The process of seed maturation is influenced by mechanical constraints

Physical forces on living tissue can elicit a developmental response. During embryogenesis in Arabidopsis thaliana and many Brassicaceae species, the expanding embryo abuts the embryo sac, forcing it to bend and fold. That this mechanical constraint appears to boost the seed’s accumulation of lipid and protein has been validated here by combining magnetic resonance imaging with various analyses carried out at the molecular and biochemical level. The evidence is that, both in planta and in vitro, the developing Brassica napus embryo indeed responds to physical restraint by an acceleration in its maturation. While the mechanistic basis of this effect remains to be fully elucidated, the present experiments have revealed the major influence exerted by growth constraints over metabolic switching during the seed maturation process.

The recognition of mechanical stimuli (referred to as mechanosensing) is a universal property of living cells, and can be critical for their health, and thus for the growth and development of the entire organism (Hamant et al., 2009; Mirabet et al., 2011; Piccolo, 2013). Animals have evolved a sophisticated mechanosensing machinery (Fratzl & Barth, 2009). Plants’ ability to sense and respond to mechanical stimuli have long been of scientific interest (Darwin & Darwin, 1880), and there exist prominent examples like the insectivorous Venus flytrap (Dionaea muscipula). In recent years, a number of authors have revealed some aspects of the mechanistic basis of this capacity (Chehab et al., 2009; Kurusu et al., 2013; Landrein & Hamant, 2013; Monshausen & Hamant, 2013; Basu & Haswell, 2017; Landrein & Ingram, 2019). The relevance of mechanical stimuli to seed development has also been recognised in recent years (Creff et al., 2015; Fourquin et al., 2016).

A perception of self is required to enable an adjustment in an organ’s shape/size to its prevailing spatial environment. It has been proposed that the mechanical stress imposed on surrounding tissue as a result of an organ’s expansion can induce a signal cascade that modulates the plant’s growth (for a review see Hamant & Moulia, 2016). The situation faced by a developing seed is a case in point: both the size and morphology of a canola/oilseed rape (Brassica napus) embryo cultured in vitro has been shown to depart substantially from what occurs in planta, not only are in vitro-grown embryos not obliged to distort in order to remain within their embryo sac (Borisyuk et al., 2013a), but their final volume can reach 10 times that of their in planta-grown equivalents. The implication is that the in planta-grown embryo is able to sense the limited space available for its growth, and adjusts its growth and metabolism accordingly. In particular, we here tested the hypothesis that the developing Brassica napus embryo boosts the accumulation of lipid and protein in response to physical restraint, and thereby accelerates its maturation (which features a massive accumulation of storage compounds).

In the first experiment, B. napus seeds were allowed to grow inside an intact silique, still attached to the plant, but the space allowed for their growth was limited by reducing the silique’s volume by constricting it (Supporting Information Fig. S1). Following Borisyuk et al. (2013b), both the morphology of the developing embryos and their accumulation of lipids were tracked using noninvasive magnetic resonance imaging (MRI) (Methods S1). As a result of the reduced space available for the embryos’ expansion, their size was reduced, while leaving their shape relatively unaffected (Fig. 1a). At the same time, after 20 d of stress they accumulated substantially more lipid than embryos which developed in a nonconstricted silique (Fig. 1b). Lipid mapping based on MRI evidenced that lipid accumulation was confined to the developing embryos (Fig. 1c,d), and that lipid levels differed clearly between the embryos that developed in a constricted silique and those that developed in a nonconstricted one (Video S1). We concluded that the space restriction clearly reduced the embryos’ size and boosted their accumulation of lipids (major seed storage product in B. napus).

An important question is whether the rate of nutrient uptake of the embryo is altered by mechanical growth constraints. This was tested by feeding 13C-labelled sucrose and 15N-labelled glutamine (Gln) to intact siliques. The outcome was that embryos that developed in a constricted silique accumulated significantly less 13C-label and 15N-label than those that developed in a nonconstricted one (Fig. S2a). Thus, the embryo adjusted its nutrient uptake to (lowered) growth rate. This might contribute to keep steady-state concentration of main sugars stable (Fig. S2b). Sugar analysis further revealed an induction of raffinose (characteristic of maturation).

To assess whether the magnitude of the resulting lipid accumulation would have been greater had the extent of the constriction been greater (and vice versa), we performed a second experiment. Brassica napus embryos were harvested from the siliques at the cotyledon stage, and were then inserted individually within a spherical, porous chamber of various volumes (Fig. S1b). The aim was to restrict the space available for the embryo’s growth without compromising its access to growth medium or limiting the exchange of gases. The set-up was designed to simulate the in planta situation in terms of imposing a space restriction for the embryo’s growth, while removing any influence of the maternal plant. The response to this space constraint was notable, both with respect to the embryo’s acquisition of biomass (Fig. 2a) and its synthesis of major storage products (Fig. 2b). The smaller the space allowed for growth, the smaller was the embryo and the greater its content.
of lipid and protein. Biomass fractionation further revealed that the embryos synthesised/accumulated less soluble metabolites but more lipid/protein as the space restriction was intensified (while their accumulation of fibre/starch was largely unaffected; data not shown). Folding of the embryos only occurred when their expansion was impeded by the chamber wall, that is under restricting chamber volumes (Fig. 2c). Lipid mapping suggested that the most prominent deposition region in the space-restricted, folded embryos was around their hypocotyl and some cotyledon tissues (Fig. 2d,e), corroborating observations made from embryos developing in planta (Borisjuk et al., 2013a). Analysis of the transcription level of a sample of genes encoding master regulators of lipid storage and seed maturation showed that the transcript abundances of WRI1 (encoding a regulator of fatty acid synthesis; To et al., 2012; Kuczynski et al., 2020), FUS3 and ABI3 were all raised by the space restriction (Fig. 2f). We tested the levels of main sugars, and did not detect significant shifts between growth conditions (Fig. S3). The overall conclusion was that the embryos were able to adjust their expansion and storage product synthesis in response to the mechanical stimulus.

**Conclusion**

The initial phase of development in a Brassicaceae seed features the expansion of the testa/endosperm, followed subsequently by that of the embryo. As the embryo’s ultimate size is constrained by the volume of the embryo sac, the embryo needs to be able to sense how much space remains available for its expansion. The most significant finding from the presented experiments was that a physical restriction to the embryo’s growth induced a metabolic shift that redirected development away from growth and into maturation. To date, the seed maturation process is generally seen to be under tight developmental and metabolic control. At the genetic level, certain transcriptional master regulators have been identified as important (Baud et al., 2008; Belmonte et al., 2013; Jo et al., 2019; Sall et al., 2019), along with several microRNAs (Willmann et al., 2011). Meanwhile, at the physiological level, the relative tissue content of specific phytohormones and/or sugars (Baud et al., 2008; Sreenivasulu & Wobus, 2013; Leprince et al., 2017; O’Neill et al., 2019) has been correlated with the switch from enlargement to maturation. The proposition here is that
Fig. 2 The effect of space restriction on the growth of embryos cultured in vitro. (a, b) The performance of embryos grown in a space of varying volume. (a) Fresh weight (FW) and dry weight (DW). (b) Lipid and protein content. Data are shown in the form mean ± SD (n = 6–10); the dotted lines show the fitted logarithmic regression curve. (c) Light micrographs of embryos grown in a spatially nonrestricted chamber (> 300 mm³) vs those grown with spatial restriction (chamber size 4.2, 22.4 or 87 mm³); the embryos shown are all of the same age. Pores in the chamber walls enabled the free flow of culture medium and allowed gas exchange. (d) Magnetic resonance imaging (MRI)-based embryo models illustrating the shape and size of spatially nonrestricted (left) and restricted embryos. (e) Lipid mapping of spatially nonrestricted and restricted embryos. (f) Transcript abundances of WRI1, ABI3 and FUS3 in embryos grown in a space of varying volume (from 4 mm³ as in planta up to >300 mm³ as free floating in vitro (nonrestricted)); values are shown in the form mean ± SD (n = 3). Expression levels were normalised to that of GAPDH.
mechanical stimuli provide a novel key element of embryo maturation: once the state of competence is defined, the onset of a physical restriction to the embryo's growth boosts the cellular maturation programme. It remains to be tested if the embryo proper has the capacity to perform mechanosensing (Landrein & Hamant, 2013; Monshausen & Haswell, 2013) and/or which molecular and biochemical features mediate the observed growth response. In particular, changes in the expression of known mechanosensitive genes need to be investigated, including experiments performed over short time periods.

There is biochemical evidence that the boost in synthesis of storage lipids (and proteins), characteristic of maturation, requires glycolysis to be strongly enhanced (Borisjuk et al., 2013; Schwender et al., 2015). The imposition of mechanical force can induce such an enhancement to glycolysis as was recently demonstrated for certain human cancer cells (for details see Park et al., 2020). The coupling of glycolysis and plant cell/embryo mechanics, as well as other hypotheses have to be tested in future work, and could provide novel avenues in leading crop research programmes.

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Author contributions

HR and LB designed the experiments and wrote the manuscript. LB performed MRI and AM performed qRT-PCR, plant cultivation, in vitro work and seed compositional analysis.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 The experimental set-up used to manipulate the space available for the growth of embryos grown in planta or cultured in vitro.

Fig. S2 Biochemical response of embryos to mechanical constraints in planta.

Fig. S3 The effect of space restriction on the soluble sugars of embryos cultured in vitro.

Methods S1 Description of experiments.

Video S1 Representative, three-dimensional models of B. napus siliques and lipid distribution based on magnetic resonance imaging.

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