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Gibberellins and flowering in long day plants, with special reference to *Lolium temulentum**

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Abstract. The relation between gibberellins (GAs) and flowering in some long day (LD) plants is reviewed, with particular emphasis on *Lolium temulentum*. Lang's early experiments with rosette plants established the effectiveness of several GAs in replacing the need for LD. Subsequent work with mutants, especially of *Arabidopsis*, has clarified genetic and environmental control points in GA synthesis, various feedback effects and some of the factors affecting responsiveness to, as well as synthesis of, GAs in the flowering process. Further complexities are revealed in the experiments with *Lolium temulentum*, which have clearly shown that the structural requirements for effectiveness of GAs in the flowering process are quite different from those for elongation growth. The precise role of GAs in the long day induction of flowering remains unclear.

Introduction

This review deals with only one aspect of the physiology of flowering, namely the role of gibberellins (GAs)† in the flowering of long day plants (LDPs), and rather selectively at that. Besides replacing the need for long days (LD), GAs may also replace the need for vernalization by prolonged exposure to low temperatures, but that issue is not considered here. Nor do I attempt a coverage of the literature on the role of GAs in the flowering of all LDP, even of those such as the garden pea for which a combination of physiological and genetic approaches has been fruitful (Ross 1994).

Experiments begun by Anton Lang with *Hyoscyamus niger* over 40 years ago seemed to provide a straight answer to clear questions: 'Does gibberellic acid replace the need for long days?' And if so, 'is GA the LD stimulus to flowering, or even the hypothetical florigen?' Many other questions and increasingly complex experiments have followed, but the role of GAs in the long day induction of flowering remains elusive.

Early experiments with rosette and other plants

The structure of gibberellic acid (GA_3) was elucidated in 1954 and the compound became available in small quantities for experiments on plants in the following year. By 1962 nine naturally-occurring GAs were known, 27 by 1969, and they now number more than 120 and have a great variety of physiological effects. One question that immediately arises, therefore, is whether different GAs perform different or specific functions, to which we shall return.

In 1956, however, only GA_3 was available and Anton Lang, who had long sought to replace the requirement for vernalization and/or LD in *Hyoscyamus niger* with auxins and other compounds, applied GA_3 to unvernalized biennial plants held in either short days (SD) or LD. Within 2 weeks of treatment in LD, and 4 weeks in SD, the rosettes began to form stems. Although the replication was small, as I saw for myself in his UCLA glasshouses, Anton knew his plant well enough to realize how significant this result was, and he didn't even wait for the plants to flower before sending a letter, on 2 May 1956, to the editor of *Naturwissenschaften*, an indication of the excitement generated by his results (Lang 1956*a*). Then, on May 30, he sent another (Lang 1956*b*) announcing that these GA treatments had also caused flower formation. By the time of his third communication in November (Lang, 1956*c*), the experiment had been repeated and extended to annual *Hyoscyamus* and several other LD-requiring plants.

In his autobiographical sketch (Lang 1980), Anton records that these results caused him 'boundless delight', as indeed they should have, being readily repeatable, extendable to many other species, and seeming to open a door at last on the biochemistry of floral induction.

Medawar (1967) once suggested that 'the spirit of John Stuart Mill glares out of the eyes of every editor of a learned journal'. Editors of plant physiology journals are no exception, acting as stern guardians of the reputation of their journal and their discipline, none more so than was Anton Lang of *Planta*. Many of us have been admonished by him — often by postcard — for insufficient replication, repetition or review. Yet, in his younger days, when excited by a result, he could be as impatient to publish as any of us.

* Dedicated to the memory of a great reviewer and editor, Anton Lang.

† Abbreviations used: AMO-1618, (4-hydroxy-5-*i*-propyl-2-methylphenyl)-trimethylammonium chloride-1-piperidine carboxylate; CCC, chlorocholine chloride; GA, gibberellin; GA_3, gibberellic acid; IAA, indol-3-yl acetic acid; LD, long day; LDP, long day plant; LSDP, long–short day plant; SD, short day; SDP, short day plant; SLDP, short–long day plant; UCLA, University of California at Los Angeles.

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There followed a veritable gold rush in which GA_3, and then other GAs, were shown to induce flowering in many species held under non-inductive daylengths. Nearly all of these were either LDP in SD, long-short day plants (LSDP) in SD, short–long day plants (SLDP) in SD, or cold requiring plants in LD at warm temperatures. Few short day plants (SDP) responded. Most of the plants that did flower were those which formed rosettes in non-inductive conditions, as emphasized by Zeevaart (1983), and stem elongation accompanied flower initiation in most of them.

However, about a third of the rosette LDP which responded to GA application with stem elongation did not initiate flowers, possibly because the wrong GA was applied. With *Myosotis alpestris,* for example, GA_3 caused only stem elongation whereas GA_7 also caused flower initiation (Michniewicz and Lang 1962). A few non-rosette LDP, such as *Lolium temulentum*, also responded to applied GA_3 with inflorescence initiation in SD. In another grass, the SLDP *Poa pratensis*, applications of GA_3 and other GAs inhibited the primary induction by SD (Heide *et al.* 1987) but replaced the secondary induction by LD (Heide *et al.* 1998). There is variation between species in the most effective GA, GA_3 for most, but GA_4, GA_5 or GA_7 for some. In several of those that did respond, an increase in endogenous GA-like substances in LD was found (Lang 1965).

Applied GAs also induced precocious or enhanced flowering in a number of conifers, GA_3 being most effective among the Cupressaceae and Taxodiaceae, and the less polar $GA_{4/7}$ among the Pinaceae (Pharis and King 1985). In fern gametophytes, antheridia formation is induced by the closely-related antheridiogens, such as the methyl ester of GA_{73} in *Lygodium japonicum*, active at a concentration as low as 10^{-14} M (Takeno *et al.* 1989).

Two questions

In reviewing these early experiments, Lang (1965) posed two important questions:

(1) Were the effects of an applied GA on flowering physiological or merely pharmacological, to use his terms?;

(2) Was an endogenous GA the postulated graft-transmissible florigen?

Concerning the first of these questions, the fact that endogenous GA levels were often greater in LD suggested that the GA effect was physiological, but analytical methods were not adequate at that stage to confirm that the content of florigenically active GAs in leaves or shoot apices was greater in LD. However, Baldev and Lang (1965) side-stepped this problem by showing that with the rosette LDP *Samolus parviflorus*, in which GA_3 induced stem elongation and flowering in SD, two inhibitors of GA biosynthesis (AMO-1618 and CCC) could inhibit both stem elongation and flowering in LD, the inhibition being reversed by applied GA_3, with both effects being proportional to dose. Lang (1980) concluded that Koch's postulates (that the effect should disappear when the agent is removed, should reappear when it is reintroduced, and should be caused only by that agent) had been satisfied and that the GA effect was physiological.

As to Lang's second question, the fact that applied GA induced flowering in many LDP, LSDP and SLDP, but in few SDP in non-inductive conditions, suggested that it was unlikely to be the hypothetical florigen. However, Chailahyjan (1961) had modified his original florigen hypothesis of 1937 in the light of the early results with GAs, and proposed that the photoperiodic stimulus consisted of two complementary groups of substances, the GAs which are more limiting in SD (particularly in LDP), and the anthesins which are more limiting in LD (particularly in SDP).

Experiments by Zeevaart and Lang (1962) with the LSDP *Bryophyllum daigremontianum* showed that GA_3 treatment could replace the need for LD and that leaves in SD from such GA-treated plants could in turn induce flowering when grafted on non-induced receptor plants. They concluded that GA is not identical with the graft-transmissible floral stimulus (florigen) but limits production of that stimulus in SD. Such an explanation could also apply to the results of King *et al.* (1987) with the SDP *Pharbitis nil*, in which GAs applied just before the long dark period promoted flowering whereas those applied during or after it were inhibitory.

The relationship between GAs and the hypothetical florigen remains unclear, but there is obviously a sequence of processes in the induction of flowering, at least one of which is promoted by a change in GA metabolism.

Stem elongation and flowering

In rosette plants stem elongation is usually coupled to flower induction. However, Cleland and Zeevaart (1970) found that growth retardants suppressed stem growth but not flower initiation in *Silene armeria*. Wellensiek (1973) was able to show that the two processes are determined by separate genes. Talon and Zeevaart (1990) found another growth retardant, tetcyclasis, which also inhibited stem elongation but not flowering in *Silene*, even though the transfer of plants to LD led to an initial increase in the concentration in the shoot tips of GAs in the later steps of the early 13-hydroxylation pathway.

This is also true of spinach (Metzger and Zeevaart 1982), in which the conversion of GA_{53} to GA_{44} and of GA_{19} to GA_{20} are both regulated by daylength (Talon *et al*. 1991). In addition, an earlier step in biosynthesis of *ent*-kaurene is also regulated by daylength (Zeevaart *et al*. 1993). Wu *et al.* (1996) subsequently showed that the level of GA_{20}-oxidase mRNA is higher in plants in LD. In yet another rosette LDP, *Agrostemma githago*, the early 13-hydroxylation pathway was transiently enhanced after 8–10 LD, but there was also an increase in the rate of turnover of endogenous GAs and in sensitivity to them (Jones and Zeevaart 1980).

Clearly the response of rosette LDPs to LDs encompasses not only several biosynthetic steps but also differences between stem elongation and flowering in the effectiveness of the various applied GAs.

Gibberellins and *Lolium temulentum*

Lolium temulentum is a grass which requires exposure to only one LD for floral induction but responds quantitatively in its rate of inflorescence development to additional LD (Evans 1960*a*). In 1956 Percy Brian gave me a sample of GA_3 to apply to *Lolium*, and other GAs later on, with which I found that single doses of several GAs were able to induce inflorescence initiation in plants in non-inductive SD comparable to that following one LD. However, they also caused immediate stem elongation with doses which elicited flowering, whereas in plants induced by one LD this does not occur until at least 3 weeks later, at about the stage when anther primordia are initiated (Fig. 1). Thus, GAs such as GA_1 and GA_3 were unlikely to be the floral stimulus in *Lolium*.

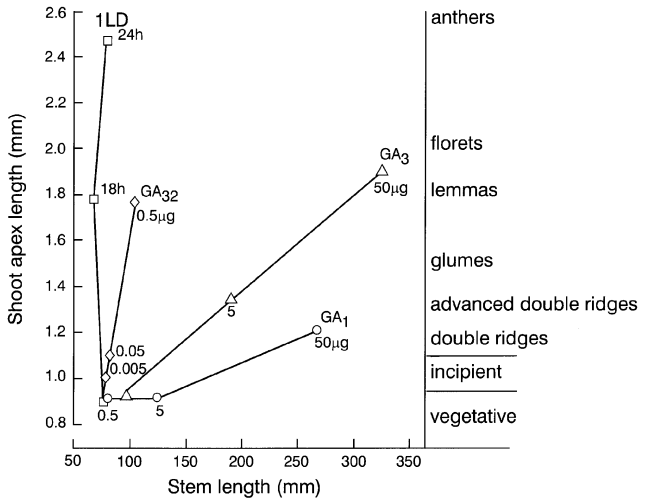

Fig. 1. The relation between flowering response (shoot apex length) and stem length three weeks after treatment for plants grown in SD with either exposure to one LD of either 18 or 24 h illumination or to a single dose (ranging from 0.005 to 50 μg) of GA_1 (○), GA_3 (△) or GA_{32} (◇). The stage of floral development reached at various apex lengths is indicated on the right hand side.

This conclusion was reinforced by the finding that the two inhibitors of GA synthesis used by Baldev and Lang (1965), namely CCC and Amo 1618, did not reduce the flowering response to one LD although they did suppress stem and leaf elongation (Evans 1964). The non-rosette *Lolium* clearly behaved very differently from the rosette LDP *Samolus*.

Even more surprising was the finding, in later experiments, that although CCC behaved as expected of an anti-gibberellin in reducing stem growth progressively at higher concentrations in plants treated with GA_3, it had the opposite effect on inflorescence initiation, GA_3 and CCC (or Amo-1618) displaying a strong synergistic, rather than antagonistic, effect at all times of application (Evans 1969). Jacques (1970) subsequently reported a similar response in another LDP, *Blitum capitatum*. In more recent experiments we have found that two acylcyclohexanedione inhibitors of 3β-hydroxylation in GAs also promote flowering when applied early on the LD (Evans *et al.* 1994*a*).

One possible explanation for these *Lolium* results is that GA could be a component of one LD process, in either the leaf or the shoot apex, but that a compound sharing an early step in the biosynthetic pathway to GAs but then branching off may also be involved, hence the GA × CCC synergism, due to substrate-level stimulation of the branch pathway.

The question therefore arose as to which GAs might be involved in LD induction of flowering in *Lolium* and which synthetic steps might be controlled by daylength. Metzger and Zeevaart (1980) had found that in spinach the step from GA_{19} to GA_{20}, mediated by GA_{20}-oxidase, was enhanced in LD. Working with Dick Pharis and his colleagues at Calgary, we made several attempts to obtain similar evidence in *Lolium* leaves harvested every 2 or 4 hours during and after one long day. GA_{20} increased relative to GA_{19} during the latter part of the LD, but the shift was not marked. However, Gocal *et al.* (1999) have since shown it to be quite pronounced after two or four LD, consonant with Metzger and Zeevaart's finding that the shift increased progressively with the number of LD up to eight.

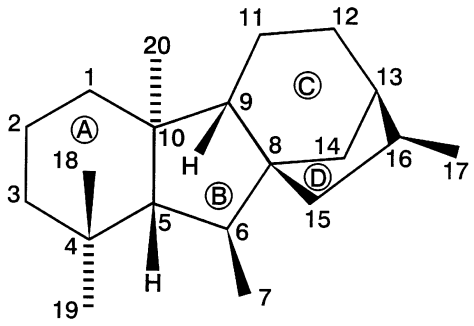

Fig. 2. The structure of *ent*-gibberellane, with numbering of the carbons and lettering of the rings.

Analyses of the GA content of vegetative and induced *Lolium* shoot apices (using bioassays with dwarf rice seedlings) suggested that some of the more highly hydroxylated C_{19} GAs increased in content by the end of one inductive LD (Pharis *et al.* 1987). The most highly hydroxylated gibberellin, GA_{32} with hydroxyls on each of carbons 3, 12, 13 and 15 (Fig. 2), was therefore applied to vegetative plants in SD and was found to induce inflorescence initiation equivalent to that after one 18 h day with single doses of only 0.5 μg per plant, without causing stem elongation (Fig. 1). Unfortunately, we have, as yet, no evidence of the occurrence of GA_{32} in *Lolium*, let alone of its control by daylength.

Structure–function relations

We therefore undertook, in collaboration with Lew Mander of the Australian National University Research School of Chemistry, an analysis of the structural requirements for florigenicity in *Lolium* among both naturally-occurring and synthetic GAs and related compounds, for clues as to what to search for among the many endogenous GAs. As the editor of *Planta*, Anton Lang didn't like this 'paper on the florigenic activity of almost innumerable GAs, with almost innumerable co-authors from almost all over the world', but he accepted it nevertheless, advising us with his usual taut post-card. Among the many GAs we tested, there was more than a 1000-fold range in the effective dose for inflorescence initiation (Evans *et al.* 1990).

The structural features enhancing florigenic activity were (cf. Fig. 2):

1. A double bond in the A ring at either C-1, 2 or C-2, 3, is essential for high florigenic activity, although not for stem elongation, hence the relative ineffectiveness of GA_1, GA_4 and GA_9 for flowering in *Lolium* although promoting stem elongation;

2. A free carboxy group is needed for both elongation and flowering;

3. Hydroxylation at C-12, -13 and -15 enhanced florigenic activity;

4. By contrast, C-3β hydroxylation reduced flowering but increased stem elongation, whereas C-3α hydroxylation retained florigenicity but greatly reduced stem elongation activity.

Clearly, the structural requirements for floral induction in *Lolium* are quite different from those for stem elongation, a clear example of possibly differential functions among the GAs. Some GAs promoted both flowering and stem elongation, some promoted one without the other, and some neither. Some derivatives even inhibited either elongation alone or both it and flowering (Mander *et al.* 1998*a*). Unfortunately, the most prominent endogenous GAs in the leaves of *L. temulentum* after 1 or 2 LD, namely GA_4, GA_8 and GA_9 (Pharis *et al.* unpublished; Gocal *et al.* 1999), are among the least florigenic.

The requirements for florigenicity listed above suggest that one category of GAs for us to seek would be highly hydroxylated and with an A ring double bond like GA_{32}. However, other candidate GAs have emerged from subsequent studies. When we examined further the effect of C-3 hydroxylation in several GAs, for example, we found that while 3α-hydroxylation greatly reduced their ability to cause stem elongation in *Lolium*, it did not reduce their florigenicity. Consequently, the naturally-occurring 3-epi-GA_1 and other 3α-hydroxy GAs became possible candidates, being quite florigenic without causing stem elongation (Evans *et al.*, 1994*a*). However, although such a GA may be needed for floral induction, experiments with inhibitors of 3β-hydroxylation have shown that 3β-hydroxylated GAs such as GA_1 and GA_3 are needed at some later stages of inflorescence development.

We also examined the effects of several changes in the D ring of the GA molecule (Fig. 2), particularly at the C-16, 17 double bond (Evans *et al.* 1994*b*). We had noticed appreciable variation between samples of GA_5 in the extent of stem elongation which they induced in *Lolium* without affecting the flowering response, and found it to be associated with contamination by C-16,17-dihydro GA_5, which lacks the double bond between carbons 16 and 17. The corresponding derivatives of several GAs were therefore synthesized, (together with $C_{16,17}$ hydroxy variants) and tested on *Lolium*. Once again we found differential effectiveness for flowering and stem elongation. For example, $C_{16,17}$ dihydro GA_5 was as effective as GA_5 for inducing flowering, but instead of being moderately promotive of stem elongation, it inhibited it by up to 40%, as well as being ineffective in promoting α-amylase production by half-seeds of *Lolium* (Evans *et al.* 1994*b*). It turned out that C-16,17-dihydro GA_5 seems to prevent stem elongation by inhibiting C-3β hydroxylation of GA_{20} to GA_1 (Junttila *et al.* 1997). The fact that GA_5 and C-16,17-dihydro GA_5 are equally florigenic in spite of the latter inhibiting the C-3β hydroxylation so crucial to activity in elongation confirms our conclusion that GA action on flowering in *Lolium* is quite independent of that on stem elongation.

As an unexpected spin-off from these experiments, some related compounds of 16,17-dihydro GA_5, particularly dichloromethano-16,17-dihydro-GA_5, have proved to be highly effective inhibitors of the growth of turf grasses (King *et al.* 1997) while other derivatives have promise for the control of height in cereal crops. The biological effects of yet other variations on GA structure have also been explored (Mander *et al.* 1998*a*, *b*).

Thus, there are several GAs and GA derivatives now known which could meet the requirements of an endogenous florigenic GA in *Lolium*, if only they could be found to occur naturally and shown to increase in its leaves or shoot apex in LD. In fact, recent unpublished experiments have turned up several other candidates, some of which have been detected in *Lolium*. However, we still need to ask ourselves the question: 'Are the gibberellins a physiological component of floral induction by LD in *L. temulentum*?'.

Evidence for the involvement of gibberellins

Several lines of evidence suggest that GAs are involved in the floral induction of *Lolium*:

1. The florigenic GAs are the only substances known to induce flowering in *Lolium* plants held in non-inductive SD, not only reproducibly but also as a result of single doses to intact plants of as little as 0.05 μg per plant for GA_{32}.

2. Moreover, florigenic GAs achieve this not only when applied to the leaves of intact plants in SD but also when applied via the culture medium for excised vegetative shoot

apices, with GA concentrations as low as 3×10^{-9} M. Initially we found that GA_3 is essential in the medium if apices from LD-induced plants are to form inflorescence primordia *in vitro*, whereas kinetin, IAA and abscisic acid are not (McDaniel *et al.* 1991). Subsequently, however, we also found that shoot apices excised from older plants maintained in SD of high irradiance can initiate inflorescences *in vitro* if GA_3 or other florigenic GAs are present in the medium (King *et al.* 1993; Evans *et al.* 1994*b*). Indeed, they can reach a much greater apex length and floral stage than apices of intact plants in SD with GAs applied *in vivo.*

3. The first outward sign of LD induction in *Lolium* is an acceleration of primordium formation at the shoot apex, beginning two days after the LD. This sign is also evident at about the same interval after GA application to the leaves of plants in SD (Evans and Blundell 1996).

4. After exposure to one LD there is a rise in the GA content of both leaves and apices of *L. temulentum* (Pharis *et al.* 1987*;* King and Moritz unpublished).

5. A homologue of the GAMyb transcription factor, whose expression is upregulated by GA and which may activate the LEAFY gene, is expressed only in the apical dome and lower leaf primordia of the *Lolium* shoot apex in SD. Following LD induction, however, GAMyb expression increases dramatically in the spikelet primordia during the early stages of inflorescence development (Gocal *et al.* 1999).

This evidence is persuasive, but not conclusive, that a gibberellin, possibly a GA with a highly florigenic structure, plays a causal role in the LD induction of flowering in *Lolium temulentum*. One of the problems with this conclusion, discussed above, namely that applied GA_3 causes stem elongation as well as inflorescence initiation, has been relieved by our finding naturally-occurring GAs and synthetic derivatives that are highly florigenic at doses which do not cause stem elongation.

Another problem is that shoot apices excised from plants induced by one LD still require GA_3 in the medium for inflorescences to develop *in vitro* (King *et al*. 1993), but this requirement may be for later steps in floral development rather than for floral induction, as discussed below.

The other problem, not unique to *Lolium*, is that inhibitors of several early steps in GA synthesis, such as CCC and Amo-1618, as well as inhibitors of 3β-hydroxylation such as the acylcyclohexanediones, can promote flowering when applied on the LD, although they are inhibitory to flowering when applied later and to stem elongation at all times (Evans *et al.* 1994*a*). Such promotion could be explained if the reduction in GA synthesis led to increased production of a related florigenic compound sharing the same early pathway of synthesis (Evans 1969). Alternatively, although GAs may replace or potentiate one component in the overall process of LD induction, they may adversely affect another one. We know, for example, that the flowering response to the application of GA_3 to the leaves of *L. temulentum* varies greatly with time of application, whereas stem elongation does not. The flowering response is often maximal for GA_3 applications on Day I (the long day) and Day VI, yet non-significant on Day II, the day after the long day (King *et al*. 1993). Consequently, inhibitors of GA synthesis are likely to have complex effects on the LD induction of flowering in *Lolium.*

Bearing on these problems is the question of where the GA acts in floral induction, in the leaf or at the shoot apex. Zeevaart and Lang's (1962) grafting experiment with *Bryophyllum* suggested that GA_3 acts within the leaf. It may also do so in *Lolium*, but our experiments with excised shoot apices show that GA is certainly required by the apex for its floral transition. In *Lolium* there is also the added complication that inflorescence initiation depends on the net effect at the shoot apex of a transmissible inhibitor of floral evocation from leaves in SD and a transmissible promoter from leaves in LD (Evans 1960*b*). Does GA overcome the inhibition, enhance the promotion or do both? Recent work with *Arabidopsis* may bear on this question.

Insights from *Arabidopsis*

In many rosette plants such as *Arabidopsis*, stem elongation usually accompanies floral initiation. GA_3 was found by Langridge (1957) to accelerate both, raising the question of whether the latter was simply a consequence of the former. However, flower buds usually appear at about the time when stem elongation begins (e.g. Xu *et al.* 1997), rather than following it. Wilson *et al*. (1992) concluded 'that higher GA levels are needed by *Arabidopsis* for elongation growth than for flowering in SD'. In *Lolium* such a conclusion depends very much on which GA is involved, and in *Arabidopsis* it is still not clear whether GA_4 as well as GA_1 is endogenously active *per se* (Ross 1994).

Exogenous GA_4 and GA_9 are more effective than GA_1 for both flower initiation and stem elongation in *Arabidopsis* (Xu *et al*. 1997), whereas these GAs are all relatively ineffective for flowering in *Lolium* despite being highly effective for stem elongation. Moreoever, neither GA_4 nor GA_9 is on the early 13-hydroxylation pathway which is known to be activated in *Arabidopsis* by LD (Xu *et al*. 1995).

The main advantage of *Arabidopsis* for flowering studies lies in the range of its mutants. Those for the various steps in floral differentiation have been particularly enlightening, but those involving various steps in GA synthesis, and action are our concern here. Unfortunately, the current system for naming them is somewhat confusing when various gibberellins such as GA_1, GA_4 and GA_5 are also being considered. The most relevant dominant genes are designated *GA1*, *GA4* and *GA5*, and their recessive mutants as *ga1*, *ga4* and *ga5*. Yet another source of confusion is *gai*, a mutant which is insensitive to GA and behaves quite differently from *ga1*.

One of the earliest acting genes is *GA1*, which controls the first committed step in GA biosynthesis, namely the formation of *ent*-kaurene by *ent*-kaurene synthase A (Sun and

Kamiya 1994). Using the *ga1-3* mutant, Wilson *et al*. (1992) found that it could not flower in SD unless treated with exogenous GA, but did flower in continuous light after some delay, i.e. the mutant made *Arabidopsis* into an obligate LDP. The failure of *ga1-3* mutants to flower in SD is paralleled by the lack of induction of the LEAFY promoter in SD and reduced expression of LEAFY in LD (Blazquez *et al*. 1998).

The 20-oxidase step, which converts C-20 to C-19 GAs, is an important environmental control point in *Arabidopsis* as in other rosette plants (Phillips *et al*. 1995; Xu *et al*. 1997). Exposure to LD leads to a rise in C-19 GAs. Expression of the *GA5* gene, which encodes GA_{20}-oxidase, correlates with the earliness of flowering and the rate of stem elongation in *Arabidopsis* (Xu *et al*. 1997) and leads to an increase in GAs in the later stages of the 13- hydroxylation pathway.

Another gene, *GA4*, controls one of these later steps in *Arabidopsis*, encoding 3β-hydroxylase which appears to control the conversion of GA_{20} to GA_1 and also of GA_9 to GA_4, but its expression is low in the stems and does not correlate with the rate of stem elongation (Xu *et al*. 1997). Cowling *et al.* (1998) have shown that active (i.e. 3β-hydroxylated) GAs regulate *GA4* transcript abundance (except in the GA response mutants *gai* and *spy5*), thus providing a sensitive feedback mechanism for the regulation of their endogenous GA levels, even after exogenous GA applications.

In contrast to the mutants which affect GA synthesis in *Arabidopsis,* SPINDLY mutants apparently activate GA signal transduction constitutively, and flower early (Jacobsen and Olszewski 1993). The non-leaky *gai* is a mutant repressor that is relatively resistant to the effects of GA (Peng *et al.* 1997) and has higher than wild type levels of endogenous active GAs (Talon *et al.* 1990). It flowers readily in continuous light but only slowly in SD, even when treated with GA_3 (Wilson *et al.* 1992).

The results of experiments with these various mutants have been interpreted by several arabidopsologists (e.g. Weigel 1995; Koornneef *et al.* 1998) as indicating two pathways to flowering in *Arabidopsis*, a slow autonomous (i.e. age-dependent) pathway and a facultative fast pathway in LD, with the suggestion that GA acts only on the 'slow' pathway. However, Wilson *et al*. (1992) observe that because the *ga1-3* mutant flowered somewhat later even in continuous light, neither pathway is likely to be completely independent of GA. Also, the behaviour of the *gai* mutant in their experiments suggests that some important change other than a rise in GA levels accelerates flowering in LD.

Thus, even with the help of a suite of mutants affecting the metabolism of, and response to, GAs, their role in the induction of flowering in *Arabidopsis* by LD remains obscure, and possibly multiple, as in *Lolium*.

Conclusion

Although it is over 40 years since Lang first elicited flowering in a long-day plant with GA_3, this admittedly selective review indicates that, in spite of Lang's (1965) invocation of Koch's postulates to conclude that the role of GA in flowering was 'physiological', we are still not clear what that role is, whether different endogenous GAs play different roles, and to what extent their roles differ between species.

Most of the LDP that flower after GA treatment form rosettes in SD, and their flowering is often confounded with concurrent stem growth. However, the experiments with the growth retardants and mutants in several rosette LDPs make it clear that floral induction can be independent of stem growth, as it clearly is in the non-rosette *Lolium*, in which the GA structures promoting floral induction are quite different from those promoting stem elongation. Thus different specific GAs may control the two processes *in vivo*, and different GAs may also be involved in the later stages of floral differentiation, as the experiments with *Lolium* also suggest.

In both *Arabidopsis* and *Lolium* there is also evidence that more than one pathway to floral induction in LD may involve GAs, and that not only the concentration and composition of GAs may change in LD, but also the responsiveness of the plants to them. We need a better understanding of biochemical pathways branching off from the GA highways, of metabolism of the florigenically-active GAs other than inactivation by 2β-hydroxylation, and of the effects of daylength not only on GA metabolism but also on the receptors for, and early responses to, the various endogenous gibberellins.

Envoi

Research papers which break new ground often continue to be cited for many years, whereas reviews tend to have a shorter working life. However, in the survey of citation classics in the plant sciences by Eugene Garfield (1987) — one in which 20% of the authors were affiliated with Australian research groups — reviews had by far the highest impact factor, in keeping with their valuable role of helping us to keep abreast of developments in related fields of research.

In writing the first of this series of eponymous reviews, I have had the sensation of walking on my own ashes, which is perhaps inherent in the nature of scientific advance. My hope is that future reviews in this series will reveal something of the human side of science, capture something of each author's unique perspective on the field, remind readers of awkward facts which seem to run counter to current paradigms, and peer forward to future possibilities.

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