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Gibberellins, Cell Division, and Plant Flowering

Recent studies on the gibberellins indicate that there is an activation of cell division as well as an activation of cell elongation (4, 14, 23, 24, 34). The discovery of the primary effects of gibberellin on stem elongation led to the idea that the action of the gibberellins was similar to that of auxin. Since that time many other activation effects have been observed (6, 21, 22, 25, 26, 33). Some are concerned with the functioning of the growing point of the stem and particularly with a modification of the rate or the direction of cell division.

It is now clear that in some cases the effect of gibberellins (GA) on stem elongation is partly due to enhanced cell division activity. Figure 1 shows longitudinal sections of *Perilla* stems in which the dimensions of the control cells are approximately the same as those of the treated cells, while the internode length of the treated plants was 2.3 times that of the control. Sometimes the stem elongation is promoted more easily in the inflorescence than in the vegetative stem. With *Iberis amara,* for instance, we obtained very little length increase in the vegetative stem, but the length of the terminal inflorescence was markedly increased (Figure 2). Such specific effects have been observed in *Begonia* (14) and in strawberry (R. Lemaitre, personal communication). Enhanced cell division plays an important role in these effects. The activated cells are those in the zone immediately under the apical meristem, as noted by Sachs and Lang (34) in vegetative plants of *Hyoscyamus niger.*

Modifications of leaf form and size induced by GA have often been observed. Two very characteristic cases are those of *Statice sinuata* and *Lepidium ruderale* (Figure 2). The continual application

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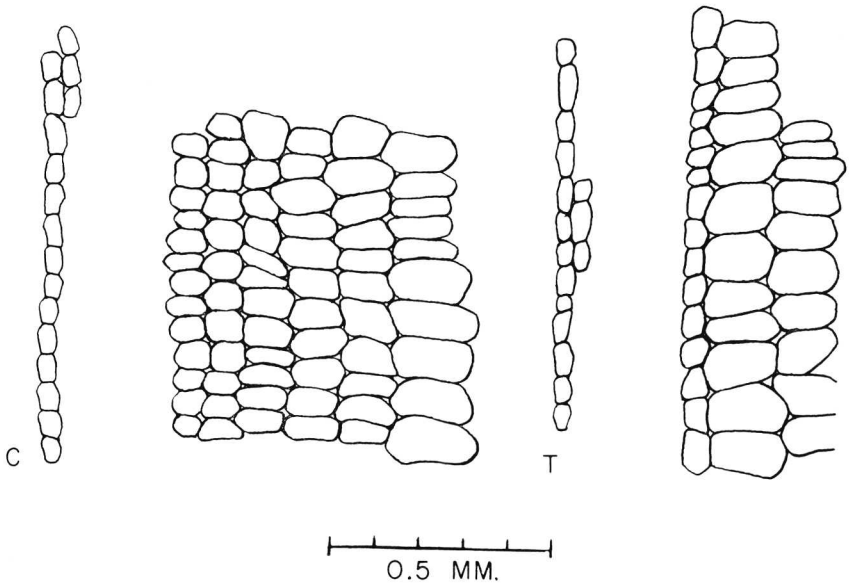

Fig. 1. Longitudinal sections of the external (**left**) and central (**right**) parenchyma of stems of *Perilla nankinensis* **C**, control; **T**, treated with 100 p.p.m. GA.

of GA to the growing point does not promote the growth of the stem, but the shape of the leaves is very strongly modified. In these two cases the leaves are larger in the treated plants but their form is more simple. This can only be explained by postulating a modification of mitotic activity within the leaf initials.

The action of GA on flowering of long-day (LD) plants grown in short days is also an effect on cell divisions in the stem apex (24). In short days the functioning of the apex is normally restricted to the formation of leaf initials. The application of GA enhances cell division in such a way that the whole meristematic region is activated, giving rise to the *"manteau de Gregoire"* (12) from which the flower primordium is formed.

The occurrence of these different effects fits relatively well with the anatomical and cytological description of distinct zones inside the meristem as proposed by Buvat (7). We can visualize that, depending on the species and upon the circumstances, GA acts selectively on one or another meristematic zone of the stem and on the young tissues initiated by the activity of the meristem. In stem elongation, the *"meristeme medullaire"* and the zone situated immediately under it would be activated. In modifications of leaf form, the activation would affect the *"anneau initial"* and the leaf initials. The formation of the flower would correspond to a more complete activation of the

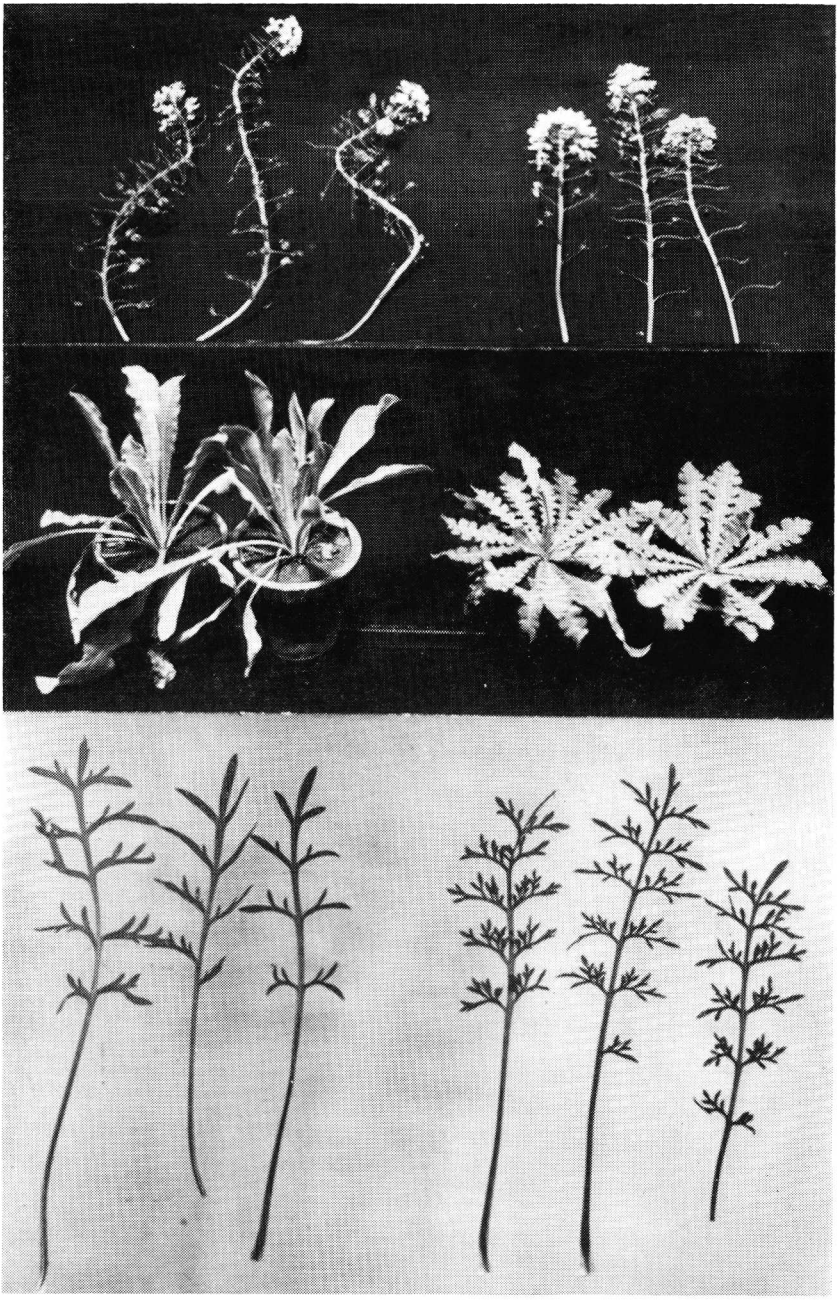

Fig. 2. Effect of gibberellic acid on morphogenesis. Elongation of the inflorescence of *Iberis amara* (***top***), modification of leaf shape in *Statice sinuata* (***middle***), and *Lepidium ruderale* (**lower**). Plants at right are controls; plants at left were treated with 100 p.p.m. GA.

mitotic capacity of the meristem as a whole including the "*meristeme d'attente*." These three effects could coincide in time.

To distinguish between the different meristematic activities of the growing point of the stem, one can compare the differential action of GA on LD and SD plants. When GA is applied to SD plants grown under long days, it cannot induce flowering but acts only on stem elongation. When applied to LD plants grown in short days, however, GA promotes both stem elongation and flowering. Using Buvat's concept, this means that the "*meristeme d'attente*" cannot be activated by GA in SD plants, while it can be activated in LD plants. Tschailachjan (42) has proposed a theory that accounts for this. He postulates that florigen is composed of two hormones, GA and anthesin. When the two hormones are both present, flowering is promoted, as evidenced by the flowering behavior of SD plants under short days and LD plants under long days. Following Tschailachjan, LD plants synthesize anthesin in short days, and the addition of GA results in the formation of florigen (GA + anthesin). On the other hand, SD plants synthesize GA in long days, and a further addition of GA has no effect on flowering because anthesin is lacking. It is clear that these differences in reaction when GA is applied indicate that an SD plant grown under long days is not identical to an LD plant in short days. There is a sort of dissymmetry which Tschailachjan's proposal attempts to interpret.

But this does not change the principal open question: How does GA activate cell division in the young tissues of the stem?

BIOCHEMICAL APPROACH TO THE ACTIVATION OF CELL DIVISION BY GA

Biochemically speaking, cell division is a very complicated phenomenon involving the synthesis of protein for which several biochemical conditions must be met. The role of ribonucleic acids in protein synthesis has been shown in animals as well as in plants. Protein synthesis also depends on the availability of a sufficient source of energy with the resulting adenosine triphosphate (ATP) playing a prominent role (1, 3, 8). This ATP may be synthesized in both respiration and in photosynthesis (16, 43). It is likely that some of these conditions are absent in meristems, particularly in the "*meristeme d'attente*" of an LD plant grown in short days or in that of an SD plant grown in long days. Protein synthesis would, therefore, be at a level insufficient for accelerated cell division. The type of block may well be different for the two groups of plants.

It is highly probable that GA can modify the rate of protein synthesis in the cells of the growing point of the stem. Further promotion of respiration (2, 19), action on several enzyme systems (40, 41), modifi-

cation of sugar content (5, 30, 40), reduced nicotine content in tobacco (45), increased ascorbic acid levels in clover (30), action on chloroplast pigments (5, 14, 30), etc., have been reported to occur after treatment by GA. In many species the effect on the pigments is grossly evident, but it is rather complex. Without a supplementary supply of mineral nutrients, as in a normal garden soil, there is generally a lowering of the pigment content. Table 1 lists nine species we have studied. In some cases the anthocyanin content is also modified. When mineral fertilizers are added in the presence of GA, the chlorophyll content does not drop much or does not drop at all. However, the drop remains evident when the treated plant flowers (30). As shown by Mosolov and Mosolova (30), redox processes are strongly enhanced in the leaves of GA-treated clover plants, and the sugar content of the leaves increases. The assimilation of mineral nutrients also increases.

All these facts show that GA profoundly affects the metabolism of plants. In spite of the fragmentary data, some of these facts clearly indicate that under adequate cultural conditions in which mineral nutrition is not limiting, GA enhances certain essential metabolic processes and increases the availability of some important metabolites. This is likely to be very favorable for protein synthesis inside the meristem and in the young tissues of the treated plants.

Another argument supports this conclusion. Photoperiodic induction of flowering, which can be replaced by the application of GA to LD plants grown in short days, seems to induce an immediate change in the capacity of meristematic cells to synthesize proteins. This appears from the following facts:

(a) Metzner (27, 28) reported that the proportion of amino acids in the protein fraction of the meristems of *Kalanchöe blossfeldiana*

Table 1. Effect of 100 p.p.m. of gibberellic acid on pigment content of plants.

Species Tested	Direction of Change in Chlorophyll Content of Leaves	Anthocyanosides	
		Direction of change in anthocyanoside content	Locus of effect
Statice sinuata	−	0	
Draba aizoides	−	0	
Capsella bursa-pastoris	0	0	
Iberis amara	−	−	Stem
Lepidium ruderale	−	0	
Beta vulgaris	−	−	Petioles
Bellis perennis	−	+	Stem
Perilla nankinensis	0	−	Leaves
Cheirantus cheiri	−	0	
Salvia splendens	−	0	
Ageratum mexicanum	0	0	
Arabidopsis arenosa	−	0	

grown in long days undergoes a rapid modification following several short days. Moreover, modifications in the nucleic acid fraction of the meristems occur during this SD induction.

(b) It is well known that photoperiodic induction rapidly changes the type of the gas exchange between the plant (in particular its leaves) and the environment (13, 35). Respiration measurements of very young isolated leaves (including the meristem) of an LD strain of *Salvia splendens* showed that during the induction phase the respiration is significantly higher under long-day conditions than under short-day conditions (10).

(c) Studying the total hematin content of leaves of LD and SD plants of *Perilla nankinensis* (SD), *Cannabis sativa* (SD), *Sinapis alba* (LD), and *Salvia splendens* (LD), we found (unpublished) that induction always causes a decrease of the molar ratio chlorophyll:hematin of the leaves. This decrease is most evident in young leaves. The modification is very rapid and is measurable a few days after the beginning of induction. We always observed that in the very young leaves the chlorophyll accumulation becomes slower upon induction, while hematin accumulates more rapidly.

(d) In flowering *Fragaria vesca* the vitamin E content of the young leaves is approximately proportional to the day length. In field experiments a maximum is found in June to July, coinciding with the increase of flower initiation (38). As shown by Nason and Lehman (32), vitamin E acts in vitro as an activator of cytochrome *c* reductase.

Points c and d directly relate to chlorophyll metabolism which is controlled by day length, although the exact site of the photoperiodic control is not yet known (9, 11, 29, 36, 37). Points b, c, and d suggest some inductive change in enzyme systems of the young tissues, a possibility which is very consistent with point a. Taken together, the four classes of facts support the following hypothesis:

In affecting chlorophyll metabolism, photoperiodic induction acts on several important metabolic processes; it enhances the respiration of the young tissues of the stem and it provides them with an improved system of hydrogen carriers passing through the series of cytochromes [the cytochrome carriers are known to be regularly associated in higher plants with meristematic activity (15)]. It therefore increases the ATP supply which is necessary for the changes in the protein fraction (27, 28) as well as for increased cell division and flowering. It would be very interesting to see if the activation of cell divisions by GA follows a scheme of this type.

ON A POSSIBLE DIFFERENCE BETWEEN SD AND LD PLANTS

Finally, we may ask why GA induces flowering of LD plants grown in short days but is ineffective in SD plants grown in long days. In

other words, why does GA activate cell divisions of the whole meristem in the first case and not in the second?

Many hypotheses are possible. We can suppose, as Tschailachjan does, that in SD plants an activator other than GA is necessary and that this activator is lacking in SD plants under long days. We can also suppose that the action of GA on metabolism is not exactly the same for LD and for SD plants, or that the necessary level of activation must be higher in SD plants than in LD plants and cannot be achieved through GA application. But there is another possibility which cannot be neglected. It is known that chloroplast structure is very delicate and that it is very rich in many enzyme systems. Within the plastid, chlorophyll is not distributed at random but is in close association with protein and lipide, the spatial organization of which is now under study in some laboratories (44). To some extent the organization protects the chlorophyll from photodestruction. The degree of protection varies from one species to another, or in the same species in accordance with the conditions of its culture. This appears evident when one studies photooxidative effects. We have found that *Chlorella pyrenoidosa* (Kandler's strain K) was relatively resistant to photooxidation, while *Chlorella vulgaris* (Pirson's strain P) was much more sensitive (39). In some mutants, photooxidation is very easy (17), but chlorophyll destruction appears to be only the final consequence of photooxidation. Long before it occurs, photosynthesis has completely ceased in high-intensity light (18, 20), phosphorylations are inhibited (18), and oxygen consumption rises probably with attendant peroxide formation (18, 20, 31). A general poisoning of metabolism occurs. Crawford (unpublished) has studied the sensitivity of the LD plant *Salvia splendens* to photooxidation by intense light. He found that photooxidation (as measured by the inhibition of photosynthesis in white light) is much more marked in the leaves of *Salvia splendens* grown in short days; the plants grown in long days are evidently more resistant to photooxidation. The high photosensitivity of *Salvia* grown in short days may be due to an insufficient protection of chlorophyll inside the chloroplasts, possibly resulting from an abnormal structure of the plastids themselves. Indeed, under short days the chloroplasts of *Salvia* do not accumulate their pigments in a normal fashion.

In practice this means that, during a given short day with light of sufficient intensity, the metabolism of an LD plant grown in short days can be partially inhibited through photooxidative processes. It can therefore be concluded that short days do not permit flowering of LD plants for two interrelated reasons: (1) suitable metabolic conditions (of the kind described above) for increased cell divisions in the meristem are lacking, and (2) photooxidation products poison metabolism during the light period.

It would be very useful to know if such a poisoning also occurs in SD plants grown in long days. In *Kalanchöe blossfeldiana,* for instance, the chlorophyll metabolism is undoubtedly different in short or long days (37). If this corresponds to a decreased level of protection, a long day with relatively intense light is likely to produce a drastic photooxidation proportional to the length of the photoperiod. Perhaps the explanation of the dissymmetry revealed by GA between the behavior of LD and SD plants is to be found here. During long days SD plants could withstand more severe metabolic inhibition of a photooxidative nature than could LD plants during short days. GA would be able to overcome this inhibition in the last case but not in the first.

ACKNOWLEDGMENT

The author is greatly indebted to the "Institut pour l'encouragement de la Recherche Scientifique appliqueé á l'Industrie et à l'Agriculture, IRSIA," Belge, for financial aid.

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