J Plant Growth Regul (2003) 22:73–81
DOI: 10.1007/s00344-003-0024-9

J P G R
Journal of Plant Growth Regulation

Hormonal Interactions in Fruit Development

Jocelyn A. Ozga* and Dennis M. Reinecke

Plant Physiology and Molecular Biology Research Group, Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada

Abstract

Fruit development involves a complex interplay of cell division, differentiation and expansion of sporophytic and gametophytic tissues that is carefully coordinated temporally and spatially. Plant hormones are signal molecules that regulate many processes of plant development, including fruit development leading to mature fruit and viable mature seed. Auxins, gibberellins, cytokinins, abscisic acid, and ethylene have been implicated at various stages of fruit development. In the past, hormone application studies and hormone analysis studies have supported the hypothesis that fruit development is in part regulated by hormonal interaction. More recently, biochemical, genetic, and molecular studies are beginning to unravel the complexities of how hormones affect fruit development. In the current work, we review selected studies that show the interplay between hormones during fruit development, with an emphasis on the interaction between auxin and gibberellin in pea fruit development.

Key words: Fruit development; Hormonal interaction; Auxin, Gibberellin; *Pisum sativum*; *Arabidopsis*; Pericarp; Ovary; Tomato

Introduction

Plant hormones play a significant role in the processes that lead to mature fruit and viable mature seed (Nitsch 1970). Auxins, gibberellins (GAs), cytokinins, abscisic acid (ABA), and ethylene have been implicated at various stages of the fruit growth cycle (Crane 1964; Nitsch 1970; Pharis and King 1985; Gillaspy and others 1993). Data supporting interactions between hormones in fruit development have come from three types of experiments: (1) hormone application studies, (2) hormone analysis studies, and more recently, (3) biochemical, genetic, and molecular studies.

To understand how hormones are involved in regulating different stages of fruit development, we first need to examine the life cycle of a fruit. Fruit development can be separated into several phases: (1) initiation of the primordia; (2) development prior to pollination and fertilization; (3) pollination, fertilization, and initial fruit set; (4) growth after fruit set; (5) ripening; and (6) senescence. Hormones have been implicated as signals that influence each of these phases (Nitsch 1970). Once floral and fruit (ovary and ovule) primordia are initiated, the ovary and ovule tissues develop until prior to the pollination/fertilization phase. At this point, ovary and ovule development slows, and will cease, and typically senescence will ensue unless events

Received: 22 January 2003; accepted: 11 March 2003; Online publication: 10 July 2003
Corresponding author; e-mail: jocelyn.ozga@ualberta.ca

triggered by pollination of the ovary and fertilization of the ovules occur. Following fertilization of the ovules, cell division and enlargement occurs in both the ovary (pericarp) and the ovules (seeds) resulting in fruit growth (fruit set). After fertilization, pollinated ovaries may still not attain sustained growth as a result of failure of syngamy (leading to embryo formation), and/or triple fusion (leading to the formation of the endosperm), or degeneration of the endosperm and embryo. In general, normal fruit growth requires the presence of developing seeds (Nitsch 1970; Eeuwens and Schwabe 1975; Ozga and others 1992). In addition, an individual fruit must effectively compete with other fruits, growing apices (shoot and root) and other plant organs for plant nutrients during growth. The final phase of fruit development is ripening and senescence. This review will focus on hormonal interactions that affect developmental events in fruit, from pollination of the ovary through fruit-set and subsequent ovary growth prior to ripening and senescence. Auxin (4-chloroindole-3-acetic acid, 4-Cl-IAA) regulation of GA biosynthesis in pea fruit will be highlighted because it is the most characterized example of hormonal interactions in the developmental stages reviewed.

From previous work on fruit development during the last 30 years, it is well established that all classes of plant hormones, auxins, GAs, cytokinins, inhibitors (for example, ABA), and ethylene play an important role in fruit development (Nitsch 1970). The precise spatial and temporal biosynthesis and action of these hormones, and their interactions to bring about normal fruit development, are only beginning to be understood.

The possible types of hormone interactions required to bring about fruit growth and development include:

1) One hormone affecting the biosynthesis of another hormone.
2) A component(s) of the signal transduction pathway of one hormone affecting a component(s) of the signal transduction pathway of another hormone.
3) Two (or more) hormone classes acting independently of one another, with their concerted action being required to bring about normal development. For example, each hormonal class affects a different cell type or tissue within the fruit but the concerted action of all hormones is required for normal fruit development.
4) One class of hormone triggers an event but another hormone class is required to bring about completion of the event.

Pollination and Fertilization

In general, the production of new cells accounts for most of the growth of fruits before pollination/fertilization (Nitsch 1970). The slowing or cessation of ovary/ovule growth and the resumption of growth following pollination/fertilization in these tissues is certainly regulated by hormones. The precise cause(s) of the cessation of growth resulting from the failure of pollination and fertilization is not understood; however, a number of events are likely involved. The level of endogenous hormone or hormone-like substances drops to a low level in the ovary/ovules at this time (auxin- and GA-like substances in grape, Coombe 1960; and other studies cited in Nitsch 1970; GAs in tomato, Fos and others 2000, 2001).

Inhibitors of ovary growth in surrounding and distant tissues appear to be present prior to pollination/fertilization events. The presence of floral whorls surrounding the ovary significantly reduced parthenocarpy (the development of a fruit without fertilization of the ovules) of the *Arabidopsis fwf* (fruit without fertilization) mutant, indicating that interfloral organ signals and functional FWF activity normally act in concert to prevent fruit initiation in the absence of pollination and fertilization (Vivian-Smith and others 2001). Vivian-Smith and coworkers (2001) speculated that FWF may inhibit auxin-dependent processes because the *fwf* mutant exhibits some auxin-related morphology. Furthermore, because GA biosynthesis is essential for pollination-induced and parthenocarpic silique growth in *fwf*, the authors suggest that the interaction of auxins and GAs is required for fruit development in *Arabidopsis,* although the specific nature of that interaction is not currently known.

Pea fruit (*Pisum sativum* L.) has been a model system to understand how GAs and more recently how auxins are involved in fruit development (Eeuwens and Schwabe 1975; Rodrigo and others 1997; Reinecke and others 1995; Reinecke 1999). Pea plants are self-pollinating, and anthesis (fully reflexed floral petals) is a standard morphological characteristic used to stage fruit development (days after anthesis [DAA]). Pollination in pea occurs approximately 24 to 36 h before anthesis whereas fertilization of the ovules occurs by anthesis (0 DAA)(Cooper 1938). Pea fruit are moderately sized allowing for easy manipulation of the ovary and seeds to investigate the roles of seeds and hormones in fruit development.

Parthenocarpic fruit can be used as a tool to understand pre- and post-pollination signals required

for ovary growth. In intact pea plants, GA treatment to unpollinated ovaries stimulates parthenocarpy (Carbonell and García-Martínez 1980). Parthenocarpy can also be induced by removing the shoot just above the unpollinated ovary (Carbonell and García-Martínez 1980), and this induced parthenocarpy can be inhibited by the application of IAA to the remaining stump (Rodrigo and García-Martínez 1998). In addition, the GA parthenocarpic mutant, *gio* (unpollinated *gio* ovaries are less sensitive to applied GA), has a higher IAA content in the shoot apex and higher IAA basipetal transport from the shoot apex (Rodrigo and others 1998). The sensitivity of *gio* ovaries to GA_3 was significantly increased by removing the apical shoot and by blocking the transport of IAA from the apical shoot with 2,3,5-triiodobenzoic acid (IAA transport inhibitor) (Rodrigo and others 1998). Therefore, these studies suggest that when GA levels are low (in the absence of pollination/fertilization of the ovary/ovules), the shoot apex acts to inhibit fruit growth and this inhibition is associated with basipetal IAA transport (Rodrigo and García-Martínez 1998; Rodrigo and others 1998). Additionally, an inhibitory role for ABA in parthenocarpy was suggested by Rodrigo and García-Martínez (1998) because ABA application to the stump of decapitated plants inhibited parthenocarpic fruit growth, and IAA treatment of the stump enhanced the content of endogenous ABA in the ovary.

Ovary response to hormones varies with pollination/fertilization status of the ovary. Unpollinated pea ovaries (pericarps) respond differently to exogenous GAs and auxin (4-Cl-IAA) than pollinated deseeded pericarps. In unpollinated pericarps, both GA_3 and 4-Cl-IAA stimulated pericarp growth, but GA_3 was significantly more active in stimulating all measured parameters of pericarp growth than 4-Cl-IAA (Ozga and Reinecke 1999). 4-Cl-IAA, GA_1, and GA_3 were similarly effective at stimulating pericarp growth in pollinated deseeded pericarps. In addition, the synergistic effect of simultaneous application of 4-Cl-IAA and GAs on pericarp growth was observed only in pollinated deseeded pericarps (Ozga and Reinecke 1999). These data suggest that pollination of the ovary or fertilization of the ovules removes an inhibition or increases sensitivity to auxin-stimulated ovary growth.

The reduction of inhibitory influences is apparently not sufficient for ovary commitment to proceed with fruit development (fruit set). For example, excised pollinated ovaries of *Cucumis anguria* transferred to sterile culture on a simple mineral salts and sugar medium were able to grow, whereas excised unpollinated ovaries, though they remained green and intact, were unable to grow on the same medium (Nitsch 1951). Therefore, fruit set is also dependent on positive growth signals generated during pollination/fertilization. These positive growth signals are derived from pollen, and/or produced by the ovary in response to pollen germination, pollen-tube growth, and/or fusion of the nuclei (fertilization) in the ovule. Growth factors by which pollination/fertilization might influence fruit set include auxins, GAs, cytokinins, and ethylene (Crane 1964; Nitsch 1970; O'Neill 1997).

In orchid (*Phalaenopsis* spp.), pollination of the ovary and fertilization of the ovules is separated in time (11 weeks) (O'Neill 1997). The pollination event initiates a cascade of responses including ovary and ovule development prior to fertilization. Auxin-stimulated ethylene has been implicated as a primary signal stimulating these processes (Zhang and O'Neill 1993; O'Neill 1997).

In many fruits, size is largely a function of cell division in the early stages and cell enlargement in the final stages of fruit growth. However, final fruit size in a number of species is a function of cell number, which is determined during the early stages of fruit growth (greater fruit cell number correlates with larger fruit at maturity) (Smith 1950; Bohner and Bangerth 1988; Scorza and others 1991). Cytokinins promote cell division in plants (Mok 1994) and cytokinin levels peak during the phase of high mitotic activity in tomato fruit (4 d after pollination) (Bohner and Bangerth 1988). Cytokinins no doubt interact with the other classes of plant hormones to stimulate and coordinate fruit development. However, their specific interactions are not currently understood.

GAs have been implicated in many aspects of reproductive development (Pharis and King 1985). Pea plants metabolize GAs by the early 13-hydroxylation pathway: $GA_{12} \rightarrow GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1$ (Sponsel 1995). In pea, *PsGA20ox1* codes for the multifunctional enzyme GA 20-oxidase that converts GA_{53} to GA_{20} (Martin and others 1996; García-Martínez and others 1997) and *PsGA3ox1* (Mendel's *LE* gene) codes for the GA 3β-hydroxylase (Lester and others 1997; Martin and others 1997) that converts GA_{20} to the biologically active GA_1. Pericarps from unpollinated pea ovaries (2 d before anthesis) contained high levels of *PsGA20ox1* mRNA (van Huizen and others 1997) and GA_{20} (5 ng· g fwt^{-1} for emasculated ovaries at the equivalent to 0 and 1 DAA) (García-Martínez and others 1991). However, pericarps from unpollinated ovaries (2 d before anthesis) contained minimally detectable levels of *PsGA3ox1* mRNA (Ozga and others 2003) and GA_1 (García-Martínez

and others 1991). After pollination, pericarp and seed *PsGA3ox1* mRNA levels increased 50-fold and 19-fold, respectively. By 1 DAA, the message level had dropped rapidly to 9- to 10-fold above pre-pollinated levels and remained at this level through 4 DAA for pollinated ovaries. Although steady-state GA_1 levels were reported to be the same in pollinated and unpollinated ovaries at 0 DAA, GA_8 levels were two times higher in pollinated than unpollinated ovaries at this time (García-Martínez and others 1991). Because GA_8 is the immediate biologically inactive product of GA_1 (as a result of 2β-hydroxylation), these data suggest that more GA_1 was synthesized in pollinated than in unpollinated pericarps and/or ovules at 0 DAA. In addition, the large increase in *PsGA3ox1* mRNA levels was not observed in emasculated pericarps at the equivalent to 0 DAA. These data show that pollination triggers the synthesis of pericarp *PsGA3ox1* mRNA message, and suggest that GA_1 is synthesized from the pool of GA_{20} present in pre-pollinated pericarps by pericarp GA 3β-hydroxylase. The resulting pulse of GA_1 could stimulate initial fruit set and development. A similar sequence of *GA20ox* (*Le20ox-1* and *-2*) and *GA3ox* (*Le3ox-2*) expression during pollination/fertilization (stage 9 and 10 of tomato flower bud development) was observed in tomato floral organs (samples were a composite of sepals, petals, anthers, pericarp, and seeds) (Rebers and others 1999). Metabolism and turnover studies are needed to clarify how GA_1 levels are regulated in these tissues and to determine the importance of GA for the growth of these tissues.

In pea, the exact timing of auxin signals with that of GA signals during pollination/fertilization is not known. However, in tobacco, appreciable quantities of an auxin-like compound start to diffuse from the base of the style after 13–20 h, from the base of the ovary after 35–40 h, and from the pedicel 60–65 h after pollination (Muir 1942). Because auxin (4-Cl-IAA) has been shown to stimulate GA biosynthesis in pea ovaries (2 to 3 DAA pollinated deseeded pericarp) (van Huizen and others 1997; Ozga and others 2003), auxin may also function as a pollination or early post-pollination signal that stimulates GA biosynthesis (via an increase in *GA3ox* mRNA levels) in the ovary, thereby stimulating initial ovary growth and fruit set.

In tomato, GA and IAA are effective in stimulating parthenocarpic fruit development when applied alone, and, when applied in combination, stimulate synergistic growth of the fruit at a low GA to auxin ratio (Wittwer and Tolbert 1960). Sastry and Muir (1963) found that GA applied to unpollinated tomato fruit increased the amount of diffusible auxin-like substance from non-detectable levels to an equivalent IAA concentration of 0.27 μM within 28 h after application. The authors concluded that the stimulus of pollination in fruit growth arose from pollen GA that brought about diffusible auxin production in the ovary. Therefore, indirect evidence also exists that supports gibberellin stimulation of auxin biosynthesis in fruit development.

Growth After Fruit Set (Enlargement of the Ovary)

Following fruit set, the pedicel/peduncle increases in size and in vascular development; existing vascular strands enlarge and new ones are differentiated (Nitsch 1952). Because development of xylem and phloem elements can be regulated by auxins, cytokinins, and GAs (Aloni 1987), it is likely that fruit-produced hormones stimulate this vascularization. Indeed, pedicel diameter increased linearly with increasing log concentration of 4-Cl-IAA when applied to 2-DAA deseeded pea fruit (Reinecke and others 1995).

In general, ovary growth following pollination/fertilization consists of both cell division and cell enlargement (Nitsch 1970). Using molecular and histological methodologies, cell division and enlargement phases were found to overlap in early pea ovary (pericarp) development (Ozga and others 2002). After pollination and fertilization, cell division in the pea pericarp was highest from anthesis (0 DAA) to 2 DAA, then subsequently decreased until the mitotic phase of fruit development was essentially completed by 7 DAA (Ozga and others 2002). Consistent with these anatomical data, histone H2A mRNA levels were highest from 2 d before anthesis (prior to pollination/fertilization) to 2 DAA and then declined rapidly (Ozga and others 2002). Expression of the H2A gene is replication dependent (Koning and others 1991) and accumulation of its mRNA is a useful marker for cell division in pea pericarp (Ozga and others 2002).

Molecular aspects of cell enlargement during early pea fruit development were studied by profiling γ-TIP (tonoplast intrinsic protein) gene expression (Ozga and others 2002). γ-TIPs are a subclass of aquaporins (Maurel 1997) that are capable of forming transmembrane channels that allow the passive transfer of water into the tonoplast (Maurel and others 1993), and their transient expression pattern is associated with cell expansion (when large central vacuoles are being formed) in plant tissues (Ludevid and others 1992). A 2-fold

increase in γ-TIP mRNA levels in pea pericarp from 0–2 DAA preceded the peak in pericarp growth rate and rapid mesocarp cell expansion (longitudinal plane) (Ozga and others 2002). After the peak in pericarp growth rate (length) and γ-TIP mRNA levels (4 DAA), γ-TIP message decreased in parallel with the reduction in the pericarp growth rate (5–7 DAA).

As the maximum length of the pericarp is approached (6–7 DAA), rapid expansion in pericarp diameter (inflation) occurs to accommodate the rapidly growing seeds (6–12 DAA) (Ozga and others 2003). Although this phase of pericarp growth has not been well documented anatomically, it is assumed that this growth is the result of cell enlargement.

Seeds are Required for Normal Fruit Development

In most fruits, normal ovary (pericarp) growth requires the presence of seeds and the final weight of the fruit is often proportional to the number of developing seeds (Nitsch 1970). This is the case in pea where pericarp growth (length, fresh weight, and dry weight) was positively correlated with initial seed number (Ozga and others 1992), and the removal or destruction of the seeds 2–3 DAA resulted in the slowing of pericarp growth and subsequently abscission (Eeuwens and Schwabe 1975; Ozga and others 1992).

After fertilization, the presence of developing seeds was required for maintenance of histone H2A and γ-TIP gene expression in the pea pericarp (Ozga and others 2002). In general, GA and 4-Cl-IAA maintained histone H2A and γ-TIP gene expression, cellular development and expansion, and pericarp growth in deseeded pericarps similarly to pericarps with seeds. Both hormones together were required to obtain mesocarp cell sizes equivalent to intact fruit (Ozga and others 2002).

Chemical signals originating from the seeds may be responsible for continued fruit development by maintaining hormone levels in the surrounding tissue (Eeuwens and Schwabe 1975; Sponsel 1982; Ozga and others 1992). Developing pea seeds and pericarps contain GAs (García-Martínez and others 1991; Rodrigo and others 1997) and auxins (4-Cl-IAA and IAA) (Marumo and others 1968; Magnus and others 1997). During early pericarp growth (2 DAA), application of the naturally occurring hormones, 4-Cl-IAA (Reinecke and others 1995) and GA (GA_1 or GA_3) (Eeuwens and Schwabe 1975; Ozga and Reinecke 1999), to deseeded pericarp can substitute for seeds and stimulate pericarp growth. However, the other naturally occurring auxin in pea fruit, IAA, was ineffective in promoting growth (Reinecke and others 1995).

Initial work comparing the growth-promoting properties of 4-, 5-, 6- and 7-Cl-IAAs and the corresponding F-IAA analogs demonstrated that a chlorine at the 4-position of the indole ring (4-Cl-IAA) was required for significant biological activity in pea pericarp growth (Reinecke and others 1995). Studies comparing the growth-promoting response and the physicochemical properties of 4-Cl-IAA and 4-substituted analogs found that the 4-substituents' size and lipophilicity were associated with the auxin's growth-promoting activity on pea pericarp (Reinecke and others 1999). Pea pericarps responded in a qualitatively different manner to two naturally occurring auxins which, in other auxin biological assays, showed only quantitative differences in activity (Reinecke 1999). These results suggest unique modes of auxin action, based on alternative molecular recognition mechanisms in this tissue, although differing auxin metabolism or cellular compartmentalization have not been entirely ruled out.

Both *PsGA20ox1* (van Huizen and others 1997) and *PsGA3ox1* (Ozga and others 2003) mRNA levels substantially decreased after pollination in the pericarp (after an initial increase in the case of *PsGA3ox1*). This decrease in message levels during this stage of ovary development may be important for a multitude of factors, including hormones (auxins and GAs), to fine-tune GA biosynthesis to coordinate ovary and seed development.

Studies using the pea split-pericarp assay (test compounds are applied to the inner walls of split, or split and deseeded 2-DAA pericarps) have shown that the presence of seeds is required to maintain both the conversion of $[^{14}C]GA_{19}$ to $[^{14}C]GA_{20}$ (van Huizen and others 1995), and the expression of *PsGA20ox1* (van Huizen and others 1997) and *PsGA3ox1* (Ozga and others 2003) in the pericarp.

The effects of hormones (4-Cl-IAA, IAA and GA_3) on the expression of *PsGA20ox1* and *PsGA3ox1* were also investigated. The application of 4-Cl-IAA to 2-DAA deseeded pea pericarp stimulated the conversion of $[^{14}C]GA_{19}$ to $[^{14}C]GA_{20}$ (van Huizen and others 1995) and the expression of *PsGA20ox1* (van Huizen and others 1997) and *PsGA3ox1* (Ozga and others 2003) in pea pericarp. IAA, which does not stimulate pericarp growth, was ineffective in stimulating expression of pericarp *PsGA20ox1* (Ngo and others 2002) or *PsGA3ox1* (Ozga and others 2003). These data suggest that similar auxin-induced

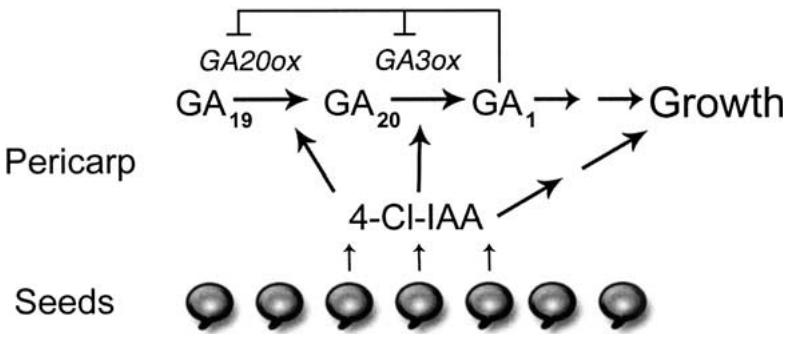

Figure 1. Model for GA-auxin interactions during pea fruit growth. 4-Cl-IAA transported from the seeds stimulates the pericarp GA biosynthetic pathway. Specifically, 4-Cl-IAA regulates pericarp GA biosynthesis by stimulating *GA20ox* ($GA_{19} \rightarrow GA_{20}$) and *GA3ox* ($GA_{20} \rightarrow GA_1$, active GA) message levels. GA_1 may feedback regulate levels of *GA20ox* and *GA3ox* message. 4-Cl-IAA also effects pericarp growth directly (independently of GA biosynthesis). GA and auxin (4-Cl-IAA) act coordinately to stimulate pea fruit growth and development.

transcription regulatory elements may operate to coordinate regulation of this part of the GA biosynthesis pathway, and that biologically active auxin acts in a concerted fashion on the GA biosynthesis pathway to stimulate production of active GAs in the fruit. Similarly, application of IAA to decapitated pea plants restored internode elongation, the message level of *PsGA3ox1*, and GA_1 level in the elongating internode (Ross and others 2000).

In addition, GA_3 reduced 4-Cl-IAA stimulation of both *PsGA20ox1* and *PsGA3ox1* message levels in deseeded pericarp. The inhibitory effect of GA_3 on 4-Cl-IAA-stimulated increases of both *PsGA20ox1* and *PsGA3ox1* message levels could be a direct effect of elevated levels of GA on the auxin-stimulation pathway or an indirect effect through the GA feedback regulation pathway (Martin and others 1996).

Interestingly, in intact fruit, pericarp *PsGA3ox1* levels increased linearly from 6 to 10 DAA. This increase coincided with the rapid increase in pericarp diameter (inflation) to accommodate the developing seeds (Ozga and others 2003). A peak in auxin levels (IAA and 4-Cl-IAA) (Eeuwens and Schwabe 1975; Katayama and others 1988; Magnus and others 1997) in the seeds precedes the increase in *PsGA3ox1* mRNA levels in the pericarp at this stage. The steady-state GA_1 levels in the pericarp (approximately 1 ng · g fw^{-1} from 4 to 12 DAA) (Rodrigo and others 1997), however, do not increase with increasing *PsGA3ox1* message level during this period. Yet, Sponsel (1982) found that 1 μg of GA_3 applied to the pericarp of ''seed-killed'' ovaries (seeds killed 2 DAA by pricking them with a needle) was sufficient to restore pericarp elongation growth, whereas 10 μg of GA_3 per pericarp was required to restore normal pericarp inflation. The greater amount of GA_3 required for stimulation of pericarp inflation coincides with a peak in *GA3ox* expression during this time. Therefore, it is possible that the turnover rate of GA_1 is substantially higher during this period, resulting in similar steady-state GA_1 levels from 4 to 12 DAA, or that a tissue localized increase in GA biosynthesis is required for pericarp inflation.

The following paragraph describes a working model for hormonal-directed fruit set, and seed and pericarp-coordinated development based on pea fruit development (Figure 1). Pollination stimulates GA_1 synthesis via an increase in *GA3ox* mRNA levels (possibly via stimulation of auxin synthesis in the ovary) in both the seeds and pericarp, resulting in initial fruit set and growth of both tissues. Subsequently, seeds maintain growth in the pericarp, at least in part, by transporting auxin (4-Cl-IAA) to the pericarp, where it stimulates both *GA20ox* and *GA3ox* message levels, maintaining a critical level of GA_1 for pericarp growth. Biologically active GA (GA_1) also can feed-back regulate its own synthesis in the pericarp by reducing *GA20ox* and *GA3ox* message levels. In addition, auxin (4-Cl-IAA) affects fruit growth directly through auxin-mediated responses (van Huizen and others 1996). Therefore, an auxin-GA interaction is required for coordination of fruit and seed development. In other plant systems (tomato, *Arabidopsis,* and so on), IAA would replace 4-Cl-IAA in the model because 4-Cl-IAA in plants has been localized (with one exception) to the Vicieae tribe from the Fabaceae family (legumes) (Reinecke 1999).

Hormones as Mobilizers of Assimilates to the Fruit

Growing fruits are very active metabolically and act as strong sinks for nutrients within the plant. Jahnke and others (1989) monitored assimilate distribution in pea plants labeled with the short-lived isotope $^{11}CO_2$ on the axil leaf subtending pollinated or unpollinated fruit. The ^{11}C-radiolabel entering the ovaries paralleled the growth rate of the fruit.

When the unpollinated ovaries stopped growing, the uptake of ^{11}C rapidly decreased. Pollination/fertilization, however, restored the strong sink activity of the ovaries. The same effect could be achieved by applying GA_3 to unpollinated ovaries. About 2 h after treatment with GA_3, the ^{11}C-radiolabel entering the ovary started to increase and, at about the same time, the ovary resumed growth. Brenner and Cheikh (1995) state that the concentration gradient of photoassimilates between source and sink tissue is likely the primary regulator for the current rate of transport and pattern of partitioning. Hormones may serve as modulators for many of the rate-limiting components in this process. Hormones may stimulate transport of nutrients through the phloem, modify the strength of the sink by stimulating its growth, increase the ability for sucrose unloading from the phloem, or act on metabolism and compartmentalization of sucrose and its metabolites (Brenner and Cheikh 1995).

Sucrose-metabolizing enzymes, such as invertases, have been implicated as playing a major role in cleaving imported sucrose, which in turn may regulate the rate of carbon import to developing fruit (Weber and others 1995). Cytokinin (6-benzylaminopurine), auxin (4-Cl-IAA; and auxin-like 2,4-dichlorophenoxyacetic acid), and GA_3 have been shown to induce parthenocarpic pea fruit (García-Martínez and Carbonell 1980; Ozga and Reinecke 1999), and these classes of hormones increased invertase activities in unpollinated fruit that correlated with the growth rate of the fruit (Estruch and Beltrán 1991). The exact roles of the endogenous hormones and possible interactions among the hormones for assimilate mobilization to the growing ovary remain to be determined by future research.

Conclusion

Fruit development is a beautifully complex process that begins with the change from a vegetative to a floral meristem and ends with mature fruit and viable seed. In the past, our understanding of hormonal interaction in fruit development has come from hormone application and hormone analysis studies. We now have powerful biochemical, genetic, and molecular techniques to continue the comprehensive understanding of fruit development. *Arabidopsis* sequencing has shown that up to 10% of its genes are likely to be involved in signal transduction, ''the diverse array of biochemical mechanisms that regulate cellular physiology'' (Chory and Wu 2001). Recent studies are showing that the interplay between signal transduction pathways for different hormones is involved in vegetative growth (for example, Francis and Sorrell 2001). Our understanding of the interactions between various plant hormones and signal transduction pathways involved in fruit development will greatly increase with careful genetic analyses and in-depth physiological studies of wild-type plants and those that have been modified using mutation techniques (Emmanuel and Levy 2002) or transgenic technology. Understanding how hormones influence gene expression during fruit development will be aided by recent research tools, including large-scale screening of gene expression using DNA microarray technology (Aharoni and O'Connell 2002) and significantly improved quantitative analysis of gene message levels using real-time RT-PCR (Ozga and others 2003). Information from model genetic systems such as *Arabidopsis* and model agronomic fruit systems (for example, strawberry, pea, and tomato) will revolutionize our understanding of how hormones interact to coordinate ovary and seed development with the mother plant to ensure continuation of the species.

Acknowledgments

The authors would like to acknowledge Dr. Frank Dennis Jr. and Dr. Robert Bandurski for their support in our initial interest in the study of fruit physiology. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC).

References

Aharoni A, O'Connell AP. 2002. Gene expression analysis of strawberry achene and receptacle maturation using DNA microarrays. J Exp Bot 53:2073–2087.

Aloni R. 1987. Differentiation of vascular tissues. Annu Rev Plant Physiol 38:179–204.

Bohner J, Bangerth F. 1988. Cell number, cell size and hormone levels in semi-isogenic mutants of *Lycopersicon pimpinellifolium* differing in fruit size. Physiol Plant 72:316–320.

Brenner ML, Cheikh N. 1995. The role of hormones in photosynthate partitioning and seed filling. In: Davies PJ, editor. Plant hormones: physiology, biochemistry, and molecular biology. 2nd ed. Dordrecht, The Netherlands: Klewer Academic Publishers, p 649–670.

Carbonell J, García-Martínez JL. 1980. Fruit-set of unpollinated ovaries of *Pisum sativum* L. Planta 147:451–456.

Chory J, Wu D. 2001. Weaving the complex web of signal transduction. Plant Physiol 125:77–80.

Coombe BG. 1960. Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. Plant Physiol 35:241–250.

Cooper DC. 1938. Embryology of *Pisum sativum*. Bot Gaz 100:123–132.

Crane J. 1964. Growth substances in fruit setting and development. Annu Rev Plant Physiol 15:303–326.

Eeuwens CJ, Schwabe WW. 1975. Seed and pod wall development in *Pisum sativum* L. in relation to extracted and applied hormones. J Exp Bot 26:1–14.

Emmanuel E, Levy AA. 2002. Tomato mutants as tools for functional genomics. Curr Opin Plant Biol 5:112–117.

Estruch JJ, Beltrán JP. 1991. Changes in invertase activities precede ovary growth induced by gibberellic acid in *Pisum sativum.* Physiol Plant 81:319–326.

Fos M, Nuez F, García-Martínez JL. 2000. The gene *pat-2,* which induces natural parthenocarpy, alters the gibberellin content in unpollinated tomato ovaries. Plant Physiol 122:471–479.

Fos M, Proaño K, Nuez F, García-Martínez JL. 2001. Role of gibberellins in parthenocarpic fruit development induced by the genetic system *pat*-3/*pat*-4 in tomato. Physiol Plant 111:545–550.

Francis D, Sorrell DA. 2001. The interface between the cell cycle and plant growth regulators: a mini review. Plant Growth Regul 33:1–12.

García-Martínez JL, Carbonell J. 1980. Fruit-set of unpollinated ovaries of *Pisum sativum* L. Planta 147:451–456.

García-Martínez JL, López-Diaz I, Sánchez-Beltrán MJ, Phillips DA, Ward DA, Gaskin P, Hedden P. 1997. Isolation and transcript analysis of gibberellin 20-oxidase genes in pea and bean in relation to fruit development. Plant Mol Biol 33:1073–1084.

García-Martínez JL, Santes C, Crocker SJ, Hedden P. 1991. Identification, quantitation and distribution of gibberellins in fruits of *Pisum sativum* L. cv. Alaska during pod development. Planta 184:53–60.

Gillaspy G, Ben-David H, Gruissem W. 1993. Fruits: a developmental perspective. Plant Cell 5:1439–1451.

Jahnke S, Bier D, Estruch JJ, Beltrán JP. 1989. Distribution of photoassimilates in the pea plant: chronology of events in non-fertilized ovaries and effects of gibberellic acid. Planta 180:53–60.

Katayama M, Thiruvikraman SV, Marumo S. 1988. Localization of 4-chloroindole-3-acetic acid in seeds of *Pisum sativum* and its absence from all other organs. Plant Cell Physiol 29:889–891.

Koning AJ, Tanimoto EY, Kiehne K, Rost T, Comai L. 1991. Cell-specific expression of plant histone H2A genes. Plant Cell 3:657–665.

Lester DR, Ross JJ, Davies PJ, Reid JB. 1997. Mendel's stem length gene (*Le*) encodes a gibberellin 3ß-hydroxylase. Plant Cell 9:1435–1443.

Ludevid D, Höfte H, Himelblau E, Chrispeels MJ. 1992. The expression pattern of the tonoplast intrinsic protein γ-TIP in *Arabidopsis thaliana* is correlated with cell enlargement. Plant Physiol 100:1633–1639.

Magnus V, Ozga JA, Reinecke DM, Pierson GL, Larue TA, Cohen ML, Brenner ML. 1997. 4-chloroindole-3-acetic acid and indole-3-acetic acid in *Pisum sativum.* Phytochemistry 46:675–681.

Martin DN, Proebsting WM, Hedden P. 1997. Mendel's dwarfing gene: cDNAs from the *Le* alleles and function of the expressed proteins. Proc Natl Acad Sci USA 94:8907–8911.

Martin DN, Proebsting WM, Parks TD, Dougherty WG, Lange T, Lewis MJ, Gaskin P, Hedden P. 1996. Feed-back regulation of gibberellin biosynthesis and gene expression in *Pisum sativum* L. Planta 200:159–166.

Marumo S, Hattori H, Abe H, Munakata K. 1968. Isolation of 4-chloroindolyl-3-acetic acid from immature seeds of *Pisum sativum.* Nature 219:959–960.

Maurel C. 1997. Aquaporins and water permeability of plant membranes. Annu Rev Plant Physiol Plant Mol Biol 48:399–429.

Maurel C, Reizer J, Schroeder JI, Chrispeels MJ. 1993. The vacuolar membrane protein γ-TIP creates water-specific channels in *Xenopus* oocytes. EMBO J 12:2241–2247.

Mok MC. 1994. Cytokinins and plant development—an overview. In: Mok DWS, Mok MC, editors. Cytokinins-chemistry, activity, and function. Boca Raton: CRC Press, p 155–166.

Muir RM. 1942. Growth hormones as related to the setting and development of fruits in *Nicotiana tobaccum.* Am J Bot 29:716–720.

Ngo P, Ozga JA, Reinecke DM. 2002. Specificity of auxin regulation of gibberellin 20-oxidase gene expression in pea pericarp. Plant Mol Biol 49:439–448.

Nitsch JP. 1951. Growth and development in vitro of excised ovaries. Am J Bot 38:566–577.

Nitsch JP. 1952. Plant hormones in the development of fruits. Q Rev Biol 27:33–57.

Nitsch JP. 1970. Hormonal factors in growth and development. In: Hulme AC, editor. The biochemistry of fruits and their products. London and New York: Academic Press, p 427–472.

O'Neill SD. 1997. Pollination regulation of flower development. Annu Rev Plant Physiol Plant Mol Biol 48:547–574.

Ozga JA, Brenner ML, Reinecke DM. 1992. Seed effects on gibberellin metabolism in pea pericarp. Plant Physiol 100:88–94.

Ozga JA, Reinecke DM. 1999. Interaction of 4-chloroindole-3-acetic acid and gibberellins in early pea fruit development. Plant Growth Regul 27:33–38.

Ozga JA, van Huizen R, Reinecke DM. 2002. Hormone and seed-specific regulation of pea fruit growth. Plant Physiol 128:1379–1389.

Ozga JA, Yu J, Reinecke DM. 2003. Pollination-, development-, and auxin-specific regulation of gibberellin 3β-hydroxylase gene expression in pea fruit and seeds. Plant Physiol 131:1137–1146.

Pharis RP, King RW. 1985. Gibberellins and reproductive development in seed plants. Annu Rev Plant Physiol 36:517–568.

Rebers M, Kaneta T, Kawaide H, Yamaguchi S, Yang Y-Y, Imai R, Sekimoto H, Kamiya Y. 1999. Regulation of gibberellin biosynthesis genes during flower and early fruit development of tomato. Plant J 17:241–250.

Reinecke DM. 1999. 4-Chloroindole-3-acetic acid and plant growth. Plant Growth Regul 27:3–13.

Reinecke DM, Ozga JA, Magnus V. 1995. Effect of halogen substitution of indole-3-acetic acid on biological activity in pea fruit. Phytochemistry 40:1361–1366.

Reinecke DM, Ozga JA, Ilić N, Magnus V, Kojić-Prodić B. 1999. Molecular properties of 4-substituted indole-3-acetic acids affecting pea pericarp elongation. Plant Growth Regul 27:39–48.

Rodrigo MJ, García-Martínez JL. 1998. Hormonal control of parthenocarpic ovary growth by the apical shoot in pea. Plant Physiol 116:511–518.

Rodrigo MJ, García-Martínez JL, Santes CM, Gaskin P, Hedden P. 1997. The role of gibberellins A_1 and A_3 in fruit growth of *Pisum sativum* L. and the identification of gibberellins A_4 and A_7 in young seeds. Planta 201:446–455.

Rodrigo MJ, López-Diaz I, García-Martínez JL. 1998. The characterization of *gio*, a new pea mutant, shows the role of indoleacetic acid in the control of fruit development by the apical shoot. Plant J 14:83–90.

Ross JJ, O'Neill DP, Smith JJ, Huub L, Kerckhoffs J, Elliott RC. 2000. Evidence that auxin promotes gibberellin A_1 biosynthesis in pea. Plant J 21:547–552.

Sastry KKS, Muir RM. 1963. Gibberellin: effect on diffusible auxin in fruit development. Science 140:494–495.

Scorza R, May LG, Purnell B, Upchurch B. 1991. Differences in number and area of mesocarp cells between small- and large-fruited peach cultivars. J Am Soc Hort Sci 116:861–864.

Smith WH. 1950. Cell-multiplication and cell-enlargement in the development of the flesh of the apple fruit. Ann Bot (London) NS 14:23–38.

Sponsel V. 1982. Effects of applied gibberellins and naphthylacetic acid on pod development in fruits of *Pisum sativum* L. cv. Progress No. 9. J Plant Growth Regul 1:147–152.

Sponsel VM. 1995. The biosynthesis and metabolism of gibberellins in higher plants. In: Davies PJ, editor. Plant hormones: physiology, biochemistry and molecular biology. 2 ed. Dordrecht, The Netherlands: Kluwer Academic Publishers, p 66–97.

van Huizen R, Ozga JA, Reinecke DM. 1996. Influence of auxin and gibberellin on in vivo protein synthesis during early pea fruit growth. Plant Physiol 112:53–59.

van Huizen R, Ozga JA, Reinecke DM. 1997. Seed and hormonal regulation of gibberellin 20-oxidase expression in pea pericarp. Plant Physiol 115:123–128.

van Huizen R, Ozga JA, Reinecke DM, Twitchin B, Mander LN. 1995. Seed and 4-chloroindole-3-acetic acid regulation of gibberellin metabolism in pea pericarp. Plant Physiol 109: 1213–1217.

Vivian-Smith A, Luo M, Chaudhury A, Koltunow A. 2001. Fruit development is actively restricted in the absence of fertilization in *Arabidopsis.* Development 128:2321–2331.

Weber H, Borisjuk L, Heim U, Buchner P, Wobus U. 1995. Seed coat-associated invertases of fava bean control both unloading and storage functions: cloning of cDNAs and cell type-specific expression. Plant Cell 7:1835–1846.

Wittwer SH, Tolbert NE. 1960. 2-Choroethyl trimethylammonium chloride and related compounds as plant growth substances. V. Growth, flowering, and fruiting responses as related to those induced by auxin and gibberellin. Plant Physiol 35:871–877.

Zhang XS, O'Neill SD. 1993. Ovary and gametophyte development are coordinately regulated by auxin and ethylene following pollination. Plant Cell 5:403–418.