A mutation at *AP2* locus of *Arabidopsis* confers spermine resistance

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Abstract

A spermine resistant mutant of *Arabidopsis thaliana* (L.) Heynh. was isolated from M_2 population of ethylmethanesulphonate-mutagenized seeds. The mutant was resistant to seed germination inhibition by spermine, but was as sensitive as the wild-type to spermidine and putrescine. In addition, the mutant displayed developmental abnormalities such as frequent cauline leaves, increased number of branches with inflorescence, reduced apical dominance, flowers subtended by bracts, disrupted floral organs with homeotic conversions. Genetic analysis indicated a single recessive nuclear mutation that was allelic to *apetala2-1* (*AP2-1*). The new mutant allele of *AP2* locus was accordingly numbered as *AP2-10*.

Additional key words: apetalous, germination, homeosis, mutant.

Introduction

Recent advances in molecular genetics have enabled clarify molecular mechanisms involved in flower development (Jack *et al.* 1993, Mandel and Yanofsky 1995). One approach has been to identify control genes by mutations that affect flower development. A number of such mutants have been isolated and characterized in *Arabidopsis thaliana* during the last several years (Meyerowitz *et al.* 1991, Jack *et al.* 1993). *Apetala2* (*AP2*) is a floral homeotic gene Meyerowitz *et al.* 1989). Mutations in *AP2* gene cause homeotic conversions in the outer two of the four whorls of the flower, the first whorl organs can be carpels, and the second whorl organs petaloid stamens, stamens, or absent. In addition, the organ number of all four whorls may be altered. Nine mutant alleles of *AP2* gene have been described in literature: *AP2-1* (Bowman *et al.* 1989), *AP2-2* (Bowman *et al.* 1991), *AP2-3* and *AP2-4* (Komaki *et al.* 1988), *AP2-5* to *AP2-7* (Kunst *et al.* 1989), *AP2-8* and *AP2-9* (Bowman *et al.* 1991).

Polyamines (putrescine, spermidine and spermine) are one group of plant growth

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substances that have been implicated in flower development. Malmberg and Rose (1987) regenerated plants from tissue culture cells of tobacco that had mutations in the polyamine biosynthesis. All of these plants had floral abnormalities including male or female sterility and homeotic conversions of floral organs. Gerates *et al.* (1988) found increased level of putrescine in a floral mutant of *Petunia*. Rastogi and Sawhney (1990) reported that floral organs of *stamenless-2* mutant of tomato contained higher levels of all three polyamines. Burtin *et al.* (1991) reported that floral abnormalities were produced in tobacco by cyclohexylamonium and methylglyoxal bis (guanylhydrazone), specific inhibitors of spermidine biosynthesis. In recent studies, free and bound polyamines were related to floral initiation and floral development in soybean, chrysanthemum and strawberry (Caffaro and Vicente 1994, Tarenghi and Martin-Tanguy 1995).

The present paper describes an independently arisen spermine-resistant mutant of *Arabidopsis* that displays *apetala2* phenotype.

Materials and methods

The wild-type strain of *Arabidopsis thaliana* race Landsberg homozygous for the *erecta* mutation was used in these studies. The mutant allele *apetala2-10* (*AP2-10*) was isolated from ethylmethanesulphonate-mutagenized (40 mM, 4 h) seeds on the basis of its ability to germinate in the presence of 0.35 mM spermine. This concentration of spermine causes 100 % inhibition of wild-type seed germination. Seeds were planted on a potting soil-sand (1:3) mixture saturated initially with the mineral solution described by Oostindier-Braaksma and Feenstra (1973). The plants were grown at 25 ± 2 °C under constant irradiance of 160 μmol m^{-2} s^{-1} at pot level white fluorescent tubes.

For dose-response experiments, wild type and mutant seeds (harvested 4 - 6 months ago) were sown on agar (1 %, m/v) plates containing a range of the growth regulator concentrations. There were 3 plates with 10 seeds of each genotype per plate for each treatment. After cold treatment (4 d, 4 °C, dark), the dishes were incubated (4 d, 25 ± 2 °C, constant light) with agar surface vertical. Spermine, spermidine and putrescine (purchased from *Sigma*) were dissolved in water.

For genetics analysis, the mutant plants were crossed with wild-type and the resulting F_1 and F_2 plants were scored for their phenotypes. For complementation analysis, plants were crossed with several marker lines displaying similar phenotypes and the resulting F_1 plants observed for their phenotypes. The marker lines NW28 (*AP1-1*) and NW29 (*AP2-1*)were gifts from the Nottingham Arabidopsis Stock Centre. Mutants *lfy-5* and *lfy-6* were a generous gift of E.M. Meyerowitz (Division of Biology, California Institute of Technology, Pasadena, U.S.A.).

Results and discussion

Mutant *AP2-10* was isolated as a spermine-resistant line of *A. thaliana*. Compared to wild-type, the mutant *AP2-10* was consistently resistant to spermine inhibition of

seed germination and root growth (Fig. 1*A*). At 0.25 mM spermine, wild-type and *AP2-10* had 40 an 100 % seed germination respectively, whereas root growth was 1.72 and 10.06 % respectively. At 0.35 mM spermine, wild-type and *AP2-10* had 0 and 100 % seed germination, whereas root growth was 0 and 2.31 %, respectively. On the other hand, the mutant line *AP2-10* was as sensitive as the wild

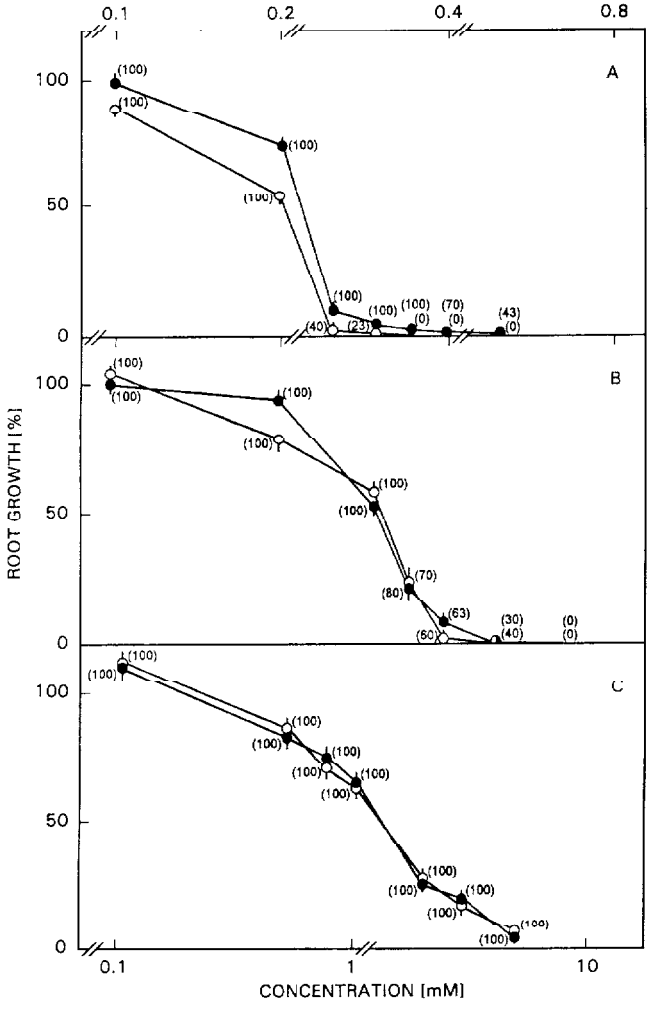

Fig. 1*A-C*. Effect of spermine (*A*), spermidine (*B*), and putriscine (*C*) on root growth of wild-type (*open circles*) and mutant *AP2-10* (*closed circles*) of *Arabidopsis*. Measurements were taken after 4 d incubation in continuous light. Mean root lengths ± SEM at each concentration are expressed as percentage of control (without growth regulator). Each value represents the mean of measurement for 30 seedlings. Control means (mm ± SEM) were (*A*) WT − 4.99 ± 0.14, *AP2 10* − 4.83 ± 0.20; (*B*) WT = 4.05 ± 0.10, *AP2-10* = 4.15 ± 0.26; (*C*) WT = 5.23 ± 0.27, *AP2-10* = 4.89 ± 0.30. Values in parentheses at each concentration are seed germination percentages.

type to spermidine and putrescine (Figs 1*B*,*C*). The mutant line showed consistent spermine resistance when the next generation was retested.

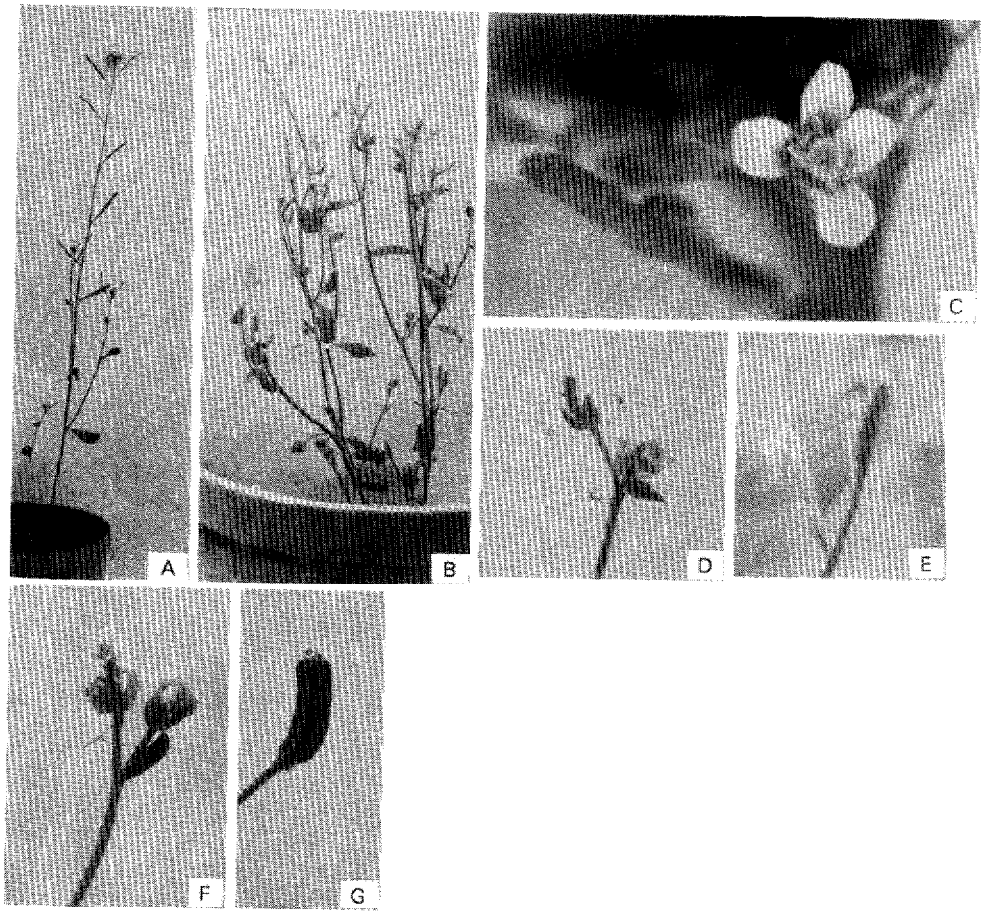

Fig. 2. Phenotypes of wild-type and mutant *AP2-10* of *Arabidopsis*. (*A*) eight week old wild type plant, (*B*) eight week-old *AP2-10* plant, (*C*) wild-type flower, (*D*) *AP2-10* flower showing staminoid petals, (*E*) *AP2-10* flower showing one bract, one petal and two carpels, (*F*) *AP2-10* flower showing one bract and several carpels, and (*G*) *AP2-10* tetralocular pod.

In addition to spermine resistance, the mutant *AP2-10* also exhibits a distinct developmental phenotype. Compared to wild-type, the mutant cauline leaves are broader and more frequent. Wild-type plants normally produce single primary inflorescence and 3 - 4 secondary floral branches (Fig. 2*A*), whereas *AP2-10* plants produce upto 7 primary inflorescences and 30 secondary floral branches (Fig 2*B*). The secondary floral branches often grow longer than the primary inflorescences. This growth pattern of *AP2-10* produces a bushy plant with corymbose raceme

inflorescence instead of a raceme of wild-type plants. The *AP2-10* phenotype thus exhibits a reduction in apical dominance. Inflorescence heads of *AP2-10* contain fewer flowers/buds compared to wild-type plants. Bracts are characteristically lacking in wild-type *Arabidopsis* whereas they are generally present upto the top of floral branches in *AP2-10* plants (Figs. 2*A,E,F*).

All four whorl organs of *AP2-10* flowers are apparently affected. The outermost whorl consist of 4 - 6 sepals. The second whorl organs are often absent, but rarely 1 - 5 petals are present. The third whorl organs are usually missing, but rarely 1 - 4 fertile stamens are present which do not elongate to the stigma level thus necessitating manual selfing. The fourth whorl organs consist of 2 - 3 or 4 locular pistil (Fig. 2*G*). Floral organs of *AP2-10* frequently display homeotic transformations such as carpeloid sepals, petaloid sepals, carpeloid petals, staminoid petals, carpeloid stamens (Figs. 2*D-F*). Carpels are thus dominantly formed in *AP2-10* and flowers usually consist of sepals and carpels only.

Table 1. Genetic analysis of spermine-resistant *Arabidopsis* line *AP2-10*.

Crosses female × male	Generation	Phenotype wild-type	*AP2-10*	intermediate
AP2-10 × wild-type	F_1	19	0	0
	F_2	76	23	0
AP2-10 × *lfy-5*	F_1	21	0	0
lfy-6 × *AP2-10*	F_1	17	0	0
AP2-10 × *AP1-1*	F_1	17	0	0
AP2-10 × *AP2-1*	F_1	0	0	18

The genetic basis for the mutant phenotype was determined by crossing the mutant line to wild-type plants and scoring the phenotype of F_1 and F_2 progeny (Table 1). For this purpose, the seeds were sown on agar without or with 0.30 mM spermine (slightly lower concentration was used to allow better survival of the resistant seedlings). After 4 d incubation (25 ± 2 °C, constant light) all the seedlings from plane agar and only resistant ones from 0.30 mM spermine were transferred to pots and grown further to score their phenotypes. All of the heterozygous F_1 progeny had wild-type phenotype, whereas F_2 progeny segregated in a ratio of three wild-type to one mutant phenotypes. This suggest a single recessive nuclear mutation controlling the mutant *AP2-10* phenotype.

The spermine-resistant mutant line *AP2-10* was also subjected to complementation analysis for determining allelism with other mutants having related phenotypes (Table 1). When *AP2-10* was crossed with apetala 1 (*ap1-1*), leafy-5 (*lfy-5*), or leafy-6 (*lfy-6*), the F_1 progeny all were normal indicating non-allelism. However, F_1 progeny of the cross *AP2-10* with *AP2-1* displayed intermediate phenotype. This suggests that *AP2-10* is allelic to *AP2-1*. Consequently, the new spermine-resistant allele of the gene *AP2* was numbered *AP2-10*.

The new mutant *AP2-10* exhibits in addition to the spermine-resistance, the developmental abnormalities similar to those of *apetala* and *leafy* mutants.

Complementation analysis indicated allelism between *AP2-10* and the floral homeotic mutant *AP2-1*. Already known *AP2* alleles range from weak to strong with respect to the degree of homeotic conversions of floral whorl organs (Bowman *et al.* 1991). Present allele *AP2-10* appears to be a moderate allele when compared with other alleles of *AP2*.

The *AP2-10* allele displays resistance to spermine but is normal in its response to spermidine and putrescine. It can be speculated that the genetic lesion may have affected either the biosynthetic pathway of polyamines (Evans and Malmberg 1989) after spermidine synthesis or spermine action. This new function of *AP2* gene indicates an involvement of spermine in floral development. Present results thus support other reports (Malmberg and Rose 1987, Caffaro and Vicente 1994, Tarenghi and Martin-Tanguy 1995) that suggest polyamine involvement in floral development. Present study further discriminates spermine from other polyamines. Specific roles of different polyamines have been indicated by few other reports (Mirza and Bagni 1991, Serrano *et al.* 1995).

References

Bowman, J.L., Smyth, D.R., Meyerowitz, E.M.: Genes directing flower development in *Arabidopsis*. - Plant Cell **1**: 37-52, 1989.

Bowman, J.L., Smyth, D.R., Meyerowitz, E.M.: Genetic interactions among floral homeotic genes of *Arabidopsis*. - Development **112**: 1-20, 1991.

Burtin, D., Martin-Tanguy, J., Tepfer, D.: DL-difluoromethylornithine, a specific, irreversible inhibitor of putrescine biosynthesis, induces a phenotype in tobacco similar to that ascribed to the root-inducing, left-hand transferred DNA of *Agrobacterium rhizogenes*. - Plant Physiol. **95**: 461-468, 1991.

Caffaro, S.V., Vicente, C.: Polyamine implication during soybean flowering induction and early reproductive transition of vegetative buds. - Plant Physiol. Biochem. **32**: 391-397,1994.

Evans, P.T., Malmberg, R.L.: Do polyamines have roles in plant development? - Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 235-269, 1989.

Gerates, A.G.M., Kaye, C., Collins, C., Malmberg, R.L.: Polyamine levels in *Petunia* genotypes with normal and abnormal floral morphologies. - Plant Physiol. **86**: 390-393, 1988.

Jack, T., Sieburth, L.E., Meyerowitz, E.M.: Genes that control flower development in *Arabidopsis*. - In: Seminars in Developmental Biology. Vol. 4. Pp. 51-63. Academic Press, New York 1993.

Komaki, M.K., Okada, K., Nishino, E., Shimura, Y.: Isolation and characterization of novel mutants of *Arabidopsis thaliana* defective in floral development. - Development **104**: 195-203, 1988.

Kunst, L., Klenz, J.E., Martinez-Zapater, J. Haughn, G.W.: *AP2* gene determines the identity of perianth organs in flowers of *Arabidopsis thaliana*. - The Plant Cell **1**: 1195-1208, 1989.

Malmberg, R.L., Rose, D.J.: Biochemical genetics of resistance to MGBG in tobacco: mutants that alter SAM decarboxylase or polyamine ratios, and floral morphology. - Mol. gen. Genet. **207**: 9-14, 1987.

Mandel, M.A., Yanofsky, M.F.: A gene triggering flower formation in *Arabidopsis*. - Nature **377**: 522-524, 1995.

Mirza, J.I., Bagni, N.: Effects of exogenous polyamines and difluoromethylornithine on seed germination and root growth of *Arabidopsis thaliana*. - Plant Growth Regul. **10**: 163-168, 1991.

Meyerowitz, E.M., Bowman, J.L., Brockman, L.L., Drews, G.N., Jack, T., Sieburth, L.E., Weigel, D.: Genetic and molecular model for flower development in *Arabidopsis thaliana*. - Development (Suppl.) **1**: 157-167, 1991.

Meyerowitz, E.M., Smyth, D.R., Bowman, J.L.: Abnormal flowers and pattern formation in floral development. - Development **106**: 209-217, 1989.

Oostinider-Braaksma, F.J., Feenstra, W.J.: Isolation and characterization of chlorate-resistance mutants of *Arabidopsis thaliana*. - Mutation Res. **19**: 175-183, 1973.

Rastogi, R., Sawhney, U.K.,: Polyamines and flower development in the male sterile stamenless-2 mutant of tomato (*Lycopersicon esculentum* Mill.). - Plant Physiol. **93**: 439-445, 1990.

Serrano, M., Martinez-Madrif, M.C., Riquelme, F., Romojaro, F.: Endogenous levels of polyamines and abscisic acid in pepper fruits during growth and ripening. - Physiol. Plant **95**: 73-76, 1995.

Tarenghi, E., Martin-Tanguy, J.: Polyamines, floral induction and floral development of strawberry (*Fragaria ananassa* Duch.). - Plant Growth Regul. **17**: 157-165, 1995.