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Abscission responses of petiolar explants of *Capsicum* varieties to treatments with growth substances and their combinations

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Abstract. The abscission responses of petiolar explants of NP 46-A and Pusa jwala cultivars of chilli (*Capsicum annuum* L) to proximal and distal applications of growth substances were investigated. Ethephon, abscisic acid and ascorbic acid proved to be accelerants of abscission irrespective of age of explants and site of application. The synthetic auxin (naphthalene acetic acid) retarded abscission in younger explants regardless of the site of application but in the older explants proximal application of the auxin promoted abscission while distal application retarded the process. Gibberellic acid showed effects opposite to those caused by the auxin. The proximal application of gibberellic acid retarded abscission irrespective of age of explants while distal application effectively accelerated abscission. The cytokinin (benzyl adenine) and morphactin delayed abscission of petiolar explants on distal as well as proximal applications. The abscission responses to combinations of growth substances were highly variable depending on the age of explants, site of applications and varietal source of experimental material. The morphactin and benzyl adenine exhibited additive interaction in retarding the abscission up to such extent that ethephon and abscisic acid could only partially counteract their effects in combination of two growth substances. The auxin and gibberellin showed irregular and mixed interactions with other bioregulants studied.

Keywords. Petiolar explant abscission; *Capsicum*; growth substances.

1. Introduction

Since the introduction of the method involving the use of petiolar explants (Addicott *et al* 1949) for the study of foliar abscission, various workers have used the technique for studying various aspects of abscission and its hormonal regulation (Biggs and Leopold 1957; Vendrig 1959; Jacobs 1968; Addicott 1970) under controlled conditions. Most of our present day knowledge about the role of plant growth substances in the control of abscission of plant parts is based on explant studies (Addicott 1981). However, contradictory results have been reported on the role of exogenous and endogenous bioregulants in abscission by various workers who used different plants but the same method. This is especially true for auxin (Addicott 1970), gibberellin (Chatterjee and Leopold 1964; Wittwer and Bukovac 1968) and cytokinin (Murty and Prakash 1975). Similar controversy exists regarding morphactin, ascorbic acid and some other plant growth regulators in relation to abscission. This situation prompted Jacobs (1979) to advocate more experimentations for the study of interactions of hormones, rather than the consideration of individual hormones. Such studies are quite uncommon under semi-controlled experimental conditions. Present experiments were, therefore, undertaken to make a quantitative appraisal of the abscission responses of chillies to applied growth substances and their combinations and also to assess the evidences of interactions of growth substances in the process.

2. Materials and methods

Seedlings of NP 46-A and Pusa jwala cultivars of chilli (*Capsicum annuum* L.) were raised in earthen pots and were maintained in a healthy, vigorous and one stemmed condition with uniform size of 6th fully unfolded leaf at the time of harvest. Before excision of explants, the required number of sterilized petri-dishes were half-filled with 1·5% agar and arranged in two steel racks which were properly sterilized by 96% alcohol spray and lined with sterilised 'absorbo' blotting paper and then covered with a thin white cotton cloth. Plants at 6 leaf stage were harvested, and quickly brought to the laboratory, washed and then surface sterilized with chlorine water. Explants from nodes 1 and 5 were excised and collected in enamel trays filled with distilled water. Each explant was finally trimmed and consisted of a petiole length of 10 mm, stem length of 10 mm below the petiolar attachment and 2 mm above the petiolar attachment. Ten explants per petri dish for distal application were inserted up to 4 mm length of petiolar stump in agar. Similarly 10 explants per petri dish for proximal application were inserted in agar medium up to 4 mm of stem length (figure 1). Into each petri dish was poured 10 ml of freshly prepared solution of growth substance. The effective concentration of each of the growth substances was determined by earlier experiments (Singh 1979). The petri dishes were placed on the racks in a randomised block design pattern. Explants treated with distilled water served as 'controls'. In these experiments, no nutrient medium was used following the recommendations of Davenport *et al* (1977).

The temperature of the experimental chambers of racks varied between 24 to 27°C. Explants were subjected to natural light intensity and photoperiod prevailing in the laboratory. High relative humidity (70–80%) was maintained in all chambers so as to minimise the dessication of explants. However, few explants (marked by†† or ††† in table 1) turned brown inspite of all care. In some treatments few explants exhibited abnormally long pre-abscission periods (marked by ** or *** in the table). Pre-abscission periods of such explants were excluded from averaging so as to minimize the experimental error. Abscission of each explant was tested daily at 0800 hr with an abscission tester by gently touching the middle part of the petiole/stem stump. Laboratory experiments were conducted twice in March 1978 and then repeated in the same month of 1979. As results obtained from all the repetitions were statistically identical, data represented in figure 2 and table 1 are those obtained from finally conducted experiments (March 1979). Data were analysed by suitable statistical methods (Chandel 1978).

3. Results

A critical analysis of the quantitative characteristics of the responses of explants of both the cultivars to externally applied growth substances individually (figure 2) and in combinations (table 1) has revealed the following facts.

3.1 *Responses to individual growth substances*

The synthetic auxin (NAA-100 mg/l) accelerated abscission of petiolar explant No. 1 on proximal application. The distal application stimulated a substantial retardation of abscission in this older explant. However, the abscission of younger petiolar explant

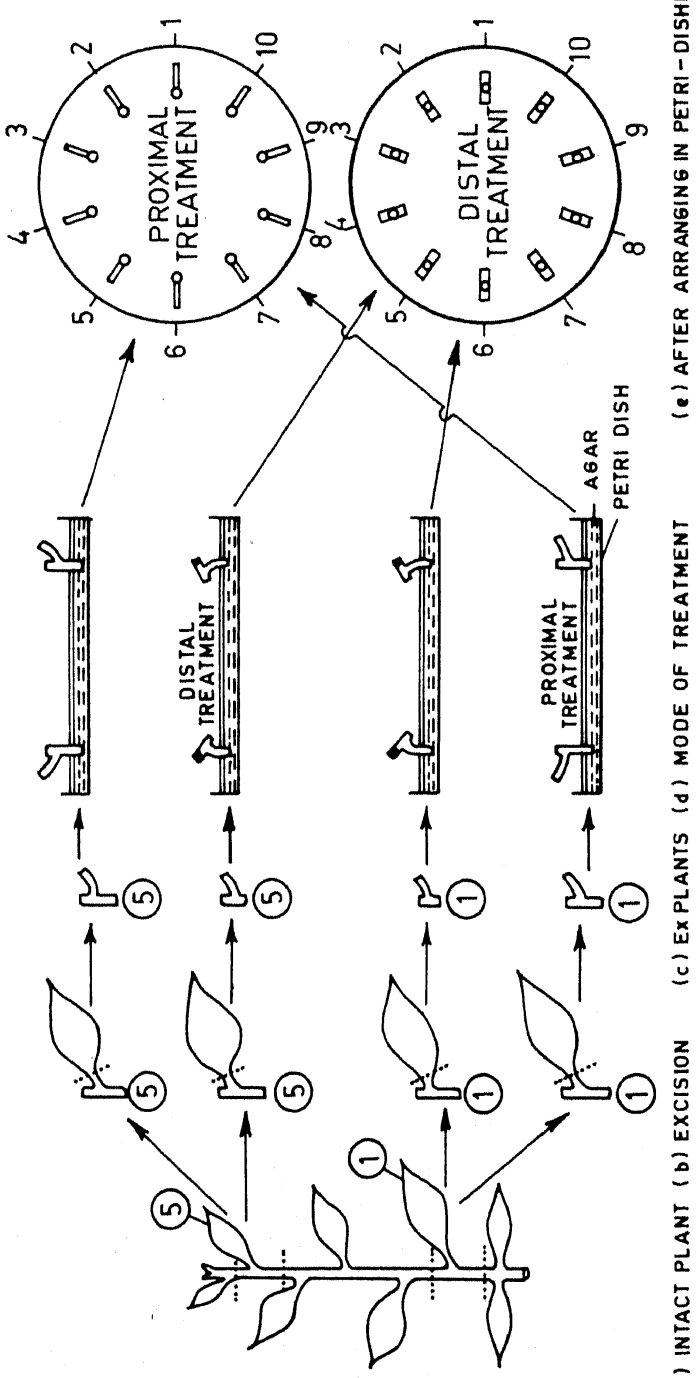

Figure 1. Diagrammatic outline of the method employed for excision, trimming and arranging petiolar explants for treatments and observations on abscission.

(No. 5) was retarded significantly by NAA applied on either sides. Benzyl adenine (BA-20 mg/l) and morphactin (CME-20 mg/l) retarded abscission of petiolar explants irrespective of explant age and mode of application in both the varieties. CME proved to be the most effective retardant of abscission (figure 2). Gibberellic acid (GA_3-100 mg/l) accelerated abscission on distal application and retarded the process when applied proximally irrespective of the age of explants. Ethephon (ETH-200 mg/l), abscisic acid (ABA-20 mg/l) and ascorbic acid (AA-200 mg/l) accelerated the abscission of petiolar explants of both ages and varieties on distal as well as proximal applications. ETH proved to be relatively the most effective accelerant of explant abscission.

3.2 *Responses to combinations of abscission retardants*

Combination of NAA and BA retarded abscission of petiolar explants of both ages and varieties on distal as well as proximal applications. The interaction of these two growth

Table 1. Pre-abscission periods (hr) of petiolar explants No. 1 and 5 in response to treatments with combinations of growth substances in *Capsicum annuum* L.

	Var. NP 46-A				Var. Pusa jwala			
	Ex. Petiole No. 1		Ex. Petiole No. 5		Ex. Petiole No. 1		Ex. petiole No. 5	
Treatments	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
Control (hr)	50·40 ±3·15	48·44†† ±3·39	56·50 ±3·19	62·20 ±3·70	46·00 ±2·59	46.66†† ±2·61	61·33 ±2·39	58·80†† ±2·49
NAA + BA	78·00 ±3·22	109·20 ±3·17	111·60 ±3·26	111·60 ±3·26	84·00 ±2·23	96·20 ±3·11	121·33†† ±0·17	106·80 ±3·14
NAA + GA	83·33†† ±3.59	30·00 ±2·21	94·20 ±3·09	19·20 ±2·78	94·80 ±2·67	25·20 ±2·16	94·80 ±2·98	13·20 ±1·26
NAA + ETH	13·20 ±1·26	14·40 ±1·31	26·40 ±2·33	18·00 ±2·67	22·80 ±2·27	13·20 ±1·26	22·80 ±3·29	36·00 ±2·33
NAA + AA	33·60 ±2·36	97·33** ±2·99	36·00 ±2·66	111·60 ±3·26	31·20 ±3·27	94·80 ±2·91	30·00 ±3·26	110·40 ±2.46
NAA + ABA	15·60 ±1·49	13·20 ±1·26	24·00 ±2·59	20·40 ±2·19	20·40 ±2·41	24·00 ±2·58	23·00 ±2·17	20·40 ±2·46
NAA + CME	130·80 ±2·91	132·00†† ±2·97	120·20 ±2·78	120·00 ±2·79	147·40 ±3·26	130·60** ±2·97	135·60 ±2·99	141·44†† ±3.2
BA + GA	86·40 ±3·61	97·20 ±2·31	111·80 ±3·41	112·80 ±3·52	96·66** ±3·01	96·00†† ±2·96	118·80 ±3·27	134·40 ±3·08
BA + ETH	24·00 ±2·59	13·20 ±1·20	24·00 ±2·69	14·40 ±1·31	13·20 ±1·26	14·40 ±1·68	14·40 ±1·68	13·20 ±1·26
BA + ABA	84·40 ±3·31	97·50*** ±3·13	115·20 ±3·33	123·60 ±2·94	88·80 ±3·17	96·00 ±3·21	121·20 ±3·03	121·40 ±3·01
BA + CME	141·33** ±3·17	139·77†† ±2·99	123·60 ±2·94	121·33†† ±3·14	130·66†† ±3·37	141·60 ±3·61	137·33†† ±3·30	120·00 ±2·98
BA + AA	97·20 ±3·18	98·40 ±3·13	122·20 ±3·18	120·00 ±3·16	134·00 ±3·41	122·40 ±3·29	124·80 −3·48	135·60 ±3·44
Control (hr)	50·40 ±3·15	48·44 ±3·39	56·40 ±3·19	62·20 ±3·70	46·00 ±2·59	46·66†† ±2·61	61·33 ±2·39	58·80†† ±2·49

Table 1 (*Continued*)

Treatments	Var. NP 46-A				Var. Pusa jwala			
	Ex. Petiole No. 1		Ex. Petiole No. 5		Ex. Petiole No. 1		Ex. petiole No. 5	
	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
GA + ETH	12·00	15·60 ±1·93	19·20 ±2·81	12·00	13·20 ±1·26	13·20 ±1·26	25·20 ±2·27	24·00 ±2·31
GA + ABA	12·00	13·20 ±1·26	24·00 ±2·59	18·00 ±2·79	22·80 ±2·11	22·80 ±2·81	13·20 ±1·26	25·20 ±2·17
GA + CME	117·80 ±2·16	121·50†† ±2·17	109·33†† ±2·98	106·80 ±2·11	93·60 ±3·38	118·80 ±3·07	91·20 ±3·00	73·33** ±2·98
ETH + AAA	84·00 ±3·19	22·80 ±2·41	96·00 ±3·17	21·60 ±2·78	73·20 ±3·10	21·60 ±2·37	96·00 ±3·21	13·20 ±1.26
ETH + ABA	32·22†† ±1·78	15·60 ±1·17	15·60 ±1·19	13·20 ±1·26	12·00	13·20 ±1·26	25·20 ±2·71	25·20 ±2·98
ETH + CME	91·20 ±3·41	88·80 ±2·91	121·20 ±3·17	122·40 ±3·41	93·80 ±3·22	88·00†† ±3·19	118·80 ±3·21	122·40 ±3·23
ETH + AA	13·20 ±1·26	13·20 ±1·26	22·80 ±2·41	23·00 ±2·36	13·20 ±1·26	21·33 ±2·79	24·00†† ±2·81	12·00
ABA + CME	121·20 ±3·17	106·50 ±2·11	94·66†† ±3·19	97·20 ±3·20	122·40 ±3·11	110·66** ±2·79	120·00 ±3·09	134·40 ±3·39
ABA + AA	21·60 ±2·39	23·60 ±2·42	12·00	26·40 ±2·39	12·00	13·26 ±1·26	21·60 ±2·13	25·20 ±3·21
CME + AA	120·00 ±3·19	117·33** ±3·25	132·00 ±3·33	97·20 ±3·28	123·60 ±3·22	95·00 ±3·42	94·55** ±3·01	78·00 ±2·78

All figures are secondary averages of 10 (except those marked by ** or *** and †† or †††).

** – Average of 9 figures
*** – Average of 8 figures
} Abnormally long pre-abscission period.

†† – Average of 9 figures
††† – Average of 8 figures
} Browned and died during experiments

LSD for NP 46-A = 6·82 (5% level of significance)
LSD for Pusa jwala = 7·39 (5% level of significance)

substances proved to be additive in enhancing the pre-abscission period. Extensive retardation of abscission by NAA + CME and BA + CME combinations also furnished the phytogerontological evidences of additive interaction of component growth substances in mixtures (table 1).

3.3 *Responses to combinations of abscission accelerants*

GA_3 when tested with ETH and ABA accelerated abscission on distal as well as proximal applications showing additive interaction in a majority of cases. However, in combination with AA, GA_3 maintained its abscission retarding action on proximal application. The accelerating action of distally applied GA_3 was not promoted by AA in NP 46-A and Pusa jwala varieties. Combinations like ETH + ABA, ETH + AA and ABA

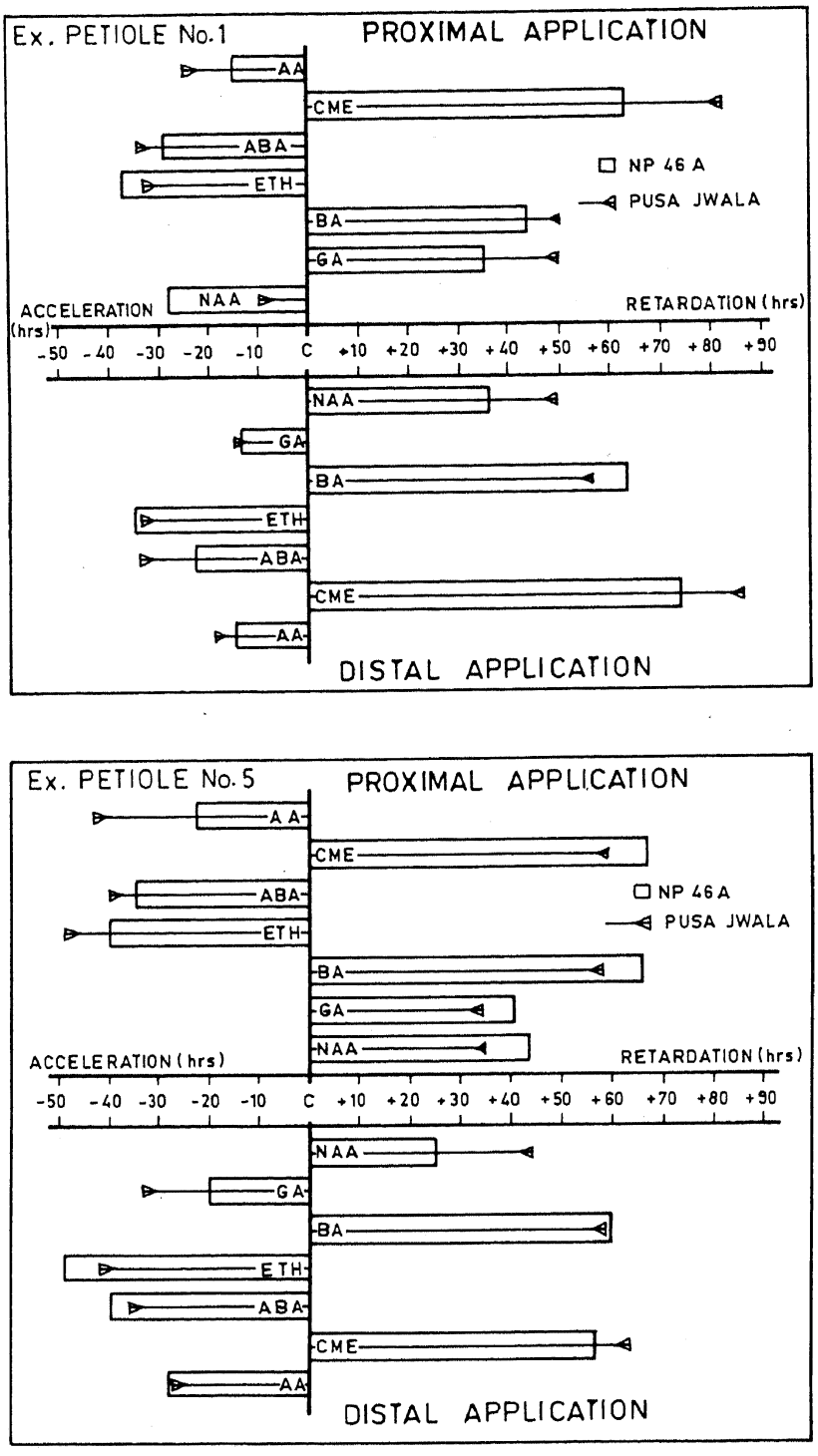

Figure 2. Percentage of changes in pre-abscission periods of *Capsicum* varieties petiolar explants in response to treatments with individual growth substances.

+ AA, though accelerated abscission significantly, none of these combinations showed additive interaction (table 1).

3.4 *Responses to combinations of abscission retardants and accelerants*

The interaction of NAA and GA_3 was much more interesting because GA_3 showed an over-riding effect on NAA action. This could be evidenced from the observations that the

retarding effect of GA_3 on proximal application and accelerating effect of GA_3 on distal application were maintained even when NAA was simultaneously applied. NAA failed to counteract significantly the strong abscission accelerating effects of ETH and ABA too. Combination of NAA with AA accelerated abscission on proximal applications while distal application retarded abscission in all cases indicating the failure of AA to reduce or modify the effect of NAA in the process of explant abscission (table 1). GA_3 could not modify the abscission retarding action of BA. BA also retarded abscission in the presence of AA and ABA, both of which could reduce its action only partially. CME strongly retarded abscission in combination with all abscission accelerants which could reduce the action of CME only insignificantly.

4. Discussion

The abscission retarding tendency of synthetic and natural auxins when applied to petiolar stumps has also been reported by several workers (Addicott 1970; Singh and Murty 1983). Louie and Addicott (1970) had concluded that factors other than auxins and auxin-gradient were involved in the regulation of abscission and Biggs and Leopold (1957) also suggested that age of explants and concentration of growth substance are important determinants of abscission response. Present studies with *Capsicum* petiolar explant system did not support the traditional auxin-gradient theory of abscission. The effects of GA_3 reported here support the findings of some earlier workers (Chatterjee and Leopold 1964; Wittwer and Bukovac 1968). Retardation of abscission by cytokinins has also been reported by Davenport *et al* (1979). Murty and Prakash (1975) have also shown a strong abscission retarding action of morphactin in cotton (*Gossypium hirsutum* L). Acceleration of abscission by ETH, ABA and AA treatments as observed in the present studies is consistent with the observations of Abeles (1973), Milborrow (1974) and Murty and Prakash (1975). The additive interaction of auxin and cytokinin in the retardation of explant abscission has also been reported by Chatterjee and Leopold (1964). The abscission responses to combinations of auxin with morphactin and of cytokinin with morphactin are in agreement with our earlier findings (Singh and Murty 1983). However, the mechanism of action and interaction of morphactin with other hormones is yet to be established (Parups 1983). The shortening of pre-abscission period of petiolar explants by gibberellin and ethylene combination was also reported by Burg (1968). The enhancement of ABA action by ETH has also been reported by Milborrow (1974). Results of interactions of GA + AA, ETH + AA and ABA + AA are in accordance with the observations of Cooper *et al* (1968) on several plant materials.

The interpretation of results of combination of NAA and GA is difficult as individually both these growth regulators have shown opposite actions in abscission on proximal and distal application. The over-riding effect of ETH in auxin and ethylene combinations was also reported by Burg (1968). The results of interactions of auxin with other abscission accelerants like ABA and AA seem to support the findings of Murty and Prakash (1975) on cotton leaf explants and of Chang and Jacobs (1973) on *Coleus* petiolar explants. The partial counteraction of the abscission retarding action of cytokinin by gibberellin was also reported by Murty and Prakash (1975) in *Catharanthus*. However, the mechanism of this interaction is still obscure (Addicott and Wiatr 1977). Partial or complete counteraction of the effect of cytokinin by abscission

accelerants like ETH, ABA and AA has also been reported by earlier workers (Abeles 1973; Milborrow 1974). Results obtained for morphactin and gibberellin interaction are similar to those reported by Murty and Prakash (1975). The responses of petiolar explants to combinations of morphactin with abscission retardants supported the findings of earlier workers (Milborrow 1974; Parups 1983). The method employed in the present investigations is simple, cheaper and can be adopted for such studies in an ordinary laboratory not having sophisticated equipments/facilities.

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