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EFFECT OF GIBBERELLIN, AUXIN AND THEIR INTERACTION ON THE PHYSIOLOGY OF ABSCISSION IN COTTON PLANT UNDER NORMAL AND WATER STRESSED CONDITION

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Abstract.

The effects of IAA and GA_3 separately and in combination were studied on abscission of debladed petiole in cotton plant (G. hirsutum L. var. M. 100). In another series of experiments water stress condition was also included in above mentioned treatments.

Gibberellin (GA_3) was found to be most effective in causing an early abscission, whereas Auxin (IAA) was found responsible for delaying this process. The effect of GA_3 was offset by higher concentration of auxin. Early abscission observed under water stress condition was not only sustained by the application of IAA but was rather delayed. Simultaneous application of GA_3 and IAA during water stress was found to be most effective in delaying abscission.

Introduction

Abscission is a physiological phenomenon and is controlled by many internal and external factors. Several classes of substances are known which promote or retard abscission in explants. These substances include various amino acids, auxins, abscisic acid and ethylene gas etc. Interaction of hormones also modify the situation. Some reviews have appeared in literature and the subject has been studied with different angles. (Jacobs, 1962, 1968; Burg, 1968; Morre, 1968; Cooper *et al.*, 1968, Addicott 1970, Morgan & Durham 1975).

Water stress also plays a leading role in the formation of abscission layer, shortage of water could be created in the plant by high ambiant temperature, wind drift or poor soil water conditions. Stress thus created may hasten the last phase of abscission causing an early separation of cellular tissue. It also controls auxin and gibberellin level in plants (Tal and Imber, 1971), and causes a dramatic accumulation of abscisic acid. Some evidences suggest that abscission induced by water stress may be due to internal ethylene production. McMichel *et al.*, 1972 reported that petioles of intact cotton plants tended to increase ethylene production within an hour, when water deficit developed and declined quickly on rewatering in some cases.

In Pakistan abscission causes a heavy damage to the cotton yield every year. Cotton plant develops the average 500 square buds and flowers, and matures on 20 to 30 bolls, the rest are shed at one stage or the other (Proc. Cent. Pak. Cotton Committee 1975). Causes for such a high rate of buds, flowers and fruits drops are still to be investigated. The present study is an effort in this direction.

Material and Method

Cotton plants (*Gossypium hirustum* L. var. M. 100) were raised in 8″ plastic pots containing sand with organic manure, irrigated with tap water. Plants were grown in controlled environment chamber having day temperature 90°F and night temperature 80°F with 12h photoperiod of 7000 lux provided by cool white fluorescent tubes supplemented with incandescent lamps.

Seedling used for different experiments were 4,5 and 6 week old having 5,7 and 9 leaves respectively. Second, third and fourth leaves were counted from the bottom leaving first and cotyledonary leaves.

Different concentrations of GA_3 or IAA (.01 to 100 ppm) depending upon the kind of experiment were applied through a drop of water to the freshly cut surface of 2nd, 3rd and 4th petiole of 4,5 and 6 weeks old plants respectively. Application of growth hormones were made only once just after the excision of leaf blades from petioles for each experiment. Cut surface was covered with grease after application to prevent water loss due to transpiration. Control plants were treated with distilled water in the same manner. Plants were keept under observation till depetiolation occurred. Petiole stumps were considered abscised when they could be dislodged with a force of approximately 5 gm applied to their ends.

In the interaction experiments 10 ppm GA_3 was added to a series of IAA (0.01-100 ppm) concentrations and applied in the same manner as mentioned above.

In the experiments dealing with water stress and hormone action, a set of pots were irrigated to saturation and water was allowed to drain down till the soil was left at the field capacity. Percentage of water left in the soil was ca'culated at every alternate day. Plants started showing wilting symptoms when the water was depleted down to approximately 8 percent, which was considered as stress condition.

Following treatments were given to the plants subjected to water stress:

(i) Stress (controls)
(ii) Stress+GA3 (10 ppm).
(iii) Stress+IAA (100 ppm).
(iv) Stress+GA_3 (10 ppm) + IAA (100 ppm)

Application of hormone and observation on abscission were made as described above.

Histological studies were performed in those treatments where formation of abscission layer was expected and defoliation was noticed. A portion of main stem with connecting petiole were fixed in FAA for histological studies after 24,32,40,48 and 56 hr. of 10 ppm GA_3 treatment. Sections were cut by rotary microtome at 10 u and stained with Heidenhain's iron hematoxylene and photomicrographs were obtained.

Observation and Results

Results of the experiment where GA_3 and IAA were applied distally (to the petiole stump) in concentration 0.01 to 100 ppm respectively shows that 10 ppm

GA_3 is most effective in promoting abscission, (fig. 1) whereas higher concentration of IAA (100 ppm) significantly delay the abscission in all the three petioles (fig. 2)

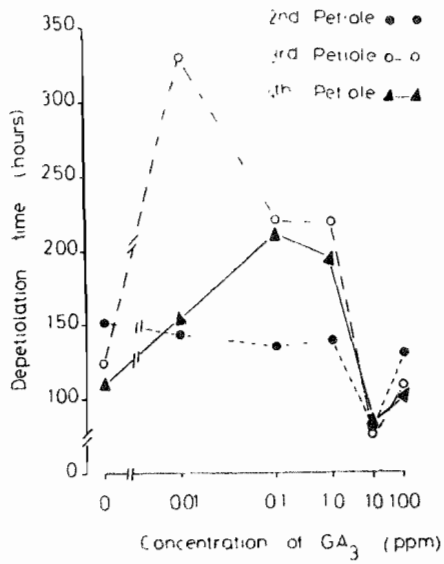

Fig. 1 Effect of various concenctrations of GA_3 on abscission of de-bladed cotton petioles.

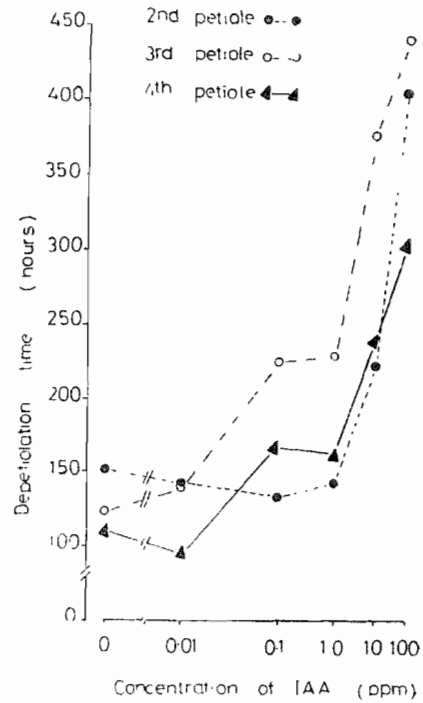

Fig. 2 Effect of various concentrations of IAA on abscission of de-bladed cotton petioles.

When 10 ppm GA_3 was applied with 0.01 to 100 ppm IAA in above mentioned experiments (Fig. No. 3), the combination of 10 ppm GA_3+IAA 100 ppm delayed abscission in all the three petioles. Effect of GA_3 was suppressed by higher concentration of IAA.

A combined effect of water stress and hormone interaction on the depetiolation is given in Fig. 4. Result show that GA_3 (10 ppm) strongly promote abscission in all the three petioles under stressed condition as well, where as IAA (100 ppm) was responsible for delaying abscission even under this condition. It is interesting to note that combination of GA3 (10 ppm) + IAA (100 ppm) is most effective in delaying abscission under water stressed condition.

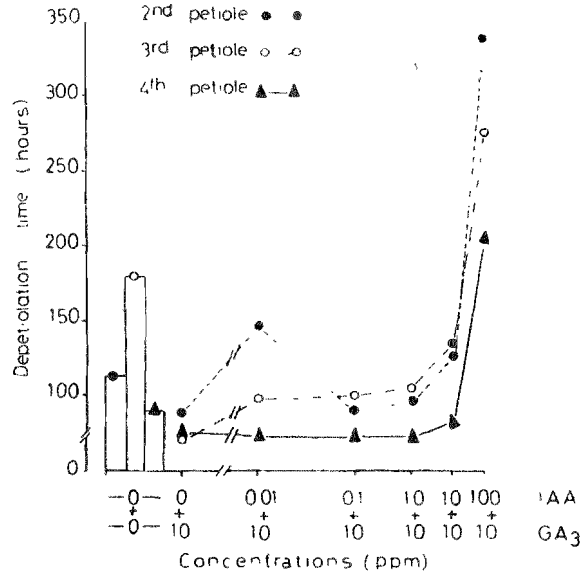

Fig. 3 Interaction of GA_3 and IAA on abscission of de-bladed cotton petioles.

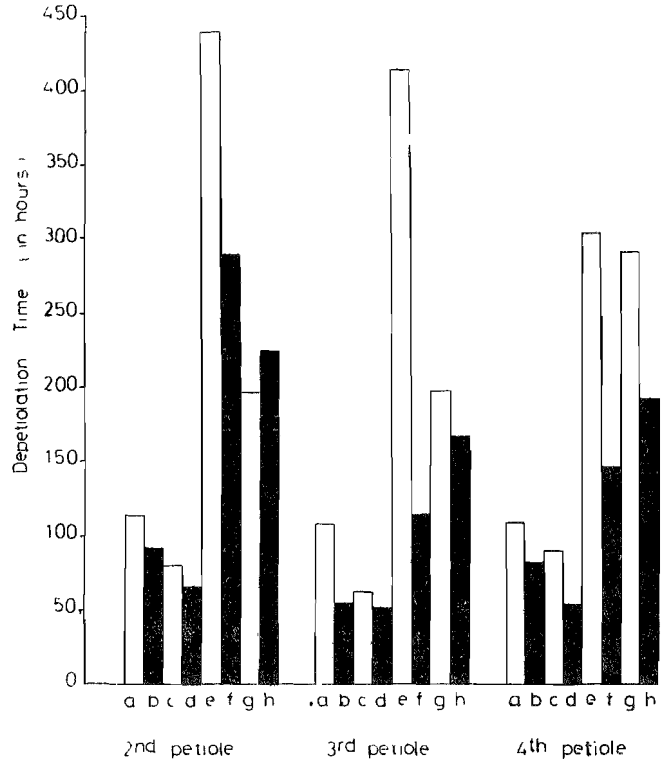

Fig. 4 Effect of phytohormones and their interaction on abscission of petioles in cotton plant under normal and water stressed condition.

Index: (a) Irrigated (Control), (b) Water stress, (c) Irrigated+GA_3 (10ppm) (d) Water stress+GA_3 (10 ppm) (e) Irrigated+IAA (10 ppm) (f) Water stress+IAA (10 ppm) (g) Irrigated+GA_3 (10 ppm)+IAA (100 ppm) (h) Water stress+GA (10 ppm)+IAA (100 ppm)

Histological Studies:

Photographs of longitudinal sections taken from the junction of the petiole after application of GA3 (10 ppm) are presented in Fig. 5.

Following stages of abscission layer formation are well illustrated.

(i) Well connected petiole through vascular bundles in normal plants (Fig. 5-A).

(ii) Induction of cell division prior to formation of abscission layer (Fig. 5-B).

(iii) Clear differenciation of abscission layer accompanied with subarization (Fig. 5-C&D).

(iv) Cleavage along cell wall of abscission layer leading to complete separation.

Discussion

GA_3 at higher concentration (10 ppm) markedly accelerated abscission in all the three intact petioles (Fig. 1). this is confirmed by photomicrographs of this zone (fig. 5). Similar result were observed by Chatterjee and Leopold (1964) on *Phaseolus vulgaris* explants, and by Lyon and Smith (1966) on cotton explants. At lower concentrations (0.01 to 1 ppm) GA3, abscission was delayed significantly except in 2nd petiole, where depetiolation was a bit early than controls. Carns *et al*, (1961) while using 14 days old cotton explants found an early abscission at higher concentration and delayed abscission at lower GA_3 concentrations.

Various therories have been put forward for the explaination of this behaviour. GA_3 is reported producing some hydrolytic enzymes (Chrespeels and Varner, 1967), which are responsible for an early abscission. This process is reported to be connected directly with metabolic destruction of endogenous IAA through the increase in IAA-oxidase activity (Schwertner and Morgan, 1966).

Following sequence may be operative:

(i) In the presence of higher concentration of gibberellic acid an increase in endogenous level of auxin occurs, (Kuraishi and Muir, 1964 and Muir and Valdovinos 1970).

(ii) Which initiate the production of ethylene (Abeles and Rubinstein, 1964 and Abeles, 1967).

(iii) As a result some hydrolytic enzymes are synthesized (Schwertner and Morgan 1971).

(iv) Hence auxin transport is inhibited (Carns, 1966, Beyer and Morgan 1971), which was otherwise responsible for delaying abscission. Enzyme synthesized in above mentioned sequence are seen deposited on cell walls in electron microscope (Sexton and Hall, 1974). These enzyme are considered responsible for the formation of separation layer (Abeles *et al*, 1971)

Experiment with 100 ppm GA_3 does not show any significant effect on abscission of all the three petioles (Fig. 1). At this concentration the effectiveness of GA_3 is reported to be blocked by anti-auxin (Cleland, 1964).

Application of IAA at debladed petiole of explant has been found effectively delaying abscission (Lewis and Bakhshi 1968; Luie and Addicott, 1970). The applied auxin fills in the gap caused by deplition of natural auxin synthesis in leaf blade (Wetmore and Jacobs 1953). Increase in applied auxin concentration is directly proportional for inhibiting abscission except in 2nd and 4th petiole where the effect is slightly delayed in lower concentrations. (Fig. 2)

A critical observation indicates that magnitude of IAA response is some what different in various petioles according to their positions. This might have to do something with the age of leaves as well. Craker *et al.* (1970) have suggested that auxin (IAA) acts as an aging retardent and inhibit abscission. It prevents the metabolic changes necessary for the formatic n of enzymes responsible for degration of cell content in the separation layer. Degrading enzymes plays a major role in controlling abscission but they act at a particular stage. Craker and Abeles (1969) have divided this phenomenon in three stages. In this connection, Rubinstein and Leopold (1963) pointed out that auxin (IAA) could either block stage No. 1 by postponing the start of aging period or stimulate No. 2 causing an early abscission. It has been shown that lower concentration of IAA (10 to 100 ppm) provide enough auxin to overcome destructive activities resulted by ethylene.

Interaction between auxin and gibberellin is reported to control the mechanism of abscission in many plants. (Chatterjee and Leopold, 1964; Jacobs and Kirk 1966). Present investigation indicates that effect of increasing concentration of IAA from 0.01 to 10 ppm was strongly suppressed by GA_3 (10 ppm) and it significantly delayed the abscission in all the three petioles (Fig. 3)

In the light of above mentioned role of GA_3 as stimulator of hydrolytic enzymes, it appear that low concentration of substrate(IAA)is present in the system is used up pretty soon and hence the auxin is not able to show off its physiological effect. Whereas if high concentration of substrate (IAA) is present, suffiicent amount of auxin is available for preventing abscission. Thinking interms of enzymes and substrate relationship, higher concentration of substrate is always inhibitory for an enzyme action.

Effect of water stress accompanied with the activities of IAA and GA_3 separately and collectively on abscission is given in Fig. 4. Following conclusion could be drawn from it.

(i) Plant under stress condition always shed their petioles earlier than controls.
(ii) Early abscission brought by GA_3 in normal condition is accelerated in stress.
(iii) Delayed abscission due to the application of IAA is offset under stress condition.
(iv) Effect of early abscission under stress condition is offset by the application of IAA upto certain extent.
(v) The net outcome of IAA and GA_3 interaction in delaying abscission is also reduced under stressed condition, excpet in 2nd petiole.

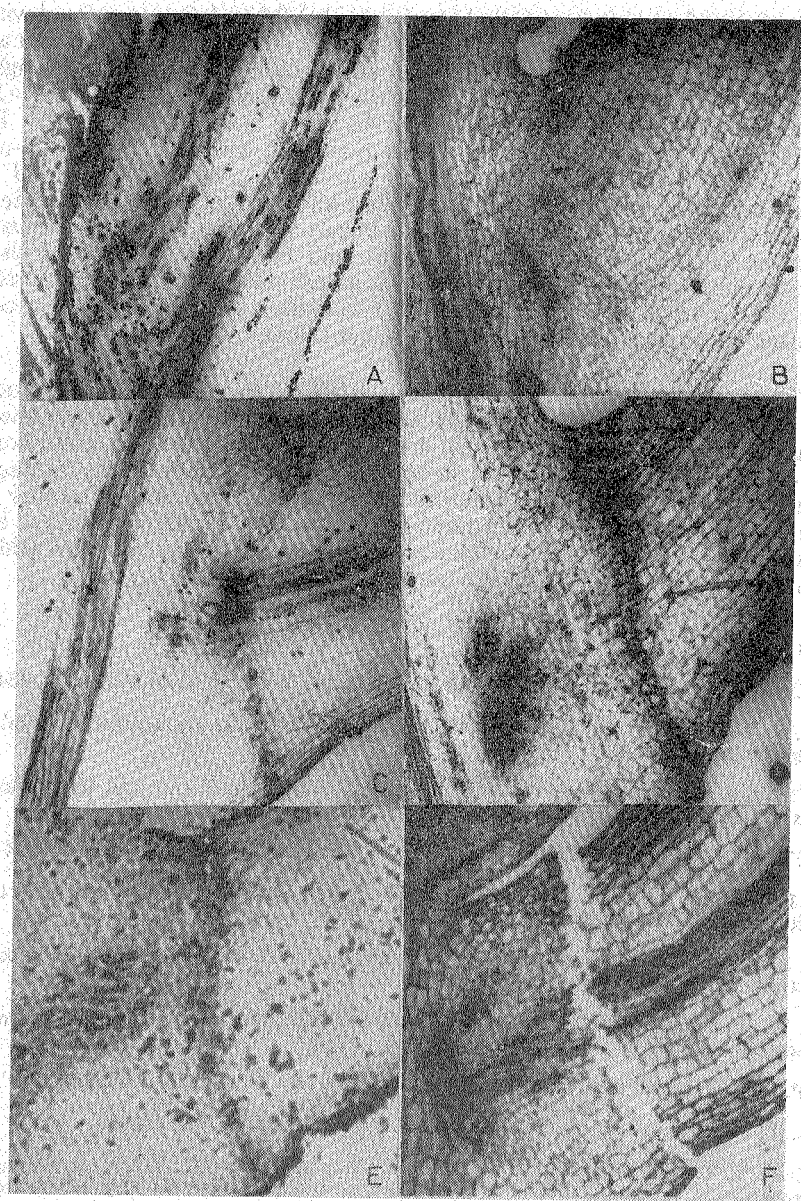

Fig. 5 Longitudinal sections showing gibberellin (GA3) induced abscission layer at joints connecting petioles with the stem of cotton plants.

A. Well connected petiole through vascular bundles in normal plants X 56.
B. Induction of cell division prior formation of abscission layer within 24 hr. X 74.
C & D. Clear differenciation of abscission layer accompanied with subarization within 32 hours to 40 hours X 90.
E & F. Cleavage along cell wall of abscission layer leading to complete separation within 48 hours to 56 hours. X 120 and X 112.

Another endogenous compound abscisic acid (ABA) is being associated with abscission since last few years (Addicott *et al*, 1962, Smith *et al*, 1968). A significant accumulation of ABA has been noticed under stressed condition (Wright, 1969, Wright and Hiron, 1969). ABA is shown responsible for the inhibition of auxin transport (Naqvi, 1972), through ethylene production (Carns 1966; Beyer and Morgan, 1969). Derbyshire (1971) found retardation in auxin levels associated with increase in IAA-oxidase activity in stress condition causing an early abscission. McMichael *et al* (1972) found that ethylene production increases within an hours of starting water deficit in the petioles of intect cotton plants. Increase in peroxidase is also found accompanied with increase in IAA-oxidase activity (Morgan and Hall, 1968). Ethylene also activate the synthesis of hydrolytic enzymes such as cellulase or pectinase, (Horton and Osborne 1967, Morcus, 1971). These enzyme secreation are deposited on the cell wall of abscission zone, thus forming separation layer (Abeles, *et al*. 1971).

The combine effect of GA_3 and stress in all the three petiole (Fig. 4) might be due to two fold production of ethylene which is responsible for an early abscission. Application of higher concentration of IAA (100 ppm) under stress condition show definite delaying effect on abscission in all the three cases. This may be due to the fact that the amount of ethylene, produced under stressed condition is smaller in comparision with amount of IAA applied and hence even if part of it is destroyed by ethylene, enough is left behind to delay the abscission. Stress in combination with IAA and GA_3 delay the abscission due to some unknown factors.

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