Plants have a sessile lifestyle and their development and growth respond to the environment.1-6 Within this context, plant morphogenesis is a plastic process that requires the integration of developmental and environmental cues. In higher plants, the reproductive phase leads to the formation of seeds, which are essential for plant dispersal and survival. Angiosperm seeds derive from a fertilized ovule and are comprised of three main compartments: the embryo derived from fertilization of a haploid egg cell, the endosperm derived from fertilization of the diploid central cell by haploid sperm cells and the integuments. Mitotic cell proliferation underpins the growth and development of all three components and is therefore essential for both embryonic and post-embryonic development.

The mitotic cell cycle of eukaryotes is composed of two alternating phases, during which DNA is first replicated (S phase) and then chromosomes are partitioned (mitosis, M), interrupted by two gaps G1 (between M and S) and G2 (between S and M). In eukaryotes, progression through the cell cycle requires the modulation of the activity of kinase complex composed of the cyclin-dependent kinase (CDK) and the regulatory subunit, cyclin (CYC).7

Cell cycle progression requires tight regulation that occurs at two main checkpoints: G1-to-S and G2-to-M. In Arabidopsis, CDKA1;1/CYCD kinases8-10 regulate the G1-to-S transition whereas G2-to-M transition requires the activity of CDKB kinases. These checkpoints are key steps to integrate developmental, environmental and nutritional cues and CYCDs have been shown to be direct or indirect integrators of mitogenic signaling triggered by phytohormones such as auxin and cytokinin and carbohydrates (e.g. sucrose and glucose) levels.8-9

We have used targeted expression of CYCD7;1 using the highly specific FWA promoter to address the consequences of altering the normal pattern of divisions early in Arabidopsis seed development.11 The FWA promoter is active pre- and post-fertilization in the central cell of the female gametophyte and developing endosperm of the seed. Interestingly, targeted expression of CYCD7;1 using the FWA promoter overcomes cell cycle arrest in the central cell of the female gametophyte before fertilization, resulting in the central cell becoming multinucleate with high frequency. Post-fertilization there is an acceleration of early endosperm and embryo development, although this slows after heart phase of embryo development, by which time the FWA promoter is no longer active. We also observed a high degree of lethality of embryos, leading to abortion.

Associated with these phenotypes provoked by CYCD7;1 expression, we recently reported that FWA::CYCD7;1 transgenic lines produce seeds with increased final size.11 These seeds display faster seedling establishment upon germination, which might benefit the fitness of this transgenic lines.11 We noted that a number of seed developmental mutants have similarly been reported to display either lethality or enlarged seeds.12-18 Previously only Fang and colleagues17 have reported both parameters and we considered whether these phenotypes might be causally related. Examining mutants with lethality but no
obvious other connection to seed growth also showed increased seed size. This suggests that drawing conclusions from observations of altered seed size must be carefully considered alongside any sibling lethality observed.12

Hence in this study, we showed evidence for the trade-off between seed size and seed number in the siliques.12-18 Targeted CYCD7;1 expression in the seed under the control of the FWA promoter was also tested using the UAS/GAL4 system,19 and using these FWA>> CYCD7;1 transgenic lines, the trade-off between seed size and number was further explored within the context of resource allocation. The number of seed pods was artificially decreased by removal of the branches of the floral stalk as well as the axillary shoots (referred as SUPRA, Fig. 1A) or increased by cutting back the primary stem to stimulate branching and therefore leading to the production of numerous seed pods (SUB, Fig. 1A). We found that these manipulations of plant architecture influenced seed size in untransformed WT plants, as well as modulating the effect conferred by FWA>> CYCD7;1. The mean seed size distribution of WT plants was not significantly different between seeds grown on NORMAL floral stalks or on SUPRA stems (i.e. when axillary stems were removed). However on SUB shoots where the main stem was removed to increase side branching, mean seed size was decreased by 4% (p = 0.76). In each case, overall mean seed size from the whole plant was determined as described.11

These results suggest that either mean seed size differs between the main inflorescence and side inflorescences or that it can be affected by limiting the resources available. However the former explanation would suggest that SUPRA seeds from the main stem only should be larger than from the NORMAL which includes both main stem and side shoot-derived seed, which was not the case, since seed size was not increased by axillary stem removal (SUPRA), in contrast to the effect of sibling abortion within the silique itself12 on seed size.

In line with our analysis of the FWA:CYCD7;1 seeds,11 we found that both FWA>> CYCD7;1 NORMAL and SUPRA plants produce larger seeds compared to WT controls (two-way ANOVA, p = 1.18 x 10^{-47}; Fig. 1). In NORMAL plants, FWA>> CYCD7;1 seeds were 8% larger than the WT (two-way ANOVA, p = 3.95 x 10^{-45}) and in SUPRA conditions FWA>> CYCD7;1 seeds showed a 9% increase in overall area. However, on the SUB plants with increased branching

Figure 1. Influence of plant architecture on seed size. (A) Cartoon depicting the experimental design: in SUPRA conditions, all axillary and secondary branches were removed resulting in a reduction of the number of seed pods produced. In NORMAL conditions plant architecture was untouched. In SUB conditions, the primary stem was cut soon after the floral transition initiating the formation of additional axillary stems and increasing the number of seed pods. (B-D) Comparison of seed size parameters between the different architectures. For each type, nine plants were grown and from each individual plant seeds were harvested and analyzed separately. For each plant, a minimum of 200 seeds was measured. Mean seed area (B), mean seed length (C) and mean seed width (D). Error bars show ± SE. (*) indicates a statistical difference in one of the seed size parameters.
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