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THE PHYSIOLOGICAL ASPECTS OF ABSCISSION WITH A REFERENCE TO COTTON PLANT

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The term abscission implies detachment of plant parts, e.g. leaves, flowers, fruits and seeds. Other examples of abscission are, the shedding of bark, and the dehiscence of anthers. Abscission is considered to be the result of a complex interaction of endogenous ethylene, auxins, senescence factors, and possibly gibberellins and kinetins.[7,19] Thus ethylene produced a negative interaction in cotton by inhibiting transport of auxin to stems and petioles while causing the plant to abscize leaves, squares, bolls and blooms.[50,51] The shedding of squares and bolls in cotton is one of the important factors affecting yield of fibre since from as low as 10% to as high as 100% of the young bolls per plant may be shed. Considering the large number of floral buds produced, as much as 5000 lb lint per acre could be expected, if all developed to maturity.[11] The shedding of floral buds and bolls may depend upon such factors as varietal differences, insect injury, environmental conditions and a host of others.

Genetic Factors

Shedding of flower buds and bolls is considered to be a heritable character.[17,37] Thus by crossing Americo–Egyptian cotton, Pima and Upland cotton Acala, for example, shedding of flower buds and young bolls in cotton was partially genetically controlled.[44,45] Harland[37] isolated by selection high and low-shedding strains of sea island cotton; he observed, however, that by continued selection of plants, which produce their first flower on the first node of the first fruiting branch, tendency to shedding could be greatly minimised. However, in selecting for non-shedding, according to Christidis and Harrison,[23] plants with high transpiration, photosynthesis, formation of vitamins, suction pressure of buds and bolls and well-developed root system be chosen.

Physiological Factors

Physiological disturbance in plant in response to soil and climatic conditions may cause shedding in cotton.[38] For example, in Upland cottons, grown in two areas of Pakistan namely Punjab and Sind, boll shedding resulted from lack of fertilization, as anthers failed to dehisce in the beginning of flowering season,[27,60] failure of bolls to mature has, in some situations, been attributed to variation in a particular photoperiod. On the other hand, boll shedding is reported to be auxin-dependent and has been reported prevented by the application of exogenous hormones.[12] Thus various studies on the mechanism of abscission have resulted in 'auxin theory', 'IAA–oxidase inhibitor system', 'abscisic acid theory, 'hormone–ethylene balance', and 'nutrient balance theory'.

Auxin Theory. Addicott, Lynch and Carns[10] proposed that auxin was the principal regulator of abscission. Thus exogenous auxin IAA applied to stem (proximal) accelerated, but that applied to petiole stumps (distal), retarded abscission.[9] Jacobs[42] and his coworkers suggested that a leaf, in the regular course of growth, has its abscission time determined by the auxin produced in its blade. When auxin production in the blade is sufficiently low, auxin production of stem takes effect to bring about abscission. This was termed as 'auxin–auxin' balance.

By using a variety of different types of explant and α-naphthalene acetic acid (NAA) as exogenous growth promoter, Leopold and his coworkers failed to confirm the major thesis of auxin-gradient theory, i.e. distal auxin retards abscission while proximal auxin accelerates it. On the other hand, Gaur and Leopold[31] suggested that both distal and proximal auxin brought about abscission; low concentrations of NAA (10^{-5}M) initiated abscission while high concentrations of NAA (10^{-3}M) retarded it. The distal effect has been shown to be smaller than the proximal one. That the differences were real has, however, been confirmed in many plants by many workers (beans: Biggs and Leopold;[14] Chatterjee and Leopold;[22] cotton: Lyon[48]). In brief, this work formed the basis of two phase theory of auxin abscission, i.e. abscission depends on the total amount of auxin reaching the abscission zone. Rubinstein and Leopold[56] divided the response of bean explants to NAA into two stages—the first or induction stage, which is inhibited by auxin and the second stage which is promoted by similar auxin concentrations. They consider the two stage effects similar to 'two phase' effects in that the promotion of abscission by distal low auxin, could be the consequence of the 'amount of auxin just low enough to allow the induction stage to proceed to completion, yet high enough to stimulate the second stage'.

However, using cotton explants and indole acetic acid (IAA), Greenblatt,[35] and Carns[19] obtained data that differed from those of Leopold and his coworkers. A necessity of work with more plant species and IAA to confirm what led to formulate the auxin gradient theory has, therefore, been suggested.[19]

Hormone–Ethylene Balance. It has been suggested that leaf abscission is regulated by a balance of hormone (auxin) and ethylene in the leaf. Working with debladed petioles, Gawady and Avery[32] pointed out

that auxin retarded abscission: it diminished though with the maturity of leaf. Ethylene may be produced by leaves and certain fruits, which accelerates agings or ripening.[13,39] Regarding the mechanism, it was suggested that auxin retards acceleration of abscission of debladed petioles by ethylene, or ethylene chlorohydrin.[36] A bulk of evidence supports the auxin part of the theory. But data supporting the role of ethylene in abscission are largely of recent origin and for the first evidence of the role of ethylene in abscission see review by Burg.[18] For example, in *Phaseolus* explants,[1] abscission correlated with increased ethylene synthesis and this received support from many observations[1,5,6,26] in that almost all abscission accelerators act by inducing ethylene production in plants. However, the evidence that substances like auxins, gibberellins and cytokinins that can induce ethylene production protect at the same time, treated tissue from the effect of ethylene, makes interpretation of the observed correlations difficult. Pratt and Goeschl[53] doubt the validity of any attempts to use results from explants to explain abscission of intact plant organs, since the production of ethylene in freshly cut explants could mask the effects of applied ethylene. For example, the explants of *Phaseolus*[55] and those of cotton[1] appeared insensitive to ethylene while the latter caused rapid abscission of intact cotton leaves[36] (including the young leaves).

The 'aging-ethylene' hypothesis stresses the primary role of ethylene being to accelerate the formation of enzymes responsible for cell separation. However, like auxins, ethylene has a good number of effects which may or may not contribute to abscission directly (e.g. breakdown of proteins, acceleration of IAA inactivation and of the loss of pectin methylesterase in the abscission zone during abscission). In addition, senescence factors, may also make difficult the understanding of the mechanism by which ethylene may induce abscission. Thus in the abscission of bean explants, the role of ethylene in accelerating aging of abscizing zone has been established in addition to its effects on inducing cellulase activity.[4]

IAA–Oxidase Inhibitor System. Schwertner[57] suggests that IAA–oxidase inhibitor system is involved in abscission. After *in vitro* studies on explant cotton, he concluded that other cofactors such as 2,4-dichlorophenol accelerated abscission while the inhibitors retarded it. It was reported, however, that enzyme inhibitors were less effective in retarding abscission than the cofactors were in stimulating it. The only other evidence in favour of involvement of IAA–oxidase inhibitor system in abscission is that from Greenblatt:[35] however, according to it the inhibitor caffeic acid, at concentrations higher than those used by Schwertner,[57] accelerated rather than retarded abscission. Depending on its concentrations, the inhibitor catechol accelerated as well as slowed down the process. The possibility of its involvement in abscission, therefore, remains to be established.

Abscisic Acid Theory. Carns, Hacskaylo and Embry[21] isolated, purified and identified two growth retarding substances of plant origin, namely abscisin I and abscisin II which appeared to be closely related.

The term abscisin was given to specific chemical compounds isolated from the burs of mature cotton. The term was replaced later by abscisic acid (ABA) at a special session of the 16th International Conference on plant growth substances, Ottawa 24–28 July 1967.

The activity of abscisic acid was found in diffusates collected from the base of cotton ovary on the day of anthesis. The activity increased in diffusates daily until a maximum was obtained between the fifth and tenth day—the period corresponding to major fruit shedding. According to Addicott *et al.*[9] cotton varieties with higher percentage of fruit drop were found to exhibit higher activity.

Most experiments on abscission in cotton involved intact foliage or leaves, intact fruits, and defruited pedicels.[9] These investigations were extended to include work on abscission acceleration of petiole stumps of cotton explants and others.

The effects of abscisic acid on cotton explants were acceleration of ethylene production and increasing of the activity of cellulase.[25] Although the effects of ABA on leaves were also similar, it usually required more than one application to cause their abscission. In addition, applications of ABA to plants which were young and vigorously growing sometimes failed to bring about abscission. For example, citrus leaves sprayed with ABA in warm weathers abscised but those sprayed in winter failed to do so.[24] Leaf responses to ABA, here, were season dependent, due perhaps to fact that endogenous hormones with which ABA may be interacting to cause abscission varied greatly with environmental factors. The evidence for its direct involvement in abscission, therefore, remains to be produced.

Gibberellins and Kinetins. Some recent work has demonstrated that gibberellic acid accelerates abscission.[20,22] For example, in bean plant, gibberellic acid at concentrations of 10^{-7}M to 10^{-3}M, promoted abscission[22] while in *Coleus* it promoted abscission when applied to petiole stump both in the presence and the absence of inhibiting amounts of IAA (Jacobs and Kirk after Carns[19]). The latter effect was particularly marked in younger petioles.

Numerous studies have suggested that gibberellic acid could be very mobile—penetrating the stem quickly and bringing about the accelerating effect of proximal auxin.

For want of substantial evidence the role of kinetin in abscission is not clear.[19]

Nutrient Balance Theory. It is considered that the percentage of fruits abscised per plant remains remarkably constant, though yield of cotton fruit per plant varies greatly with the nutrition of plant. However, under certain conditions extreme imbalance of carbohydrate and nitrogen may affect abscission. In some cases, cotton abscised the young fruits that it could not supply with adequate carbohydrate and nitrogen:[29,49] in others, abscission was quite high even though there was no evidence of the shortage of carbohydrates and nitrogen.[29]

It is observed that some amino acids acted under certain circumstances as promoters of abscission (glutamic acid and alanine: Rubinstein and Leopold;[56] methionine: Yager and Muir[63]). Yager[62] suggested that pectin methylesterase retarded abscission when stimulated by high IAA and that the effect of me-

thionine in accelerating abscission could be due to its acting as 'a methyl donor in the solubilization of pectic substances in cell walls'. By contrast, the effect of auxin stimulation of pectin methylesterase in retarding abscission is considered to help make sites available for calcium, thus stabilising cell walls and retarding abscission. While methionine is one precursor of ethylene, its abscission accelerating effects could arise from stimulation of ethylene synthesis. On the other hand, the effects of ethylene in abscission have been suggested to arise from its control of specific RNA's and protein synthesis.[40,2,61] Thus it seems, as viewed by Carns;[19] that at least certain amino acids could be directly involved in abscission while others could be serving to promote or retard the process.

Elsewhere, hormone production in developing fruit has been related to transport to, and concentration of nutrients in the fruits. According to Luckwill[47] older fruits produced more auxin than the younger fruits and nutrients were transported to and concentrated in the regions containing more auxin. As a consequence younger fruits would receive few nutrients and produce less auxin, thus causing abscission.

Carbohydrates affect abscission over a wide range of experimental conditions.[14,34] Carns[19] views that the abscission process is energy dependent and the role of carbohydrates could be one of participation in the biosynthesis of specific intermediates.

Anatomy

Anatomical studies on abscission report the presence of abscission zone at the base of abscizing organs. An abscission zone is differentiated prior to the onset of abscission—being characterised by smaller cells towards the maturity of the abscizing organ. The intercellular spaces of most cells in the abscission zone disappear. The protoplasm becomes denser with cell division commencing (cotton: Bornman,[15,16] *Phaseolus vulgaris*: Webster[61]). The separation is accompanied by softening of cell walls and the solubilization of certain cell wall constituents. The process of cell wall breakdown is well established for cotton and many other plants.[8] In some studies the occurrence of cell division in the separation zone (leaf abscission: Gawady and Avery;[32] style abscission in citrus: Goldschmidt and Leshem[33]) has not been reported to be a part of abscission. However, the changes involved in the separation layer include swelling of cells proximal to the separation layer[19,61,33] and of increased activities of hydrolytic enzymes such as cellulase[3,41] and pectin esterase[54] in the separation layers. Thus dissolution of cementing substances between the cells takes place to affect separation. This may involve from a single layer of cells to that of many layers.[19]

The cell separation resulting from the chemical alteration of cell walls and their breakdown is well-documented.[30] Thus staining of freshly-cut cotton explants with alkaline hydroxylamine–$FeCl_3$ showed localization of pectic substances mainly in the middle lamella. The staining of pectic substances in the walls of the cells of separation zone decreased prior to breakage (though some golgibodies and mitochondria were as yet present). Similarly, the loss of pectic substances was marked in cotton explants treated with gibberellic acid and indole acetic acid. The loss of a proportion of hemicelluloses from cells of separation zone has also been reported. Thus cell wall breakdown in cotton occurred firstly, by the loss of pectic substances from cells of separation zone and secondly, by the breakdown of cell wall polysaccharides, thus bringing about weakening of cell walls and their ultimate rupture.[15,16]

In *Nicotiana*, Jensen and Valdovinos[43] noted plasmodesmata branching into the middle lamella of cell wall and postulated that dissolution of middle lamella could be brought about by plasmodesmata in the cells of separation zone.

Other aspects of interest in the fine structure of cell wall studies are, the occasional presence of certain cellular inclusions such as numerous microbodies with crystalloid cores in continuity with the cell wall in *Nicotiana*. The crystalloid cores in animal cells are reported to contain hydrolytic enzymes (rat liver cells: de Duve[28]). Thus much remains to be known regarding the nature of wall fractions involved in the separation of cells.

In addition, the observation that cell wall may invariably be involved in abscission stresses the importance also of such inorganic ions as would participate in the structural composition of cell wall. For instance calcium could be important for its well known role in the formation of pectin and other components of plant cell wall, protein synthesis, and meristematic activity in plants.[59] Whereas, in cotton an increased production of flowers and bolls has been related to high calcium nutrition of plants,[58] the observation that the plant requirement of calcium for growth in a number of dicots was twice as much as that of monocots,[46] assumes great significance. In addition the observation that 2,4-D had a tendency to induce ethylene production rather strongly in dicots (e.g. cotton) but only slightly in monocots (e.g. sorghum[52]) parallels the observation on calcium nutrition of dicots as compared to that of monocots. The other chemical element of equal importance in the nutrition of cotton could be boron for its role in cell division and pectin synthesis.[59]

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