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Tansley Review

The Role of the Pod in Seed Development: Strategies for Manipulating Yield

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Summary

Pods play a key role in encapsulating the developing seeds and protecting them from pests and pathogens. In addition to this protective function it has been shown that the photosynthetically active pod wall contributes assimilates and nutrients to fuel seed growth. Recent work has revealed that signals originating from the pod may also act to co-ordinate grain filling and regulate the reallocation of reserves from damaged seeds to those that have retained viability. In this review we consider the evidence that pods can regulate seed growth and maturation, particularly in members of the Brassicaceae family, and explore how the timing and duration of pod development might be manipulated to enhance either the quantity of crop yield or its nutritional properties.

I. Introduction

A pod, or silique as it is known in the Brassicaceae, is a photosynthetically active organ that encloses the seeds during their development. Once seed maturation is complete the bivalve pod splits longitudinally and its contents are released. In this review we will focus our discussions primarily on members of the Brassicaceae family and seek to demonstrate that the function of the pod extends far beyond simply safeguarding the maturing seeds. In this review we will refer to siliques by the more generic term pod, as much of the data generated from the study of Brasicaceous species has wider relevance across other families in the plant kingdom that reproduce through the formation of pods which enclose their seeds. Whilst pods are not essential for an individual's existence they play a paramount role in the survival of a species and could therefore be considered as one of the most important organs of a plant. In light of this it is perhaps surprising that relatively little research has been carried

out on pod growth and development and how this might be manipulated to enhance crop yield.

Seeds constitute an important source of dietary protein due to their high concentrations of seed storage proteins (SSPs) which can help alleviate malnutrition under circumstances when the consumption of animal protein is low. In the Brassicaceae family the main SSPs can be classified into either 12S globulins or 2S albumins, precursors of which are synthesised on the rough endoplasmic reticulum and, after maturation, reside in protein storage vacuoles within the seed (Herman & Larkins, 1999). Despite their nutritional benefits, seeds are deficient in some essential amino acids (see Mandal & Mandal, 2000 for a review) and are therefore incapable of completely alleviating protein malnutrition without supplementation. Hence yield enhancement is not only concerned with net increases in marketable produce but also in strategies to improve nutritional quality. The latter term encompasses many different aspects of seed composition but with regard to protein it represents the correlation between the amino acid profile of a seed and a balanced diet as recommended by the World Health Organisation (WHO) (Mandal & Mandal, 2000). Recent figures from WHO estimate that whilst the number of underweight children has fallen to 16% of the global population a simultaneous increase in inhabitants means that this still represents 104 million undernourished children worldwide (World Health Organisation, 2010). In stark contrast, a global obesity epidemic is also occurring and as of the year 2000 WHO estimated that 300 million adults worldwide were obese (World Health Organization, 2000), a larger group than those considered to be undernourished. Intriguingly, there is evidence that one approach to help alleviate obesity might be to improve SSP levels and seed nutritional quality as high protein diets have been shown to assist in reducing and maintaining a healthy body mass (Claessens *et al.*, 2009).

The increasing acknowledgement that food security is a growing global problem was highlighted by Ban Ki-moon, speaking at a UN summit on solving the world's food crisis in 2008, when he predicted that there would need to be a 50% increase in global food production by 2030. Thus, a rising world population (predicted to reach around 9 billion by 2050), against a backdrop of climate change, makes the need to optimise yield of paramount importance. Maximising the efficiency of crop growth is one way global food demands can be met, and with a growing body of evidence that the pod directly influences seed composition it is bringing our ability to manipulate pod growth and development to the forefront of yield enhancement strategies.

II. Pod Structure and Development

The Brassicaceae family contains more than 3000 species and these produce non-fleshy fruit in the form of a silique that emerges from the gynoecium following ovule fertilisation. Recent transcript profiling analyses has provided evidence to support the assertion that the pod wall represents a modified leaf (Ma *et al.*, 2005; Scutt *et al.*, 2006; Wagstaff *et al.*, 2009). In the model *Brassica* species Arabidopsis the pod wall (pericarp) is composed of two fused carpels that undergo cell expansion between fertilisation and maturity, causing the pod to elongate about seven times its initial length between fertilisation and maturity (Sessions & Zambryski, 1995; Louvet *et al.*, 2006) . In contrast, the pods from members of the Fabaceae family are formed from a single carpel so, whilst the fruit from both families is commonly referred to as a pod, the term silique is reserved for members of the Brassicaceae family.

2.1. The Pod Wall Structure

The pericarp has been classified into three functional cell layers; the exocarp, mesocarp and endocarp, which are all characteristic components of fruit cell walls. The

exocarp comprises a single celled epidermal layer that is populated with stomata to facilitate gaseous exchange. The mesocarp is composed of layers of chlorenchyma cells that are rich in chloroplasts (Sessions & Zambryski, 1995). Finally, the endocarp consists of two dissimilar cell layers, a surface layer (ena) made up of large thin walled cells and an inner layer (enb) formed from small tightly packed cells as a result of several anticlinal cell divisions (Spence *et al.*, 1996). The silique wall is not entirely uniform and a narrow dehiscence zone (DZ), approximately two cell layers in width, spans the length of the silique between the valve and the replum (for review see Ferrandiz *et al.*, 1999; Ferrandiz, 2002). Such pericarp differentiation is necessary to target cellular degradation to the middle lamella between DZ cells, thus allowing the pod to shatter and release its mature seeds (Meakin & Roberts, 1990). Ultrastructural analysis of mature green silique walls has revealed developmental patterns associated with the onset of senescence, as they contain fewer thylakoids per granum than would normally be observed in leaf plastids (Wagstaff *et al.*, 2009). This feature, often associated with the structural reorganisation of thylakoid membranes, accompanies reduced PSI and PSII activity during senescence (Prakash *et al.*, 2001). Additionally, the decrease in chlorophyll in the pod wall precedes the decline observed in seeds which remain photosynthetic for longer (Wagstaff *et al.*, 2009); together these ultrastructural and physiological features may be required for optimisation of photosynthate accumulation in seeds. The consequences of such structural changes may be to enable a greater percentage of incident light to reach the seeds. In a crop such as oilseed rape (*Brassica napus*) this could potentially enhance its yield as ATP and NADPH are predicted to be required for the biosynthesis of seed storage products such as lipids (Fuhrmann *et al.*, 1994; Aach & Heise, 1998; Schwender *et al.*, 2004; Goffman *et al.*, 2005). Given that only 20-30% of the incident light passes through the silique wall, and the spectral quality also changes in favour of the far red (FR) wavelengths, oilseed rape seeds develop within a shaded environment. These FR

wavelengths may also trigger the induction of seed dormancy, which is a common feature of freshly harvested seed from this crop, since such wavelengths are known to inhibit seed germination (Borthwick *et al.*, 1951). Using similar strategies to shade leaves, seeds have a lower chlorophyll a/b ratio to enable them to capture a greater amount of the available light (Eastmond *et al.*, 1996; King *et al.*, 1998) and it is reasonable to assume that selection pressures would have favoured a temporal separation of the loss of photosynthetic capacity between pods and seeds. One advantage of having a temporal division in photosynthetic maximums is that a greater amount of incident light is capable of reaching the developing seeds which can enhance ATP production leading to increased oil synthesis (Ruuska *et al.*, 2002). High levels of anthocyanins are contained within the testa of *M. truncatula* seeds, which not only give the seeds colour (Abirached-Darmency *et al.*, 2005) but potentially impart protection against photo-oxidative stress; for a review of anthocycanin function see Archetti *et al.* (2009). As the silique wall begins the process of senescence and chlorophyll catabolism the enclosed seeds, which have matured within a shaded environment, are slowly exposed to higher levels of incident light. Consequentially the anthocyanins in the seed testa may afford some protection against increased UVB exposure and associated build up of reactive oxygen species.

2.2. Pod Wall Development

Despite being characteristically viewed merely as a protective organ, for instance the pod morphology of alfalfa has been shown to be instrumental in safeguarding seeds against chaliced wasps (Small & Brookes, 1984), the role of the pod appears to alter during the course of development. For instance, transcriptional profiling of the pod wall at different developmental stages has revealed that the observed changes in pod anatomy and chlorophyll levels throughout pod maturation correlated with alterations in transcription factor expression patterns (Wagstaff *et al.*, 2009). Indeed there is a strong association between pod

development and seed size (Pechan & Morgan, 1985) which has prompted the suggestion that pod length could be used as an indication of crop yield (Diepenbrock, 2000).

III. The Pod as a Sink in Plant Resource Allocation

The allocation of resources to developing siliques remains poorly understood, with the majority of research concentrating on nutrient remobilisation out of senescing leaves. Nevertheless, it has been established that at anthesis the pod becomes a resource sink capable of storing remobilised nitrogen (N) and carbon (C) for utilisation upon germination (Harvey, 1973; BuchananWollaston, 1997; Diepenbrock, 2000; Rossato *et al.*, 2001; Schiltz *et al.*, 2005). This recycling of nutrients is essential for producing seeds that contain high concentrations of storage compounds such as proteins, lipids and starch, which is why the phloem remains functional throughout senescence (Feller & Fischer, 1994). Unlike other plant organs, such as taproots, the pod is considered a sink throughout development (Rossato *et al.*, 2001) although in practice the pod wall contributes assimilates to the developing embryo during the final stages of seed maturation (Rochat & Boutin, 1991), for example, 20% of the N accumulated in pea seeds has been shown to be remobilised from the adjacent pod wall (Schiltz *et al.*, 2005).

3.1. Nitrogen Uptake

There is a considerable body of evidence to support the view that resources from vegetative parts of the plant are remobilised into the pod (Harvey, 1973; Flinn *et al.*, 1977; Schiltz *et al.*, 2005). ^{15}N labelling experiments have demonstrated that about 48% of the N cycling through oilseed rape ends up in mature pods (Rossato *et al.*, 2001) and it is presumed that most of this originates from vegetative tissues, since little N uptake occurs during flowering and pod development. This observation indicates that N fertilisation after flowering would only have minimal effects on plant yield. Our understanding of the pathways involved

in remobilising N from the leaves to developing pods are superficial, however, recent insights into the metabolic role of the enzyme pyruvate orthophosphate dikinase (PPDK) in metabolism may have shed some light on this problem. PPDK interconverts pyruvate and phosphoenolpyruvate and is central to photosynthesis in C4 plants, but it is also up-regulated during leaf senescence of C3 plants where it functions in a pathway that generates the transport amino acid glutamine, which is then loaded into the phloem. Over-expressing cytosolic PPDK results in more efficient amino acid transport and hence N remobilisation from the leaves is accelerated during senescence, leading to increases in Arabidopsis seed weight and N content, but not seed number (Taylor *et al.*, 2010). One possible explanation for these observations is that an extended growth period and larger rosette size might provide a greater initial resource pool in the vegetative tissues from which to reallocate storage compounds. However, while elevating the soil N content has been shown to enhance crop yield by increasing the number of pods per plant such an approach does not impact upon pod or seed weight (Allen & Morgan, 1972). This implies that the reproductive strategy of oilseed rape is to direct assimilates into additional pods when resources are plentiful rather than to produce seeds containing a greater concentration of storage products (Gammelvind *et al.*, 1996). Combining the increased N remobilisation efficiency of PPDK over-expressers with higher soil N might substantially enhance yield in terms of both pod quantity and quality.

3.2. Leaf Senescence

Leaf senescence is a highly co-ordinated process that enables maximum recovery and remobilisation of nutrients from the leaves. At the onset of Arabidopsis leaf senescence there is an increase in the transcription of genes such as the ABC, sugar, peptide, amino acid and cation transporters in addition to the potential mobilisation of sulphur released upon protein degradation (Buchanan-Wollaston & Ainsworth, 1997). The start of leaf senescence is also accompanied by a concomitant increase in pod CO_2 metabolism indicating that this organ has

an elevated rate of photosynthesis (Gammelvind *et al.*, 1996; Robinson & Hill, 1999), potentially to enable nutrient uptake into the pod. As pods are photosynthetic organs, capable of generating reducing energy and ATP, their exact sink requirements are still a matter of debate. In oilseed rape, Allen and Morgan (1972) predicted that pods were capable of supporting their own growth, but subsequent examination of different *Brassica* species indicates that the photoassimilate contribution by the pod wall to developing seeds might be species specific (Ramana & Ghildiyal, 1997). The mechanisms by which resources are allocated into individual seeds is unknown for, whilst *Arabidopsis* fills its seeds in a uniform, co-ordinated manner, other species such as peas have larger and heavier seeds in the middle of their pods compared to those at the distal and proximal extremes of the pod (Harvey, 1973). A fuller understanding of the source-sink relationship could prove to be crucial in improving crop yield as the sinks compete for the available resources.

3.3. Leaf-Pod Push-Pull Export and Import System

In some species the import of resources into the developing seeds is closely correlated with the capacity of leaves to export assimilates (Wittenbach & Vernon, 1983). However, in Arabidopsis the development of reproductive structures only minimally influences leaf senescence with organ age having a far greater effect. Selective pod removal had almost no impact on individual leaf senescence in Arabidopsis, but overall plant longevity was increased by 20-50 days according to Nooden & Penney (2001), but in our hands the rosette leaves remained green if all but the main inflorescence was removed (Figure 1). Whole plant senescence can be delayed through the removal of seeds from both pea and soybean pods (Lockhart & Gottschall, 1961; Lindoo & Nooden, 1977) and it has been proposed that the pea seed coat determines sink strength (Rochat & Boutin, 1992). Further work implies that this 'pulling power' is coordinated with the breakdown of leaf storage products (Taylor *et al.*, 2010) and it is likely that the pod sink strength is not a fixed entity but instead co-ordinated

throughout development to balance the 'needs' of the seeds, for instance N remobilisation decreases during the later stages of seed filling (Schiltz *et al.*, 2005). The plastic nature of whole plant resource allocation can be observed in many of the soybean de-podding experiments performed by Nooden and co-workers (Lindoo & Nooden, 1976; Lindoo & Nooden, 1977; Nooden *et al.*, 1978; Nooden & Murray, 1982) and from studies examining sterile mutants (Nooden & Penney, 2001). The number of pods also has the capacity to affect leaf photosynthesis, for instance selective pod removal in soybeans leads to reduced rates of CO_2 exchange within these plants, probably due to stomatal closure, a consequence of increased photoassimilate accumulation within the leaves brought about by having fewer sinks to export resources to (Setter & Brun, 1980; Wittenbach & Vernon, 1983). This indicates the presence of a dynamic feedback loop in which the pods signal their resource requirements to the leaves, causing the remobilisation of photoassimilates relative to the signal strength received (Figure 1). Hence, when fewer pods are present in de-podded plants the leaves temporarily halt photoassimilate production in response to an accumulation of carbohydrate in the leaves and an absence of 'pull' from the pods. An alternative view is that the sinks do not pull in resources but instead 'free load' by altering the conductance of plasmodesmata at the phloem-sink interface to affect the rate of nutrient unloading, matching this to their resource requirements (see Lalonde *et al.*, 2003 for a review).

Maximum remobilisation capacity is critical for R selected species, such as Arabidopsis, whose reproductive strategy is to produce viable seeds as quickly as possible. A potential response to such a life history trait is that Arabidopsis determines its seed set based upon the nutritional supply during the reproductive stage, rather than it being predetermined by growth and development during the vegetative phase, a trait associated with weeds growing in unpredictable environments (Schulze *et al.*, 1990). Such a trait could be desirable for commercial *Brassica* and legume crops to improve seed set.

IV. Resource Transport into the Seeds via the Pod

4.1. Transport from the pod wall

The ability to manipulate resource partitioning and assimilate transport into the seeds and pods could help maximise overall yield (Wardlaw, 1990). Studies using detached pods have shown that the photosynthetic tissues of the pod wall are capable of generating 60% of seed assimilates (reviewed in Diepenbrock, 2000), although it must be noted that *in vitro* pod growth results in a decrease in internal pod O_2 concentrations compared to growth *in vivo*, which can alter the amount of storage compound within a seed (Musgrave *et al.*, 2008). As development progresses there is an increase in the compounds exported from the pod wall into the seeds. During this period the pod wall efficiently remobilises any accumulated N into the seeds such that upon harvest 80% of the total shoot N in oilseed rape has been relocated into the seeds. Seeds are capable of receiving the majority of their amino acids through the phloem-mediated pathway(Okumoto *et al.*, 2002), which can come from the pod wall as well as vegetative organs. Such remobilisation decreases during the later stages of development (Schjoerring *et al.*, 1995; Schiltz *et al.*, 2005), potentially due to the absence of sucrose synthase (SUS) activity in the pod wall and funiculus (Fallahi *et al.*, 2008) which is predicted to provide energy for phloem loading and unloading of solutes at this site (Fallahi *et al.*, 2008). Whether photoassimilates generated during pod wall photosynthesis can be re-allocated to other pods is not yet clear, but such a mechanism could prove valuable given that pods higher up in the canopy receive a greater amount of incident light and are thus capable of increased photosynthetic rates whilst being largely exempt from the problems of self shading.

4.2. Phloem unloading at the pod

The import of resources into the pod is primarily concerned with phloem unloading, but this area has received little attention compared with phloem loading at the source site (Patrick, 1997), which is partly due to the great diversity between different sink types. Seeds are well adapted for the uptake of photoassimilates translocated from the pod wall. In *Medicago truncatula* the micropylar region of the seed coat, a small opening in the outer epidermis of the ovule located at one end of the seed, contains a vascular system believed to be instrumental in nutrient transport into the developing seed (Abirached-Darmency *et al.*, 2005). Photoassimilates enter the funiculus which leads to the vascular bundle in the seed coat where unloading can occur (Van Dongen, 2003; Stadler *et al.*, 2005).

Whilst there is a comprehensive understanding of how resources are transported via the xylem and phloem to the pod junction subsequent steps describing the mechanism of transfer from the pod petiole to the seed-funiculus are still poorly defined. The current notion is that transport initially occurs through the symplastic pathway using plasmodesmata and is driven by simple diffusion and or bulk flow. However solutes must be subsequently translocated via apoplastic pathways to move between seed and pod tissues, plus the presence of a selectively permeable apoplastic pathway connecting the maternal tissue and phloem helps prevent nutrient loss. In addition, an apoplastic pathway can run in parallel to the symplastic pathway between the phloem and maternal tissues, but the exact details surrounding resource transport into the seeds are still under review (Patrick & Offler, 2001; Lalonde *et al.*, 2003).

It is predicted that the concentration of storage products within the seeds helps regulate the efficiency with which resources are transported around the plant (Schulze *et al.*, 1994). One such mechanism of regulating supply and demand between the phloem and pod involves the reduction of apoplastic sucrose levels within sink tissues (reviewed in Patrick, 1997), a further indication that the pods act as sinks to pull resources in from the surrounding

tissues. This flow of solutes is driven along the phloem by passive transport caused by differences in turgor pressure between the source and sink organs (Patrick, 1997).

4.3. Nutrient transporters

Numerous nutrient transporters are located within the funiculus and at the base of the pod in the pedicel. One of the many Arabidopsis sulphate transporters, *SULTR2-1,* controls the translocation of sulphur into seeds and is potentially capable of regulating the import of this element into seed storage proteins, (Awazuhara *et al.*, 2005). Regulating sulphate uptake into pods can directly impact upon both seed quality and yield yet *SULTR2-1* mRNA levels do not alter regardless of the sulphur concentration that plants are grown in, highlighting the fundamental importance of this transporter in maintaining an import system (Awazuhara *et al.*, 2005).

Since only small amounts of nitrate are directly translocated from the roots into the seeds (Chopin *et al.*, 2007), by itself nitrate is unlikely to contribute much N towards seed nutrition but instead it is predicted to serve as a signalling molecule, or to alter the osmotic balance during the early stages of seed filling (McIntyre, 1997; Chopin *et al.*, 2007), although during periods of nitrogen deficiency it was postulated that nitrates might have a greater role to play in enhancing seed nutrition (Fan *et al.*, 2009). As the Arabidopsis amino acid transporter *AAP8*, which has a similar expression pattern to *SULTR2-1* (Awazuhara *et al.*, 2005), is present in the funiculus and pod vascular tissue it is predicted to be responsible for enabling the import of organic nitrogen into the seeds (Okumoto *et al.*, 2002), leading to the hypothesis that amino acids enter the pod vascular tissue and are transported though the funiculus where they are imported into seeds at the micropylar region (Awazuhara *et al.*, 2005).

4.4. Impact of seed wounding

Although seeds clearly function as a sink for assimilates and nutrients during development the impact of seed wounding or abortion on transport processes has received little attention. All seeds within a pod are connected to the vascular trace by the funiculus. In Arabidopsis, the response regulator gene *ARR22* has been shown to be expressed specifically at the junction between the funiculus and chalazal tissues (Gattolin *et al.*, 2006; Horak *et al.*, 2008). Despite chalazal tissues important role in supplying nutrients to the developing seeds, silencing of *ARR22* does not give rise to a morphologically detectable phenotype (Gattolin *et al.*, 2006; Horak *et al.*, 2008), although ectopically expressing the gene results in the generation of extremely dwarfed plants. If seeds from Pro_{ARR22}::GUS plants are punctured then expression of the reporter is rapidly up-regulated in the chlazal region indicating that the *ARR22* transcript accumulates as a result of injury to the seed (Gattolin *et al.*, 2006). Recent research has revealed that the wounding of Arabidopsis pods is accompanied by rapid changes in transcript profile, causing mRNAs encoding seed storage proteins to dramatically decline, whereas those encoding proteins involved in proteolysis substantially increase, suggesting that damaged seeds may initiate a resource remobilisation programme (Naomab and Roberts unpublished). Intriguingly these changes do not occur in plants where the *ARR22* gene has been silenced, indicating that this response regulator protein plays an important role in signalling the presence of tissue damage within a seed. The demonstration that wounding causes an increase in the expression of genes involved in protein breakdown suggests that there may be a mechanism by which aborted seeds could redistribute their assimilates to support the development of those that will progress to maturation, indicating that one possible role for ARR22 is to modulate assimilate partitioning into seeds contained within a pod. The precise mechanism that co-ordinates seed filling within an individual pod is unknown and, whilst in a weed such as Arabidopsis this is uniform, the trait may have been lost in some domesticated crops where seed size within an individual pod can vary considerably. The

spatial and temporal expression of *ARR22* make it a strong candidate for having a role in the co-ordination process and by manipulating its expression it might be possible to extend or reduce the seed filling period.

V. Pod Senescence and Dehiscence

5.1. Method of Seed Dispersal

At a plant level, uncoordinated pod senescence and dehiscence is advantageous and limits the seed loss that can occur if ripe pods shatter during temporarily adverse environmental conditions that subsequently inhibit germination of the next generation of plants. However, in a commercial setting, premature and uncoordinated pod shattering results in substantial pre-harvest losses and therefore significantly reduces net yield. For instance up to 20% of oilseed is lost per annum due to premature pod shatter whilst in Birdsfoot trefoil (*Lotus corniculatus* L.) this can be as high as 50% during adverse weather conditions (MacLeod, 1981). Such events can also impede subsequent crop growth due to the emergence of volunteer plants in the following growing season. Hence preventing premature pod shatter would instantly increase net crop yield, which, aside from the economic implications, would undoubtedly contribute to a viable solution of sustaining an escalating world population. Due to its economic importance, *Brassica napus* is a popular candidate species for investigating methods aimed at preventing or delaying pod shatter. Nevertheless, the development of a genotype that fails to shatter may not provide an ideal solution to this problem as this could compromise the postharvest processing chain due to difficulties in removing seeds from the pods without damage. A more amenable approach might be to delay or suspend the final stages of pod development after seed maturation to prevent individual pods shattering until all the pods have fully developed, at which time an internal or external stimulus could be applied to co-ordinate pod shattering across the crop.

364 *5.2.1 Dehiscence*

365 The pod wall of Brassicaceae family members is typically composed of two valves
366 connected by a replum. In between these lignified cell types is a narrow band of valve margin
367 cells that forms the dehiscence zone (DZ) and remains a non-lignified separation layer (SL)
368 throughout pod development (Ferrandiz, 2002). Pod shattering is either initiated at the base of
369 the pod where the pedicel meets the replum, as in oilseed rape (Morgan *et al.*, 2000), or at the
370 pod tip and will continue along the DZ until the valves have completely separated (Davies &
371 Bruce, 1997). A highly co-ordinated sequence of cellular and molecular events are required
372 to bring about the dissolution of the middle lamella between cells of the DZ, and separation is
373 precipitated in part by water loss from the pod wall, causing the valve cells to shrink and
374 creating the tension necessary to pull them apart (Meakin & Roberts, 1990; Liljegren *et al.*,
375 2004).

376 *5.2.2 Genetic dissection of dehiscence*

377 Much of the research undertaken in this area has focused on the differentiation and
378 development of the DZ in the model species Arabidopsis. Formation of this non-lignified
379 region is controlled by several MADS-Box genes which are capable of not only negatively
380 regulating each other's expression but also acting independently in the valve, valve margin
381 and replum cell layers. Expression of the functionally redundant *SHATTERPROOF 1*
382 (*SHP1*) and *SHATTERPROOF 2* (*SHP2*) genes specifies the DZ and promotes lignification of
383 adjacent cells (Flanagan *et al.*, 1996; Liljegren *et al.*, 2000). To confine the DZ to the valve
384 margin *FRUITFULL* (*FUL*) represses *INDEHISCENT* (*IND*), *ALCATRAZ* (*ALC*) *SHP1* and
385 *SHP2* expression in the adjacent valve cells as well as preventing lignification of the DZ (Gu
386 *et al.*, 1998; Ferrándiz, 2000; Rajani & Sundaresan, 2001; Liljegren *et al.*, 2004). The
387 transcription factor *SPATULA* (*SPT*) might also function in pod dehiscence since its
388 expression is identical to that of *FUL* from mid pod development leading the authors to

propose that these two genes share regulatory roles (Heisler *et al.*, 2001), although more recent work from the same group has suggested that *SPT* is regulated by *IND* (Groszmann *et al*., 2010). *SHP1* and *SHP2* transcriptionally activate *ALC* expression, which is required for establishing the SL between the valve margin and repulm (Rajani & Sundaresan, 2001). At the onset of the pod shatter process, pectin in the SL cell walls is degraded by hydrolytic enzymes, such as ADPG1 and ADPG2 polygalacturonase (PG) enzymes which, in combination with increased pod wall tension, enables dehiscence to proceed (Ogawa *et al.*, 2009). In addition to the PG enzymes it is predicted that a cyclic nucleotide-gated ion channel, *AtCNGC2*, might be involved in regulating programmed cell death within the DZ cells in Arabidopsis *(*Köhler *et al., 2001)*.The *IND* gene also functions downstream of the SHP transcription factors and is similarly required for differentiation of the valve margin cells, as well as lignification of the adjacent valve and replum cell layers (Liljegren *et al.,* 2004). However *IND* also appears to be transcriptionally activated by factors other than *SHP1* and *SHP2*, since low expression levels can be detected in the valves in the *shp/shp2/ful* triple mutant (Ferrándiz, 2000; Liljegren *et al.*, 2004). *IND* forms a self regulating network and is also required for the expression of *ADPG1* in the DZ (Ogawa *et al.*, 2009); thus if *IND* is not expressed to define the valve margins then the PG enzyme which would breakdown this cell layer also fails to be produced. All five transcription factors involved in patterning the silique: *IND*, *ALC, SHP1, SHP2*, and *FUL* are required for lignification of the valve layer and hence seed dispersal (Liljegren *et al.*, 2004). Furthermore, these MADS-box genes are predicted to be repressed by *REPLUMLESS* (*RPL*) which functions to specify the replum cell layer adjacent to the valve margin and hence maintain the DZ at the valve margin (Roeder *et al.*, 2003).

Mutating the transcription factors involved in specifying the Arabidopsis DZ can create an indehiscence phenotype, for instance in the *FUL* gain of function (Ferrándiz, 2000),

414 *shp1shp2* double knockout (Liljegren *et al.*, 2000), *IND* loss of function (Liljegren *et al.*,
415 2004) and *ALC* loss of function (Rajani & Sundaresan, 2001) plants the DZ is prevented
416 from forming properly, demonstrating that the genetic manipulation of orthologues in
417 *Brassica* species might be a suitable strategy for controlling pod shatter. However, there are
418 potential limitations to this strategy as ectopic expression of the *FUL* gene in *B. juncea* has
419 been revealed to make pods resistant to threshing (Østergaard *et al.*, 2006). A more recent
420 approach showed that it is possible to fine-tune the severity of the shatter phenotype through
421 inducing point mutations in *Brassica* orthologues of the *IND* gene and selecting those
422 variants with a commercially useful degree of valve margin disruption (Girin et al., 2010).
423 Whilst wheat has been cultivated for thousands of years, the large scale commercialisation of
424 oilseed rape is a more recent development and, within the genetic diversity of material that
425 exists, some cultivars exhibit a greater resistance to pod shatter than commercial varieties
426 (Morgan *et al.*, 1998). This diversity provides an extensive genetic pool that can be
427 investigated and should aid the identification of better mechanisms for successfully
428 controlling pod dehiscence.

429 *5.3. Seed abscission*

430 For seed dispersal to take place, not only does the pod have to 'unzip', but the seed
431 must also detach from the funiculus. Like dehiscence, the regulation of seed abscission is a
432 highly co-ordinated event culminating in wall dissolution at the hilum. Work on Arabidopsis
433 has shown that *HECATE3* (*HEC3*), which directs the expression of *ADPG1* in the seed
434 abscission zone, and *SEEDSTICK* (*STK*) are required for normal seed shedding (Pinyopich *et*
435 *al.*, 2003; Ogawa *et al.*, 2009).. This aspect of plant development provides a further avenue
436 for manipulating and potentially co-ordinating crop yield.

437 *5.4. Hormonal regulation of dehiscence and seed abscission*

A role for the plant hormones ethylene and auxin in regulating the timing of abscission has been documented extensively, with ethylene promoting and auxin inhibiting the process (Sexton & Roberts, 1982). Although a peak in ethylene production has been shown to precede dehiscence, exposure to the gas does not hasten pod shatter (Meakin & Roberts, 1990). Changes in auxin levels during pod development have also been identified but it is not clear to what extent the hormone regulates the dehiscence process (Johnson-Flanagan & Spencer, 1994; Chauvaux *et al.*, 1997; Child *et al.*, 1998). A recent publication (Sorefan *et al.*, 2009) demonstrated that local changes in pod auxin concentration are crucial for the differentiation of the DZ. The local auxin minimum generated at the valve margins seems to be produced by *IND* which acts to regulate auxin transport and as such increasing indole-3-acetic acid (IAA) levels at the valve margin leads to the development of an indehiscent phenotype due to the absence of this cell layer (Sorefan *et al.*, 2009). A recent publication by Arnaud *et al.* (2010) showed that gibberellin is a direct target, and is absolutely required, for the correct functioning of *IND*. The same authors concluded that *ALC* interacts directly with DELLA repressors, which antagonize *ALC* function but are destabilized by gibberellin. Taken together, these findings show that the gibberellin/DELLA pathway has a key role in patterning the Arabidopsis fruit and its eventual dehiscence.

Seeds are a major source of ethylene synthesis but their climateric production of the gas may only accelerate the onset of senescence rather than promote dehiscence *per se* (John *et al.*, 1995). This is highlighted by examining parthenocarpic pods which also produce a peak in ethylene and undergo shatter, albeit at a delayed rate, indicating that the pod wall has the capacity to produce the gas (Meakin & Roberts, 1990; Child *et al.*, 1998). The Arabidopsis protein AtTRP1, an orthologue of a tomato protein which interacts with the tomato ethylene receptors LeETR1 and NR, is highly expressed in the seed abscission zone

(Lin *et al.*, 2009). This observation suggests a possible role for AtTRP1 in regulating seed shedding which could be tested in lines where the gene is silenced or over-expressed.

VI. The Role of Plant Phytohormones in Pod Development

6.1.1 The influence of salicylic acid on seed yield

Plant phytohormones function in many aspects of development including cell differentiation, elongation, pattern formation and coping with abiotic and biotic stresses, all of which help maintain a high reproductive capacity. Depending on the tissue location and developmental stage phytohormones can either act synergistically or antagonistically towards one other which, in addition to pleiotropic effects, make their roles in pod and seed development difficult to discern.

Phytohormones can directly influence yield, for instance decreasing salicylic acid (SA) levels in Arabidopsis *NahG* transgenic lines and *sid2* mutants increases both the number of seeds per pod and the number of pods per plant, the latter resulting from an enhanced branching phenotype (Abreu & Munné-Bosch, 2009). Such physiological alterations were also correlated with a change in seed composition whereby N, vitamin E and pro-vitamin A content were enriched. This increase is a likely consequence of the late flowering and delayed senescent phenotype associated with SA deficient plants, thus enabling a longer period for resource translocation into the developing seeds (Martinez *et al.*, 2004; Abreu & Munné-Bosch, 2009). Such findings complement other studies which demonstrate that the constitutive overproduction of SA reduces seed yield (Mauch *et al.*, 2001). A decrease in seed weight was however reported in plants with reduced SA abundance (Abreu & Munné-Bosch, 2009) and, since germination potential was never measured, it is unknown how these altered ratios of nutritional compounds in the seed affect viability. Despite the implications for improving crop yield the effects of manipulating SA levels are not fully understood and,

in light of the fact that exogenous application increased seed yield in a grass species (Joaquin *et al.*, 2007), in contrast to the findings in Arabidopsis, this highlights a potentially species specific role for SA. In addition to this SA has a fundamental role in plant defence against microbial pathogen attack (reviewed in Vlot *et al.*, 2009) and environmental stresses (reviewed in Horváth *et al.*, 2007), hence complete KOs are unlikely to be commercially viable. Nevertheless, SA knockout lines that are regulated by a pod-specific promoter might extend the pod developmental period and allow more resource reallocation into the seeds, without compromising innate resistance strategies.

6.1.2 Ethylene mutants

Since the discovery of ethylene as a biologically active and readily diffusible plant growth regulator (Neljubov, 1901) it has been associated with many processes including, but not restricted to, seed germination, growth, timing of organ senescence, fruit ripening and abscission (Abeles *et al.*, 1992). Ethylene can temporally and spatially regulate numerous aspects of plant development, with fleshy and dehiscent fruits becoming more competent to respond to ethylene ripening signals as they age (Joaquin *et al.*, 2007). For instance a burst in seed ethylene production correlates with the onset of pod dehiscence (Oeller *et al.*, 1991), highlighting the importance of ethylene in regulating the timing of developmental events, even if it is not necessarily inducing such responses. The interaction between different hormone pathways remains largely undiscovered, although ethylene is currently known to assist in plant responses to JA, SA, auxin ABA and cytokinin signalling and together they play an important role in responding to biotic and abiotic stresses. This has led to considerable effort being invested in uncovering the ethylene response pathway (for reviews see Ecker & Stepanova, 2000; Guo & Ecker, 2004) with recent studies focusing on the mechanisms of sensing and reacting to ethylene signals through a family of cell surface receptors. Arabidopsis has five ethylene receptors (encoded by *ERS1, ERS2, ETR1, ETR2* and

512 *EIN4*) and mutations conferring dominant ethylene insensitivity all occur in the hydrophobic
513 regions of the N-terminal ethylene sensor domain (Bleecker *et al.*, 1988; Hua *et al.*, 1995;
514 Ecker *et al.*, 1998), implying that there are only a limited number of genetic locations in
515 which mutations are capable of causing ethylene insensitivity (Bleecker *et al.*, 1998). The fact
516 that ethylene receptor mutants have subtly altered phenotypes and encode distinct proteins
517 implies a functional specificity for the different receptors. This view was upheld by Zhou *et*
518 *al.* (2007) who argued that they are not functionally redundant but, as previously suggested,
519 may mediate the response of more than one signal (Bleecker *et al.*, 1998). For instance a link
520 between glucose sensitivity and the ethylene pathway (Zhou *et al.*, 1998) has been made
521 since glucose acts to decrease *EIN3* levels (Yanagisawa *et al.*, 2003), whilst in the monocot
522 rice it has been shown that reduced expression of the ethylene receptor *ETR2* can increase
523 thousand grain weight by up to 4% through altering starch acclimation and increasing sugar
524 translocation into the filling grains (Wuriyanghan *et al.*, 2009). The ethylene insensitive
525 receptor mutant *etr1-1* demonstrates a retarded leaf senescence phenotype which corresponds
526 to a delay in the expression of other senescence associated genes (SAGs). However, this
527 extended visual longevity does not correlate with functionality of the photosynthetic
528 apparatus, so *etr1-1* leaves have entered into the senescence programme despite retaining
529 higher chlorophyll levels for longer (Grbic & Bleecker, 1995), potentially indicating that
530 *etr1-1* mutants are unlikely to positively affect seed yield.
531 Examination of microarray data for Arabidopsis (Table 1; developmental data from
532 Wagstaff *et al*., 2009; wound response data taken from Naomab, 2008) revealed that the only
533 ethylene receptor showing at least a doubling of transcript levels during developmental pod
534 wall senescence was *ETR2* which increased 2.8-fold from mature green to yellow senescent
535 pod walls. This gene did not produce any signal on the wounded tissue arrays, indicating that
536 it does not have a role in the wound response at the transcriptional level; the same could be

said for *EIN4* but to a lesser extent. *ERS1* and *ERS2* were up-regulated 1.9-fold and 1.5-fold respectively in wild type pods 90min after wounding the intact pods multiple times with a pin, although only *ERS1* showed any developmental response, perhaps indicating that there is a segregation of ethylene receptors with respect to the signals they respond to. Most *ACC Synthase* (*ACS*) and *ACC Oxidase* (*ACO*) genes present in the Arabidopsis genome did not produce a signal on the microarrays, indicating that the process of ethylene biosynthesis is regulated at the post-transcriptional level. The exception was *ACO4* which was 19-fold up-regulated during developmental senescence and 11.4-fold increased 90 minutes after wounding. In contrast, *ACS2* does not appear to change during developmental senescence but it was 4.7-fold induced by the wound signal. Wounding also increased expression of genes encoding sugar transporters/signalling molecules, although these did not change during senescence. The glucose transporter *GPT1*, the hexokinase glucose sensor *HXK1, SUC2, SUC3, SUC4* and *SUC5* were all up-regulated by wounding. Of these *SUC2* increases the most (8-fold) within the 90 minute response period. It would appear therefore, that developmentally programmed resource allocation is regulated slightly differently to resource re-allocation that occurs after an unexpected event such as wounding which will compromise the viability of the seeds within that pod. Ethylene appears to have a stronger association with the wound response, despite its traditional links with the senescence process in other plant organs, and genes encoding sugar transporters within the pod only appear to be up-regulated after wounding, indicating that they may be more involved with resource export than import.

6.2. A role for ABA in seed development

Abscisic acid (ABA) is traditionally associated with stress responses, and consequentially growth retardation, but in the absence of such environmental insults it is required for normal seed maturation and is able to promote cellular growth, including in the

pod (Cheng *et al.*, 2002). In wheat the ABA:ethylene ratio affects the rate of grain filling and, since this is quite sensitive, imposing a small stress such as mild drought amplifies ABA levels within wheat grains and correlates with an increase in grain filling (Yang *et al.*, 2006). Similar results have been observed in oilseed rape (*B. napus*) and *Medicago truncatula* where raised ABA levels induced by osmotic stress stimulated a higher production of SSP transcripts and accumulation of free amino acids respectively (Wilen *et al.*, 1990; Planchet *et al.*, 2010). Plant sensitivity to ABA is controlled by *ABI3*, which in turn is required for the accumulation of SSPs within the seed (Nambara *et al.*, 1992).Whilst the oilseed rape experiments described above were performed on excised embryos, it does suggest that a controlled application of ABA to the pods may increase the abundance of SSPs in the seeds without the need to implement a water stress.

6.3 A role for other phytohormones

Gibberellins (GAs) are another class of phytohormone that have numerous functions within the plant including helping to break seed dormancy, regulating plant growth and floral induction. In Arabidopsis normal pod development requires GA levels to be kept within a confined range, as increased concentrations result in fewer seeds per pod (Rieu *et al.*, 2008) and a decrease in pod wall length and weight (Srinivasan & Morgan, 1996). Correlations between GA and cytokinin levels also appear crucial for regulating pod wall growth (Srinivasan & Morgan, 1996). Fertilisation triggers an auxin-mediated promotion of GA synthesis specifically in the ovule which is then transported to the valves where GA targets DELLA proteins for degradation and therefore releases the repression of fruit growth seen in unfertilised pods (Marti *et al.,* 2007; Dorcey *et al*., 2009).

Despite not being classed as a hormone, glucose also helps to regulate phytohormone levels and it is capable of functioning like a hormonal signalling molecule by indicating the plant's nutrient status (Arenas-Huertero *et al.*, 2000; Cheng *et al.*, 2002; Rolland *et al.*, 2002).

For instance, there are interactions between sugar and nitrogen signalling that can affect the carbon-nitrogen balance, (Sheen *et al.*, 1999), indicating that the capacity of a plant to sense changes in the glucose concentration within individual organs can regulate phytohormone production and consequentially mediate the source-sink nutrient balance (Cheng *et al.*, 2002). This theory is further supported by the observation that the Arabidopsis glucose insensitive/ABA-deficient mutant *gin1/aba2* has smaller pods than wild type and consequentially produces far more aborted embryos per pod than wild type, although any mature seeds are the normal size (Cheng *et al.*, 2002).

6.3. Altering the developmental period

Cytokinins help to regulate the timing of senescence and, since their levels fall at the onset of this process, exogenous application can delay senescence (Nooden *et al.*, 1979). This knowledge has enabled leaf senescence to be postponed by attaching a promoter from the senescence specific gene *SAG12* to the gene encoding isopentyl transferase (*IPT*), which catalyses cytokinin biosynthesis, generating auto-inhibition of senescence through the maintenance of pre-senescence cytokinin levels. In tobacco this prolonged the flowering period and photosynthetic lifespan which together resulted in a 50% increase in dry weight and seed yield, although it was not reported whether this also affected seed composition (Gan & Amasino, 1995). However, the SAG12:IPT construct in wheat only resulted in delayed senescence and not an increase in seed yield which the authors suggested was due to interference by the construct with the normally extremely rapid relocation of resources from senescing leaves to reproductive sinks (Sýkorová *et al.*, 2008). The tight correlation between developmental period and seed yield raises the possibility that a similar system of auto-regulation of senescence could be implemented to co-ordinate pod development, at least in dicotyledonous plants. Theoretically, delaying senescence and extending the photosynthetic period would increase the potential for seed filling and prevent the onset of dehiscence. The

process of senescence and dehiscence could subsequently be coordinated across the whole plant if the inhibition provided by cytokinin could be turned off in a controlled manner, for example by using an inducible promoter system.

VII. Silique biosynthesis of compounds for the seed

7.1.Silique and seed photosynthesis

Since the onset of leaf senescence occurs prior to the last pod forming, and before seed fill is complete, embryos have to rely upon pod or seed wall and stem photosynthesis to generate the remainder of their photoassimilates required for viable seed production. Enclosure within a pod limits the photosynthetic capacity of the seed itself; in contrast the pod has a photosynthetic potential far greater than that of a leaf if assessed on the assimilate produced per unit of chlorophyll basis (King *et al.*, 1997). Carbon photosynthates stored within the pod wall are thought to be remobilised to developing seeds as a decrease in hexose levels corresponds with a concomitant increase in seed growth (King *et al.*, 1997). Additionally *de novo* starch synthesis within oilseeds is presumed to be insufficient to account for the final oil levels observed, indicating the importance of translocating carbohydrates, such as sucrose and hexose, across the pod wall. To enable seeds to generate some of their own photoassimilates the pod wall in oilseed rape has a sclerencyma cell layer nearest to the inner pod cavity that is predicted to act as a barrier to gas diffusion and therefore aid a build-up of CO_2 around the seeds (King *et al.*, 1998). Developing seeds are capable of fixing this CO_2 and consequentially generating energy for the synthesis of seed storage products, although predictions suggest that the quantity of CO_2 is not enough to sustain photosynthesis in the seed itself. In the pea pod, for example, it has been calculated that respiration accounts for the loss of more C than is incorporated into the fruit during the photosynthetic period. In the second half of seed development the pod is only capable of

producing about 10% of the carbon required by the seed (Flinn *et al.*, 1977) most likely due to onset of chlorophyll catabolism in the pod wall, but this reinforces the absolute necessity to re-allocate resources around the plant.

7.2.1 Seed storage proteins

SSPs accumulate within both protein storage vacuoles and the endoplasmic reticulum (Crofts *et al.*, 2004). During the seed filling phase the import of amino acids into the embryo only occurs via the phloem and requires the amino acid co-transporter located within the pod vascular system that is encoded by *AAP2* (Hirner *et al.*, 1998). *AAP2* might therefore have an important role in transporting amino acids from vegetative plant organs and the pod wall into the seed. Should it emerge that SSP transcripts found in the pod wall (Wagstaff *et al.*, 2009) are transcribed into proteins which are subsequently translocated to the seeds *AAP2* might be involved in their transport. This leads to the possibility that targeting pod wall transcripts could increase the concentration of nutritionally essential sulphur proteins normally lacking within oil seeds.

7.2.2 Synthesising molecules in the pod wall

A microarray analysis in *B. napus* revealed that seeds express genes encoding many essential storage compounds (Yu *et al.*, 2010). This finding does not automatically signify that all these genes were translated into proteins *in situ* but it does indicate that the seed does not necessarily need to import them from the pod wall. This needs to be weighed against the observation that, despite seeds containing the enzymes for synthesising some of their own compounds such as glucosinolates, they are still produced in the pod wall and translocated into the developing embryos (Bilsborrow *et al.*, 1993; Zhao *et al.*, 1993; Du & Halkier, 1998). The reasons for a plant utilising this strategy is unclear; energetically it would be more efficient for the seeds to synthesise their own storage compounds so the factors that determine whether the seeds, pod wall, or both, translate these remain to be elucidated.

7.3. Lipids and oils

At higher light intensities seeds are capable of synthesising more fatty acids, indicating that an increased rate of photosynthesis might be responsible for this (Schwender *et al.*, 2004; Goffman *et al.*, 2005). Therefore it follows that either making the pod walls thinner to improve light penetration, or providing the seeds with more energy, could improve oil synthesis. This topic is laced with controversy, for whilst Eastmond *et al.* (1996) believes that the low level of incident light reaching a seed is insufficient to generate enough reducing power in the form of NADPH for lipid biosynthesis, two independent studies (Willms *et al.*, 1999; Schwender *et al.*, 2004) refute this claim. Instead both support the notion that green oilseeds are well adapted to low light levels and as such can produce enough energy for fatty acid synthesis. Regardless of who is correct, the implication of this is that an increased oil content could be achieved by having seeds and or pod walls which remained photosynthetically active for longer, potentially by utilising the *SAG12:IPT* auto-regulation system discussed above or a stay green phenotype which remains functionally photosynthetic and has delayed senescence.

7.4. Translocation of molecules from the pod wall into the developing seeds

The transport of molecules from the pod wall into the seeds represents a centripedal mode of transport towards the inner integuments of the pod presumably via the single entry point at the base of the pod in the vascular system. Seeds of the legume species *Medicago truncatula* are highly specialised for the importation of nutrients with their micropylar region containing a vascular system organised into tracheids (Abirached-Darmency *et al.*, 2005). The presence of sucrose synthase (SUS) in phloem associated companion cells (Fallahi *et al.*, 2008) when the siliques are fully mature supports the previously identified role of SUS in phloem loading/unloading (Martin *et al.*, 1993; Nolte & Koch, 1993). It is predicted that SUS could be vital for transporting assimilates generated in the pod wall into developing seeds via

687 the pod wall phloem (Fallahi *et al.*, 2008) especially since the SUS protein exhibits a spatial
688 change throughout development. Initially SUS is highly expressed within the pod wall and
689 funiculus, but by the later stages of development it is only found in the embryo and aleurone
690 layer of the seed (Fallahi *et al.*, 2008), indicating a translocation of assimilates from the pod
691 wall into the seeds via the phloem tissue.
692
693 **VIII. Conclusion**
694 This review has highlighted the contributions that a pod can make to the development
695 of the encapsulated seed and identified strategies for manipulating resource allocation
696 (summarised in Figure 2). In addition to providing protection from biotic and abiotic stresses
697 it is evident that the photosynthetically active pod can contribute assimilates and nutrients
698 that are subsequently imported into the developing seeds and a 'push-pull' model is proposed
699 where the strength of the sink exerted by the seeds determines the degree to which resources
700 are remobilised from other parts of the plant. Transcript profiling of the pod wall during
701 development has revealed that the tissue shares features in common with leaf material and it
702 is possible that shared events take place during pod and leaf senescence. Manipulation of the
703 timing of pod senescence may make it possible to enhance the duration of grain filling and
704 increase protein, carbohydrate or lipid content into the developing seed. In weed species, such
705 as Arabidopsis, the partitioning of assimilates is highly co-ordinated within a pod so that at
706 dehiscence all seeds are at an equivalent stage of maturation; although the timing of
707 dehiscence across the plant, or a population of plants, is generally uncoordinated so that pods
708 release their contents over a long period of time. In domesticated crops, such as peas and
709 beans, grain filling may be less well orchestrated within a pod or an ear, and some seeds may
710 act a stronger sinks than others. In contrast, coordination across the whole plant, and between
711 plants in a monoculture, is extremely good as a consequence of the strong selection imposed

712 by man for uniformity as crops have been domesticated. The mechanism that co-ordinates
713 assimilate import into seeds positioned at different sites in a pod is unclear. However, recent
714 transcriptional analyses in Arabidopsis has identified a response regulator, *ARR22*, expressed
715 within the micropylar tissues, that plays a key role in regulating the response of seeds to
716 wounding and could contribute to regulating the assimilate import/export. Further 'omic
717 analyses of pod tissues, particularly those of the pod wall, will assist in dissecting the
718 contribution of the pod to the development of the seed. Armed with this information it
719 should be possible to devise strategies to manipulate pod development so that we can not
720 only enhance seed yield but also, and perhaps even more importantly, its nutritional value.

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Table 1. Expression of genes involved in ethylene and sugar transport/signalling during developmental pod senescence (green to yellow pods) and wound response (0-90min after wounding)[1].

Function	Gene	AGI	Fold change in senescent pods	Fold change in wounded pods
Ethylene receptors	ETR1	At1g66340	0.94	1.50
	ETR2	At3g23150	2.83	NP
	ERS1	At2g40940	1.70	1.99
	ERS2	At1g04310	1.01	1.55

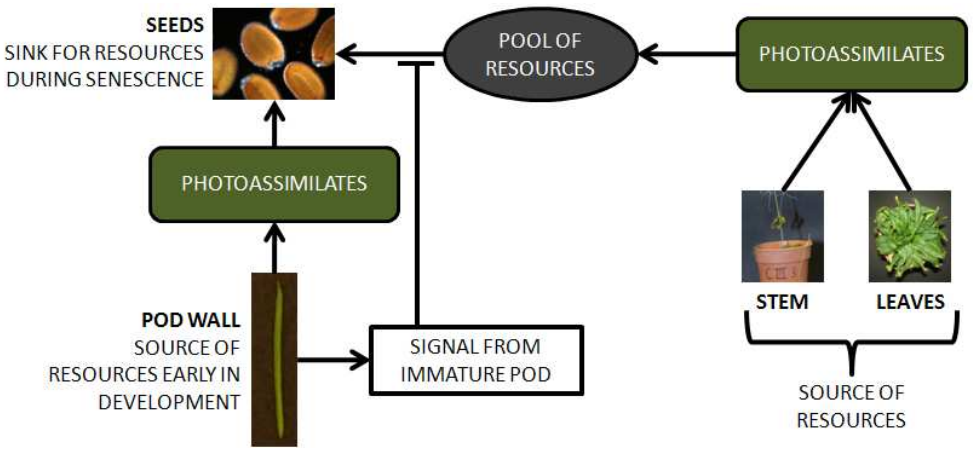
	EIN4	At3g04580	1.93	0.86
Ethylene biosynthesis	ACS2	At1g01480	NP	4.73
	ACS4	At2g22810	NP	NP
	ACS9	At3g49700	NP	NP
	ACS11	At4g08040	NP	NP
	ACO4	At1g05010	19.80	11.39
Sugar transport	GPT1	At5g54800	0.84	2.17
	GPT2	At1g61800	0.18	NP
	GLT1	At5g16150	1.12	1.11
	SUC2	At1g22710	0.92	8.31
	SUC3	At2g02860	1.24	1.52
	SUC4	At1g09960	0.77	3.54
	SUC5	At1g71890	0.10	1.33
	HXK1	At4g29130	0.84	2.45

[1]Shaded values indicate a fold increase greater than 1.5; NP indicates no signal present on array.

Figure legends

Figure 1. Push-Pull model of resource allocation. Vegetative green organs such as leaves and stems produce assimilates which are pushed into the central pool of resources for that plant. Photosynthetic activity of the pod also contributes to the resource pool early in development, but it becomes a sink during senescence and seed maturation. A negative feedback loop, hypothesised to be mediated by an unknown signal originating from the immature pod, prevents early remobilisation of resources away from photosynthetic organs which is only broken as the pull from the maturing seeds becomes strong enough to initiate remobilisation from the central pool. The strength of the pull is proportional to the number of maturing pods on the plant; hence selective pod removal prevents senescence of the rosette leaves as the number of sinks is reduced.

Figure 2. Ways to manipulate yield. Blue boxes indicate targets strategies for yield manipulation; green boxes indicate the tools that could be used; pink boxes show the consequence of manipulation for each strategy. Coloured edges of blue boxes are linked with lines of the same colour. References in brackets are listed below and in full within the reference section of the main manuscript. (1) Allen & Morgan, 1972; (2) Taylor et al., 2010; (3) Abreu & Munné-Bosch, 2009; (4) Wuriyanghan et al., 2009; (5) Yang et al., 2006; (6) Wilen et al., 1990; (7) Maia-Grondard & Limami, 2010; (8) Gan & Amasino, 1995; (9) Sorefan et al., 2009.



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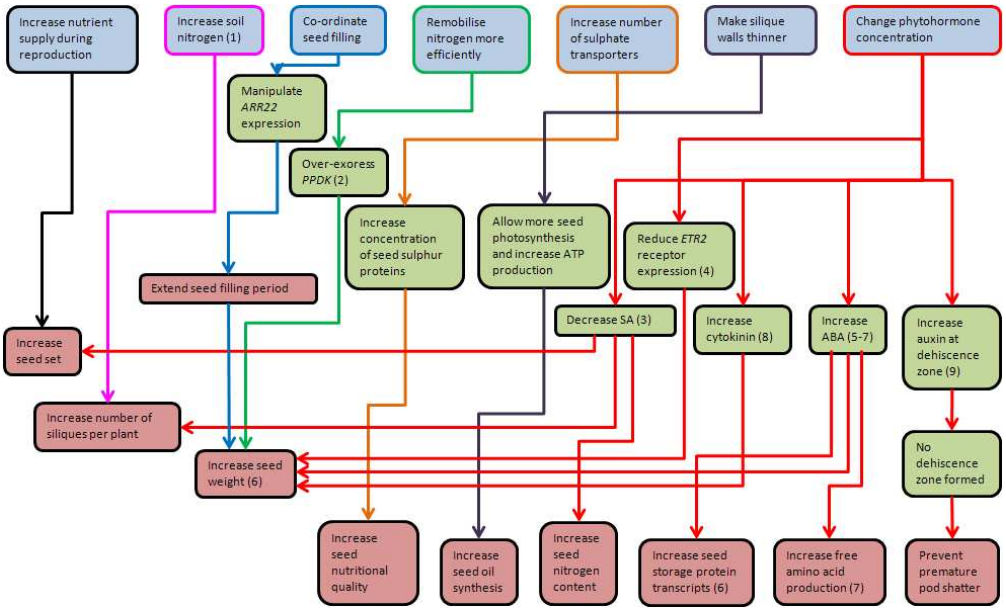
34x15mm (600 x 600 DPI)



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41x25mm (600 x 600 DPI)