Gibberellins and grain development in barley

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Abstract

This review deals with gibberellins (GAs) and their possible physiological activity in developing cereal grains, particularly barley (*Hordeum vulgare* L.). Data on the occurrence of GAs in developing barley grains, and their metabolic interconversions, are summarised. These results are discussed in relation to other aspects of grain development, including the developmental profiles of the other major hormones. The first convincing evidence that GAs are involved in seed development and growth came from studies on GA-deficient mutants of pea (*Pisum sativum* L.), and similar mutants in barley will allow such studies to be extended to this species. The well-known induction of α-amylase by GA treatment of aleurone tissue from mature barley grains provides a valuable conceptual framework for investigating GA (and ABA) activity in late stages of grain development. It potentially allows data on hormone contents, and target tissue sensitivity and response capacity, to be integrated with physiological responses critical in the balance between grain maturation and sprouting.

Additional key words: *abscisic acid, α-amylase, aleurone, auxin, cytokinin, GA-deficient dwarf mutant*

Introduction

Gibberellins (GAs) are important hormonal regulators of vegetative growth in plants. Their effects are most clearly revealed by the dwarf stature of GA-deficient mutants, and by the rapid growth observed when such mutants are treated with an active GA. This growth-promotive activity of GAs has prompted an interest in whether they play a similar role in regulating the growth of seeds. A number of lines of evidence are consistent with such a possibility, including the fact that developing seeds are often a very rich source of GAs, and that peak GA contents may occur when seed growth rates are high. It is only recently, however, that more direct evidence for a role of GAs in seed development has been obtained. Swain *et al.* (1995; 1997) used a GA-deficient mutant (*lh*) of pea (*Pisum sativum* L.) that affects the production of GAs in both vegetative tissue and developing seeds. A comparison was made between seeds of different genotype developing on *lhlh* mutant plants after pollination with either wild type (*Lh*) or mutant (*lh*) pollen. Homozygous (*lhlh*) seeds had an increased rate of abortion when compared to heterozygous (*Lhlh*) seeds. Furthermore, the *lhlh* seeds that survived had a reduced rate of embryo and seed growth, associated with a much lower content of bioactive GAs than either the wild type or heterozygous seeds. The conclusion from these studies was that seed GAs are required for early seed development and growth, most probably by promoting assimilate unloading. This conclusion is based on evidence from two mutants at a single GA biosynthetic locus in pea, and further studies using other mutants and other species are required before its generality is established. Nevertheless, these studies establish a strong case for the importance of GA biosynthesis in seed growth and development. In this review I would like to summarise what is currently known about GAs in developing grains of cereals, particularly barley, and address potential physiological roles. Other more general reviews on related topics include those of Pharis and King (1985), and Garcia-Martinez and Hedden (1997).

Patterns in Grain Growth and Development

Early expansion of the barley grain is dominated by elongation of maternal tissues, especially the pericarp, which elongates to attain final grain length in the first 7-10 days post-anthesis (dpa). Growth of the enclosed embryo and endosperm begins during this time, and after the first week or so, the endosperm becomes the major component of growth. Development of barley endosperm has been divided into four stages (Bosnes *et al.*, 1992): a syncytial stage, covering the first 5 or 6 dpa, a cellularisation stage lasting about 2 days, a differentiation stage (from 8 to 22 dpa) when further division of endosperm cells occurs and different sub-types of aleurone and starchy endosperm cells are

formed, and finally a maturation stage during which grain filling is completed and mature sub-types of endosperm cells become recognisable. Growth of the embryo occurs predominantly during the latter half of endosperm growth, but is quantitatively minor because it comprises only a few percent of grain mass.

Against the background of these events, the accumulation profiles of the major grain hormones can be superimposed. First we will look briefly at cytokinin, auxin and abscisic acid (ABA) before considering GAs in greater detail. Morris (1997) provides an excellent summary of cytokinins and auxins in developing caryopses of cereals. An early peak of cytokinin (predominantly zeatin and zeatin riboside) activity occurs in the first week following anthesis, and has been found in several cereal species including barley. In maize, rice and wheat the timing of this early peak varies slightly, but coincides with the peak in endosperm mitotic index, perhaps indicative of a causal relationship. The rapid decline in cytokinin levels that follows the peak may result from increased cytokinin oxidase activity.

Auxin content increases at a later stage in grain development; for instance, in both barley and wheat IAA increases rapidly from about 8 to 20 dpa, coinciding with the early stages of rapid grain-filling (Bangerth *et al.*, 1985). The vast majority of the IAA formed exists as conjugates. Morris (1997) and Bandurski *et al.* (1995) have discussed possible physiological roles for free IAA in the endoreduplication of endosperm nuclei, and for IAA-conjugates in providing a source of free IAA for post-germinative growth of the shoot. Darussalam *et al.* (1998) studied the effect of IAA applied to either the stem internode lacuna of wheat plants or to detached cultured ears, and observed considerable increases in grain growth rates at 10, 20 and 30 dpa. They concluded that IAA stimulated photoassimilate transport both to and within developing wheat grains.

ABA accumulation occurs late during development of wheat and barley grains (King, 1976; Walker-Simmons and Sesing, 1990; Morris *et al.*, 1991). Grains developing under cool conditions show a peak in ABA content at 40-60 dpa, before a decline that accompanies water loss. The developmental profile of embryo ABA content was similar to that of ABA in the remnant of the grain, although on a dry weight basis the embryo ABA content tended to be higher than the ABA content of endosperm. ABA plays an important role in promoting development of wheat and barley embryos, at least in culture, and in regulating the onset of gene expression associated with embryo maturation and desiccation tolerance (Bartels *et al.*, 1988; Rock and Quatrano, 1995). The relationship of ABA content to dormancy is also of considerable interest, although changes in responsiveness to ABA may be more important than changes in ABA content (Walker-Simmons and Sesing, 1990). In maize there is a very clear association between kernel ABA and germination, since both ABA-deficient mutants and mutants with reduced ABA sensitivity show vivipary (Smith and Fong, 1993). In wheat and barley the evidence is not as convincing, partly because the corresponding mutants have not been isolated. However, Kawakami *et al.* (1997) recently described non-dormant mutants of wheat that had reduced embryo sensitivity to ABA, as well as a reduced ABA content at 30 dpa.

GAs in Developing Grains of Barley

Early studies on GAs in developing grains used analytical procedures that failed to distinguish between many of the GAs that we now know to be present. There have been several studies of endogenous GAs in developing barley grains that have used GC-MS or GC-SIM procedures, and only these will be considered. Developing grains of Proctor barley 3 weeks post anthesis (milk ripeness) contained GA_{12}, GA_{17}, GA_{25}, GA_{34}, GA_{48}, GA_{69}, 18-OH-GA_4, and traces of GA_1, GA_4, GA_8 and GA_{20}. A fraction that would have contained conjugates was treated enzymatically, and GA_{17}, GA_{20}, and GA_{48} were identified. In addition, a tentative identification of 18-OH-GA_{34} was made in both the original acidic ethyl acetate fraction, and in the putative conjugate fraction (Gaskin *et al.*, 1984). These GAs include members of the early 13-hydroxylation pathway (GA_1, GA_8, GA_{20}), known to be synthesised in germinated barley grains (Grosselindemann *et al.*, 1992), as well as non-3,13-hydroxylated GAs (GA_{12}) and 3-hydroxylated GAs (GA_4, GA_{34}). In addition, the presence of 12β-hydroxylated and 18-hydroxylated GAs (GA_{48}, GA_{69} and 18-OH-GA_4, 18-OH-GA_{34} respectively) indicates that other hydroxylases are present and active in developing grains, or that there is a considerable lack of specificity in the grain isozymes that carry out these hydroxylations.

In a follow-up study, metabolism experiments were carried out by injecting double-labelled GAs into developing endosperm (4 to17 days post-anthesis) and examining the metabolites present after a further 7 days (Gilmour *et al.*, 1984). Evidence for a functioning early 13-hydroxylation pathway in very young grains (4 to 7 days post-anthesis) was provided by feeds of GA_{12}-aldehyde (products: GA_1, GA_8), GA_{20} (products: GA_1, GA_8, GA_{29}) and GA_1 (product: GA_8). Feeds of other intermediates showed, in addition to the 2β- and 3β-

hydroxylations seen above, single or multiple hydroxylations at positions 12β and 18, consistent with the analyses of endogenous GAs (Gaskin *et al.*, 1984). Several conclusions were made: (i) 13-hydroxylation was observed with early intermediates (GA_{12}-aldehyde), but not with later intermediates (GA_4, GA_9), (ii) there was a degree of specificity in hydroxylation patterns, for instance 13-hydroxylated GAs were not 12β- or 18-hydroxylated (but note the possible presence of 18-OH-GA_1 later reported by Green *et al.*, 1997), and 18-hydroxylation was observed only on 3β-hydroxylated substrates, and (iii) 2β-hydroxylated GAs underwent extensive further hydroxylation. Although both of these studies involved qualitative analyses, 18-OH-GA_4 was regarded as a major GA on the basis of ion intensity. Furthermore, the GA contents in developing grains were much higher than those found in grains germinated for 3 days (Gaskin *et al.*, 1984).

GAs in developing grains of Triumph barley were analysed by Boother *et al.*, 1991. GAs in the early 13-hydroxylation pathway (GA_{20}, GA_1) were detected in grains at 26 dpa, but not at 33 or 54 dpa. A variety of other GAs was detected, similar to those found in Proctor barley above, including the characteristic 18-hydroxylated derivatives of GA_4, GA_{34} and GA_{48}.

A study of GAs in developing grains of Himalaya barley and of a GA-deficient dwarf mutant in this variety (M117, mutant at the *grd1* locus) revealed a major reduction in grain GAs in the dwarf mutant relative to the tall parent. Developing grains of Himalaya had their highest content of bioactive GAs (GA_1 and GA_4) at 20 dpa, and these declined thereafter, as the content of the respective 2β-hydroxylated derivatives (GA_8 and GA_{34}) increased (Table 1). Throughout development the GA content of the dwarf mutant was only a few percent of the wild type. Associated with a reduced GA content, the growth rate of grains of the dwarf mutant was considerably less than that of Himalaya, and final grain size was reduced by 20 % on a dry weight basis.

The presence of GA conjugates in developing barley grains was alluded to above, but Senns *et al.* (1998) have now identified and quantified GA-glucosides including GA_1-3-*O*-Glc, GA_{20}-13-*O*-Glc, GA_8-2-*O*-Glc, and GA_{29}-2-*O*-Glc. In barley varieties Himalaya and Salome, the contents of GA_{20}, GA_8, and their corresponding glucosides have been followed through grain development and maturation. In Himalaya, the GA_{20} content was greatest at milk ripeness, and generally declined thereafter. The corresponding GA_{20}-13-*O*-Glc was at lower levels through most of development, but in the mature grain was at levels (0.4 ng per g f. wt.) that were 3-4 times higher than those of GA_{20}. The content of GA_8 was greater than that of GA_{20}, and the peak was observed later in development (early ripeness). Typically, there was much more GA_8-2-*O*-Glc than GA_8, and at maturity the excess was about 17-fold (Senns *et al.*, 1998).

The GA-glucosides are of particular interest from the perspective of whether they can be a source of bioactive GAs following germination; for instance, conjugates of GA_{20} and GA_1 could hydrolyse following imbibition to provide active or potentially active GAs that stimulate post-germinative growth and α-amylase production. Opinion is divided as to whether de novo GA biosynthesis is required for α-amylase production following germination, and conjugates could provide a supply of active GAs that might explain differences in results between research groups (Grosselindemann *et al.*, 1991; Zwar and Chandler, 1995). A vivid example of the potential for precursors of active GAs accumulated in

Table 1. GA contents of developing grains of Himalaya barley and of a GA-responsive dwarf mutant (M117).

		Content of GA (pg/grain)[1]			
Line	DPA	GA_1	GA_4	GA_8	GA_{34}
Himalaya	20	114	1360	528	68700
Himalaya	30	58	813	1460	15900
Himalaya	40	3.0	309	3420	20700
M117	20	5.5	18.5	35	645
M117	30	1.5	50.0	<23	302
M117	40	2.0	84.0	23.5	844

[1] Determined from calibration curves for GA_1, GA_4, GA_8, and from peak area (corrected) for GA_{34}. Data from P.M. Chandler and A. Poole, unpublished.

seeds to influence post-germinative growth is provided by the slender mutant in pea, especially in a vegetative dwarfing background (Ross *et al.*, 1995).

Green *et al.* (1997) analysed the GAs in developing grains (40 dpa) of a shrivelled-grain mutant of barley that accumulates high levels of α-amylase activity in the mutant grains as they mature. They compared normal and shrivelled grains that were segregating in the ears of heterozygous parent plants, to investigate whether differences in the contents of active GAs might account for the considerable production of α-amylase that begins in the shrivelled grains at this stage of development. There was previous evidence suggesting that GAs were involved, as there was a 10- to 20-fold reduction in the α-amylase content of shrivelled grains when this mutation was crossed into a GA-deficient dwarfing background, now known to have a very low content of grain GAs (M117, see above, and Table 1). The analysis of a range of GAs in the normal and mutant grains revealed considerable differences. For instance, the content of 2β-hydroxylated GAs was about 10-fold higher in normal grains than in mutant grains. In contrast, the content of GA_1 (bioactive) in mutant grains was 5.8-fold higher than in normal grains, and 18-OH-GA_1 was present at 28-fold the level in normal grains. This latter GA may have intrinsic activity, since the activity of GA_{22} (18-OH-GA_5) has been reported to be equal to that of GA_5 (Crozier *et al.*, 1970) in a barley half-grain assay. It was concluded that the higher than normal levels of active GAs in the shrivelled grains could potentially account for their premature expression of α-amylase. These high levels of active GAs may be a consequence of reduced levels of 2β-hydroxylation in the mutant grains, but this requires confirmation by metabolic studies.

Interpreting Data on Hormone Content

Some general comments should first be made on interpreting the data on GA contents in developing seeds, and, to varying extents, these comments also apply to the other hormones discussed above. First, grain GAs are often present at very high levels, and often include structures that are considered to have low or zero biological activity. It seems a general feature of developing seeds that hormones are present at high levels, e.g., cytokinins are often at levels 10 times higher than in vegetative tissue (Morris, 1997). Most attention is naturally given to analysing those GAs (e.g., GA_1) that are known to be active in established assays, for instance growth promotion or α-amylase induction. The very high contents of "inactive" GAs, such as GA_8, GA_{34} and their hydroxylated derivatives are difficult to interpret, but until we know more about the role of GAs in developing grains we may lack the appropriate assay to determine whether these GAs are truly "inactive" from a grain perspective.

Second, the developmental profile of hormone content does not always relate in a simple manner to the potential activity of the hormone, since this will depend on its local concentration surrounding an appropriately responsive target tissue. There have been major technical advances that now allow reliable and accurate determinations of many GAs, but currently there is little information on sites in the developing grain where these different GAs occur. A pertinent example to consider is the response of aleurone to GA, especially α-amylase production, since this has been extensively studied in developing grains of wheat and barley. Ideally we would like to know the concentration of GAs surrounding aleurone tissue, rather than in the whole grain. Although the peak content of active GAs occurs about 3 weeks post-anthesis, aleurone tissue does not appear to respond to GAs earlier than about 4 weeks post-anthesis, and it may not achieve maximal responsiveness until much later (Green *et al.*, 1997). Even in relatively well-defined systems there are still major impediments to predicting behaviour based on hormone analyses. A further consideration is that there are large changes in water content as grains develop and mature. If a hormone is distributed uniformly through tissue water, its concentration may actually increase, even while the hormone content is declining, if the rate of water loss exceeds that of hormone loss.

Third, it is common to find that the activity of a hormone does not depend solely on its own concentration, but also on the concentrations of antagonistic factors. GAs are no exception, and the classic effects of GA on α-amylase production by aleurone, and on growth, are both antagonised by ABA. Thus, to develop the aleurone example (above) still further, it is not sufficient to know the concentrations and relative activities of different GAs in the vicinity of aleurone, and the responsiveness of the tissue to GA. We also need the same information concerning ABA, and perhaps other antagonists and agonists as well.

Fourth, most studies analysing hormone contents in developing seeds have used cultivated or bred varieties, rather than wild relatives. The conclusions reached about the possible roles of hormones in seed development and/or growth may relate more to the changes that have accompanied domestication and breeding (e.g., increased seed size, reduced seed dormancy), than to their original biological roles.

Possible Physiological Roles of GAs in Developing Grains

It is commonly assumed that GAs have some role to play in developing grains, because of their widespread occurrence in developing seeds, their importance in regulating vegetative growth, and their importance in mobilising endosperm reserves in germinated barley grains (Jacobsen *et al.*, 1995; Zwar and Chandler, 1995). It is much more difficult to demonstrate a specific role. The obvious possibility to consider is that GAs are important for grain growth, since at least for Himalaya barley, the maximum content of bioactive GAs occurs when growth rates are high. Further support for this view is provided by the observation that grains of the GA-deficient dwarf M117 (see Table 1) grow more slowly than wild type grains, and achieve a smaller final dry weight. However, there are other GA-deficient loci in barley that also have smaller grain sizes, yet have normal grain GA contents (P. M. Chandler, unpublished observations). In these cases the smaller grains on dwarf parent plants might result from the limited grain-filling capacity of the parental plant. The experiments necessary to resolve the possible influences of grain GA content and maternal factors have not yet been carried out in barley. In wheat, the dominant *Rht* mutations are associated with reduced sensitivity to GA, and with smaller grain size. Pinthus and Gale (1990) have shown by the analysis of F_2 grains born on *Rht/rht* F_1 spikes that there are maternal factors that account for the reduced grain size, although their nature has not been determined.

The availability of mutants such as M117 that affect grain GA content provides several experimental options for studying the role of GAs in grain growth.

i Is there an early peak in GA biosynthesis that is not affected in the mutants? In pea, it was the early capacity for GA biosynthesis that seemed to be important for seed formation and early growth rates, rather than the late peak in GA content. Most of the barley studies have concentrated on the mid-developmental stages, and the possible relationship of GA content to grain growth rate is only just emerging.

ii Reference was made above to elongation of the pericarp, maternal tissue that could potentially limit the length attained by a developing grain. Little is known regarding the GA dependence of this elongation. Does treatment of the parent plant with GA stimulate grain growth rates? Under normal conditions, GAs may be required for grain growth, but may not be limiting. A GA-deficient mutant provides ideal material for such investigations.

iii Are GAs in the grain of maternal or filial origin? By analysing crossed grains it should be possible to distinguish whether grain GAs are derived from maternal tissue, or depend on embryo/endosperm genotype.

Another important issue related to grain GAs is their role in determining the α-amylase contents of developing grains. In early work, Duffus (1969) showed considerable α-amylase activity present in aleurone and starchy endosperm of maturing barley grains at 20-40 dpa. Evidence that this might be due to grain GAs came from the use of an inhibitor of GA biosynthesis, chlorocholine chloride (CCC), which prevented α-amylase production, and by restoration of α-amylase production in the presence of CCC plus GA_3. We have already discussed the study on a shrivelled-grain mutant of barley that produces α-amylase late in grain development, and the possibility that this results from abnormally high levels of GA_1 and possibly18-OH-GA_1 (Green *et al.*, 1997). Another mutant of interest is slender, *sln1* (Foster, 1977), which is interpreted as a constitutive GA response mutant (Chandler, 1988; Lanahan and Ho, 1988). In a Himalaya barley background, plants that are heterozygous at this locus produce grains that give rise to normal and slender seedlings in a 3:1 ratio. The grains that are homozygous at *sln1* frequently show visible sprouting in the ear, and have a considerable α-amylase content when assayed in the mature grain (P.M. Chandler, unpublished data). There is variation in the degree to which these two processes occur, probably related to environmental factors that alter the rate of grain maturation. In this respect, the behaviour of the slender mutant is akin to pre-harvest sprouting observed in both wheat and barley. Both situations involve a modification to the normal maturation program, with the result that early events in germination occur before final grain maturity is attained. In wheat, there is also the distinct phenomenon of late maturity α-amylase production. This differs from the classic sprouting situation in the lack of visible germination, and in a uniform distribution of α-amylase throughout the endosperm. Nevertheless, it appears that GAs are potentially involved, since the presence of the *Rht* dwarfing loci reduces the levels of α-amylase at maturity (Mrva and Mares, 1996). In all these examples where differences in grain α-amylase content are attributed to differences in GA content or sensitivity, the possible influence of ABA on α-amylase content is often only poorly understood.

Conclusions

We are just beginning to understand the role of GAs (and other hormones) in cereal grain development, but it is likely that there will be considerable progress in the next decade. This will depend on reliable assays for the hormones, which are now available, coupled with experiments that are biologically refined; these may involve sampling precise parts of the grain, the use of well-defined mutants, and concurrent analyses of particular aspects of grain physiology and gene expression related to the hormone(s) under investigation. It is also likely that approaches using transformation and reporter genes will begin to contribute to our scientific understanding of cereals, as they have done for several years for dicotyledonous plants.

Cereals account for 54 % of current total world food production, amounting to nearly one billion tonnes of grain per year, mostly of wheat and rice (Evans, 1998). Clearly, there is much progress to be made in defining the physiological roles of hormones in developing cereal grains, as even small effects assume significance on such a large scale. This knowledge will be of intrinsic interest to our minds, and perhaps of more practical interest to our stomachs.

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