Control of seed mass and seed yield by the floral homeotic gene *APETALA2*

K. Diane Jofuku, Pamela K. Omidyar*, Zorana Gee, and Jack K. Okamuro†

Biology Board, Sinsheimer Laboratories, University of California, Santa Cruz, CA 95064

Communicated by Robert B. Goldberg, University of California, Los Angeles, CA, December 30, 2004 (received for review December 20, 2004)

***APETALA2* (*AP2*) is best known for its role in the regulation of flower meristem and flower organ identity and development in *Arabidopsis*. We show here that *AP2* also plays an important role in determining seed size, seed weight, and the accumulation of seed oil and protein. We demonstrate genetically that *AP2* acts through the maternal sporophyte and endosperm genomes to control seed weight and seed yield. Thus, *AP2* functions outside the boundaries of flower meristem and flower organ development to affect agronomically relevant traits in *Arabidopsis*.**

Apetala2 | *Arabidopsis* | seed protein and oil contents | seed size

Genetic and molecular studies have revealed an evolutionarily conserved network of regulatory genes that orchestrate flower development in *Arabidopsis* and in other plant species. Included in this core group of flower-promoting and floral organ identity genes is the homeotic regulatory gene *APETALA2* (*AP2*). AP2 is the founding member of a large family of transcription factors in *Arabidopsis* characterized by the presence of a 68-aa repeated domain referred to as the AP2 domain (1–7). Loss of *AP2* activity has been shown to effect qualitative and quantitative changes not only in floral meristem and floral organ identity and development (1, 8–11) but also in seed coat development (1, 12, 13). Molecular studies have shown that the *ap2* flower phenotypes result in part as a consequence of ectopic expression of the MADS domain containing transcription factor *AGAMOUS* (*AG*) during flower development (14, 15), indicating that one important function of *AP2* is to negatively regulate *AG* transcription.

In flower development, the expression of most of the core regulatory genes is both temporally and spatially restricted to developing flowers (14, 16–18). These include the MADS domain-containing transcription factors *AG*, *APETALA1* (*AP1*), *PISTILLATA* (*PI*), and *APETALA3* (*AP3*). In contrast, *AP2* transcripts have been detected at every stage of *Arabidopsis* development and in virtually every organ type examined, including young seedlings, vegetative leaf, stem and root, the inflorescence meristem, all four types of floral organs, ovules, and developing seeds and embryos (1, 19), suggesting that *AP2* may be active at other times in development. Recent studies, however, have shown that the detection of *AP2* transcripts may not be a sufficient indicator of *AP2* activity. For example, *AP2* activity can be controlled at the translational level by the microRNA mi172 (20, 21) or subject to posttranslational modification by phosphorylation, similar to that observed for other AP2 domain-containing proteins (ref. 22; R. Khush and J.K.O., unpublished data). We demonstrate here that *AP2* has functions outside the boundaries of floral meristem identity, floral organ identity and the control of floral organ number. Our analysis of *AP2* activity and its effects on seed size and seed mass in mutant and transgenic *Arabidopsis* identify *AP2* as a significant player in the control of seed mass and seed yield.

Materials and Methods

Plant Material and Growth Conditions. *Arabidopsis thaliana* Landsberg *erecta* (Ler), Columbia (Col), and Columbia C24 were used as control seeds. *ap2-1* and *ap2-9* seeds (Ler) were provided by M. Koornneef (Wageninen University, Wageninen, The Netherlands) and G. Drews (University of Utah, Salt Lake City), respectively; *ap2-3* and *ap2-4* seeds (Ler) were provided by K. Okada (Kyoto University, Kyoto); and *ap2-5* and *ap2-6* seeds (Col) were provided by G. Haughn (University of British Columbia, Vancouver). *ap2-10* is in the C24 genetic background (1). *malesterile1* (*ms1*) seeds (Ler) were obtained from the *Arabidopsis* Biological Resource Center (Columbus, OH). Plants were grown in either a greenhouse or a growth chamber in a 1:1:1 mixture containing vermiculite, perlite, and peat moss. Plants grown in the growth chamber were exposed to 8 h of darkness and 16 h of light in a Conviron E15 chamber (Controlled Environments, Asheville, NC) as described (23). Plants were watered with a one-quarter strength Peter's solution (Grace-Sierra Co., Milpitas, CA). Mature brown seeds were harvested, dried for 7 days at 25°C, and stratified for 7 days at 7°C before being analyzed.

Plant Transformation. Wild-type *Arabidopsis* root explants were transformed with Ti plasmid vectors pPW9, pPW14.4, and pPW15 according to standard procedures (24). pPW9, pPW14.4, and pPW15 contain the 1.68-kb *AP2* gene coding region (1) cloned in a transcriptional fusion in the sense (pPW9) and antisense (pPW14.4 and pPW15) orientations with the cauliflower mosaic virus 35S constitutive promoter. The Ti plasmid vector used for these constructs pGSJ780A (Plant Genetic Systems N.V., Ghent, Belgium) contains the plant selectable marker gene neomycin phosphotransferase (*NPTII*) that confers kanamycin resistance to transformed plant cells. Independently transformed *Arabidopsis* lines were selected for kanamycin resistance and the presence of flowers displaying the *ap2* mutant phenotype.

Seed Size and Seed Mass Analysis. Average seed mass was determined by weighing mature dry seeds in batches of 100. The weights of at least five sample batches were measured for each seed lot. Size distributions of wild-type and mutant seed populations were analyzed by separating batches of ≈0.1–0.2 g of seeds by using a series of fine wire sieves. Sieve mesh sizes 35, 40, 45, 50, 60, 70, and 80 (Fisher Scientific) with exclusion sizes of 500, 425, 355, 300, 250, 212, and 180 μm, respectively, were used for each analysis. Seeds retained by each sieve were weighed by using an analytical balance (Mettler-Toledo AG, Greifensee, Switzerland) with mass expressed as a percent of the total weight of the seed sample analyzed.

Transmission Electron Microscopy and Image Processing. Plastic sections of *Arabidopsis* seeds were generated as described by Yadegari *et al.* (25) and were examined in a JEOL JEM-100B transmission electron microscope operating with an accelerator

Abbreviations: *AP2*, *APETALA2*; Col, Columbia; Ler, Landsberg *erecta*; *ms1*, *malesterile1*.

*Present address: 720 University Avenue, Suite 200, Los Gatos, CA 95032.

†To whom correspondence should be sent at the present address: Ceres, Inc., 1535 Rancho Conejo Boulevard, Thousand Oaks, CA 91320. E-mail: jkokamuro@ceres-inc.com.

voltage of 80 kV. All images were scanned and digitized by using a Polaroid Sprintscan 35 or an AGFA Arcus II flatbed scanner (AGFA Division, Miles). Contrast and brightness were adjusted by using PHOTOSHOP 8.0 (Adobe Systems, Mountain View, CA).

Seed Yield Analysis. Plants were grown in the greenhouse in individual 3.5-inch square pots filled to the top with a 1:1:1 mixture containing vermiculite, perlite, and peat moss. Plants were bottom-watered with a one-quarter strength Peter's solution. When the seedlings reached the four-leaf stage, each seedling was fitted with an 11- × 11-inch "collar" consisting of an inverted plastic humidity tray to catch all seeds released by dehiscing siliques. All seeds from a single plant were harvested when the plant was mature and the last siliques produced on the inflorescence had elongated, turned brown, dried, and were ready to dehisce. Mature plants were cut at the crown and crushed by hand to release seeds from all remaining siliques. These hand-harvested seeds were combined with those caught in the humidity tray, sieved to remove plant debris, dried at 25°C for 7 days, and weighed.

Determination of Seed Protein and Fatty Acid Content. Total seed protein extracts were prepared as described (26). Protein concentrations were determined by using a colorimetric assay (Bio-Rad). Total seed fatty acids from wild-type and mutant *Arabidopsis* seeds produced and harvested under similar growth conditions were extracted and converted to methyl esters by using a 1:2 dilution of methanolic-3N HCL (Supelco) in absolute methanol at 85°C for 16 h. The free fatty acid C15:0 (Sigma) was used as an internal control to assess efficiency of extraction. Seed fatty acid methyl esters were analyzed, and total seed fatty acid composition was determined by gas chromatography with an Omega Wax 250 column (Supelco) and a Sigma 300 gas chromatograph (PerkinElmer). Total fatty acid content was determined by comparing the estimated total fatty acid methyl ester peak area to that of the C15:0 internal standard.

Results

***APETALA2* Contributes to the Determination of Seed Weight and Seed Size in *Arabidopsis*.** Previous studies showed that *ap2* mutant seeds lack a distinctive seed coat epidermal cell structure called the columella and are more irregular in shape than wild-type seeds (1, 12, 13), indicating that *AP2* is required for normal seed coat development. We found that *AP2* is also involved in seed mass control. Table 1 shows that all seven *ap2* mutants examined produced seeds that displayed significant increases in average seed mass, ranging from 27% to 104% greater than wild-type controls. Seeds produced by the weak partial loss-of-function mutants *ap2-1* (27) and *ap2-5* (10) showed the smallest gains in average seed mass ranging from 27% to 39% greater than that of parental wild-type Ler and Col, respectively (Table 1). In contrast, seeds produced by the strong *ap2* mutants *ap2-4* (8), *ap2-6* (10), and *ap2-10* (1) showed 69–104% gains in average seed weight compared with wild type (Table 1).

It has been suggested that seed fill, and therefore seed weight, is determined in part by the availability of assimilates to the developing seed. If true, then a decrease in total seed number may result in an increase in average seed weight. Because total seed number depends on many factors, including fertility, and because strong *ap2* mutants are reduced in male fertility (1, 8–11) and total seed yield, we could not rule out the possibility that the observed increase in *ap2* seed weight was produced at the expense of total seed number and yield. To test this hypothesis, we assessed the extent to which male infertility can affect *Arabidopsis* seed weight. *ms1* flowers produce no seeds unless hand pollinated with wild-type pollen (28, 29). We therefore hand pollinated five flowers on five *ms1* plants with wild-type pollen and then determined the average weight of the seeds produced. Seeds resulting from this forced pollination develop in a background of unfertilized ovules and therefore, according to the hypothesis, should show an increase in average seed weight. As predicted, the average weight of F_1 [*ms1* (−/−) × wild type] seeds was increased, but the average gain was only 22% greater than that of wild-type seeds, far lower than the 69–104% gains observed for severe *ap2* mutants (Table 1). Even weak *ap2* mutants with good fertility and seed set such as *ap2-1* showed statistically higher increases in weight (27–31%) (Table 1). Thus, although male infertility can enhance seed weight slightly, it is not solely responsible for the dramatic increase in seed weight displayed by weak or strong *ap2* mutants. Consistent with this result, Alonso-Blanco *et al.* (30) concluded that reduced fertility does not dramatically impact *Arabidopsis* seed size and weight.

Table 1. Genetic control of *Arabidopsis* seed weight

Plant	Average seed weight	% increase over wild type
ap2-1 (Ler)	2.1 (0.1)	+27
	2.2 (0.1)	+33
	2.1 (0.2)	+31
	2.8 (0.2)	+33
ap2-3 (Ler)	2.6 (0.1)	+27
ap2-4 (Ler)	3.5 (0.3)	+69
	3.5 (0.2)	+69
ap2-5 (Col)	2.9 (0.1)	+39
ap2-6 (Col)	3.5 (0.2)	+69
ap2-9 (Ler)	2.9 (0.1)	+40
ap2-10 (C24)	3.7 (0.4)	+79
	3.9 (0.3)	+90
	4.2 (0.5)	+104
ms1 × Ler	2.9	+22
Col	1.8 (0.1)	
	2.1 (0.1)	
Ler	1.6 (0.1)	
	2.1 (0.1)	
	2.3 (0.1)	
C24	2.0 (0.1)	
	2.3 (0.1)	

Average seed weight is given in mg per 100 seeds. Standard deviation values are given in parentheses. Percent increases in seed weight were calculated based on comparison with that of wild-type seeds produced under similar growth conditions.

We examined *ap2* mutant seed size by fractionating seeds produced by individual wild-type and mutant plants by using a series of wire mesh screens. Fig. 1 shows that seeds produced by a wild-type plant typically displayed a narrow range of exclusion sizes compared with *ap2* mutant seeds. For example, wild-type Col seeds ranged in exclusion size from 250 μm to 300 μm (Fig. 1*A*), whereas wild-type Ler seeds showed a slightly broader range of 212 μm to 355 μm (Fig. 1*B*). Both seed types have an average exclusion size between 212 and 250 μm (Fig. 1 *A* and *B*). In contrast, both *ap2-6* (Col) (Fig. 1*C*) and *ap2-9* (Ler) (Fig. 1*D*) mutant seeds displayed a shift in range to larger exclusion sizes. In the case of *ap2-6*, mutant seeds ranged in size from 250 μm to as high as 425 μm (Fig. 1*C*). In addition, both *ap2* mutant seeds showed an increase in average exclusion size compared with wild type. Taken together, these results demonstrate that reducing *AP2* gene activity consistently increases both seed size and seed weight in *Arabidopsis*.

To determine the cellular basis for the difference in seed size between wild-type and *ap2* mutant seeds, we examined cotyledon cell size in mature *ap2-9* and Ler seeds by transmission electron microscopy. Fig. 2 shows that *ap2-9* cotyledon cells are larger than those of wild type. Similar results were obtained for

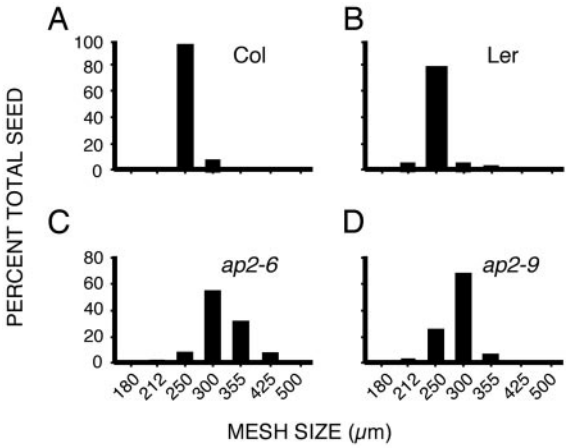

Fig. 1. *ap2* seeds are larger in size than wild-type seeds. Preweighed batches of wild-type Col (*A*), Ler (*B*), and *ap2-6* (Col) (*C*) and *ap2-9* (Ler) (*D*) mutant seeds from single plants were passed through a series of wire sieves of decreasing mesh size (in μm) as described in *Materials and Methods*. *ap2-6* (10) and *ap2-9* (11) are severe *ap2* mutants. Boxes designate the percent total seeds by weight retained by each sieve.

ap2-6 (data not shown). We conclude that the increase in seed size and seed weight observed for *ap2* seeds are due in part to an increase in embryo cell size.

***AP2* Activity Can Be Manipulated to Control Seed Mass in Transgenic *Arabidopsis* Plants.** We used antisense and sense cosuppression strategies to suppress *AP2* activity *in planta* to test whether seed mass could be manipulated in transgenic wild-type *Arabidopsis* plants. Nine independent lines of transgenic plants containing a chimeric *AP2* antisense gene construct and eight lines containing an *AP2* sense gene construct tested positive for kanamycin resistance and the presence of one or more copies of T-DNA (data not shown). Seven of the nine *AP2* antisense and two of the eight sense cosuppression lines produced *ap2*-like flowers and seeds that were significantly larger when compared with seeds produced by control transgenic plants. Table 2 shows that the gains in seed weight ranged from 22% for antisense line PW15-2 (C24) to 89% for PW15-3 (C24) compared with controls. Increased seed weight was observed for T_1, T_2, and T_3 generation seeds (Table 2), indicating that the seed size phenotypes are heritable. Similarly, seeds produced by the two *AP2* sense cosuppression mutant lines PW9-1 (Ler) and PW9-1 (C24) showed gains in seed weight that ranged from 26% and 86% higher than that of control transgenic seeds, respectively (Table 2). Together, these results demonstrate that *AP2* gene sequences can be used to genetically engineer significant increases in *Arabidopsis* seed weight.

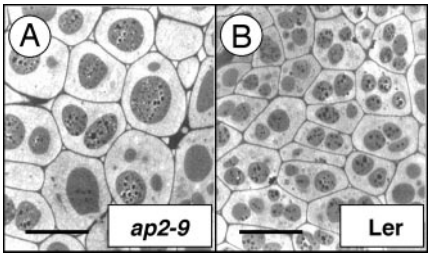

Fig. 2. The *ap2* mutation affects embryo cell size in *Arabidopsis*. Transmission electron micrographs of mature *ap2-9* (*A*) and Ler (*B*) *Arabidopsis* seeds show cotyledon cell size and morphology. *ap2-9* represents a severe loss-of-function mutant in the Ler background (11). Images were taken at the same magnification. (Bar: 12 μM.)

Table 2. Suppression of *AP2* activity in transgenic *Arabidopsis* plants results in increased seed mass

Transgenic line	Average seed weight	% increase over wild type
AP2 antisense lines		
PW14.4-1 (C24) (T_1-15)	3.1	+35
PW14.4-1 (C24) (T_1-15) T_3 seeds	3.4 (0.3)	+47
PW15-1 (C24) T_1 seeds	3.6 (0.1)	+76
PW15-2 (C24) (T_1-2)	2.6 (0.1)	+25
PW15-2 (C24) (T_1-7)	2.5 (0.2)	+22
PW15-3 (C24) T_1 seeds	3.9 (0.1)	+89
PW15-1 (Ler) T_1 seeds	2.4 (0.1)	+42
PW15-2 (Ler) (T_1-3)	2.8 (0.0)	+33
PW15-2 (Ler) (T_1-17)	2.7 (0.0)	+28
AP2 cosuppression lines		
PW9-1 (C24) T_1 seeds	3.8 (0.0)	+86
PW9-1 (Ler) (T_1-19)	2.7 (0.2)	+26
PW9-1 (Ler) (T_1-24)	2.7 (0.1)	+26
pGSJ780A vector control lines		
PW3-1 (C24) T_1 seeds	2.2 (0.1)	+9
PW3-2 (C24) T_1 seeds	2.3 (0.0)	+13
PW3-1 (Ler) T_1 seeds	2.3 (0.1)	+7
PW3-2 (Ler) (T_1-4)	2.4 (0.2)	+12
PW3-2 (Ler) (T_1-6)	2.3 (0.0)	+9
PW3-2 (Ler) (T_1-8)	2.1 (0.0)	−0.5

Transgenic lines were in the C24 or Landsberg *erecta* (Ler) backgrounds. Average seed weight reflects that of T_2 seeds produced by a single kanamycin-resistant T_1 transgenic plant unless otherwise stated. Standard deviation values are given in parentheses. Percent increases in seed weight were calculated based on comparison to that of wild-type seeds produced under similar growth conditions.

AP2 Controls *Arabidopsis* Seed Mass in Part Through the Maternal Sporophytic and Endosperm Genomes. As in all angiosperms, seed development in *Arabidopsis* depends on the interaction between the triploid endosperm and the diploid sporophytic and embryonic genomes to orchestrate morphogenesis and the deposition of seed reserves in the developing seed (31). Previously, we and others (1, 12, 13) showed that *AP2* functions through the sporophytic genome to regulate seed coat development. We carried out reciprocal crosses by using *ap2-10* and wild-type plants to determine whether *AP2* functions through the sporophytic genome to control seed mass. Table 3 shows that *ap2-10* (+/−) seeds produced by *ap2* mutant flowers pollinated with wild-type pollen were larger by weight than wild-type seeds but still smaller than *ap2-10* (−/−) seeds produced by mutant flowers pollinated with *ap2* pollen. As expected, seeds produced by an *ap2* mother, regardless of the genotype of the pollen donor, displayed the distinctive *ap2* seed coat phenotype (data not shown; refs. 1, 12, 13). In contrast, *ap2-10* (+/−) seeds produced by wild-type flowers pollinated with *ap2-10* pollen were comparable in weight to wild-type seeds (Table 3) and had normal seed coats (data not shown). Together, these results suggest that *AP2* controls seed mass in part through its activity in the maternal sporophyte and endosperm and not in the embryo.

To further test the genetic contribution of *AP2* activity in the maternal sporophyte and endosperm, we generated seeds with *ap2* mutant endosperm (−/−/−) by crossing *ap2-10* (+/−) flowers with *ap2-10* mutant pollen. If *AP2* activity in the endosperm is required for seed mass control, then the prediction is that 50% of the seeds produced from this cross will be similar in mass to those produced by a homozygous *ap2* mutant. Table 3 shows that the average weight of seeds produced by this cross

PLANT BIOLOGY

Table 3. Genetic control of seed mass and yield by *AP2*

Parental genotypes		F_1 seed genotype			Average seed mass		
♀	♂	*SP*	*EN*	*EM*	F_1	F_2	Average F_2 seed yield
−/−	−/−	−/−	−/−/−	−/−	4.75 (0.07)	ND	ND
−/−	+/+	−/−	−/−/+	−/+	3.65 (0.14)	2.84 (0.21)	2.70 (0.35)
+/+	−/−	+/+	+/+/−	+/−	2.60 (0.04)	ND	ND
+/+	+/+	+/+	+/+/+	+/+	2.30 (0.10)	2.46 (0.15)	2.00 (0.22)
+/−	−/−	+/−	+/+/−	+/−	3.13 (0.12)	ND	ND
		+/−	−/−/−	−/−			
+/+	AS/AS	+/+	+/+/AS	+/AS	3.50 (0.26)	ND	ND

The + and − designations indicate the presence of the wild-type *AP2* and the recessive mutant *ap2-10* alleles, respectively. AS indicates the *AP2* antisense transgene in pollen donor PW15-3 (C24) that confers an *ap2* seed size phenotype as described in Table 2. Standard deviation values are shown in parentheses. Seed mass is given in units of mg per 100 seeds. Seed yield is the average weight (g) of the total number of F_2 seeds produced per plant for WT ($n = 7$) and *ap2-10* (+/−) ($n = 9$) F_1 plants. SP, EN, and EM refer to the maternal sporophyte, endosperm, and embryo genotypes, respectively. ND, not determined.

was intermediate between that observed for seeds produced by a homozygous *ap2-10* mutant and by a wild-type plant. However, these seeds did not segregate as predicted for *ap2-10* mutant seed size when assayed by microscopic and seed sieve analyses (data not shown). We conclude that *AP2* activity in the maternal sporophyte is critical for the control of *Arabidopsis* seed mass.

To definitively test for *AP2* seed mass control activity in the endosperm, we generated seeds by crossing wild-type flowers with pollen containing a 35S-*AP2* antisense transgene [PW15-3 (C24); Table 2]. We reasoned that the transgene would suppress *AP2* gene activity in the endosperm and embryo of the developing seeds but not in the maternal sporophyte. Table 3 shows that the seeds produced by this cross were significantly greater in weight than control seeds (Table 3), but less than those produced by *ap2* mutants grown under similar conditions. Because *AP2* activity in the embryo does not appear to be critical for seed size control (Table 3), these results indicate that *AP2* activity in the endosperm is also critical for the control of seed mass. Taken together, these studies demonstrate that *AP2* functions in at least two of the three genomes necessary for *Arabidopsis* seed production.

***ap2* Can Increase Seed Weight Without a Compensatory Decrease in Total Seed Yield.** To test whether increased seed weight negatively affects total seed yield, we took advantage of the fact that *AP2* controls seed mass in part through the sporophytic and endosperm genomes (Table 3) and compared average seed weight and total seed yield for *ap2-10* (+/−) and control plants. As shown previously, F_1 [*ap2-10* (−/−) × C24 (+/+)] seeds displayed increased seed weight (Table 3). These seeds were germinated, and the resulting plants were found to be fully fertile; they produced F_2 seeds that were larger than those of wild type by 15% ($P < 0.01$) and produced 35% more seed by weight per plant than wild-type C24 ($P < 0.01$) (Table 3). These results suggest that *AP2* controls both seed mass and total seed yield in *Arabidopsis*.

Increase in *ap2* Seed Weight Is Due in Part to Increases in Total Seed Protein and Oil Contents. We extracted total protein from wild-type and *ap2* mutant seeds to determine whether increases in *ap2* seed mass are due in part to increases in seed reserves. Table 4 shows that total *ap2* mutant seed protein content increased by 13–78% when compared with wild type. In addition, the spectrum of soluble proteins extracted from mature wild-type and *ap2* mutant seeds under denaturing conditions were qualitatively indistinguishable, with no detectable difference in the relative representation of the two major classes of *Arabidopsis* seed storage proteins, the 12S cruciferins (32, 33) or the 2S albumins (ref. 34 and data not shown). Similar results were obtained by using wild-type and *ap2* seed protein extracts generated by aqueous salt extraction procedures (data not shown). Taken together, these results indicate that the observed increases in seed protein content in *ap2* mutant seeds are not due to selective increases in seed storage protein reserves.

We also compared total seed fatty acid content and composition in wild-type and *ap2* mutant seeds. Like its close relative oilseed rape (*Brassica napus*), *Arabidopsis* seeds produce a small number of fatty acid species that collectively represent as much as 80% of the total seed fatty acids (35–37). These fatty acids include palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosenoic (C20:1), and erucic (C22:1) acids. Table 4 shows that there is ≈680–730 μg of these fatty acid species per 100 *Arabidopsis* wild-type seeds. In contrast, *ap2* mutant seeds as well as seeds produced by *ap2-10*

Table 4. Genetic control of total seed protein and fatty acid contents in *Arabidopsis* by *AP2*

Genotype	Total protein, μg per 100 seeds	Total FA, μg per 100 seeds	% FA methylesters (area % by GC)									
			16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0
ap2-4	729 (107) (+33%)	910 (70) (+25%)	6.6	3.7	16.5	26.9	23.7	2.8	19.6	>0.5	>0.5	2.8
Ler	545 (22)	730 (20)	6.0	4.1	22.7	26.7	17.4	2.2	19.3	>0.5	>0.5	1.6
ap2-5	617 (24) (+13%)	920 (70) (+35%)	6.9	4.1	14.8	30.7	20.8	2.2	17.0	>0.4	>0.4	2.2
Col	548 (42)	680 (70)	8.1	4.0	13.6	33.7	20.9	2.1	16.6	>0.5	>0.5	1.4
ap2-10	836 (15) (+78%)	1450 (90) (+113%)	7.9	4.6	12.1	25.2	24.3	3.3	19.5	>0.4	>0.4	2.5
ap2-10(+/−)	ND	870 (70) (+32%)	7.0	5.2	15.1	26.5	23.2	3.1	18.2	>0.5	>0.4	1.8
C24	469 (19)	680 (10)	8.0	5.7	15.1	26.5	22.5	3.0	17.6	>0.5	>0.5	1.6

Standard deviation values are given in parentheses, and percent increases with respect to wild-type controls are shown. FA, fatty acids; ND, not determined.

(+/−) plants showed increases in fatty acid content of 25–113% when compared with wild type, increases that are roughly proportional to the observed increases in *ap2* seed weight and total seed protein content (Tables 1 and 4 and data not shown). In addition, we detected no statistically relevant differences in fatty acid composition between wild-type and *ap2* mutant seeds with some notable exceptions. For example, Table 4 shows that *ap2* mutant seeds showed a 37–57% increase in the levels of lignoceric acid (C24:0) when compared with that in parental wild-type seeds. In the case of *ap2-4* and *ap2-10* seeds, these observed increases were accompanied by compensatory 27% and 20% decreases in oleic acid (C18:1) levels, respectively (Table 4). We conclude that reducing *AP2* gene activity increases both seed size and contents, and consequently seed yield, without substantial changes in seed protein and fatty acid composition.

Discussion

In many plant species, seed size and seed mass have been implicated as important determinants in seedling survival and vigor upon germination (38). Field studies have shown that large seeds can confer enhanced seedling establishment and survival (39–42), increased tolerance to flooding (43), and tolerance to insect predation (44, 45). Because of the advantages associated with larger seeds and because of the potential of increasing total seed yield through seed size, it is of agronomic importance to identify the genes involved in the determination of seed size and seed mass. We demonstrate here that *AP2* plays an important role in the control of seed mass and seed yield in *Arabidopsis*. The loss of *ap2* activity resulted in seed mass increases as much as 100% greater than that of wild-type seeds (Table 1). The increases in seed mass observed for *ap2* mutant seeds are not solely due to the homeotic nature and reduced fertility of *ap2* flowers because the mass of seeds produced by severe *ap2* mutants like *ap2-10* greatly surpassed that observed for seeds produced by *ms1* mutant plants (Table 1).

How does *AP2* carry out its functions to affect seed size, embryo size, seed weight, and the accumulation of seed reserves? Our conclusion that *AP2* is acting in the maternal sporophyte and endosperm (Table 3) is supported by RNA gel blot and RT-PCR analyses that show that *AP2* is expressed in all vegetative organs examined and in the developing seed (1, 19). The data presented here also suggest that *AP2* activity affects source–sink relations. Consistent with this hypothesis, Ohto *et al.* (46) have shown that *ap2* mutants are altered in sucrose sensing, flowering time, leaf number, and soluble sucrose metabolism in developing seeds. Genetic and physiological studies suggest that *AP2* acts in part by suppressing gibberellin signaling to ensure uniformity of flower development against normal physiological fluxes (23). Gibberellins are plant growth regulators that have been shown to promote flowering, seed development, and growth in *Arabidopsis* and in many other plant systems (47, 48). Our observation that *ap2* seeds are consistently more heterogeneous in size whereas wild-type seeds are consistently uniform (Fig. 1) suggests that *AP2* could play a similar role in the maintenance of seed size uniformity. Although we show here that the major effect of *ap2* on mature seeds is increased cell size (Fig. 2), Ohto *et al.* (46) have recently shown that *ap2* also increases embryo cell number. Consistent with these data, the data of Alonso-Blanco *et al.* (30) show that at least 11 seed size and seed weight quantitative trait loci contribute to seed size variation in *Arabidopsis* by affecting both cell number and cell size, with one major quantitative trait locus mapping to the region of chromosome IV that contains *AP2.* We propose that *AP2* may negatively affect the ability of gibberellins to regulate metabolism in both source and sink tissues and in so doing, affect cell size and cell number during seed growth.

Efforts to increase agronomic traits like seed protein or seed oil content in *Brassica*, soybean, and other seed crops have shown that changes in total seed protein levels are often inversely proportional to changes in seed oil levels in the presence of a fixed supply of assimilates (49–51). That is, total seed protein or total seed oil levels per seed can increase but usually at the expense of the other. In contrast, we observed that seed weight increases were accompanied by increases in both total seed protein and total seed oil contents (Tables 1 and 4). To date the potential impact of homeotic genes like *ap2* on agronomic traits like seed weight, seed yield, and seed composition have received little attention from traditional plant breeding efforts because of their strong negative effects on fertility, plant growth, and development. The discovery that such traits can be enhanced in *Arabidopsis* by a genetic reduction in *AP2* copy number may be a useful strategy for identifying new yield-limiting genes in *Arabidopsis* for analysis in crop plants.

We thank Chris Robles for technical assistance and Drs. Tony Kinney and Angus Murphy for assistance with the fatty acid analysis. We also express our appreciation to Drs. John Harada, Jane Silverthorne, and Lincoln Taiz for their encouragement during the course of this study. P.K.O. was supported by a University of California Systemwide Biotechnology Training Grant. This study was supported by the National Institutes of Health (Grant GM46309) and the National Science Foundation (Grant 9604507) and by a gift from Ceres, Inc., to J.K.O.

1. Jofuku, K. D., den Boer, B. G., Van Montagu, M. & Okamuro, J. K. (1994) *Plant Cell* **6,** 1211–1225.
2. Okamuro, J. K., Caster, B., Villarroel, R., Van Montagu, M. & Jofuku, K. D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7076–7081.
3. Ohme-Takagi, M. & Shinshi, H. (1995) *Plant Cell* **7,** 173–182.
4. Wilson, K., Long, D., Swinburne, J. & Coupland, G. (1996) *Plant Cell* **8,** 659–671.
5. Stockinger, E. J., Gilmour, S. J. & Thomashow, M. F. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 1035–1040.
6. Zhou, J., Tang, X. & Martin, G. B. (1997) *EMBO J.* **16,** 3207–3218.
7. Magnani, E., Sjolander, K. & Hake, S. (2004) *Plant Cell* **16**, 2265–2277.
8. Komaki, M. L., Okada, K., Nishino, E. & Shimura, Y. (1988) *Development (Cambridge, U.K.)* **104,** 195–203.
9. Bowman, J. L., Smyth, D. R. & Meyerowitz, E. M. (1989) *Plant Cell* **1,** 37–52.
10. Kunst, L., Klenz, J. E., Martinez-Zapater, J. & Haughn, G. W. (1989) *Plant Cell* **1,** 1195–1208.
11. Bowman, J. L., Smyth, D. R. & Meyerowitz, E. M. (1991) *Development (Cambridge, U.K.)* **112,** 1–20.
12. Léon-Kloosterziel, K. M., Keijzer, C. J. & Koornneef, M. (1994) *Plant Cell* **6,** 385–392.
13. Western, T. L., Burn, J., Tan, W. L., Skinner, D. J., Martin-McCaffrey, L., Moffatt, B. A. & Haughn, G. W. (2001) *Plant Physiol.* **127,** 998–1011.
14. Drews, G. N., Bowman, J. L. & Meyerowitz, E. M. (1991) *Cell* **65,** 991–1002.
15. Mizukami, Y. & Ma, H. (1992) *Cell* **71,** 119–131.
16. Jack, T., Brockman, L. L. & Meyerowitz. E. M. (1992) *Cell* **68**, 683–697.
17. Mandel, M. A., Gustafson-Brown, C., Savidge, B. & Yanofsky, M. F. (1992) *Nature* **360,** 273–277.
18. Goto, K. & Meyerowitz, E. M. (1994) *Genes Dev.* **8,** 1548–1560.
19. Kinoshita, T., Miura, A., Choi, Y., Kinoshita, Y., Cao, X., Jacobsen, S. E., Fischer, R. L. & Kakutani, T. (2004) *Science* **303,** 521–523.
20. Aukerman, M. J. & Sakai, H. (2003) *Plant Cell* **15,** 2730–2741.
21. Chen, X. (2004) *Science* **303,** 2022–2025.
22. Gu, Y. Q., Yang, C., Thara, V. K., Zhou, J. & Martin, G. B. (2000) *Plant Cell* **12,** 771–786.
23. Okamuro, J. K., Szeto, W., Lotys-Prass, C. & Jofuku, K. D. (1997) *Plant Cell* **9,** 37–47.
24. Valvekens, D., Van Montagu, M. & Van Lijsebettens, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5536–5540.
25. Yadegari, R., Paiva, G., Laux, T., Koltunow, A. M., Apuya, N., Zimmerman, J. L., Fischer, R. L., Harada, J. J. & Goldberg, R. B. (1994) *Plant Cell* **6,** 1713–1729.
26. Naito, S., Dubè, P. H. & Beachy, R. N. (1988) *Plant Mol. Biol.* **11,** 109–123.
27. Koornneef, M., de Bruine, J.-H. & Goettsch, P. (1980) *Arab. Inf. Serv.* **17,** 11–18.
28. Van der Veen, J. H. & Wirtz, P. (1968) *Euphytica* **17,** 371–377.
29. Dawson, J., Wilson, Z. A., Aarts, M. G. M., Braithwaite, A. F., Briarty, L. G. & Mulligan, B. J. (1993) *Can. J. Bot.* **71,** 629–638.

30. Alonso-Blanco, C., Blankestijn-de Vries, H., Hanhart, C. J. & Koornneef, M. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 4710–4717.
31. Esau, K. (1997) *Anatomy of Seed Plants* (Wiley, New York), 2nd Ed.
32. Heath, J. D., Weldon, R., Monnot, C. & Meinke, D. W. (1986) *Planta* **169,** 304–312.
33. Pang, P. P., Pruitt, R. E. & Meyerowitz, E. M. (1988) *Plant Mol. Biol.* **11,** 805–820.
34. van der Klei, H., Van Damme, J., Casteels, P. & Krebbers, E. (1993) *Plant Physiol.* **101,** 1415–1416.
35. Downey, R. K. (1983) in *High and Low Erucic Acid Rapeseed Oils*, eds. Kamer, J., Sauer, F. & Pigden, W., (Academic, New York).
36. James, D. W., Jr., & Dooner, H. K. (1990) *Theor. Appl. Genet.* **80,** 241–245.
37. Taylor, D. C., Giblin, E. M., Reed, D. W., Hogge, L. R., Olson, D. J. & MacKenzie, S. L. (1995) *J. Am. Oil Chem. Soc.* **72,** 305–308.
38. Westoby, M., Leishman, M. & Lord, J. (1996) *Philos. Trans. R. Soc. London B.* **351,** 1309–1318.
39. Armstrong, D. P. & Westoby, M. (1993) *Ecology* **74,** 1092–1100.
40. Kolawole, G. O. & Kang, B. T. (1997) *Commun. Soil Sci. Plant Anal.* **28,** 223–1235.
41. Milberg, P. & Lamont, B. B. (1997) *New Phytol.* **137,** 665–672.
42. Vera, M. L. (1997) *Plant Ecol.* **133,** 101–106.
43. Fenster, C. B. (1997) *Aquat. Bot.* **56,** 215–231.
44. Moegenburg, S. M. (1996) *Oecologia* **106,** 539–543.
45. Lopes, E. C. A., Destro, D., Montalvan, R., Ventura, M. U. & Perez-Guerra, E. (1997) *Euphytica* **97,** 161–166.
46. Ohto, M.-a., Fischer, R. L., Goldberg, R. B., Nakamura, K. & Harada, J. J. (2005) *Proc. Natl. Acad. Sci. USA* **102,** 3123–3128.
47. Zeevaart, J. A. D. (1983) in *The Biochemistry and Physiology of Gibberellins,* ed. Crozier, A. (Praeger Publishers, New York), Vol. 2.
48. Taiz, L. & Zeiger, E. (2002) *Plant Physiology* (Sinauer Assoc., Sunderland, MA), 3rd Ed.
49. Denis, L., Dominguez, J., Baldini, M. & Vear, F. (1994) *Euphytica* **79,** 29–38.
50. Eriksson, I., Westerlund, E. & Aman, P. (1994) *J. Sci. Food Agric.* **66,** 233–240.
51. Hartwig, E. E., Kuo, T. M. & Kenty, M. M. (1997) *Crop Sci.* **37,** 770–773.