Mutation of the transcription factor LEAFY COTYLEDON 2 alters the chemical composition of Arabidopsis seeds, decreasing oil and protein content, while maintaining high levels of starch and sucrose in mature seeds

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The transcription factor LEAFY COTYLEDON 2 (LEC2; At1g28300) is preferentially expressed in developing seeds of Arabidopsis. Detailed biochemical analysis of a loss-of-function lec2 mutant was carried out in seeds 6–21 days after flowering (DAF). In comparison to wild type controls, lec2 seeds had 15% less protein and 30% less oil, but accumulated 140% more sucrose and >5-fold more starch. We also quantified biomass and carbohydrates in the seed coat and embryo. The lec2 mutant had smaller seeds and an altered proportion of dry weight (bigger seed coat and smaller embryos). Mutant plants produced less mature seeds per silique and the harvest index was reduced. Soluble sugars (glucose, fructose and sucrose) was accumulated in the seed coat of the lec2 mutant, whereas the opposite effect was observed in the embryos (decrease in comparison to wild type). The rate of starch synthesis increased during early development, whereas the rate of starch degradation was diminished during late development, leading to higher residual starch in mature seed of the mutant. Starch accumulated in both seed coat and embryo. Homozygous mutant plants produced seeds that could germinate well if they were harvested immaturely, whereas seeds that became dry during maturity lost their germination efficiency very rapidly. We conclude that the LEC2 transcription factor not only controls cotyledon identity and morphology as previously reported, but also alters: (1) the delivery of photosynthates from the seed coat to the embryo (sink strength), (2) carbon partitioning towards different storage compounds (oil, proteins and carbohydrates), (3) the rate of starch synthesis and degradation in developing seeds and (4) germination capacity of dry seeds.

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Introduction
Seed development is a crucial process in the life cycle of all plants, particularly of weed plants like Arabidopsis thaliana that produce many seeds and have a rapid generation cycle. The accumulation of storage compounds in the embryo, endosperm and seed coat is important for seed survival and dispersion, since it contributes to efficient development and desiccation tolerance of seeds, and germination. During the early stage of Arabidopsis embryo development (until 6 days after flowering (DAF)), cell division occurs together with pattern formation leading to the torpedo stage embryo (Baud et al., 2002). During the maturation stage, storage compounds are synthesized and deposited mainly in the embryo (Hills, 2004). The maturation phase (9–21 DAF) is characterized by a linear increase in seed dry weight due to the accumulation of large amounts of storage products and a concomitant decrease in relative water content of the seed (Angeles-Núñez and Tiessen, 2010; Baud et al., 2002; Fait et al., 2006). During this stage, the embryo becomes metabolically quiescent and tolerant to desiccation.

The carbon reserves of the endosperm and embryo are believed to fuel the establishment of the seedling following germination (Bewley and Black, 1994; Eastmond et al., 2000). The accumulation of total harvestable biomass and the chemical composition of seeds are not only relevant aspects of evolutionary plant fitness, but are also important traits for agricultural purposes. In crop species like canola, maize and soya, oil storage or starch storage in seeds is economically attractive, since it can be used both for food or biofuels. In Arabidopsis seeds, carbon and nitrogen resources are mainly stored in the form of lipids and proteins, with very little starch and sucrose remaining in the dry seed (Angeles-Núñez and Tiessen, 2010; Baud et al., 2002). In contrast, in maize kernels approximately 75% of the seed biomass is starch, with a protein content of ~10% and an oil content of around 3–5% (FAO, 1992). An overview of the metabolic routes leading to different storage compounds in seeds

Abbreviations: DAF, days after flowering; FA, fatty acid; hexose-P, hexose phosphate; lec2, leafy cotyledon 2 mutant.
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is shown in Fig. 1. Photoassimilates such as sucrose arrive systemically via the phloem to the maternal tissue of the seed coat, and from there they are passed apoplastically to the endosperm and embryo (filial tissues). Sucrose is metabolized to generate different storage compounds via the major carbon pathways (Fig. 1).

To date, a number of transcription factors have been identified in Arabidopsis that are involved in the control of seed development (Gutierrez et al., 2007; North et al., 2010; Santos-Mendoza et al., 2008). Four major regulators ABSCISIC ACID-INSITIVE 3 (ABI3; At3g54320), FUSCA 3 (FUS3; At3g26790), LEAFY COTYLEDON 1 (LEC1; At1g21970) and LEAFY COTYLEDON 2 (LEC2; At1g28300) control many aspects of seed maturation, such as cotyledon identity, the accumulation of oil, and the acquisition of desiccation tolerance and dormancy (Stone et al., 2001; To et al., 2006). The Arabidopsis LEAFY COTYLEDON genes LEC1 and LEC2 were identified originally as loss-of-function mutations resulting in defects in both embryo and cellular identity (Harada, 2001). LEC2 is required for the maintenance of suspensor morphology, specification of cotyledon identity, progression through the maturation phase, and suppression of premature germination (Stone et al., 2001). Both FUS3 and LEC2 encode plant-specific transcription factors that possess a VP1/ABI3-like B3 domain, which are central regulators of seed maturation (Luerssen et al., 1998; Stone et al., 2001). LEC1 encodes a HAP3 subunit of a transcription factor that binds to CCAAT (Kwong et al., 2003). It has been postulated that a master regulator of the LEC genes is PICKLE (PKL), which encodes a CHD3-chromatin-remodeling factor responsible for repression of regulators of embryonic identity during germination (Rider et al., 2003). In the pkl mutant, roots have the capacity to undergo somatic embryogenesis due to derepression of the LEC genes controlling embryonic identity.

The mutants lec1 and lec2 produce morphologically normal plants, except for the notable presence of trichomes on the adaxial surface of the seed cotyledons (therefore the term “leafy cotyledons”). The lec1 mutant seed presents defects in suspensor morphology during early embryogenesis, it stores less proteins and lipids in the embryos, and it produces defective seeds (desiccation-intolerant) which have to be rescued before seed maturation (Lotan et al., 1998; Luerssen et al., 1998; Meinke, 1992; Meinke et al., 1994). The lec2 phenotype is less severe, since the seeds of the mutants are less sensitive to desiccation and only slightly affected in the accumulation of oil (Meinke et al., 1994; Stone et al., 2001, 2008). Previous studies have focused on the morphological and developmental phenotypes of the abi3, fus3, lec1 and lec2 mutants. Less is known about the metabolic phenotypes of these transcription factors. It has been reported that ectopic LEC2 expression in Arabidopsis induces accumulation of seed storage proteins in roots, increases oil content in ovules (Stone et al., 2008) and modifies lipid composition of leaves (Mendoza et al., 2005), events that normally occur during the maturation phase of embryogenesis. However, no quantitative data was presented about sucrose–starch metabolism in any organ of the overexpressor or the loss of function mutant.

In this article, we investigate the role of the transcription factor LEC2 in controlling sink strength and starch levels in developing seeds. We therefore decided to measure the accumulation of dry weight, proteins, oil, sucrose and starch during development and in mature seeds. We analyzed the mutant alleles lec2-4 and lec2-1 which have similar loss of function phenotypes. We asked whether there was an increased rate of starch synthesis at early stages, or if there was an attenuated rate of starch degradation at later stages in the lec2 mutants. We also tested the hypothesis whether LEC2 has an effect on the sink strength of the seed, i.e. if the seed biomass ratio between seed coat and embryo is altered. This would mean that in addition to controlling seed developmental processes of cellular differentiation and embryo growth, LEC2 is also involved in photoassimilate delivery between phloem, seed coat and embryo. Quantifying carbon partitioning between storage compounds during development could give some hints to whether LEC2 is a transcriptional regulator of some key enzymes for the route of sucrose–starch interconversion in Arabidopsis.

Materials and methods

Plant material and culture conditions

The lec2-1 and lec2-4 (Stone et al., 2001) mutants in the Wassilewskija (Ws) background of Arabidopsis thaliana L. (thale cress) were generated by the project of the Versailles T-DNA project (Bechtold et al., 1993). Both mutants have similar phenotypes, with the allele lec2-1 having a bit more severe phenotype than the allele lec2-4. For practical reasons (seed availability), we made experiments either with the lec2-1 or the lec2-4 mutants.

Seeds were surface-sterilized and sown on Murashige and Skoog (MS) medium (M02 555, pH 5.6; Duchefa Biochemie, Haarlem, The Netherlands). After a cold treatment (48-h at 4°C) in the dark, the plates were transferred to a growth chamber and incubated at 20°C/15°C (day/night) temperatures under a 16-h/8-h light/dark regime. After 15 days, the plantlets were transferred to sterile compost, grown under a 16-h/8-h light/dark regime associated with 21°C/18°C (day/night) temperature. The plants were irrigated twice a week with fertilization solution (Plant-prod, fertile, http://www.fertilpot.com).
**Metabolic analyses**

Samples of 20–50 lyophilized seeds for each replica and developmental stage were used for protein, carbohydrate and lipid analyses, as described for Arabidopsis seeds by Angeles-Núñez and Tiessen (2010). Due to practical reason (poor germination of dry seeds), seed availability of homozygous plants was sometimes limiting. When heterozygous mutant plants were grown, siliques were harvested and mutant seeds were selected individually for the lec2 phenotype before metabolic measurements were performed on those samples.

**Separation of seed tissues**

Seeds were harvested at 21 days after flowering (DAF) and then separated under a stereoscope into two parts: embryo and seed coat. In Arabidopsis, the endosperm is so tightly attached to the integument, that it is impractical to separate them. Thus, we use name “seed coat” for the bulk of two tissues: the integument (seed envelope of maternal origin) and the gelatinous seed endosperm (triploid filial tissue). Measurements of dry weight and starch levels were done on sufficiently large bulbs (5–30 mg) of seeds with replicates.

**Determination of harvest index**

Wild type (Ws) and leafy cotyledon 2 mutant (lec2-1) plants were grown in the greenhouse under semi-controlled conditions. Plants grew with sufficient sunlight, optimal fertilization, irrigation and pest control. Mature Arabidopsis plants were carefully harvested and weighed. The plant harvested was crushed by hand to release seeds from all remaining siliques. All seeds from a single plant were harvested and weighed when the plant was mature and the last siliques produced on the inflorescence had elongated and turned brown. Plant biomass was dried by exposing to sunlight and heat. The harvest index (HI) was calculated according to the formula HI=(dry weight of seeds/dry weight of aboveground plant biomass)×100.

**Results**

**Dry weight during seed development**

Homozygous mutant plants with loss of function for the leafy cotyledon 2 (lec2) gene grew normally and produced leaves and inflorescences of almost the same size as wild type controls (data not shown). Since lec2 is preferentially expressed in seeds (Schmid et al., 2005), we analyzed seeds more carefully during development. The increase of seed dry weight (DW) in both wild type (WT) and the lec2 mutant followed a linear-sigmoid pattern with a continuous increase between morphogenesis and mid-maturation phases (Fig. 2). During the early phases (6–9 DAF), the mutant seeds had similar dry weight than the WT controls. At later stages (12–21 DAF) the mutant had significantly lower DW than the WT (12 d P=1.8×10^{-5}; 15 d P=0.0005; 18 d P=0.0009; 21 d P=9.02×10^{-7}). At 21 DAF (mature seed stage) the mutant had ~12% less DW than the WT controls (P=9.0×10^{-7}). Seed color in the lec2 mutant was altered, showing a stronger accumulation of dark phenolic compounds in mature seeds (Fig. 3). The seed coat was bigger but the color of the integument was similar between mutant and WT (Fig. 3). The lec2 embryos were different from the WT with respect to both appearance (leafy phenotype) and color (Fig. 3). This particular pattern of phenolic compound accumulation in the embryo suggested that the lec2 mutation affected metabolism in different seed tissues differentially.

**Carbohydrate levels during seed development**

To determine whether the transcription factor LEC2 had an influence on the carbohydrate levels during seed development, measurements were performed on carefully harvested, selected and lyophilized seeds. Marked changes in the levels of starch, sucrose, glucose and fructose were detected in the mutant during seed development in comparison to the WT. Wild type seeds transiently accumulated high amounts of starch during the morphogenesis phase (6–12 DAF), whereas starch was later degraded during the maturation phase (12–21 DAF) (Fig. 4). In the mutant seeds, starch levels were much higher throughout seed development (Fig. 4). Starch was increased by 62% at 9 DAF, by 70% at 15 DAF, by 320% at 18 DAF and by 1550% at 21 DAF (Fig. 4). The soluble sugar levels in the mutant seeds were also significantly affected during seed development when compared with the WT control (Fig. 5). In the mutant seeds, the fructose content was first increased by 60% from 9 to 12 DAF, but then it was decreased by
Fructose (A) and glucose (B) accumulation in wild-type (W) and the lec2 mutant. Each data point is the mean ± SE (n = 3). Each sample consisted of an independent pool of 20 seeds from a bulk of siliques harvested from 40 plants.

42% from 15 to 18 DAF (Fig 5A). The difference was less marked at 21 DAF (Fig 5A). The glucose levels of the lec2 mutant were similar to the WT except at 18–21 DAF when glucose was increased by 55% (Fig. 5B). The sucrose content behaved similarly, with similar values as the WT early during development and a drastic increase at later stages (Fig 5C). Sucrose content was increased by 6-fold at 15 DAF and 2.5-fold at 18 DAF. At 21 DAF (dry seed stage) the lec2 seeds had still 1.4-fold higher levels of sucrose (Fig. 5C). The hexoses/sucrose ratio in the mutant decreased significantly by 40% and 80%, respectively, at 9 and 15 DAF (Fig. 5D). All differences on metabolite levels between WT and mutant remained significant whether the values were expressed on a per seed basis (Figs. 4, 6 and 7) or dry weight basis (data not shown).

**Fatty acid content and protein during seed development**

To determine the quantitative impact of the lec2 mutation on the accumulation of seed storage compounds, we also analyzed the time-course of lipid and protein accumulation in developing seeds (Figs. 6 and 7). Compared to WT, the total fatty acid content in the mutant seeds was decreased by 50% and 20% at 12 and 15 DAF, respectively, and by 30% during late maturation period (18–21 DAF).
(Fig. 6A). Similarly, significant differences in the very long chain fatty acid (VLFA)/C16-C18 ratio were detected especially from 12 to 21 DAF (decreased by 10%) (Fig. 6B).

The total protein content in mutant seeds was also reduced (Fig. 7). In lec2 mutant seeds, the protein content was 27% and 38% lower at 12 and 15 DAF, respectively. The difference was still significant at late stages of seed development (18–21 DAF) (Fig. 7).

Analysis of embryo and seed coat of mature seeds

The results indicated that there were significant differences regarding the metabolic status of lec2 mutant seeds during development (6–18 DAF), and that those biochemical differences were still observable at the mature seed stage (21 DAF). Since all previous measurements were done on whole seeds (Figs. 2–7), we decided to investigate the different types of seed tissues in more detail. Arabidopsis seeds consist of an embryo, endosperm and integument. For technical reasons, we were able to separate Arabidopsis seeds in only two parts: embryo and seed coat, the latter including the maternal seed envelope (integument) and the gelatinous seed endosperm. We first measured the dry weight of mature seeds of the lec2 mutant (Fig. 8A) and the proportion each of these two tissues separately (Fig. 8B). The mature seeds of WT store 66% of the dry biomass in the embryo and 34% in the seed coat. The percentage distribution of seeds from the Ws ecotype is similar to the value of other wild type ecotypes (e.g. Col-0) (see also Angeles-Nuñez and Tiessen, 2010). In the lec2 mutant, the proportion of dry weight was reversed, with 40% of the dry biomass being stored in the embryo and 60% in the seed coat (Fig. 8B). The lec2 seeds were significantly smaller than the wild type controls (Fig. 8A, Figs. 2 and 3). The color of the seeds was heterogeneous, but there was a tendency to accumulate dark compounds, mainly in the embryo and not so much in the seed coat (Fig. 3).

We also measured carbohydrate levels in the two seed tissues separately (Fig. 9). We found that the sugar content in embryo and seed coat of the lec2 mutants behaved reciprocally, i.e. general increase in the seed coat and decrease in the embryo (Fig. 9). The fructose levels were increased by 3-fold in seed coat but not in the embryo of the lec2 mutant (Fig. 9A). The glucose levels were decreased by 68% in embryo mutant and increased by 200% in seed coat (Fig. 9B). The sucrose content were decreased by 37% in embryo mutants and increased by 250% in seed coat (Fig. 9C). Starch levels in mature seed of the lec2 mutant were higher in both tissues: seed coat (2.3-fold) and embryo (3-fold) (Fig. 9D).

Analysis of harvest index

The marked differences of biomass partitioning within seeds could be indicative of altered sink strength within different tissues of the lec2 mutant plants. In order to test this hypothesis, we measured the number of seeds per silique and the harvest index, referring to the yield of seeds that can be obtained per dry weight of total mature plant biomass. The lec2 mutants had significantly less seeds per silique than the WT (Fig. 10A). The mutants had also slightly smaller siliques (Fig. 10B). But more importantly,
**Fig. 8.** Dry weight in different tissues of dry Arabidopsis seeds (21 DAF). Wild-type plants (Ws) and lec2-1 mutant were grown in a greenhouse under semi-controlled conditions. Seed were harvested at 21 days after flowering (DAF). (A) Percentage weight of seed embryo or seed coat (% of whole seed dry weight). (B) Absolute weight per seed. Double stars indicate level of significance ($p < 0.01$). Values are the mean ± SE ($n=4$). Each sample consisted of independent pool of ∼100 seeds from a bulk of siliques harvested from ten plants.

**Fig. 9.** Carbohydrate content in different tissues of Arabidopsis seeds (embryo and seed coat). Wild-type plants (Ws) and lec2-1 mutants were grown in a greenhouse under semi-controlled conditions. Siliques were harvested at 21 days after flowering (DAF) which correspond to the physiological state of mature dry seeds. (A) Fructose content (B) Glucose content (C) Sucrose content (D) Starch content. Values are the mean ± SE ($n=4$). Each sample consisted of independent pool of ∼500 seeds from a bulk of siliques harvested from ten plants.
Germination efficiency of mature seeds

Throughout development, the mutant seed had less oil (Fig. 6), less protein (Fig. 7) but more starch (Fig. 3), and this was observed in the two seed tissues (Fig. 9D). The ler2 seeds accumulated less dry mass (Figs. 2 and 8A) and had much smaller embryos (Fig. 8B). It is known that the size of seeds and their chemical composition can alter the efficiency of germination and plant fitness. For example, the alteration of storage partitioning can affect the physiological maturity of Arabidopsis seeds (Angeles-Núñez and Tiessen, 2010). We therefore decided to evaluate quantitatively how much was seed germination compromised in the ler2 mutant. Germination efficiencies were 98 ± 2% for wild type and less than 5% for the ler2 mutant for seeds harvested after 21 DAF (p < 0.001) (data not shown).

Discussion

The main objective of this work was to gain a better understanding of the physiological importance of the transcription factor LEC2 for determining biomass accumulation and carbohydrate levels within the seed coat and embryo. Previous work had already well established that LEC2 plays an important role during Arabidopsis seed development. Most reports had focused on the morphological and molecular phenotypes of loss or overexpression of the LEC2 transcription factor (Meinke et al., 1994; Stone et al., 2001, 2008). In this work we characterized the biochemical effects of ler2 loss of function mutations, mainly focusing on sucrose–starch metabolism during seed development.

Mutation of LEC2 leads to a perturbed oil and protein content

The maturation period of Arabidopsis seeds is divided in early, mid and late maturation phases. In early maturation period (9–12 DAF), the levels of storage lipids and proteins were still low (Figs. 6 and 7). During the mid phase (12–18 DAF), high rates of fatty acid and protein synthesis occur in the embryo (Figs. 6 and 7). During late maturation (18–21 DAF) storage compound synthesis end (Figs. 6 and 7). At this stage, the embryo becomes metabolically quiescent and acquires the ability to germinate after desiccation (Angeles-Núñez and Tiessen, 2010; Baud et al., 2002; Fait et al., 2006). In Arabidopsis seeds, the lipid and protein reserves represent 30–40% of the seed dry weight (DW) each, with starch remaining only as a minor component. Seed storage compounds have to be accumulated and then degraded for a normal process of germination (Bewley and Black, 1994; Eastmond and Graham, 2001). In this work, we have documented that mutation of the transcription factor LEAFY COTYLEDON 2 (LEC2) affects many of the previously mentioned processes.

The two main storage compounds in Arabidopsis seeds (lipids and proteins) were decreased in the ler2 mutants throughout development (Figs. 6 and 7). This observation is consistent with previously reported data for mature seeds of ler2 (Meinke et al., 1994; Stone et al., 2001). The biochemical differences are small during early stages, but become gradually larger during later stages of development (Figs. 5–7). This fits nicely with the expression pattern of LEC2 (Atg28300) and its putative importance for seed development and maturation. In addition to fatty acid and proteins, we also measured carbohydrates quantitatively during seed development, and in different tissues separately, and found some significant differences as discussed in the following sections.

Mutation of LEC2 modifies the soluble sugar ratio in developing seeds

During the period from 9 to 15 DAF the levels of hexoses were high (Fig. 5A and B) and the levels of sucrose were low (Fig. 5C) (see also Angeles-Núñez and Tiessen, 2010; Baud et al., 2002). During the period from 15 to 21 DAF, sucrose began to accumulate (Fig. 5C) and the hexose/sucrose ratio changed (Fig. 5D). In the ler2 mutant, the pattern of soluble sugar levels was altered throughout development but mostly at latter stages (Fig. 5). It seems that glucose, fructose and sucrose rather accumulate in the seed coat, but not in the mutant embryo (Fig. 9).

In many plants, soluble sugar metabolism is essential for the control of seed development, mainly through the regulation of source/sink relations (Herbers and Sonnewald, 1998). Mutation of invertase and other sucrolytic enzymes that alter the hexose/sucrose ratio does lead to marked effects in plant development (Barratt et al., 2009) or seed development in many species (Angeles-Núñez and Tiessen, 2010; Chourey et al., 2006; Jain et al., 2008; Turner et al., 2009; Welham et al., 2009).
In Arabidopsis seeds, the transition from the pre-storage phase to the maturation (storage) phase (where cell elongation and differentiation occur), is also characterized by a clear metabolic switch, from a high to low hexose/sucrose ratio (Fig. 5D; see also Baud et al., 2002; Fait et al., 2006). In potato tubers, both sucrose and hexose can generate signals that activate ADP-glucose pyrophosphorylase (AGPase), a key enzyme of starch metabolism (Tiessen et al., 2002, 2003). Since sugar metabolism and transport can be highly compartmentalized in seeds (Morley-Smith et al., 2008), even small differences in hexose/sucrose ratio can have dramatic effects on seed development and storage metabolism. For example, an altered hexose/sucrose ratio in sucrose synthase (sus) mutants was reported to modify carbon partitioning and the maturation in Arabidopsis seeds (Angelés-Núñez and Tiessen, 2010). The lec2 mutant had also a perturbed hexose/sucrose ratio (Fig. 5D) which was correlated to a modified pattern of storage compound accumulation (Figs. 4–7).

The soluble sugar ratio might influence biomass partitioning and seed size

It has been previously reported that seed size in Arabidopsis can be partially controlled by transcription factors that act in different tissues (siliques, seed coat, endosperm and embryo). For example, the APETALA2 (ap2) gene influences seed size via a maternal effect (Jofuku et al., 2005; Ohto et al., 2005). In the ap2 mutant, seed size was increased 100% and this correlated with a marked increase of the hexose/sucrose ratio throughout seed development (Ohto et al., 2005). Seeds in the lec2 mutant were smaller (Figs. 2 and 3) and the hexose/sucrose ratio was lower during most time of seed development (Fig. 5B). This makes it tempting to speculate that the hexose/sucrose ratio can influence seed size via an osmotic effect, while storage product deposition might be altered by a sugar signaling effect (Angelés-Núñez and Tiessen, 2010; Tiessen et al., 2003; Weber et al., 1998). The osmotic effects could be due to hexoses having a higher osmolarity than sucrose on a per weight basis. This in turn may permit a greater influx of water leading to larger cell volume in tissues where the hexose/sucrose ratio is higher. Higher hexose/sucrose ratios may also stimulate mitotic activity, promoting cellular proliferation and could therefore lead to greater seed size (Ohto et al., 2005; Weber et al., 1996). Most of the knowledge on seed development has been derived from the study of legume species (Weber et al., 2005). More work is required in order to determine how carbon metabolism and more specifically the different sucrolytic activities influence seed size in Arabidopsis.

Mutation of LEC2 affects starch metabolism

The pathways of embryo starch metabolism could be similar in several respects to those in Arabidopsis leaves (Andriotis et al., 2010), but starch turnover in the developing seed is spatially and temporally more complex. Starch accumulation in Arabidopsis seeds could be functionally linked to cell division and differentiation rather than to storage function (Andriotis et al., 2010).

In Arabidopsis seeds, starch is transiently accumulated during the first half of development and very low amounts remain in the dry seed (Fig. 4; see also Baud et al., 2002; Fait et al., 2006). The mutant seeds exhibited elevated starch content at 9 DAF (Fig. 4). At 18–21 DAF starch dropped to very low levels in the wild type plants but not in the lec2 mutants (Fig. 4).

The reduced rate of starch degradation in the lec2 mutant might be indicative of a restricted expression of hydrolytic enzymes that are required for normal seed germination. This could explain that homozygous lec2 seeds had a greatly reduced lifetime and germination efficiency.

Little is known about the importance of starch metabolism in oilseed embryos. Previous reports had argued that the temporary storage of starch during early stages of seed development maintains constant and strong sink strength by preventing accumulation of sucrose and hexoses in the seed coat and embryo (Lin et al., 2006). In oilseed rape, it was proposed that transient starch accumulation could strengthen the seed as a principal sink organ prior to the synthesis of the storage compound (oil) and that it could finely regulate nutrient allocation (daSilva et al., 1997). Higher sucrose levels usually lead to higher rates of starch synthesis (Tiessen et al., 2002, 2003). The biochemical interactions between the different pathways are not fully understood. Several studies have shown that seed starch levels behave reciprocally with the other main storage compounds, oil and proteins (Figs. 4, 6 and 7; see also Angelés-Núñez and Tiessen, 2010; Lin et al., 2006). The concept of metabolic competition between the starch and oil pathways is further supported by the phenotype of other seed mutants like ssel/per16 (Lin et al., 2004) and wr1 (Cernac and Benning, 2004; Focks and Benning, 1998) and other high starch mutants that also have reduced seed lipid content (Andriotis et al., 2010).

The bulk of the sucrose needed in reproductive tissues has to be imported via the phloem through different tissue layers and cell types (Figs. 1 and 3). In Arabidopsis, sucrose arrives first at the seed coat (maternal origin) and then it is taken up apoplasitically by the developing embryo (Fig. 1). The physiological function of the seed coat is believed to be the protection of the integrity of the embryo, mainly, since no role has been described for storage metabolism during seed maturation or germination. Typically, the seed coat tissue represents ~34% of seed DW (see Fig. 8; Angelés-Núñez and Tiessen, 2010) and contains low levels of starch, hexoses and sucrose (Fig. 7; Angelés-Núñez and Tiessen, 2010). The embryo represents ~66% of seed DW (Fig. 9D; Angelés-Núñez and Tiessen, 2010) and contains high amounts of storage compounds (lipids and protein), which represent more than 80% of embryo DW. The rest is made up by sugars, residual starch and other components (Fig. 7). The lec2 mutation reversed the biomass proportion of mature seeds. The lec2 seeds had 60% weight in the seed coat and only 40% of dry weight was represented by the embryo (Fig. 8B). In mature seeds of lec2, there was a decrease of soluble sugars in the embryo, but an increase of glucose, fructose and sucrose in the seed coat (Fig. 9).

We postulate that the metabolic changes caused by the lec2 mutation within seed coat and embryo led to an altered sink strength of the embryo. Since the lec2 mutation strongly affected the sucrose/hexose ratio and also impaired starch degradation in the seed coat, this may have limited the growth of the embryo.

Several mutants can alter seed starch

Besides the wr1 mutant (Focks and Benning, 1998), the lec2 mutant is the second example of a transcription factor that increases Arabidopsis seed starch. Most previous reports have documented starch phenotypes caused by the lack of certain metabolic enzymes (Andriotis et al., 2010). Many single and double mutants affected in leaf starch metabolism show altered starch accumulation in Arabidopsis seeds (Andriotis et al., 2010). The lec2 mutant is not altered in the leaves (data not shown), but increases soluble sugars in the seed coat (Fig. 9) and accumulates high starch in the embryo and seed coat (Fig. 7A and D).

The interaction of LEC2 with other genes

LEC2 is a transcription factor believed to regulate multiple genes of seed development. For example, this transcription factor has
been shown to regulate AtS23 gene implicated in protein synthet- 

sis (Kro et al., 2003), and the WRINKLED1 gene implicated in lipid synthesis (Baud et al., 2007, 2009). It has also been suggested that the mRNA levels of SUCROSE SYN-THASE 2 (SUS2) can be increased by LEC2 overexpression (Mendoza et al., 2005). Complementary studies indicate that SUS2, SUS3 and other metabolic enzymes are spatially and temporally regulated by LEC2, FUS3 and AB1 in developing seed of Arabidopsis (unpublished data). Mutants lacking either SUS2 or SUS3 activity have reduced levels of starch in developing seeds (Angéles–Núñez and Tiessen, 2010). LEC2 could influence seed starch levels indirectly through regulation of key sucrolytic enzymes such as SUS2, or directly by the transcriptional regulation of starch metabolic enzymes such as AGPase and amylases.

It is tempting to speculate that the effects of LEC2 on metabolism could be partially accounted for by the fact that it controls the expression of WR11, since the transcription factor WR11 can directly regulate some enzymes of central metabolism (Baud et al., 2007; Ruska et al., 2002), and its loss has major effects on starch, sugar, lipid and protein metabolism (Focks and Benning, 1998). Unfor- 

tunately, neither the seed coat nor the embryo biomass ratio was analyzed in the wr1 mutant that could provide explanatory hints about sink strength and seed physiology (Baud et al., 2007; Focks and Benning, 1998). In order to reveal the regulatory network of starch metabolism in Arabidopsis seeds, more detailed experiments are needed with a side-by-side comparison of single and double mutants wr1 and lec2.

Final conclusions

Our data suggest that LEC2 plays role in primary carbon metabolism in Arabidopsis seeds. We conclude that developing seeds of lec2 mutants are affected in the sucrose/hexose ratio and the starch pathway, and this in turn altered the sink strength of the embryo and the harvest index of the plant. The lec2 mutant has higher rates of starch synthesis at early stages, and lower rates of starch degradation at later stages (Fig. 4).

The results expand the view of the LEC2 transcription factor not only as a regulator of morphogenesis, but also as a sink strength factor and a master regulator of sugar and starch metabolism in seeds. We postulate that LEC2 plays a key role in the transcriptional control of genes implicated in sugar import, starch synthesis and hydrolysis. The question of whether it controls the expression of several enzymes directly, or if it acts indirectly through other fac- 

tors or proteins is an exciting topic that remains open for further research.

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