Joint DPGRG and BPGRG Symposium - "Aspects and Prospects of Plant Growth Regulators" (1980) Monograph 6

THE ROLE OF PLANT GROWTH REGULATORS IN DRY MATTER DISTRIBUTION IN BULBOUS PLANTS

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1. Introduction

Bulbous plants are an extremely heterogeneous group sharing a characteristic ability to form subterranean storage organs, i.e., bulbs and corms. The stored material is composed predominantly of carbohydrates, about 65% of them on a dry matter basis in hyacinths and up to 90% in tulips. The main component of the carbohydrates is starch. The content of non-reducing sugars varies, ranging from 3 to 20% in tulips and from 11 to 17% in hyacinths. The amount of reducing sugars is rather low: for tulips in the range of 1 to 4% and for hyacinths 0.2 to 1.3%. The variation is dependent on the developmental stage of the bulbs and the environmental conditions, among which temperature is of decisive importance for development (1).

The storage organs grow mainly in a post-floral period of high photosynthetic activity. After this phase, the aerial parts of the plants usually die off and the bulbs can be lifted and stored in a dry condition. During storage the bulbs seem to be in a dormant state because they show hardly any change externally, but true dormancy occurs only in lilies and gladioli under certain conditions (2), the internal development proceeds continuously. During the period of dry storage, new roots, shoots, and daugther bulbs are re-initiated, after which the size of the organs increases steadily. In tulips, hyacinths, and daffodils, all leaves and the complete inflorescence and primordial stem internodes are present shortly after lifting, whereas in irises, gladioli, and lilies the initiation proceeds until most of the leaves are formed but the inflorescence is not formed until after replanting of the bulbs (3, 4).

The internal development is controlled by temperature: high and low temperature (25-30°C and 0-5°C) retard the formation and extension of the new organs and have a strong influence on dormancy release as well as on the induction of flowering or bulbing in newly formed buds.

Furthermore, cooling is obligatory to satisfy the cold requirement for maximal and rapid elongation of the shoot after the advent of warmer conditions.

The effects of temperature on the development have been studied extensively by Blaauw et al. (5); Beijer (6), Rees (7), LeNard and Cohat (8), and Slootweg (9). The principles of thermoperiodicity are applied for flower

production by forcing of the plants in glasshouses (10).

The first attempt to analyse the effects of temperature on the metabolism of bulbous plants were made by Algera (1, 11) who studied the carbohydrate metabolism and respiration of tulips and hyacinths in relation to development. After the discovery of plant hormones, several investigations were performed to analyse the effects of temperature on growth-regulator levels. This paper represents an attempt to arrive at a synthesis of the available information on developmental phenomena, dry-matter allocation, and the growth regulator status of the plants. Allocation of dry matter is indicated by the pull function of growing organs, which form sinks with a competitive relationship, and by the push function of not only the bulb scales, which mobilize and export their stored material, but also after leaf extension, of the photosynthetically active leaves. To answer the question as to the degree to which endogenous plant hormones control the distribution of dry matter, a review is given of the literature on endogenous growth regulators and effects of externally applied growth substances on dry matter distribution.

2. Endogenous growth substances

The presence and varying levels of endogenous growth regulators have been investigated in relation to the stage of development and temperature by several investigators. It is, however, difficult to distinguish relationships between developmental stage, growth-regulator status, and temperature, because of the complexity of developmental phenomena and problems encountered in the quantification of growth-substance activity in extracts. An increase of growth-promoting substances sometimes coincides with a corresponding increase of inhibitors, and an over-all increase of a given substance in all organs does not tell much about organ correlations.

2.1. Auxins

The first report on endogenous auxins in Lilium longiflorum (12) described a gradiënt of auxin activity in the bulb from a maximum in the stem tip to a minimum in the outer and older bulb scales. The level in the stem tip was about 1,000 times higher than that in the scales, and the levels in the stem, the basal plate, and the inner bulb scales were intermediate. Storage of the bulbs in moist peat moss, which activated shoot growth, led to increased auxin activity in regions with meristematic activity, e.g. the shoot and the basal plate were root primordia are formed.

Tsukamoto (13) found a considerable increase of auxin-like substances in the outer scales of Lilium longiflorum after cooling, whereas only very small amounts were present before cooling Rahkimbayev et al. (14) analysed tulip bulbs during cooling and found 'unimportant changes' of auxin levels

in the scales, the shoot, and the basal tissue. The most thorough investigations were made by Kaldewey (15, 16) and Edelbluth and Kaldewey (17) in Fritillaria meleagris and narcissus with respect to stalk elongation. Auxins were initially rather difficult to detect due to the interaction with inhibitors, but were eventually found in the flower stalk, synthesized mainly by the gynoecium but also by the more basipetally. located parts of the stem. A relationship with temperature treatments cannot be established from these studies, because only actively growing tissues were analysed.

In general, it can be concluded that auxin activity is stimulated in the floral organs and regions with meristematic activity by a rise in temperature after cooling.

2.2. Gibberellin-like substances

The gibberellin-like substances (GLS) have been given the most intensive investigation, i.e., with respect to flower formation in irises (18, 19, 20); floral stalk elongation and bud development in tulips (21, 22, 23, 24); and in dormancy release and development of Lilium longiflorum (25).

An increase in GLS was found by Aung and De Hertogh and by Rodrigues Pereira (18) in iris bulbs after cooling, the increase of the free compound being correlated with a decrease of the bound fraction. Recently, Hanks and Rees (24) analysed the GLS contents of the various organs of the tulip bulb after planting in autumn, and found two peaks of GLS activity, the first occurring prior to satisfaction of the cold requirement and the second around the time of rapid shoot elongation and flower maturation. Young shoots and developing daughter bulbs (DB) showed higher GLS activity than mother bulb scales. There are, however, some discrepancies between the findings of Aung et al. and Hanks and Rees as to the time of peak occurrence, the amount of activity, and the identity of GLS. There might not only be a redistribution of GLS from scales over the newly formed organs, but also synthesis in young roots, daughter bulbs, and flower buds.

Alpi et al. (20) obtained similar results for irises, i.e., an increase of GLS activity in developing bulblets and young floral organs after cooling. The activity in the roots and scales of the mother bulb was low, but increased at the end of the cooling period.

Lin et al. (25) reported a rise in GLS in Lilium longiflorum due to synthesis of the substances during the cooling treatment. Their data are in agreement with the findings in tulips and irises.

Finally, Rudnicki and Nowak (26) and Rudnicki (27) found a maximum level of GLS activity in hyacinth bulbs after a 6-week high-temperature treatment. The activity then decreased to undetectable amounts and reappeared after a 6-week cooling treatment.

In general, it can be concluded that cold treatment of bulbs leads to increased GLS activity, especially in the newly formed organs (shoot with flower bud and daughter bulbs). The increases occur prior to the actual rapid growth and are due partially to new synthesis and partially to redistribution from the mother-bulb scales.

2.3 Cytokinins

Little is known about cytokinins. From the author's findings and those of others (personal communication, Vonk and van Bragt) it is clear that these substances are present in tulips and irises. Tsukamoto (28) found a coincidental increase of cytokinins in gladioli after a dormancy-breaking heat treatment and planting. For hyacinths Rudnicki (27) reported the presence of four cytokinin-like compounds showing the highest activity after lifting of the bulbs during flower formation. Rahkimbayev et al. (14) found an increase of cytokinin activity in shoots and roots of tulip bulbs during cooling and no increase in the bulb scales. During storage at room temperature only low levels of cytokinin activity could be detected in all organs.

The information on the metabolism of this kind of plant hormones is still too limited to permit conclusions about relationships with temperature treatments and developmental phenomena.

2.4. Inhibitors

Endogenous inhibitors have been analysed in various crops by Alpi et al. (20), Lin et al. (25), Tsukamoto (13, 28), Aung and Rees (29) and Rudnicki and Nowak (26). These authors identified several inhibitory substances, including abscisic acid (ABA), fatty acids, and phenolic compounds.

Alpi et al. (20) found an increase of non-acidic inhibitors in scales, sheath leaves, and roots in irises during cooling; acidic inhibitors decreased. After planting, the levels of inhibitory substances declined except in the roots; in the scales, floral organs, bulblets, and leaves both acidic and non-acidic fractions disappeared almost completely.

Tsukamoto (13, 28) and Lin et al. (25) analysed the growth-inhibitor content of Lilium longiflorum and found a decrease of inhibitors during cold treatment coinciding with an increase of auxin-like growth-promoting substances. The level of inhibitors decreased. Also, at room temperature, but the level of promotors did not rise in this condition.

Hyacinths, too, showed a decrease of ABA during cold treatment (26, 27). For tulip bulbs, Rahkimbayev et al. (14) found a decrease of the inhibitor content during cooling of the bulbs, but the activity did not disappear.

2.5. Ethylene

Ethylene is produced in bulbous plants under special conditions (30).

A peak in ethylene production in flower buds of Lilium 'Enchantment' occurs at a bud length of 3 to 4 cm. Reduction of the amount of light, which induces bud abscission, stimulates ethylene production. In tulips, desiccation of flower buds (blasting) due to exposure to high temperatures late in the storage season (blindstoken treatment, (7)) is accompanied by increased ethylene production. Recently, Moe et al. (31) established a peak in ethylene production by intact tulip bulbs after cooling followed by a rise of the temperature to 21°C.

Correlations between endogenous ethylene contents and development have not been established so far.

In summary, it can be said that low-temperature treatment of bulbs, which enhances the growth rate of newly formed organs, results in an increase of promoting substances (GLS, auxins, and perhaps also cytokinins) and a decrease of inhibitory substances (ABA, fatty acids, and phenolic compounds). Because promoters and inhibitors interact, balanced patterns of PGR activity are assumed to be correlated with each developmental stage. Subtle changes in such dynamic balances in individual organs will exert an influence on the sequence of developmental phenomena. Determinations of this kind of condition can hardly be made. The effects of temperature via growth regulators on developmental phenomena such as flower formation, induction of bulbing and growth of bulbs, and retardation of development are unknown. In this respect the importance of auxin activity is very poorly understood.

3. The effect of applied growth substances

In section 2 the conclusion was drawn that interactions between growth substances control development. Specific activities of a particular growth regulator are not clear. Externally applied growth substances can give indications as to the ways in which specific endogenous growth substances are active. To quote Rees (7): 'A number of workers have applied some auxin, gibberellin or kinetin - often in desperation - but results are usually negative, have not been followed up in detail and are widely scattered through the literature, so that no coherent picture emerges'. The following positive results can be mentioned with respect to dry matter distribution.

3.1. Translocation of dry matter to the inflorescence

Mae and Vonk (32) who studied the development of flower buds of irises, found that the translocation of assimilates in the apical direction was suppressed to an extreme degree under conditions of poor lighting. These conditions led to complete stagnation of the growth of the flower buds and upper internode of the stem, resulting in desiccation of these organs (blasting). This blasting could be overcome to considerable extent by the injection of growth substances. BAP (benzyl amino purine) was the most

effective, followed by kinetin, adenine, and GA_3; IAA was not effective. The authors suggested that the growth substances might play a part in the distribution of carbohydrates.

Similar results have been obtained by Ginzburg (33) and Halevy (34) for gladioli, which also show blasting under reduced light intensity. Application of GA prevented this aberration and promoted the movement of labelled assimilates towards the inflorescence at the expense of the corm. Furthermore, these authors found that the conditions that induce the blasting are accompanied by a decrease of the endogenous content of GA in the leaves. Ginzburg (33) found that spraying of gladiolus plants with CCC, which stimulates GA synthesis (35), promoted the growth of the inflorescence by directing assimilate movement towards the inflorescence at the expense of the corm. After anthesis GA is ineffective in influencing the direction of assimilate movement from the labelled leaf towards the corm, but CCC is still active.

The development of the inflorescence of both irises and gladioli strongly depends on the newly formed assimilates. The development of flower buds of tulips is predominantly dependent on the mobilization and translocation of carbohydrates from the mother bulb (36, 37). Stagnation of development occurs after improper temperature treatments or exposure of the bulbs or plants to ethylene (38). The effect of such unfavourable conditions can be overcome by injections of a solution of kinetin, BA, GA_3, or GA_{4+7} or application to the flower bud of these substances in lanolin paste (39, 40, 41).

It has been suggested that the flower bud has to compete with the young daughter bulbs for substrate. Growth of the flower bud can be enhanced at the expense of daughter-bulb growth by the application of GA or BA, or in gladioli with GA and CCC (42). On the other hand, the growth of daughter-buds can be enhanced by the external application of BA to these buds, which gives some inhibition of growth of the flower (43).

Moe (41) injected GA_{4+7} together with ^{14}C-sucrose into the outer scales of a tulip bulb and found increased ^{14}C import into the flower bud and other plant organs. Translocation of ^{14}C from the scales was inhibited by the injection of CEPA. This inhibition could not be overcome by application of GA_{4+7}, but the CEPA-induced blasting of the flower bud was reduced to very low percentages by the administration of both GA_{4+7} and kinetin. This may be an indication that the flower can just manage to grow on newly synthesized material.

Another kind of translocation was observed after application of auxins to decapitated stems of tulips (40, 43, 44, 45). Growth of the stem was totally inhibited after dissection of the leaves and flower bud. Applica-

tion of lanolin paste containing 0.2% IAA to the cut surface of the stem or the scars of the dissected leaves completely restored elongation of the internodes below the site of application. This means that the influence of the flower as sink on acropetal translocation of dry matter from the mother bulb can be replaced by auxin. This effect was achieved only with auxins, not with GA_3 or BA. It should also be mentioned that application of auxin to the flower bud causes stagnation of the development of the floral organs (39) and thus inhibition of the acropetal translocation of substrate. This is understandable, because auxin application stimulates the production of ethylene by the flower buds (unpublished), which is known to cause growth stagnation of the flower bud (blasting).

If auxin activity is the principal mechanism for directing the substrate flow, what is the effect of cytokinins and gibberellins? Do they stimulate auxin activity, auxin release from conjugates, or auxin synthesis? In this connection it is interesting to note that auxin application to uncooled tulips also promotes stem elongation to a certain extent (unpublished results). GA application, which is known to be a partial substitute for the cold requirement (23, 46, 47) might have some effect leading to the stimulation of auxin activity.

In summary cytokinins , gibberellins, and auxins applied under special conditions strengthen the sink activity at the site of application. Auxin activity might be the primary requisite for the direction of the flow of dry matter.

3.2. Effects of plant-growth regulators on bulbing

The data mentioned so far concern the effects of PGR's on the outgrowth of the shoot and the inflorescence. However, appreciable, amounts of carbohydrate are transported to buds, which form the new storage organs. Heath and Holdsworth (48) postulated the existence of hormones that induce bulbing in onions via auxin formation. To the best of my knowledge, specific hormones of this kind have not been isolated yet. Bulbing in tulips and irises requires a low-temperature treatment (8). It is conceivable that the increase of GLS seen in daughter bulbs after cooling (20, 24) stimulates bulbing, but the question of causal relationships is highly speculative, because the endogenous growth regulator content undergoes so many changes.

Cormel formation in stolons of gladioli in vitro has been induced with kinetin, whereas GA_3 inhibited this process. ABA did not affect cormel formation, and NAA had no direct effect (49). It is conceivable that the assimilate supply induces bulbing. Assimilates are directed to sinks by hormone activity.

From own experiments I have doubts about bulbing induction by kinetin. Application of BA to buds of tulip bulbs that were expected not to produce

bulbs because the material had been stored at a relatively high temperature, caused a tremendous growth which simulated bulb development. In fact the outermost primordium had developed into an inflated organ, whereas the inner primordia had remained small and did not have a distinctly scale-like nature.

Bulbing in lilies (50) and in onions (51) has been shown to be associated with the activity of auxins. Maximum fresh weight increases of young bulblets in explants of scale tissue of Lilium speciosum were found at an optimum of 0.03 to 0.10 mg NAA/l in the nutrient medium (50). Bulbing in onions was induced by applied auxins and extracts of plants exposed to LD for the induction of bulbing (51). Furthermore, in onions bulbing was stimulated by the application of ethephon under conditions of SD (52). GA administered to plants by injection retarded bulb formation (53).

In addition, it is generally known that bulbs show enhanced growth after anthesis or removal of the flower. This can be explained by the elimination of auxin-producing organs or correlative inhibition exerted by the flower on the young daughter bulbs. In the conception of Ginzburg and Ziv (49), cormel formation in gladioli is dependent on the assimilate supply, which can be explained as a push function of the sources. However, it is also possible that newly formed organs give the signal for substrate supply to the source organs, which can be explained as a pull function of the sink organs. Unfortunately, too little information is available on endogenous growth regulators, and above all on auxins, to permit conclusions.

3.3. Dormancy release

The development of shoots or bulblets and consequently the distribution of dry matter can fail completely due to dormancy, especially in lilies and gladioli (2). Low- and sometimes high-temperature treatments can release dormancy. Lin et al (54) broke dormancy of Lilium longiflorum by the application of GA_3. ABA counteracted the effect of GA_3. Analysis of the bulbs revealed that cold treatment leads to the synthesis of growth-promoting substances resembling gibberellin (25) or auxin (13). The concentration of growth inhibitors (one of which resembled ABA) was higher in the new bulb scales than in the old ones, but the amount decreased under low-temperature storage.

In gladioli, Tsukamoto (28) induced dormancy release by soaking the corms in a solution of BA. It is known also that ethylene and ethephon (2) can cause dormancy release in gladioli corms.

4. Final remarks

I would like to suggest that in bulbous plants sink activity is under the influence of the growth promoters of auxin-like, gibberellin-like, and

cytokinin-like substances. The growth of young bulbs is suppressed by correlative inhibition until anthesis. Cooling of the bulbs stimulates the mobilization of material stored in the bulb scales via a change in the equilibrium between growth promotors and growth inhibitors: in general gibberrellins, cytokinins, and auxins increase and inhibitors decrease. Auxins, cytokinins and gibberellins strengthen the sink activity of the inflorescence and counteract the influence of inhibitors. Ethylene weakens the sink activity. Auxin application to the inflorescenee causes stagnation of the growth of the inflorescence because it leads to long lasting ethylene production in the floral organs.

Flower-bud blasting and abscission of flower bud (lilies) occur during forcing of bulbous plants. Both are thought to be due to inadequate substrate supply to the inflorescence. Application of growth regulators offers possibilities for the prevention of these disorders, but is not yet feasible on a large scale. The problem is how to apply such substances in the required concentration to a particular organ, and how to suppres unwanted side effects.

Practical use can be made of dwarfing agents (ancymidol and ethephon), which reduce stem height, a desirable effect for pot-plant production. Some authors (27, 55, 56, 57) have described encouraging prospects for replacement of cold treatment by PGR's to control dormancy, speed up flower development, improve the quality of cut flowers, and make more efficient use of the material stored in the bulb scales, thus saving energy during the glasshouse period. For many of the proposed uses current knowledge is scanty and scattered. There is an urgent need for coördination and coöperation in fundamental research and investigations on application techniques.

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