negative action on auxin (Jackson and Osborne 1970), up-regulation in ethylene synthesis (Riov *et al.* 1990) and induction cellulases and hydrolases (Abeles *et al.* 1992). Cytokinins are involved in growth promoting activity (Brzobohaty *et al.* 1994), thus it is speculated that the declining cytokinins levels may be of significance in promoting abscission. Mor *et al.* (1983) reported decline in carnation petal abscission by the cytokinin application and the effect rendered was due to decline in ethylene production. Similarly, Khalil *et al.* (2006) reported decline in abscission of flowers by kinetin application in lentil plants and the effect was implicated as the outcome of increased IAA, cytokinins and decreased ABA. Thus the parallel increase in ABA and ethylene and decline in IAA and cytokinins in abscising floral organs under high temperature contributed to floral abscission in capsicum under high temperature.

In the plants grown under 25/20°C, the putrescine content was high in cv. Arka Mohini and spermine and spermidine in cv. Arka Gaurav. Following the high temperature treatment, the contents of polyamines increased in the flowers (49.6-181.7%) and floral buds

Table 2. IAA, ABA and cytokinins-ZR and DHZR contents in the floral buds and flowers of capsicum cultivars at two temperatures (data represents mean±SE, n=4)

Parameters	Floral parts	Arka Mohini		Arka Gaurav	
		25/20°C	35/25°C	25/20 °C	35/25°C
IAA (ng g^{-1})	Buds	71.3±8.54	49.0±5.36	91.2±7.75	63.3±5.39
	Flowers	43.9±3.55	28.2±1.98	56.6±5.31	38.4±4.14
ABA (ng g^{-1})	Buds	31.2±2.14	65.9±6.11	26.3±2.87	38.2±2.87
	Flowers	21.7±2.98	58.7±6.46	32.4±3.96	56.5±3.86
ZR (ng g^{-1})	Buds	102.4±8.41	57.4±5.24	152.3±12.11	87.9±9.17
	Flowers	112.1±12.45	71.6±6.27	87.7±9.62	46.6±5.35
DHZR (ng g^{-1})	Buds	156.5±11.22	104.2±9.07	142.3±10.41	121.2±13.56
	Flowers	92.5±8.06	61.3±5.41	99.8±8.55	76.3±8.28

Table 3. Polyamine contents in the floral buds and flowers of capsicum cultivars at two temperatures (data represents mean±SE, n=4)

Polyamines (nmol g^{-1})	Floral parts	Arka Mohini		Arka Gaurav	
		25/20°C	35/25°C	25/20 °C	35/25°C
Putrescine	Buds	112.3±10.32	266.9±21.42	80.3±8.04	112.8±13.04
	Flowers	69.9±5.27	129.2±10.63	50.4±3.97	75.4±6.41
Spermidine	Buds	65.5±6.55	108.9±7.98	72.4±8.65	175.4±12.74
	Flowers	34.7±2.32	75.5±8.12	42.5±5.39	119.7±9.20
Spermine	Buds	70.9±5.11	99.6±12.07	95.8±13.22	201.1±15.62
	Flowers	57.2±4.32	98.5±8.54	75.5±9.04	137.4±14.44

(40.5-137.7%), and the magnitude of increase in putrescine and its content under high temperature (35/25°C) were witnessed high in abscission susceptible cv. Arka Mohini, and of spermidine and spermine in the abscission tolerant cv. Arka Gaurav. Amongst floral buds and flowers, the flowers in both the cultivars recorded low concentration of polyamines under high temperature. The low polyamines contents are stated to be of significance in observed high incidence of flower drop in capsicum under high temperature. Putrescine, spermidine and spermine are the commonly occurring free polyamines responsible for most of the polyamine dependent responses in plants. The polyamines similar to cytokinins have been shown to possess anti-abscission activity (Altman 1989), and polyamines biosynthesis is reported to compete with the ethylene biosynthesis by sharing common precursor, *S*-adenosylmethionine (Turano *et al*. 1997); described as a stimulator of abscission (Ruperti *et al*. 1998). Mattoo *et al.* (2010) using molecular approach showed putrerscine as negative regulator while spermidine and spermine as positive regulators of cellular amino acid metabolism and thus the protein biosynthesis. The declined protein concentration and altered protein expression pattern contributed to abscission (Tripathi *et al.* 2009). The stimulation in ethylene is expected to decrease polyamine levels, which in turn may trigger abscission by stimulating the production of free radicals and superoxide ions (Drolet *et al.* 1986) and altering the membrane stability and functionality (Altman *et al.* 1989). Kumar *et al.* (1997) reported that polyamine levels were modified significantly under wide range of environmental conditions including under high temperature. The pattern of trend observed under stress in polyamines has been shown dependent on species, cultivars, and stress severity (Kumar *et al.* 1997). Our study also showed that the polyamine changes under high temperature in capsicum are cultivar dependent. Aziz *et al.* (2001) reported that susceptibility of grapevine to flower or fruitlet abscission is greatly dependent on the modulation of polyamine concentrations in the ûoral organs early during development. Among the polyamines, it is appeared that component polyamines do not have similar role in high temperature induced response mechanism. The spermidine and spermine contents showing greater increases in abscission tolerant cultivar indicated their involvement in imparting of thermotolerance in capsicum. The high efficacy of spermine and spermidine in high temperature tolerance is suggested by virtue of their respective tetravalent and trivalent natures; the physical property that equip them with better capacity towards cell membrane stabilization. The spermine and spermidine being contributory to thermotolerance has been reported earlier (Cheng *et al.* 2009). Aziz *et al.* (2001) in grape reported that spermidine application prior to anthesis prevented abscission. Similarly, spermine application has been shown to control the abscission of inflorescence buds in pistachio (Khezri *et al.* 2010). Ameliorative effects of spermidine and spermine towards high temperature induced injury as evident from pollen germinability has been reported in tomato (Song *et al.* 1999) and *Prunus mume* (Wolukau *et al.* 2004). Cheng *et al.* (2009) showed that the transgenic tomato over expressing S-adenosyl-l-methionine decarboxylase (SAMDC), the key regulatory enzymes in the biosynthesis of polyamines over produced spermidine and spermine, and induced high temperature tolerance (38°C).

From the above findings it appeared that the floral abscission in capsicum sensitized by high temperature is dependent on the relative content of growth inhibitor, ethylene and ABA and growth promoters, cytokinins and auxins, and polyamines. The higher levels of ethylene and ABA in combination with lower levels of cytokinins, IAA and the polyamines, spermidine and spermine as recorded in high abscission cv. Arka Mohini were critical for cultivar sensitivity to floral organ abscission in capsicum under high temperature.

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REFERENCES

Abeles, F.B., Morgan, P.W. and Saltveit, M.E. (1992). Ethylene in plant biology, 2nd edn, Academic Press, San Diego, 414p.

Altman, A. (1989). Polyamines and plant hormones. In: Bachrach, U. and Heimer, Y.M. (eds.), The Physiology of Polyamines, Vol. **31**, pp. 121-145, CRC Press Inc, Boca Raton, Florida.

Aneja, M., Gianfagna, T. and Ng, E. (1999). The roles of abscisic acid and ethylene in the abscission and senescence of cocoa flowers. *Plant Growth Regul.* **27:** 149-155.

Aziz, A., Brun, O. and Audran, J.C. (2001). Involvement of polyamines in the control of fruitlet physiological abscission in grapevine (*Vitis vinifera*). *Physiol. Plant.* **113:** 50–58.

Banowetz, G.M., Ammar, K. and Chen, D.D. (1999). Temperature effects on cytokinin accumulation and kernel mass in dwarf pea. *Ann. Bot.* **83:** 303-307.

Barthe, G.A. and Stewart, I. (1985). Enzyme immunoassay (EIA) of endogenous cytokinins in citrus. *J. Agr. Food Chem.* **33:** 293-297.

Bhatt, R.M., Srinivasa Rao, N.K., Upreti, K.K. and Jyothi, L. (2009). Hormonal activity in tomato flowers in relation to their abscission under water stress. *Ind. J. Hort.* **66:** 492-495.

Brown, K.M. (1997). Ethylene and abscission. *Physiol. Plant.* **100:** 567-576.

Brzobohaty, B., Moore, I. and Palme, K. (1994). Cytokinin metabolism: implications for regulation of plant growth and development. *Plant Mol. Biol.* **26:** 1483-1497.

Campillo, E. del and Bennet, A.B. (1996). Pedicel breakstrength and cellulose gene expression during tomato flower abscission. *Plant Physiol.* **111:** 813-820.

Cheng, L., Zou, Y.J., Ding, S.L., Zhang, J.J., Yu, X.L., Cao, J.S. and Lu, G. (2009). Polyamine accumulation in transgenic tomato enhances the tolerance to high temperature stress. *J. Integr. Plant Biol.* **51:** 489-499.

Doorn, W.G. van and Stead, A.D. (1997). Abscission of flowers and floral parts. *J. Exptl. Bot.* **48:** 821-837.

Drolet, G., Dumbroff, E.B., Legge, R.L. and Thompson, J.E. (1986). Radical scavenging properties of polyamines. *Phytochem.* **25:** 367-371.

Erickson, A.N. and Markhart, A.H. (2002). Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. *Plant Cell Environ.* **25:** 123–130.

Flores, H.E. and Galston, A.W. (1982). Analysis of polyamines in higher plants by high performance liquid-chromatography. *Plant Physiol.* **69:** 701–706.

Galliard, T. and Grey, T.C. (1969). A rapid method for the determination of ethylene in the presence of other volatile natural products. *J. Chromat.* **41:** 442-452.

Goren, R. (2010). Anatomical, physiological, and hormonal aspects of abscission in citrus. *Hort. Rev.* **15:** 145-182.

Gruinn, G. and Brummett, D.L. (1988). Changes in free and conjugated indole-3-acetic acid and abscisic acid in young cotton fruits and their abscission zone in relation to fruit retention during and after moisture stress. *Plant Physiol.* **50:** 313-318.

Huberman, M., Riov, J., Aloni, B. and Goren, R. (1997). Role of ethylene biosynthesis and auxin content and transport in high temperature-induced abscission of pepper reproductive organs. *J. Plant Growth Regul.* **16:** 129-135.

Iglesias, D.J., Tadeo, F.R., Primo-Millo, E. and Talon, M. (2006). Carbohydrate and ethylene levels related to fruitlet drop through abscission zone A in citrus. *Tree* **20:** 348-355.

Jackson, M.B. and Osborne, D.J. (1970). Ethylene, the natural regulator of leaf abscission. *Nature* **255:** 1019-1022.

Ketsa, S. and Rungruchkanont, K. (2007). Effect of inhibitors of auxin and ethylene on abscission development of Dendrobium inflorescences. *Adv. Plant Ethylene Res.* **5:** 297-299.

Khalil, S., El-Saeid, H.M. and Shalaby, M. (2006). The role of kinetin in flower abscission and yield of lentil plant. *J. Applied Sci. Res.* **2:** 587-591.

Khezri, M., Talaie, A., Javanshah, A. and Hadavi, F. (2010). Effect of exogenous application of free polyamines on physiological disorders and yield of 'Kaleh-Ghoochi' pistachio shoots (*Pistacia vera* L.). *Scientia Hort.* **125:** 270-276

Klueva, N.Y., Maestri, E., Marmiroli, N. and Nguyen, H.T. (2001). Mechanisms of thermotolerance in crops. In: A.S. Basra A.S. (ed.), Crop Responses and Adaptations to Temperature Stress, pp. 177-217, Food Products Press, Binghamton, New York.

Kumar, A., Altabella, T., Taylor, M.A. and Tiburcio, A.F. (1997). Recent advances in polyamine research. *Trends in Plant Sci.* **2:** 124-130.

Kuznetsov, V.V. and Shevyakova, N.I. (2007). Polyamines and stress tolerance in plants. *Plant Stress* **1**: 50-71.

Kuznetsov, V.V., Radyukina, N.L. and Shevyakova, N.I. (2006). Polyamines and stress: Biological role, metabolism and regulation. *Russ. J. Plant Physiol.* **53:** 583-604.

Lizada, M.C.C. and Yang, S.F. (1979). A simple and sensitive assay for aminocyclopropane-1-carboxylic acid. *Anal. Biochem.* **100:** 140–145.

Mattoo, A.K., Minocha, S.C., Minocha, R. and Handa A.K. (2010). Polyamines and cellular metabolism in plants: transgenic approaches reveal different responses to diamine putrescine versus higher polyamines spermidine and spermine. *Amino Acids* **38:** 405-413.

Mor, Y., Spiegelstein, H. and Halvy, A. (1983). Inhibition of ethylene biosynthesis in carnation petals by cytokinins. *Plant Physiol.* **71:** 541-546.

Rahman, A., Amakawa, T., Goto, N. and Tsurum, S. (2001). Auxin is a positive regulator for ethylene-mediated responses in the growth of *Arabidopsis* roots. *Plant Cell Physiol.* **42:** 301-307.

Riov, J., Dagan, E., Goren, R. and Yang, S.F. (1990). Characterization of abscisic acid-induced ethylene production in citrus leaf and tomato fruit tissues. *Plant Physiol.* **92:** 48-53.

Rungruchkanont, K., Ketsa, S., Chatchawankanphanich, O. and Doorn, W.G. van (2007). Endogenous auxin regulates the sensitivity of Dendrobium (cv. Miss Teen) flower pedicel abscission to ethylene. *Funct. Plant Biol.* **34:** 885-894.

Ruperti, B., Bonghi, C., Tonutti, P. and Ramina, A. (1998). Ethylene biosynthesis in peach fruitlet abscission. *Plant Cell Environ.* **21:** 731-737.

Sakata, T., Yagihashi, N. and Higashitani, A. (2010). Tissue-specific auxin signaling in response to temperature fluctuation. *Plant Signaling Behavior.* **5:** 1510-1512.

Sexton, R.A. (1995). Abscission. In: M. Pessarakli (ed.), Handbook of Plant and Crop Physiology, pp. 497-525, Dekker, New York.

Song, J., Nada, K. and Tachibana, S. (1999). Ameliorative effect of polyamines on the high temperature inhibition of *in vitro* pollen germination in tomato (*Lycopersicon esculentum* Mill.). *Scientia Hort.* **80:** 203-212

Toh, S., Imamura, A., Atanabe, A., Nakabayashi, K., Okamoto, M., Jikumaru, Y., Hanada, A., Aso, Y., Ishiyama, K., Tamura, K., Iuchi, S., Kobayashi, M., Yamaguchi, S., Kamiya, Y., Nambara, E. and Kawakami, N. (2008). High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in arabidopsis seeds. *Plant Physiol.* **146:** 1024-1025.

Tripathi, S.K., Singh, A.P., Sane, A.P. and Nath, P. (2009). Transcriptional activation of a 37 kDa ethylene responsive cysteine protease gene, RbCP1, is associated with protein degradation during petal abscission in rose. *J. Exptl. Bot*. **60:** 2035-2044.

Turano, F.J., Kramer, G.F. and Wang, C.Y. (1997). The effect of methionine, ethylene and polyamine catabolic intermediates on polyamine accumulation in detached soybean leaves. *Physiol. Plant*. **101:** 510–518.

Upreti, K.K. and Murti, G.S.R. (2004). Effect of brassinosteroids on growth, nodulation, phytohormones content and nitrogenase activity in French bean under water stress. *Biol. Plant*. **48:** 407-411

Wahid, A., Gelani, S., Ashraf, M. and Foolad, M.R. (2007). Heat tolerance in plants: An overview. *Environ. Exptl. Bot.* **61:** 199-223.

Weiler, E.W. (1982). An enzyme-immunoassay for *cis* (+)-abscisic acid. *Physiol. Plant.* **54:** 510-514.

Weiler, E.W., Jourdan, P.S. and Conrad, W. (1981). Levels of indole-3-acetic acid in intact and decapitated coleoptiles as determined by specific and sensitive solid-phase enzyme immunoassay. *Planta.* **153:** 561-571.

Wein, H.C., Tripp, K.E., Hernandez-Armenta, R. and Turner, A.D. (1989). Abscission of reproductive structures in pepper: Causes, mechanism and control. In: Green, S.K. (ed.), Tomato and Pepper Production in the Tropics, pp. 150-165, Asian Vegetable Research and Development Centre, Taipei, Taiwan.

Wien, H.C. and Zhang, Y. (1991). Prevention of flower abscission in bell pepper. *J. Amer. Soc. Hort. Sci.* **166:** 516-519.

Wolukau, J.N., Zhang, S., Xu, G. and Chen, D. (2004). The effect of temperature, polyamines and polyamine synthesis inhibitor on *in vitro* pollen germination and pollen tube growth of *Prunus mume. Scientia Hort.* **99:** 289-299.

Woodward, A.W. and Bartel, B. (2005). Auxin: Regulation, action and interaction. *Ann. Bot.* **95:** 707-735.

Yang, S.F. and Oetiker, J.H. (1998). Molecular biology of ethylene biosynthesis and its application in horticulture. *J. Jap. Soc. Hort. Sci.* **67:** 1209-1214.