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Seed Development and Reserve Compound Accumulation in Common Bean (*Phaseolus vulgaris* L.)

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ABSTRACT

Much of what we know today on the molecular aspects of seed development comes from basic studies carried out in the model species Arabidopsis. However, many differences exist between the developmental programs of the small seeds of Arabidopsis, basically without nutritional reserves, and the crop legume seeds, which are bigger and accumulate nutrients designated to germination. Since seed nutritional reserves are essential to guarantee food production, it is important to understand the genetic, biochemical and physiological mechanisms favouring a better incorporation rate of the main reserve compounds in seeds, such as proteins, carbohydrates, lipids and minerals, which will foster breeding programs towards more productive and efficient genotypes. Here, we review the current understanding of seed development of common beans as well as refer to conserved developmental mechanisms between this and other related species. The state of the art of biochemistry, genetics and physiology, including protein, hormonal and nutritional interactions, during seed development of common beans and the perspectives to further understand and control it are discussed with emphases on metabolic pathways, and nutrient transport and storage compounds. The text also alludes to crop management favouring incorporation of certain substances or altering the proportion of storage compounds and genotypes that store more efficient forms of phosphate than phytates. Additionally, we discuss the major challenges and perspectives for future investigation on controlling mechanisms of the main reserve compounds in common bean seeds.

Keywords: legumes, metabolic pathways, model species, nutrient transport, phytate, storage compounds

CONTENTS

INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) are tropical legumes that diverged from temperate legumes 35-55 million years ago in the Tertiary period (Doyle and Luckow 2003). It presents a great diversity of phenotypes regarding seed size, colours and composition, reflecting the great genetic diversity within the species (Papa *et al*. 2005; Gonçalves Ceolin *et al*. 2007). Although evolutionarily advantageous, this genetic plasticity puzzles plant biologists in understanding the bases behind developmental interactions, especially of those divergent characteristics from established model species, such as seed development, since the most studied plant model, *Arabidopsis*, did not develop the capacity of accumulating significant nutritional reserves in their seeds.

One purpose of studying seed development in grain species is to specify the most adequate harvesting moment in terms of maximum nutrient accumulation and seed vigour, which coincide with physiological maturity and further dehydration to reach the harvest point. Considering that seed nutrient reserves are essential to guarantee food production, it is essential to understand the genetic, biochemical and physiological mechanisms favouring a higher rate of incorporation of the main reserve compounds in the seeds, such as protein, carbohydrates, lipids or minerals and other nutritional factors. A better understanding of phloem discharge may lead to the optimization of reserve compound synthe-

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ses in seeds as well as the study of metabolic pathways, especially on key enzymatic steps, can potentially lead to a better control of amounts or types of substances stored in the seeds. These effects are efficiently addressed by using genetic mutants with more efficient transport of sugars, amino acids and P to the seeds (Raboy *et al*. 2000). Understanding the metabolic changes associated with structural changes during seed development will ultimately indicate the processes related to yield, since a desirable genotype would present at first, a rapid seed filling at the beginning of the seed development program leading to an early seed fixation, and after, a short maturation period, which may be linked to the drain strength of the developing organ. In this review, besides genetic and biochemical interactions during development important to seed production.

PHASES OF SEED DEVELOPMENT

Seed development in legumes is highly related to nutrient metabolism and transport as an intense sink activity. In order to understand and analyse this process, it is fundamental to study the genetic, physiological and biochemical interactions during seed maturation. The phases of seed development are well established (Weber *et al*. 2005); however, little is known yet about the dynamic interactions and the complexity of the processes involved. Seed is a complex organ regarding its genetics: the seed coat is genetically maternal, the endosperm is filial (but in diploid species, it is triploid due to the double fertilization that gives origin to the development of this tissue) and the embryo is purely filial. The development of a legume seed follows a series of events divided in three phases according to dry matter accumulation. Initially, seed development is characterized by a relatively slow mass accumulation during histodifferentiation or embryogenesis. The maturation phase is followed by a continuous and fast increase in dry matter that coincides with an augmentation of germination and vigour potentials, until reaching the maximum content of dry matter at physiological maturity. This phase comprehends cell expansion and reserve compound allocation concomitantly to embryo growth. Seed dehydration is characteristic of the third phase, together with biological mechanisms leading to embryo desiccation resistance and, at last, germination viability. Sugars and nitrogen are signals regulating seed development (Wobus and Weber 1999) and metabolic exchanges, as well as signalling crosstalk between seed and mother plant, are intermediated by the seed coat (Borisjuk *et al*. 2003).

Hormones play fundamental roles during seed development. They are involved in processes since the beginning of embryo formation, tissue development, reserve accumulation and its mobilization during the germination. As exhaustively studied during pea seed development, high levels of cytokinin, auxins, abscisic acid and gibberellin were found with their maxima varying at different moments. Cytokinins are present in the beginning of seed development, during an intense cellular division process, increasing at a rapid speed after the fecundation, diminishing as the seed develops. Auxins are synthesized from the amino acid tryptophan, and in seeds it is present since the initial phases of development, being responsible for compound assimilation from the mother-plant. Gibberellins are associated with cell expansion and, in association with auxins, with driving reserve synthesis. ABA impairs early germination in the pod (viviparity) and is associated with LEA protein synthesis and desiccation resistance (Koornneef *et al*. 2002; Ali-Rachedi *et al*. 2004).

Embryogenesis or embryo histodifferentiation

This phase is specifically marked by cell division and differentiation that will form the embryo tissues and the endosperm. After the sexual fusion (syngamy), there is a brief period of reorganization, during which the pronounced vacuole near the zygote gradually disintegrates, the zygote cytoplasm becomes homogenous and the nucleus increases in size. The zygote becomes elongated and a few vacuoles are homogenously distributed in the cytoplasm. Cell division of the zygote will not initiate until a small portion of the endosperm is already formed, the polarity lines are established in the embryo sac in preparation for future divisions and growth (Marcos Filho 2005).

The embryo develops in the interior of the ovule from the fertilized egg cell (zygote). Although the initial growth of the embryo seems fair, there is already a moderate demand for energy (Borisjuk *et al*. 2003). Initially, the pro-embryo assumes a claviform or cylindrical form, then, its distal portion becomes the active site of cellular divisions, increasing in volume and becoming roughly spherical. This modification distinguishes the body of the embryo and the suspensor. In following events, there is a change in the embryo symmetry: from the spherical form with radial symmetry it develops into a distally flattened structure, acquiring bilateral symmetry. On the other hand, the suspensor is hereby degenerated by programmed cell death events (Lombardi *et al*. 2007). Initially, the cotyledons seem like small protrusions, and after a series of divisions and cell expansion, they later acquire their characteristic aspect similar to leaves. The terminal portion of the axis below the cotyledons differentiates into root meristem or radicle, forming the hypocotyl-radicle axis (Marcos Filho 2005).

In a more advanced developmental stage of the plant embryo, all structures that will form the plantlet can be easily identified: the apical meristem (in some embryos, it may be located in the epicotyl – above the cotyledons); both cotyledons (in the case of dicots); the hypocotyl (located below the cotyledons); the radicle meristem; and the embryonic root or radicle (when the radicle cannot be distinguished in the embryo axis, the whole structure below the cotyledons is called hypocotyl-radicle axis). In most dicot species, the developing embryo feeds from the reserves in the endosperm and perisperm (whenever present). This is the reason that beans develop large and fleshy cotyledons that store energy for plantlet development. The endosperm contents are hydrolyzed and transported to the embryo during the seed filling phase. Whereas the embryo and the endosperm are developing, the teguments also go through visible modifications, especially increasing in thickness. The funiculus usually suffers abscission leaving behind a scar named hilum.

Cytokinins are involved in early embryogenesis especially by regulating growth through cell division and sugar metabolism. In pea, cytokinin peaks during the heart-shape stage of embryo development (Quesnelle and Emery 2007). Additionally, the expression of genes involved in the last step of active gibberellin synthesis (GA-oxidases) was localized in the suspensor neck of developing seeds of runner bean (*P. coccineus*) between the late globular and heart stages, in the embryo epidermal cells, in the endosperm during the transition from globular to heart stages, and during cotyledon and inner tegument development (Solfanelli *et al*. 2005). Auxin has also been shown to be important for controlling seed development of common bean. PvIAP1, a functional enzyme involved in auxin conjugation (thus, auxin homeostasis control) (Walz *et al*. 2008) was detected during the rapid growth period of seed development in common beans (Walz *et al*. 2002).

During the early stages of embryo development, there is a nutritional competition between the embryo, the endosperm and the suspensor. In genetically incompatible crosses of related species, such as common bean (*P. vulgaris*) and runner bean (*P. coccineus*), embryos are aborted during the initial developmental phases, from globular to early cotyledon, probably due abnormal endosperm development or suspensor hypertrophy, with physiological influence of the seed coat (Ndoutoumou *et al*. 2007). *In vitro* embryo rescue at the globular stage was suggested to overcome this incompatibility in breeding programs and a protocol for was proposed for *Phaseolus* interspecific crosses (Schryer *et al*. 2005).

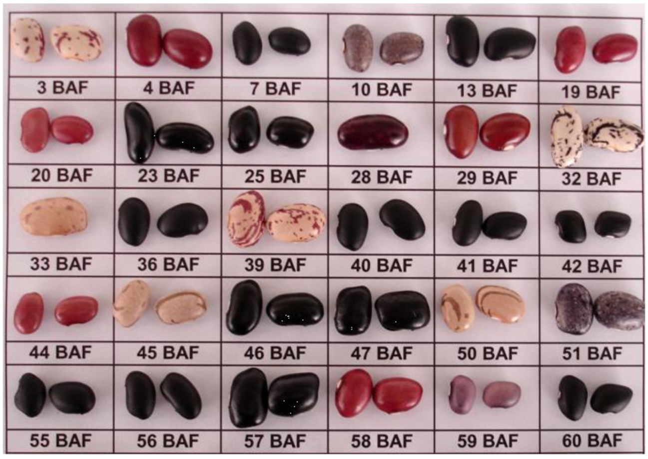

Fig. 1 Diversity of seed coat colour in common bean (*Phaseolus vulgaris*) in South Brazil. BAF: Banco Ativo de Feijão (Active Bank of Beans).

Maturation and control points in the metabolism of sucrose, nitrogen and phytic acid

During seed maturation, the embryo stop growing, mitosis is less intense, tegument tissue differentiates, storage compounds start to accumulate and the seed develops tolerance to desiccation (Gutierrez *et al*. 2007) in a clear developmental switch from cell proliferation and differentiation to cell adaptation. In general, at the end of the maturation phase, maximum accumulation of dry matter in the seed is reached, representing the physiological maturity (**Fig. 1**). During the desiccation phase and even at the end of the maturation phase, a high dehydration rate and the rupture of trophic connexions with the mother plant is observed. Whereas the metabolism decreases drastically, the embryo remains viable, with abscisic acid concentration remaining high to guarantee dry matter flow and enzyme activity acting on anabolic processes (Pammenter and Berjak 1999). In *Arabidopsis*, metabolic profiling indicated that the preparation for germination starts already during seed desiccation (Fait *et al*. 2006). In this stage, gibberellins and brassinosteroid synthesis are locally active and exert important functions during maturation (Radchuk *et al*. 2006). Although relatively still understudied in legume seeds, abscisic acid (ABA) has been regarded as essential for embryo tolerance to desiccation (Buitink *et al*. 2006).

The accumulation of reserve compounds in the seeds is meant to feed the embryo during development and guarantee seed germination and plantlet emergence. Among the main seed reserve compounds are carbohydrates, proteins, lipids and phytic acid (phytin). Although containing chlorophyll and being of limited photosynthetic capacity, legume seeds are essentially sink organs. The main reserve substances are compounds derived from carbon fixation by leaf mesophyll cells into sucrose, which is transported via phloem, discharged in the apoplastic region between the mother-plant and the seed cells and later incorporated by the seed through the seed coat (Weber *et al*. 1997; Patrick and Offler 2001). During the maturation phase, sucrose arrives to the seeds through a ventral vascular system localized along the pod, in a region delimited around the hilum which forms a vascular net around the tegument seed. There is no symplastic contact between the tegument and the cotyledons either and carbohydrates are discharged from the tegument vascular system to an apoplastic region between the tegument and the embryo (Borisjuk *et al*. 2003). Simple molecules are transported to the developing seed, such as sucrose, amino acids (especially asparagine and glutamine) and mineral ions (Golombek *et al*. 2001). All reserve compounds found in a mature seed are derived from these simple molecules and follow this transportation mechanism by a concentration gradient of metabolites that favours the differentiation events (Wobus and Weber 1999). A duo of sucrose transporters expressed in the coat of developing seeds of common beans, a symporter (PvSUT1) and a facilitator (PvSUF1), were functionally characterized recently (Zhou *et al*. 2007).

Genotypic differences in the rate of developmental seed growth have been used to identify control points in the photoassimilate accumulation in this organ (Tegeder *et al*. 2000). Sucrose uptake was found more variable among genotypes than dry matter flow when labelled sucrose was measured around the plasma membrane whereas the dry matter flow is the combination of net transport through the membrane and the metabolic distribution of the accumulated sucrose so that genotypic differences might be due to sucrose sequestration and allocation of reduced carbon from sucrose to protein and starch biosynthesis. This effect was also observed in other species during the filling phase with a low conversion rate from hexoses to sucrose in cotyledons of *Vicia faba* and pea, in which high levels of sucrose and low of hexoses may be promoted by sucrose hydrolysis or by the synthesis of other storage compounds through the activity of sucrose synthase (Koch 1996).

Seed maturation genes are controlled by master regulatory transcription factors that in legumes may be regulated by sugars, as suggested by studies in Arabidopsis mutants showing leafy cotyledon phenotypes (Tsukagoshi *et al*. 2007). PvALF is a seed-specific B3 transcription factor induced by ABA in common bean involved in chromatin remodelling and activation of protein storage genes, such as phaseolin (Bobb *et al*. 1995, 1997). An evolutionary conservation of known *Arabidopsis* mechanisms in legume embryogenesis, such as the involvement of FUS3 and ABI5 functions onto seed-specific gene expression of a legume involving PvALF was demonstrated (Ng and Hall 2007). Metabolic profiling of seeds during maturation showed reduction of sugars, organic acids and amino acids towards incorporation into storage compounds when compared with the previous phase (Fait *et al*. 2006). Recently, a comprehensive list of classified transcription factors in a legume genome, the model *Medicago truncatula*, has been reported (Udvardi *et al*. 2007). This, together with whole-genome transcriptional profiling will enable to identify most of the master regulatory genes involved in seed development in the near future.

Some proteins and carbohydrates are synthesized in the seed only later during the development. Among them, there are LEA (Late Embryogenesis Abundant) proteins, which may be associated with the embryo capability of withstanding dehydration, although the mechanism is still unveiled. They are characterized by a hydrophilic amino acid composition, highly soluble in water and resistant to high heat. Functionally, they act to protect the embryo from dehydration and other environmental stresses, such as salt and heat (Wang *et al*. 2003b).

Specific sugars that are synthesized later during embryo development were also found associated with stress tolerance. Sucrose, for example can confer a glass state to the mature seed, impairing membrane fusion and increasing seed longevity (Gurusinghe and Bradford 2001).

Common bean seeds accumulate great amounts of proteins and starch, indicating a regulated integration of carbohydrate and nitrogen metabolisms. During legume seed development, amides are imported from other organs and converted into amino acids (Golombek *et al*. 2001). The rate of each metabolism is defined by the concentration of starch and sucrose. An example of this regulation can be exemplified by the transcription of legumins in the seeds of *rugosus* (*rug*) pea mutants, which present wrinkled seeds and less starch in the seeds than the wild-type, leading to higher nitrogen contents and a higher sucrose/starch ratio (Turner *et al*. 1990).

Amino acid uptake by parenchymatic cells of pea cotyledons may be mediated by specific H^+-amino acid transporters, although this is not proved yet (Tegeder *et al*. 2000). In pea seed coats, amino acids are metabolized and restructured, especially glutamine, alanine and threonine and later,

Fig. 2 Phases of pod and seed development after fecundation in relation to dry matter accumulation in the seeds. From left to right: 0.04; 0.08; 0.17; 0.24; 0.22 g/grain and 17; 19; 23; 28; 35 days after flowering (DAF), respectively. Bar = 2 cm.

asparagine, are released (Rochat and Boutin 1991). In a saturable system, co-transport of amino acids with H^+ is thought to be important and has been shown as an important control point, such as the VfAAP1 observed in parenchyma cells of faba beans (*Vicia faba*) cotyledons, which is expressed at its maximum during storage protein accumulation phase (Miranda *et al*. 2001).

During seed development, phosphorus accumulation as phytate (phytic acid) follows the same pattern in legumes as in cereals, generally following dry matter accumulation (**Fig. 2**) (Asada *et al*. 1969; Makower 1969; Ogawa *et al*. 1979; Raboy and Dickinson 1987; Coelho *et al*. 2005). Some species differ in which tissues phytate accumulation occurs and how it is distributed in protein bodies. In some cereals, as in rice and wheat, phytate is deposited in the aleurone layer, which is easily removed during grain processing (Ogawa *et al*. 1979). In soybean and common bean, phytate is dispersed in the cotyledons, associated to the proteins in protein bodies (Lott *et al*. 1985).

In some studies with developing seeds, intermediates of inositol phosphates were not detected, as in rice and wheat (Asada and Kasai 1962; Saio 1964; Cosgrove 1966) or soybean (Raboy and Dickinson 1987), whereas in other studies, intermediates with different phosphorylation levels (tris-, tetrakis- and pentakisphosphate) were found in maize, sunflower and common beans (Sobolev and Rodionova 1966; Loewus and Loewus 1983) as well as mono- and pentakisphosphate in Wolffiella floridana (Roberts and Loews 1968), monophosphate in rice (Tanaka *et al*. 1971) and tetrakis- and pentakisphosphate in cotton embryos (Sharma and Dieckert 1974). There are relatively few reports on phytate contents and its intermediates in seeds of common beans, with even fewer during seed development.

Commercial varieties of common beans were reported to present a higher rate of phytate biosynthesis from 16 to 26 days after flowering (DAF). Small inositol phosphates, IP_3 to IP_5, being clearly detected during different stages of seed development, being IP_3 contents much higher than IP_4 or IP_5 (Coelho *et al*. 2005). *Myo*-inositol kinases, enzymes that phosphorylate myo-inositol until IP_6 are present during seed development. In soybean seeds incubated with labeled substrate, the larger substrate used by a inositol (1,3,4)-P_3 kinase was inositol (1,3,4,5)-P_3, which was converted to inositol (1,3,4,5,6)-P_5 (Phillippy 1998). Other studies reported that the expression of inositol (1,3,4)-$P_{3\text{-}5/6}$ kinase in Arabidopsis was correlated with the synthesis of inositol (1,3,4,6)-P_4 and inositol (1,3,4,5)-P_4 at the ratio 1:3 (Wilson and Majerus 1997). Inositol phosphates smaller than IP_6 were detected in mature seeds, however it may be a hydrolysis product of phytate during seed storage (Brearley and Hanke 1996a; Coelho *et al*. 2005). In mature cereal grains, its intermediates represent only 2% of the total inositol phosphate. Notwithstanding, in legume grains, this proportion is around 28% (Lehrfeld 1989; Burbano *et al*. 1995; Kasim and Hardy 1998; Harland and Narula 1999).

Strategies to decrease phytate contents in seeds could be achieved through increasing pools of other phosphate forms, such as inorganic phosphate or inositol tetra- and pentakisphosphates, such as carried out in maize (Raboy *et al*. 2000). Thse forms of phosphate are believed to have a lesser effect as antinutrient (Persson *et al*. 1998; Sandberg *et al*. 1989; Lonnerdal 2002).

CARBON AND NITROGEN METABOLISMS DURING SEED DEVELOPMENT

Seed development is dependent upon nutrient supply delivered through the phloem. To arrive at the seed cells, a stream of nutrients involving a suite of membrane transporters, such as aquaporins, channels and secondary transporters takes place in all phases of seed development (Zhang *et al*. 2007). A symplastic movement was revealed in great details in Arabidopsis embryos by using localized expression of green fluorescent protein (GFP) and following its symplastic mobilization throughout seeds and embryos (Stadler *et al*. 2005), revealing that the outer integument is a symplastic extension of the phloem as well as characterizing as symplastic domains: the inner integument, and the embryo at the globular stage plus the suspensor.

The sink strength of the seed will at last determinate the yield potential for seed production. The ectopic overexpression of phosphoenolpyruvate carboxylase (PEPC) in *Vicia narbonensis* resulted in a higher storage potential and increased protein content in the seeds (Radchuk *et al*. 2007). Besides the photosynthetic efficiency determining solute concentration in the phloem sap, changes in the seed physiology, the activity and quantity of membrane transporters are key factors establishing the sink strength of the seed. Seed-specific amino acid permeases are well studied in legumes. The pea AAP1 and 2 were shown to be expressed in the seed coat, cotyledon epidermis and parenchymatic storage cells especially during the protein storage phase (Tegeder *et al*. 2007). These authors also showed evidence that α-ketoglutarate and oxaloacetate may provide signals leading to upregulation of amino acid synthesis. In another study, seed overexpression of AAP12, a seed-specific amino acid permease of *Vicia narbonensis* increased uptake and allocation of fixed carbon and nitrogen to seeds, resulting in greater seeds and higher yields (Gotz *et al*. 2007).

Interestingly, vegetative organs were not compromised, but rather stimulated by this seed sink strength, leading to a stronger vegetative growth. In lupin, the activity of a neutral invertase was demonstrated to be predominant during the early stages of seed development whereas a sucrose synthase activity was pronounced during the growing and storage phases. Water status influences this metabolism and interestingly, a mild drought during the maturation phase in lupin led to an increase in stachyose and raffinose oligosaccharides although other metabolites analysed were unchanged in mature lupin seeds (Pinheiro *et al*. 2005). These storage carbohydrates are involved in the acquisition of desiccation tolerance, conferring a low cellular osmotic potential; however they have also been implicated as antinutritional factors and as source of flatulence effects (Viana *et* *al*. 2006), opposing nutritional properties and agronomic trait.

In a study of seed traits among genotypes of mungbean (*Vicia radiata*, previously *Phaseolus aureus* and *Phaseolus radiatus*), the authors reported differential activity of enzymes involved in controlling the carbon flux: sucrose synthase, UDP-glucose, ADP-glucose pyrophosphorylases and hexokinase, and concluded that the development of larger seeds is concerted with a prolonged activity of enzymes of carbon metabolism control (Chopra *et al*. 2007). Hexokinase is also thought to play a key role as a glucose sensor in the plant cell, leading to control gene transcription, translation, protein turnover and enzymatic activity (Rolland *et al*. 2006). The Sucrose Nonfermenting-1-Related Protein Kinase (Snrk1) was demonstrated in pea to regulate carbon energy flow in maturating cotyledons by repressing other energetically expensive cellular processes, deviating available resources towards storage compound synthesis and stress tolerance (Radchuk *et al*. 2006). The importance of glucose-6-phosphate (Glu-6-P) in seed development was shown through the identification and functional characterization of the plastidial Glu-6-P/phosphate translocator (GPT1) of *Vicia narbonensis*. This gene is expressed in vegetative sink tissues, flowers and young seeds. In the embryo, its expression overlaps the starch accumulation phase. This translocator was shown to perform a rate-limiting step for starch synthesis in developing seeds, to control the partitioning into storage proteins and being essential to embryo plastids and during seed maturation (Rolletschek *et al*. 2007). In pea, phosphoenolpyruvate carboxykinase (PEPCK) was found throughout seed development, with maximum activity in coat and cotyledon tissues before protein accumulation in the cotyledons. This enzyme was strongly induced by nitrogen supply under organic or inorganic forms, indicating its involvement on nitrogen metabolism in developing seeds (Delgado-Alvarado *et al*. 2007).

Many legumes, through a mutualistic symbiosis with rhizobia in the root nodules, are able to fix atmospheric nitrogen, being largely independent of the soil nitrogen. Tropical legumes, such as common beans, incorporate the fixed nitrogen primarily as ammonia into ureides (allantoin or allantoate). A common bean allantoin permease (PvUPS1) was reported (Pélissier *et al*. 2004) and its expression profile indicates a function in the allantoin phloem loading towards sink organs (Pélissier and Tegeder 2007). Much of the transporter systems involved in nutrient exchange between the symbionts have been revealed biochemically, but still awaits largely the identification and functional characterization of their genetic entities (Benedito *et al*. 2006).

PHOSPHATE METABOLISM DURING SEED DEVELOPMENT

The most abundant form of phosphorus in seeds is phytate (*myo*-inositol hexakisphosphate, IP_6) where up to 80% of total seed phosphorus content may be stored in this form (Raboy 1990). During seed development, phytic acid is deposited within single-membrane storage organelles referred to as protein bodies (Lott *et al*. 1985), forming insoluble, crystal complexes of minerals such as K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+} and Zn^{2+} via ionic associations with phosphate groups, known as phytate or phytin (Lonnerdal 2002). Large amounts of phytate stored in seeds as these very stable complexes results in a notorious antinutrional property, critical to the consumption by humans and monogastric animals, which lack the hydrolytic enzyme phytase in their digestive tract (Cheryan 1980). Nutritional reserves accumulated during seed development are intended to be relocated during germination and the early seedling growth stages, being phytate an important source of inorganic phosphate, minerals and *myo*-inositol. Nevertheless, it was also claimed to be beneficial to human health with antioxidant and anticarcinogenic effects (Zhou and Erdman 1995).

The first step in the synthesis of phytate is the production of 1-L-*myo*-inositol-1-phosphate (Ins(1)P) from D-glucose-6-phosphate catalyzed by the isomerase D-*myo*-inositol-3-phosphate (Ins(3)P) synthase (MIPS, E.C. 5.5.1.4). From this point, one possible pathway involves the metabolism of Ins(3)P to IP_3 through a lipid- and phospholipase C-dependent pathway (Majerus *et al*. 1988). IP_3 is subsequently metabolized to IP_6 by the action of several kinases and possibly some phosphatases (Wilson and Majerus 1997; Phillippy 1998), which may alternatively synthesize phytate directly from inositol-phosphates (InsP) (Biswas *et al*. 1978). In either pathway, there are different reports on the identification of the exact isomers and kinases or phosphatases involved in the metabolism of InsP (Brearley and Hanke 1996b). This subject is difficult to evaluate because phytate biosynthesis may occur through multiple and simultaneous pathways (Stevenson-Paulik *et al*. 2002; Shi *et al*. 2003; Coelho *et al*. 2005; Shi *et al*. 2005), and may not be linear, involving both phosphorylation and dephosphorylation events, and being some of the enzymes even multifunctional, acting on more than one substrate of the pathway (**Fig. 3**). There is also evidence supporting that a control exists upstream IP_3 (Coelho *et al*. 2005).

The biochemical pathway of phytic acid synthesis, or its regulation, has not been completely elucidated as yet. However, it is clear that during the synthesis of phytic acid, phosphate, *myo*-inositol or glucose may be the initial substrates to be incorporated in the phytate synthesis. Phosphate is incorporated from ATP, glucose and *myo*-inositol are carbon skeletons (Loewus and Loewus 1983), being both able to generate *myo*-inositol-1P through *myo*-inositol-1P synthase and *myo*-inositol kinase, respectively (Loewus and Murthy 2000).

Some reports provided important evidences that MIPS play a regulatory role in phytate synthesis of developing legume seeds. In mungbean, the sum of inositol-phosphate pools (IP_1 to IP_5) represents around 20% of the amount of IP_6, whereas MIPS activity was detected between 7 and 14 days after flowering, following a decline in its activity afterwards (Majumder and Biswas 1973). Soybean was reported to have four MIPS isoforms, but GmMIPS1 is the one expressed especially during early seed development (Hegeman *et al*. 2001; Chappell *et al*. 2006), more specifically in the outer integument layer (Chiera and Grabau 2007). Silencing the soybean MIPS (*GmMIPS1*) through RNA interfering (RNAi) showed an effective reduction of phytate contents and improved phosphorus availability in seeds, although also affected germination (Nunes *et al*. 2005). Most recently, rice *low phytic acid* (*lpa*) mutants were described. Among eight mutants, five showed to be non lethal in homozygosity for these loci. Among the mutated loci, a putative *myo*-inositol kinase (E.C. 2.7.1.64) was identified, showing it as a potential candidate for molecular breeding (Liu *et al*. 2007). In soybean, two *lpa* mutants were described recently, being one characterized as a GmMIPS1 mutant (Yuan *et al*. 2007).

The seed-specific form of common bean MIPS is 94% identical to its soybean orthologue (Hegeman *et al*. 2001; Nunes *et al*. 2005; Sparvoli and Fileppi 2005). The MIPS activity in common bean seed is dependent on its transcript level and represents a rate limiting step in phytic acid synthesis, with an important control point regulating this metabolic route upstream of IP_3. In favor of this hypothesis, there are reports not only assessing InsP levels in mature seeds, but also exploring temporal variations during seed development. We observed that the flux between IP_3 and IP_5 during seed development in three cultivars of common bean seemed to be determined in part by substrate concentration rather than only by variations in enzymatic activity (Coelho *et al*. 2005). Therefore, there is evidence supporting that a control exists upstream IP_3. In this scenario, we indicate an important role for MIPS, which could be the cause of increased input into the pathway upstream IP_3. More recently we examined myo-inositol-3-phosphate synthase (MIPS) (EC 5.5.1.4) activity and gene expression during seed development of common bean. Phytate concentration was low at the initial stage of seed development, coinciding with a pe-

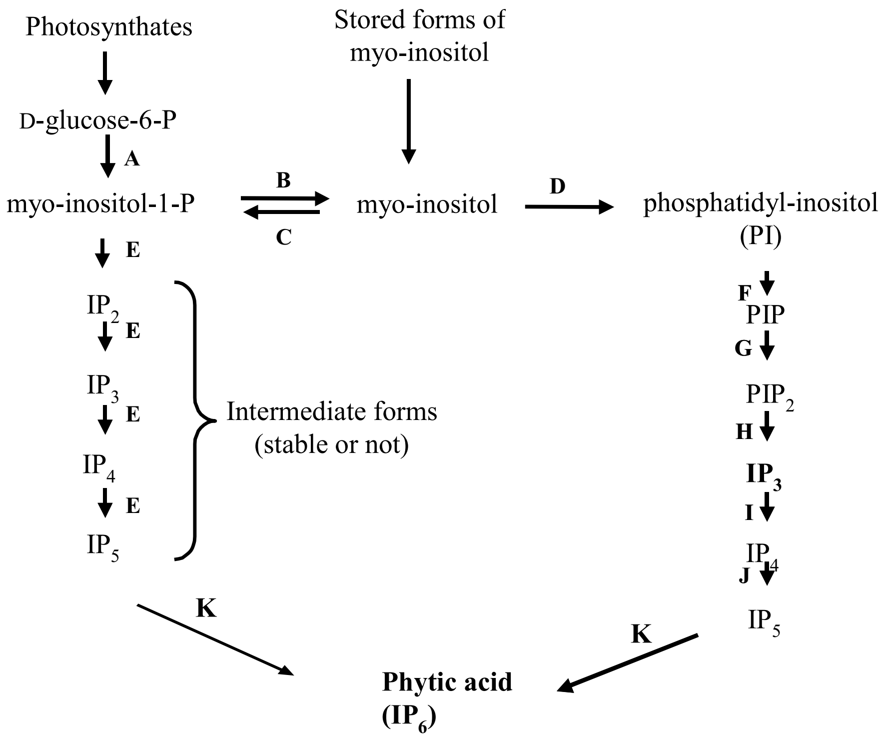

Fig. 3 Possible metabolic pathways for phytic acid biosynthesis. On the left, route proposed by Biswas et al. (1978), in the absence of stable intermediates, and Brearly and Hanke (1996) when stable intermediates are present. On the right, route proposed by Majerus *et al.* (1988). **A**: 1-L-*myo*-inositol-1P synthase; **B**: 1- L-*myo*-inositol-1P phosphatase; **C**: *myo*-inositol kinase; **D**: phosphatidyl-inositol synthase; **E**: phosphoinositol kinase; **F**: PI kinase; **G**: PIP kinase; **H**: phospholipase C; **I**: IP_3 kinase; **J**: IP_4 kinase; **K**: pentakisphosphate kinase.

riod of the most intense seed metabolism, but followed by a period of high enzymatic activity and gene expression of MIPS when a decrease in its specific activity and transcription was detected throughout seed development until 20 days after flowering; however, the specific activity of MIPS dropped more expressively than the gene expression, matching with higher phytate concentration. Hence, we show that there is evidence of one control point regulating phytate synthesis with MIPS enzyme (Coelho *et al*. 2007).

In another study, *in vitro* synthesis of phytate was studied in common bean fruit explants with different sucrose concentrations, phosphorus, *myo*-inositol, abscisic acid, glutamine, and methionine with fixed concentrations tested during a time course (0-9 days after cultivation). Phytate variation coincided with different concentrations of sucrose, *myo*-inositol, P and ABA throughout the period for the duration tested. P, sucrose, ABA, and *myo*-inositol caused an increase of phytate in the seed, showing that it could be possible to alter its content by cultivating fruit explants *in vitro* (Coelho *et al*. 2008).

An important aspect to be tackled in this area is the understanding of transcriptional dynamics of MIPS paralogues in a legume species as well as the genetic variability of MIPS activity and gene expression associated with phytate concentration in seed development in common beans and other legumes, as well as activity and transcriptional profiling of MIPS paralogues in common beans. Genotypic diversity was found in MIPS activity in landraces varieties of common bean during the maximum of phytate synthesis (unpublished results), indicating possible MIPS isoforms or differential gene promoter regulation. Yet, with increasing evidence that phytate synthesis has other regulation points than MIPS, it is also attractive the analysis of other enzymes related to inositol biosynthesis during seed development, such as *myo*-inositol kinase (MIK), responsible for multiple *myo*-inositol phosphorylation, in order to gather more evidences on alternative routes of phytate that do not include Ins(1)P via MIPS. Interestingly, phytate contents did not seem to be influenced by period or dose of N supply (Coelho *et al*. 2008).

Grain characteristics influenced by crop management

Besides, a positive correlation was observed between cooking time and N supplied at later stages of grain filling stage (R6), in agreement with previous results showing that N was essential to yield and protein contents in the grain, that it was a function of N availability and developmental stage of the plant (Silveira and Damasceno 1993), and that the genotype exerted a strong influence over this trait (Furtini *et al*. 2006), whereas N side dressing or Mo application did not affect bean yield, but they influenced grain technological characteristics, such as increased protein contents, cooking time and maximum hydration time (Silva *et al*. 2006). Another recent finding is the relationship between nodulation and seed quality (germination and vigour) through higher levels of trehalose contents (Altamirano-Hernandez *et al*. 2007).

Phytate contents in seeds are dependent on phosphate availability in the soil (Raboy and Dickinson 1993). Thus, genetic improvement may benefit of genotypes with less influenced by environmental aspects in this regard. Another report showed that phosphate availability does not necessarily affect phytate contents, since there is a negative relationship between up taken phosphate by the plant and the phosphate stored as phytate in the seeds (Santos 1998). Other reports show that the reduction of phytate contents did not have negative consequences on germination rate or seed vigor in soybean (Raboy *et al*. 1985). However, a selection aiming at reducing phytate contents would probably lead to a concomitant reduction in protein contents in the seed (Raboy *et al*. 1990).

Phosphate uptake is affected by phosphate availability in the soil and the genetic background of the plant (Raboy *et al*. 1984). Whereas phosphate availability will depend upon soil aspects, its physical, chemical and biological characteristics, the plant root system can secrete phosphatases to the soil in order to hydrolyze organic phosphate to inorganic form (Pi), in order to allow root uptake. Phosphatases are also present in plant tissues and are associated with phosphate remobilization within the plant (Duff *et al*. 1994).

It is interesting to notice that the strategies used by the plant to regulate phosphate levels in its tissues may affect phytate levels in the seed. When plants are adequately supplied with Pi from the soil, they can absorb more than its developmental requirements. Raboy and Dickinson (1984) described different levels of phytic acid in seeds of soybean genotypes, which were classified as sensitive or tolerant to high phosphates in the soil. A sensitive genotype accumulated more phytic acid in grains with the increase of phosphate contents in the leaves, whereas a tolerant genotype did not show this relationship.

One way of preventing Pi accumulation is to convert it to organic forms, so that phytate accumulation in seeds could be a way of regulating excessive Pi, as suggested by Raboy and colleagues (1985), who reported differences in germination rate among soybean genotypes with contrasting levels of phytic acid in seeds (0.19 to 1.0 mg phytate/seed). In moderate deficiency of Pi in the substrate, plants keep phosphate homeostasis, regulating Pi concentrations in the cytosol through controlling Pi levels in the vacuole. However, the level of this regulation was reported to be dependent on genotypes (Mimura *et al*. 1990).

Phosphate utilization efficiency, since uptake to assimilation, is a highly inheritable trait, which means that it can be manipulated in breeding programs. This is an indirect way of controlling phosphate accumulation in the seeds (Thung 1990). However, the best way is determining the proportion of this phosphate which is accumulated as phytic acid and the biochemical and physiological mechanisms behind it. Unfortunately, to date these factors are still unknown. The genetic variability for phytic acid contents has been demonstrated with great potential to obtain potential genotypes for breeding programs. One work reported analysis of twelve millet diallelic parentals with segregating phenotype for this trait in the progeny (Satija and Thukral 1985). Raboy *et al*. (1990) and Larson *et al*. (1998) showed that maize and oat mutants with low levels of phytic acid presented dramatic reduction in the phytate P: Pi ratio. More recently, the maize mutant ipa2 was shown to have a decreased phosphate level as phytic acid (IP_6) in seeds, accumulating instead other forms of inositol phosphates, such as IP_3, IP_4 and IP_5 (Raboy *et al*. 2000). In previous studies with legumes (Coelho *et al*. 2002), eleven common bean genotypes were compared for phytate contents in mature seeds, which resulted in detection of a great genetic variability among genotypes (0.7-1.4%), indicating that it is possible to select contrasting genotypes to study phytate content regulation at the biochemical level as well as to incorporate this trait into breeding programs.

THE QUALITY PROBLEMS OF COMMON BEAN GRAINS – BREEDING CHALLENGES

Wang *et al*. (2003a) reviewed the nutritional problematic of legume seeds concerning protein quality, antinutritional compounds, carbohydrates and minerals, and breeding possibilities regarding each category. Many of these traits concern common beans.

Most legume seeds are known for their high protein contents and serve as staple food in many regions of the globe. However, the proteins present in many legume seeds are poor in sulphur-containing amino acids (methionine and cysteine), as well as tryptophan, which are essential for humans and need to be provided by the diet (Andrade *et al*. 2004). Arabidopsis mutants have been useful to elucidate the biochemical pathways of amino acid synthesis and conversions so that we can engineer a stronger anabolism of essential amino acids in seeds. This could be done by forging biosynthetic pathways, such as by deviating lysine synthesis towards methionine by down regulating *S*-adenosylmethionine synthase transcription, which uses methionine as a substrate (Hacham *et al*. 2007). It is important, however, to realize that increasing the levels of sulphur-containing amino acids will also elevate the nutritional demand of sulphate by the plant.

Another nutritional problem already cited elsewhere in this review is the presence of galactooligosaccharides that causes flatulence in monogastric animals, such as raffinose, stachyose, and verbascose (McPhee *et al*. 2002). In common beans, these oligosaccharides can account to almost 6% of the grain fresh weight (da Silva *et al*. 2006) and may correlate with drought tolerance (Kavar *et al*. 2008).

Common beans are also a major source of dietary iron, especially in developing countries. Playing a bigger role than phytate, polyphenols (tannins) present in the coat of mature seeds were shown *in vitro* as a more important antifactor than phytate for chelating iron and making it non digestible (Ariza-Nieto *et al*. 2007). On the other hand, polyphenols are significantly correlated with biotic resistances (Islam *et al*. 2003), leaving breeders with a difficult equation to solve. Ascorbate was shown to improve iron digestibility of common beans (Ariza-Nieto *et al*. 2007) and may be a counterbalance concerning iron availability.

LIPIDS AND OTHER STORAGE COMPOUNDS IN COMMON BEAN SEEDS

Lipid is not the preferential storage compound in common beans as it is in other legumes, such as soybean. We reckon that a few transcription factors may play key roles in determining the preferential biosynthetic pathways of storage compounds and deciphering the genetic players involved in this process may enable a more controlled manipulation of composition in legume seeds. Comprehensive transcriptional profiling of soybean and common beans may reveal differential genes to generate hypotheses into this matter.

It will be interesting to unveil the polyphenol biosynthesis pathway in seed coat of common beans and whether the manipulation of its levels will be associated with other traits of economical importance.

IMPORTANT TRAITS OF COMMON BEAN SEEDS FOR BREEDING PROGRAMS

A striking feature of common bean is a great plasticity of the seed coat colour and patterns. As expected, it presents a complex genetic inheritance (McClean *et al*. 2002; Bassett 2007) and is a major characteristic in breeding programs. Nevertheless, in the variety Pinto, the post-harvest darkening of seed coats is an important, undesirable trait, which has been reported recently to be due to a single, recessive gene (Junk-Knievel *et al*. 2008).

Another important trait is the content of raffinose in the coat, since this compound is related to the flatulence effect in humans and is thought to be the cause of rejection by monogastric animals (Aranda *et al*. 2001). The recently found positive correlation between polyphenols in the mature seed coat and iron bioavailability, in addition to great genotypic differences (Ariza-Nieto *et al*. 2007), indicates that this may be a trait to be incorporated in breeding programs to improve nutrient availability.

Understanding how seeds of common bean develop and the major genes involved in this process may potentially lead to molecular breeding tools, either transgenic or not. The exact characterization temporal and spatial expression of seed-specific genes may reveal interesting promoters to be use with genes of interest that will not be expressed in other parts of the plant, leading to energy economy of the entire physiology, reflecting, thus, in higher yields. A well-known and much used common bean seed promoter is the one driving the *phaseolin* (*phas*) gene expression (Ng and Hall 2007), that can be used to drive transgene overexpression in legume seeds. The characterization of other common bean promoters and the test of known promoters from other species, especially the models will potentially allow a more directed expression, temporally and spatially, with potential energy savings for the plant.

CHALLENGES AND PERSPECTIVES

It is remarkable the phenotypic variability presented within the common bean genetics. Unlike pea, the scientific research of common beans has not benefited of many mutants for gene discovery in the species. Most of what is known on its molecular level of common beans comes from hypothesis raised from knowledge gathered in other species and tested in this species.

Mutants of *Arabidopsis thaliana* have proved to be valuable for functional analyses of seed-specific genes in genetic complementation approaches. For instance, the *Arabidopsis* high-affinity amino acid permease AAP8 was shown to be expressed during early seed development and to play a central role in amino acid uptake in endospermic cells, supplying the developing embryo with amino acids (Schmidt *et al*. 2007). *Arabidopsis* mutants for this gene are impaired in normal seed development, and given the similarity of the developmental processes between legumes and this model species (at least during the early embryogenesis) this mutant is a valuable tool for heterologous complementation of legume amino acid permeases.

The understanding of seed development in legumes has taken advantage of model species, such as *Arabidopsis*, as well as with alternative legume systems, such as *Vicia*, and transgenic plants with modification in metabolic routes, which make them ideal systems to study the control points of nutrient accumulation in the seeds (Weber *et al*. 2005).

Legume seeds are important sources of human food. However, the improvement of legumes protein quality is highly desirable. Metabolic engineering is key in this process and the understanding of how seed biochemistry and physiology work will help manipulating metabolic routes towards increasing desirable compounds in the seed.

Genomics of common beans and model systems

Legume biology is currently flourishing with new model species, such as *Medicago truncatula* and *Lotus japonicus*, as well as an important tropical legume model, as much as an important crop, soybean. The genome sequencing of these three legumes is nearly complete, comprehensive transcriptomics studies are being made available and the genetic map of soybean, which is expected to be the most syntenic to common beans among these model species, is saturated. A global initiative for sequencing ESTs (expressed sequence tags) of common beans is under development (www.phaseolus.net). Common beans present an estimated genome size of 630 Mb divided into 11 haploid chromosomes (whereas the soybean genome is 1,200 Mb divided into 20 chromosomes, the *M. truncatula* genome is 450 Mb in 8 chromosomes, and the genome of the model species *Arabidopsis thaliana* is only 120 Mb in 5 chromosomes; all with genome sequencing projects complete or near completion), and there is no expectation for having the its genome sequenced in the near future. Yet, due to its huge phenotypical plasticity, analyses of natural variation at the genomic level of genes potentially linked to agronomic traits will be a great asset as a molecular tool in breeding programs. With the new generation of high-throughput sequencing techniques, a project for deep sequencing ESTs could, instead, create comprehensive microarrays to assess gene expression.

OTHER LEGUMES AS REFERENCES TO COMMON BEANS

A transcriptomic study of developing seeds of guar (*Cyamopsis tetragonoloba*) was recently published (Naoumkina *et al*. 2007). Since guar seeds are rich in a mucilage composed mainly of galactomannan, this study can offer clues on differential gene regulation and preferential biochemical pathways during seed development between this and other legume species, leading to a better understand about the dynamics of reserve compound storage in this family. Another study that will impact the field is the transcriptomic and proteomic analysis of *M. truncatula* developing seeds (Gallardo *et al*. 2007). This conjugated analysis can potentially reveal another layer of functional regulation: post-transcriptional gene regulation. *M. truncatula* seeds present a balanced composition of storage compounds (carbohydrates, proteins and lipids). *M. truncatula* is a temperate grain legume counting with a comprehensive gene expression atlas (Benedito *et al*. 2008) that includes a complete seed developmental series (http://bioinfo.noble.org/gene-atlas) that may be of relevance for comparative analyses or creating hypotheses on gene expression of common bean as well.

MOLECULAR BREEDING OF COMMON BEANS

Most legumes are recalcitrant for genetic transformation. A recent protocol on transformation of *Phaseolus* spp. roots mediated by *Agrobacterium rhizogenes* (Estrada-Navarrete *et al*. 2006) was published and will allow molecular studies of root genes. Alternatively, a highly efficient protocol using particle bombardment was reported recently (Rech *et al*. 2008).

The seed coat was proposed to be engineered molecularly to enhance the quality of the seed and its yield or even to serve as biofactory of novel compounds (Moise *et al*. 2005). In common beans, once the genetic transformation is effective, this goal can be pursued to understand better and manipulate coat composition.

Since common beans are much used in developing countries as an important source of nutrients, including proteins, it is important to understand the nature of nutrient accumulation in their seeds in order to enable quantitative and qualitative nutritional improvement of beans for human consumption. With many crops gathering resources to reach a level of incorporating molecular tools into their breeding programs, common beans must take its share for speeding up elite genotype generation. For that, genomics and biochemical studies must tackle the genetic elements controlling the most important traits in the species, as well as a genotypic survey into its great genetic diversity. Potentially, farmers and consumers will benefit from this basic knowledge with stronger plants in the field and better grains in their plates.

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REFERENCES

Ali-Rachedi S, Bouinot D, Wagner MH, Bonnet M, Sotta B, Grappin P, Jullien M (2004) Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. *Planta* **219**, 479-488

Altamirano-Hernandez J, Lopez MG, Acosta-Gallegos JA, Farias-Rodriguez R, Pena-Cabriales JJ (2007) Influence of soluble sugars on seed quality in nodulated common bean (*Phaseolus vulgaris* L.): the case of trehalose. *Crop Science* **47**, 1193-1205

Andrade CAdB, Patroni SMS, Clemente E, Scapim CA (2004) Produtividade e qualidade nutricional de cultivares de feijão em diferentes adubações. *Ciência e Agrotecnologia* **28**, 1077-1086

Aranda P, Dostalova J, Frias J, Lopez-Jurado M, Kozlowska H, Pokorny J, Urbano G, Vidal-Valverde C, Zdyunczyk Z (2001) Carbohydrates in grain legume seeds: Improving nutritional quality and agronomic characteristics. CAB International, Wallingford, UK, pp 61-87

Ariza-Nieto M, Blair MW, Welch RM, Glahn RP (2007) Screening of iron bioavailability patterns in eight bean (*Phaseolus vulgaris* L.) genotypes using the Caco-2 cell *in vitro* model. *Journal of Agricultural and Food Chemistry* **55**, 7950-7956

Asada K, Kasai Z (1962) Formation of *myo*-inositol and phytin in ripening rice grains. *Plant and Cell Physiology* **9**, 397-401

Asada K, Tanaka K, Kasai Z (1969) Formation of phytic acid in cereal grains. *Annals of the New York Academy of Sciences* **165**, 801-814

Bassett MJ (2007) Genetics of seed coat color and pattern in common bean. *Plant Breeding Reviews* **28**, 239-315

Benedito VA, Dai X, He J, Zhao PX, Udvardi MK (2006) Functional genomics of plant transporters in legume nodules. *Plant Functional Biology* **33**, 731-736

Benedito VA, Torres-Jerez I, Murray JD, Andriankaja A, Allen S, Kakar K, Wandrey M, Verdier J, Zuber H, Ott T, Moreau S, Niebel A, Frickey T, Weiller G, He J, Dai X, Zhao PX, Tang Y, Udvardi MK (2008) A gene expression atlas of the model legume *Medicago truncatula*. *Plant Journal* **55**, 504-513

Biswas S, Maiti SB, Chakrabarti S, Biswas BB (1978) Purification and characterization of myoinositol hexaphosphate adenosine diphosphate phosphotransferase from *Phaseolus aureus*. *Archives of Biochemistry and Biophysics* **185**, 557-566

Bobb AJ, Chern MS, Bustos MM (1997) Conserved RY-repeats mediate transactivation of seed-specific promoters by the developmental regulator PvALF. *Nucleic Acids Research* **25**, 641-647

Bobb AJ, Eiben HG, Bustos MM (1995) PvAlf, an embryo-specific acidic transcriptional activator enhances gene expression from phaseolin and phytohemagglutinin promoters. *Plant Journal* **8**, 331-343

Borisjuk L, Rolletschek H, Wobus U, Weber H (2003) Differentiation of legume cotyledons as related to metabolic gradients and assimilate transport into seeds. *Journal of Experimental Botany* **54**, 503-512

Brearley CA, Hanke DE (1996a) Inositol phosphate in barley (*Hordeum vulgare* L.) aleurone tissue are stereochemically similar to the products of breakdown of InsP6 *in vitro* by wheat-bran phytase. *Biochemical Journal* **318**, 279-286

Brearley CA, Hanke DE (1996b) Metabolic evidence for the order of addition of individual phosphate esters to the *myo*-inositol moiety of inositol hexakisphosphate in the duckweed *Spirodela polyrhiza* L. *Biochemical Journal* **314**, 227-233

Buitink J, Leger JJ, Guisle I, Vu BL, Wuillème S, Lamirault G, Le Bars A, Le Meur N, Becker A, Küster H, Leprince O (2006) Transcriptome profiling uncovers metabolic and regulatory processes occurring during the transition from desiccation-sensitive to desiccation-tolerant stages in *Medicago truncatula* seeds. *Plant Journal* **47**, 735-750

Burbano C, Muzquiz M, Osagie A, Ayet G, Cuadrado C (1995) Determination of phytate and lower inositol phosphate in Spanish legumes by HPLC. *Methodology* **52**, 321-325

Chappell AS, Scaboo AM, Wu X, Nguyen H, Pantalone VR, Bilyeu KD (2006) Characterization of the *MIPS* gene family in *Glycine max*. *Plant Breeding* **125**, 493-500

Cheryan M (1980) Phytic acid interations in food systems. *Critical Reviews in Food Science and Nutrition* **13**, 297-335

Chiera JM, Grabau EA (2007) Localization of *myo*-inositol phosphate synthase (GmMIPS-1) during the early stages of soybean seed development. *Journal of Experimental Botany* **58**, 2261-2268

Chopra J, Kaur N, Gupta AK (2007) Sustained activities of carbon metabolizing enzymes determine seed size in *Vigna radiata* (mungbean). *Current Science* **92**, 1420-1424

Coelho CMM, Bellato CM, Garcia AKM, Vitorello VA, Azevedo RA (2008) Variation in phytate accumulation in common bean (*Phaseolus vulgaris* L.) fruit explants. *Brazilian Archives of Biology and Biotechnology* **51**, 163-173

Coelho CMM, Benedito VA, Figueira A, Vitorello VA, Azevedo RA (2007) Variation in the enzyme activity and gene expression of *myo*-inositol-3-phosphate synthase (MIPS) and phytate accumulation during seed development in common bean (*Phaseolus vulgaris* L.). *Acta Physiologiae Plantarum* **29**, 265-271

Coelho CMM, Santos JCP, Tsai SM, Vitorello VA (2002) Seed phytate content and phosphorus uptake and distribution in dry bean genotypes. *Brazilian Journal of Plant Physiology* **14**, 51-58

Coelho CMM, Tsai SM, Vitorello VA (2005) Dynamics of inositol phosphate pools (tris-, tetrakis- and pentakisphosphate) in relation to the rate of phytate synthesis during seed development in common bean (*Phaseolus vulgaris* L.). *Journal of Plant Physiology* **162**, 1-9

Cosgrove DJ (1966) The chemistry and biochemistry of inositol polyphosphates. *Reviews of Pure and Applied Chemistry* **16**, 209-224

da Silva FL, Guimarães VM, de Barros EG, Moreira MA, dos Santos Dias LA, de Almeida OMG, José IC, de Rezende ST (2006) Biochemical composition and indigestible oligosaccharides in *Phaseolus vulgaris* L. seeds. *Plant Foods Human Nutrition* **61**, 87-89

Delgado-Alvarado A, Walker RP, Leegood RC (2007) Phosphoenolpyruvate carboxykinase in developing pea seeds is associated with tissues involved in solute transport and is nitrogen-responsive. *Plant Cell and Environment* **30**, 225-235

Doyle JJ, Luckow MA (2003) The rest of the iceberg-legume diversity and evolution in a phylogenetic context. *Plant Physiology* **131**, 900-910

Duff SMG, Sarath G, Plaxton WC (1994) The role of acid phosphatases in plant phosphorus metabolism. *Physiologia Plantarum* **90**, 791-800

Estrada-Navarrete G, Alvarado-Affantranger X, Olivares JE, Diaz-Camino C, Santana O, Murillo E, Guillen G, Sanchez-Guevara N, Acosta J, Quinto C, Li DX, Gresshoff PM, Sanchez F (2006) *Agrobacterium rhizogenes* transformation of the *Phaseolus* spp.: a tool for functional genomics. *Molecular Plant-Microbe Interactions* **19**, 1385-1393

Fait A, Angelovici R, Less H, Ohad I, Urbanczyk-Wochniak E, Fernie AR, Galili G (2006) *Arabidopsis* seed development and germination is associated with temporally distinct metabolic switches. *Plant Physiology* **142**, 839-854

Furtini IV, Ramalho MAP, Abreu ÂdFB, Neto AEF (2006) Resposta diferencial de linhagens de feijoeiro ao nitrogênio. *Ciência Rural* **36**, 1696-1700

Gallardo K, Firnhaber C, Zuber H, Hericher D, Belghazi M, Henry C, Kuster H, Thompson R (2007) A combined proteome and transcriptome analysis of developing *Medicago truncatula* seeds: evidence for metabolic specialization of maternal and filial tissues. *Molecular and Cellular Proteomics* **6**, 2165-2179

Golombek S, Rolletschek H, Wobus U, Weber H (2001) Control of storage protein accumulation during legume seed development. *Journal of Plant Physiology* **158**, 457-464

Gonçalves Ceolin AC, Gonçalves-Vidigal MC, Soares Vidigal Filho P, Vinícius Kvitschal M, Gonela A, Alberto Scapim C (2007) Genetic divergence of the common bean (*Phaseolus vulgaris* L.) group Carioca using morpho-agronomic traits by multivariate analysis. *Hereditas* **144**, 1-9

Gotz KP, Staroske N, Radchuk R, Emery RJN, Wutzke KD, Herzog H, Weber H (2007) Uptake and allocation of carbon and nitrogen in *Vicia narbonensis* plants with increased seed sink strength achieved by seed-specific expression of an amino acid permease. *Journal of Experimental Botany* **58**, 3183-3195

Gurusinghe S, Bradford KJ (2001) Galactosyl-sucrose oligosaccarides and potential longevity of primed seeds. *Seed Science Research* **11**, 121-133

Gutierrez L, Van Wuytswinkel O, Castelain M, Bellini C (2007) Combined networks regulating seed maturation. *Trends in Plant Science* **12**, 294-300

Hacham Y, Song L, Schuster G, Amir R (2007) Lysine enhances methionine content by modulating the expression of *S*-adenosylmethionine synthase. *The Plant Journal* **51**, 850-861

Harland BF, Narula G (1999) Food phytate and its hydrolysis products. *Nutrition Research* **19**, 947-961

Hegeman CE, Good LL, Grabau EA (2001) Expression of D-*myo*-inositol-3-phosphate synthase in soybean. Implications for phytic acid biosynthesis. *Plant Physiology* **125**, 1941-1948

Islam FM, Rengifo J, Redden RJ, Basford KE, Beebe SE (2003) Association between seed coat polyphenolics (tannins) and disease resistance in common bean. *Plant Foods Human Nutrition* **58**, 285-297

Junk-Knievel DC, Vanderberg A, Bett KE (2008) Slow darkening in pinto bean (*Phaseolus vulgaris* L.) seed coats is controlled by a single major gene. *Crop Science* **49**, 189-193

Kasim AB, Hardy ME Jr. (1998) The analysis for inositol phosphate forms in feed ingredients. *Journal of the Science of Food and Agriculture* **76**, 1-9

Kavar T, Maras M, Kidric M, Sustar-Vozlic J, Meglic V (2008) Identification of genes involved in the response of leaves of *Phaseolus vulgaris* to drought stress. *Molecular Breeding* **21**, 159-172

Koch KE (1996) Carbohydrate modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 509-540

Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. *Current Opinion in Plant Biology* **5**, 33-36

Larson SR, Young KA, Cook A (1998) Linkage mapping of two mutations that reduced phytic acid content of barley grain. *Theoretical and Applied Genetics* **97**, 141-146

Lehrfeld J (1989) High performance liquid chromatography analysis of phytic acid on a pH stable, macroporous polymer column. *Cereal Chemistry* **66**, 510-515

Liu QL, Xu XH, Ren XL, Fu HW, Wu DX, Shu QY (2007) Generation and characterization of low phytic acid germplasm in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **114**, 803-814

Loewus FA, Loewus MW (1983) *Myo*-inositol: its biosynthesis and metabolism. *Annual Review of Plant Physiology* **34**, 137-161

Loewus FA, Murthy PPN (2000) *Myo*-inositol metabolism in plants. *Plant Science* **150**, 1-19

Lombardi L, Ceccarelli N, Picciarelli P, Lorenzi R (2007) DNA degradation during programmed cell death in *Phaseolus coccineus* suspensor. *Plant Physiology and Biochemistry* **45**, 221-227

Lonnerdal B (2002) Phytic acid-trace element (Zn, Cu, Mn) interactions. *International Journal of Food Science and Technology* **37**, 749-758

Lott JNA, Randal PJ, Goodchild DJ, Craig S (1985) Occurrence of globoid crystals in cotyledonary bodies of *Pisum sativum* as influenced changes in experimentally induced changes in Mg, Ca and K contents of seeds. *Australian Journal of Plant Physiology* **12**, 341-353

Majerus PW, Connoly TM, Bansal VS, Inhorn RC, Ross TS, Lips D (1988) Inositol phosphates: synthesis and degradation. *The Journal of Biological Chemistry* **263**, 3051-3054

Majumder AL, Biswas BB (1973) Metabolism of inositol phosphates: Part V – Biosynthesis of inositol phosphates during ripening of mung bean (*Phaseolus aureus*) seeds. *Indian Journal of Experimental Biology* **11**, 120-123

Makower RU (1969) Changes in phytic acid and acid-soluble phosphorus in maturing pinto beans. *Journal of Science Food and Agriculture* **20**, 82-84

Marcos Filho J (2005) *Fisiologia de Sementes de Plantas Cultivadas* (1), FEALQ, Piracicaba 495 pp

McClean PE, Lee RK, Otto C, Gepts P, Bassett MJ (2002) Molecular and phenotypic mapping of genes controlling seed coat pattern and color in common bean (*Phaseolus vulgaris* L.). *Journal of Heredity* **93**, 148-152

McPhee KE, Zemetra RS, Brown J, Myers JR (2002) Genetic analysis of the raffinose family oligosaccharides in common bean. *Journal of the American Society for Horticultural Science* **127**, 376-382

Mimura T, Dietz KJ, Kaiser W, Schramm MJ, Kaiser G, Heber U (1990) Phosphate transport across biomembranes and cytosolic phosphate homeostasis in barley leaves. *Planta* **180**, 139-146

Miranda M, Borisjuk L, Tewes A, Heim U, Sauer N (2001) Amino acid permeases in developing seeds of *Vicia faba* L.: expression precedes storage protein synthesis and is regulated by amino acid supply. *The Plant Journal* **28**,

61-72
Moise JA, Han S, Gudynaite-Savitch L, Johnson DA, Miki BLA (2005) Seed coats: structure, development, composition, and biotechnology. *In Vitro Cellular and Developmental Biology – Plant* **41**, 620-644
Naoumkina M, Torres-Jerez I, Allen S, He J, Zhao PX, Dixon RA, May GD (2007) Analysis of cDNA libraries from developing seeds of guar (*Cyamopsis tetragonoloba* (L.) Taub). *BMC Plant Biology* **7**, 62
Ndoutoumou PN, Toussaint A, Baudoin JP (2007) Embryo abortion and histological features in the interspecific cross between *Phaseolus vulgaris* L. and *P. coccineus* L. *Plant Cell, Tissue and Organ Culture* **88**, 329-332
Ng DWK, Hall TC (2007) PvALF and FUS3 activate expression from the *phaseolin* promoter by different mechanisms. *Plant Molecular Biology* **66**, 233-244
Nunes ACS, Cuneo F, Amaya-Farfán J, Capdeville Gd, Rech EL, Aragão FJL (2005) RNAi- mediated silencing of the myo-inositol-1-phosphate synthase gene (*GmMIPS1*) in transgenic soybean inhibited seed development and reduced phytate content. *Planta* **223**, 45-51
Ogawa M, Tanaka K, Kasai Z (1979) Accumulation of phosphorus, magnesium and potassium in developing rice grains: followed by electron probe X-ray analysis focusing on the aleurone layer. *Plant and Cell Physiology* **20**, 19-27
Pammenter NW, Berjak PA (1999) A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research* **9**, 13-37
Papa R, Acosta J, Delgado-Salinas A, Gepts P (2005) A genome-wide analysis of differentiation between wild and domesticated *Phaseolus vulgaris* from Mesoamerica. *Theoretical and Applied Genetics* **111**, 1147-1158
Patrick JW, Offler CE (2001) Compartmentation of transport and transfer events in developing seeds. *Journal of Experimental Botany* **52**, 551-564
Pélissier HC, Frerich A, Desimone M, Schumacher K, Tegeder M (2004) PvUPS, an allantoin transporter in nodulated roots of French beans. *Plant Physiology* **134**, 664-675
Pélissier HC, Tegeder M (2007) PVUPS1 plays a role in source-sink transport of allantoin in French bean (*Phaseolus vulgaris*). *Functional Plant Biology* **34**, 282-291
Persson H, Turk M, Nyman M, Sandberg A-S (1998) Binding of Cu^{+2}, and Cd^{+2} to inositol tri-, tetra-, penta-, and hexaphosphates. *Journal of Agricultural and Food Chemistry* **46**, 3194-3200
Phillippy BQ (1998) Identification of inositol 1,3,4-trisphosphate 5-kinase and inositol 1,3,4,5-tetrakisphosphate 6-kinase in immature soybean seeds. *Plant Physiology* **116**, 291-297
Pinheiro C, Rodrigues AP, De Carvalho IS, Chaves MM, Ricardo CP (2005) Sugar metabolism in developing lupin seeds is affected by a short-term water deficit. *Journal of Experimental Botany* **56**, 2705-2712
Quesnelle PE, Emery RJN (2007) *Cis*-cytokinins that predominate in *Pisum sativum* during early embryogenesis will accelerate embryo growth *in vitro*. *Canadian Journal of Botany* **85**, 91-103
Raboy V (1990) The biochemistry and genetics of phytic acid synthesis in higher plants. In: Morre EJ, Boss WS, Loewus FA (Eds) *Inositol Metabolism in Plants*, John Wiley & Sons, New York, pp 55-76
Raboy V, Dickinson DB (1984) Effect of phosphorus and zinc nutrition on soybean seed phytic acid and zinc. *Plant Physiology* **75**, 1094-1098
Raboy V, Dickinson DB (1987) The timing and rate of phytic acid accumulation in developing soybean seeds. *Plant Physiology* **85**, 841-844
Raboy V, Dickinson DB (1993) Phytic acid levels in seeds of *Glycine max* and *G. soja* as influenced by phosphorus status. *Crop Science* **33**, 1300-1305
Raboy V, Dickinson DB, Below FE (1984) Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium and protein among lines of *Glycine max* and *G. Soja*. *Crop Science* **24**, 431-434
Raboy V, Dickinson DB, Neuffer MG (1990) A survey of maize kernel mutants for variation in phytic acid. *Maydica* **35**, 383-390
Raboy V, Gerbasi PF, Young KA, Stoneberg SD, Pickett SG, Bauman AT, Murthy PPN, Sheridan WF, Ertl DS (2000) Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. *Plant Physiology* **124**, 355-368
Raboy V, Hudson SJ, Dickinson DB (1985) Reduced phytic acid content does not have an adverse effect on germination of soybean seeds. *Plant Physiology* **79,** 323-325
Radchuk R, Radchuk V, Goetz KP, Weichert H, Richter A, Emery RJN, Weschke W, Weber H (2007) Ectopic expression of phosphoenolpyruvate carboxylate in *Vicia narbonensis* seeds: effects of improved nutrient status on seed maturation and transcriptional regulatory networks. *The Plant Journal* **51**, 819-839
Radchuk R, Radchuk V, Weschke W, Borisjuk L, Weber H (2006) Repressing the expression of the SUCROSE NONFERMENTING-1-RELATED PROTEIN KINASE gene in pea embryo causes pleiotropic defects of maturation similar to an abscisic acid-insensitive phenotype. *Plant Physiology* **140**, 263-278
Rech EL, Vianna GR, Aragão FJ (2008) High-efficiency transformation by biolistics of soybean, common bean and cotton transgenic plants. *Nature Protocols* **3**, 410-418
Roberts RM, Loews FA (1968) Inositol metabolism in plants. VI Conversion of *myo*-inositol to phytic acid in *Wolffiella floridana*. *Plant Physiology* **43**, 1710-1716
Rochat C, Boutin JP (1991) Methabolism of phloem-borne amino acids in maternal tissues of fruit of nodulated or nitrate feed pea plants. *Journal of Experimental Botany* **42**, 207-214
Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology* **57**, 675-709
Rolletschek H, Nguyen TH, Hausler RE, Rutten T, Gobel C, Feussner I, Radchuk R, Tewes A, Claus B, Klukas C, Linemann U, Weber H, Wobus U, Borisjuk L (2007) Antisense inhibition of the plastidial glucose-6-phosphate/phosphate translocator in *Vicia* seeds shifts cellular differentiation and promotes protein storage. *Plant Journal* **51**, 468-484
Saio K (1964) The change in inositol phosphates during the ripening of rice grains. *Plant and Cell Physiology* **5**, 393-400
Sandberg AS, Carlsson NG, Svanberg U (1989) Effect of inositol tri-, tetra-, and hexaphosphates on *in vitro* estimation of iron available. *Journal of Food Science* **54**, 159-161
Santos JCP (1998) Estado nutricional do feijoeiro (*Phaseolus vulgaris* L.) e teores de nutrientes e fitatos nos grãos. PhD thesis, Universidade de São Paulo, Piracicaba, São Paulo, Brasil, 120 pp
Satija DR, Thukral SK (1985) Genetic analysis of phytic acid content in pearl millet. *Theoretical and Applied Genetics* **70**, 693-696
Schmidt R, Stransky H, Koch W (2007) The amino acid permease AAP8 is important for early seed development in *Arabidopsis thaliana*. *Planta* **226**, 805-813
Schryer PA, Lu Q, Vanderberg A, Bett KE (2005) Rapid regeneration of *Phaseolus angustissimus* and *P. vulgaris* from very young zygotic embryos. *Plant Cell, Tissue and Organ Culture* **83**, 67-74
Sharma CB, Dieckert JW (1974) Free and bound myoinositol phosphates in mature cotton embryos. *Phytochemistry* **13**, 2529-2533
Shi J, Wang H, Hazebroek J, Ertl DS, Harp T (2005) The maize low-phytic acid 3 encodes a *myo*-inositol kinase that plays a role in phytic acid biosynthesis in development seed. *The Plant Journal* **42**, 708-719
Shi J, Wang H, Wu Y, Hazebroek J, Meeley RB, Ertl DS (2003) The maize low-phytic acid mutant lpa2 is caused by mutation in an inositol phosphate kinase gene. *Plant Physiology* **131**, 507-515
Silva TRB, Lemos LB, Tavares CA (2006) Produtividade e característica tecnológica de grãos em feijoeiro adubado com nitrogênio e molibdênio. *Pesquisa Agropecuária Brasileira* **41**, 739-745
Silveira PM, Damasceno MA (1993) Doses e parcelamento de K e de N na cultura do feijoeiro. *Pesquisa Agropecuária Brasileira* **28**, 1269-1276
Sobolev AM, Rodionova MA (1966) Phytin synthesis by aleurone grains in ripening sunflower seeds. *Sovietic Plant Physiology* **13**, 958-961
Solfanelli C, Ceron F, Paolicchi F, Giorgetti L, Geri C, Ceccarelli N, Kamiya Y, Picciarelli P (2005) Expression of two genes encoding gibberellin 2- and 3-oxidases in developing seeds of *Phaseolus coccineus*. *Plant and Cell Physiology* **46**, 1116-1124
Sparvoli F, Fileppi M (2005) Isolation and characterization of the seed *myo*-inositol-3-phosphate synthase gene from common bean. *Biologia e Biotecnologia Agrária* **20133**, NCBI - AJ853494
Stadler R, Lauterbach C, Sauer N (2005) Cell-to-cell movement of green fluorescent protein reveals post-phloem transport in the outer integument and identifies symplastic domains in Arabidopsis seeds and embryos. *Plant Physiology* **139**, 701-712
Stevenson-Paulik J, Odom AR, York JD (2002) Molecular and biochemical characterization of two plant inositol polyphosphate 6-/3-/5-kinases. *The Journal of Biological Chemistry* **277**, 42711-42718
Tanaka K, Watanabe K, Asada K, Kasai Z (1971) Ocorrence of myo-inositol monophosphate and its role in ripening rice grains. *Agricultural and Biological Chemistry* **35**, 314-320
Tegeder M, Tan Q, Grennan AK, Patrick JW (2007) Amino acid transporter expression and localization studies in pea (*Pisum sativum*). *Functional Plant Biology* **34**, 1019-1028
Tegeder M, Thomas M, Hetherington L, Wang X-D, Offler CE, Patrick JW (2000) Genotypic differences in seed growth rate of *Phaseolus vulgaris* L. II. Factor contributing to cotyledon sink activity and sink size. *Australian Journal of Plant Physiology* **27**, 119-128
Thung M (1990) Phosphorus: a limiting nutrient in bean (*Phaseolus vulgaris* L.) production in Latin America and field screening for efficiency and response. *Genetic Aspects Plant Mineral Nutrition* **3**, 501-521
Tsukagoshi H, Morikami A, Nakamura K (2007) Two B3 domain transcriptional repressors prevent sugar-inducible expression of seed maturation genes in *Arabidopsis* seedlings. *Proceedings of the National Academy of Sciences USA* **104**, 2543-2547
Turner RT, Barratt DHP, Casey R (1990) The effect of different alleles at the r locus on the synthesis of seed storage proteins in *Pisum sativum*. *Plant Molecular Biology* **14**, 793-803
Udvardi MK, Kakar K, Wandrey M, Montanari O, Murray J, Andriankaja A, Zhang JY, Benedito V, Hofer JM, Chueng F, Town CD (2007) Legume transcription factors: global regulators of plant development and response to the environment. *Plant Physiology* **144**, 538-549
Viana PA, De Rezende ST, Marques VM, Trevizano LM, Passos FM, Oliveira MG, Bemquerer MP, Oliveira JS, Guimarães VM (2006) Extra-

cellular alpha-galactosidase from *Debaryomyces hansenii* UFV-1 and its use in the hydrolysis of raffinose oligosaccharides. *Journal of Agriculture and Food Chemistry* **54**, 2385-2391

Walz A, Park S, Slovin JP, Ludwig-Müller J, Momonoki YS, Cohen JD (2002) A gene encoding a protein modified by the phytohormone indoleacetic acid. *Proceedings of the National Academy of Sciences USA* **99**, 1718-1723

Walz A, Seidel C, Rusak G, Park S, Cohen JD, Ludwig-Müller J (2008) Heterologous expression of IAP1, a seed protein from bean modified by indole-3-acetic acid, in *Arabidopsis thaliana* and *Medicago truncatula*. *Planta* **227**, 1047-1061

Wang TL, Domoney C, Hedley CL, Casey R, Grusak MA (2003a) Can we improve the nutritional quality of legume seeds. *Plant Physiology* **131**, 886-891

Wang WX, Vinocur B, Altman A (2003b) Plant response to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218**, 1-14

Weber H, Borisjuk L, Wobus U (1997) Sugar import and metabolism during seed development. *Trends in Plant Science* **2**, 169-174

Weber H, Borisjuk L, Wobus U (2005) Molecular physiology of legume seed development. *Annual Review of Plant Biology* **56**, 253–279

Wilson MP, Majerus PW (1997) Characterization of a cDNA encoding *Arabidopsis thaliana* inositol 1,3,4-trisphosphate 5/6-kinase. *Biochemical Biophysical Research Communication* **232**, 678-681

Wobus U, Weber H (1999) Sugars as signal molecules in plant seed development. *Biological Chemistry* **380**, 937-944

Yuan FJ, Zhao HJ, Ren XL, Zhu SL, Fu XJ, Shu QY (2007) Generation and characterization of two novel low phytate mutations in soybean (*Glycine max* L. Merr.). *Theoretical and Applied Genetics* **115**, 945-957

Zhang WH, Zhou YC, Dibley KE, Tyerman SD, Furbank RT, Patrick JW (2007) Nutrient loading of developing seeds. *Functional Plant Biology* **34**, 314-331

Zhou JR, Erdman JW (1995) Phytic acid in health and disease. *Critical Reviews in Food Science and Nutrition* **35**, 495-508

Zhou Y, Qu H, Dibley KE, Offler CE, Patrick JW (2007) A suite of sucrose transporters expressed in coats of developing legume seeds includes novel pH-independent facilitators. *The Plant Journal* **49**, 750-764