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PLANT HORMONE MUTANTS OF *ARABIDOPSIS THALIANA*

JAVED I. MIRZA

Institute of Biology,
Bahauddin Zakariya University, Multan, Pakistan.

Abstract

Mutant lines of *Arabidopsis thaliana* resistant to either spermine (a polyamine), naphthalene acetic acid (an auxin), or benzyl adenine (a cytokinin) were isolated by screening for growth of seedlings in the presence of growth inhibitory concentrations of the respective growth regulator. In addition to growth regulator resistance, mutant lines displayed distinct developmental phenotypes including alterations of the stature, seed dormancy, hypocotyls, roots, leaves, flowers and pods. Genetic evidence indicated that the mutant phenotype in 19 mutant lines was due to single recessive nuclear mutations.

Introduction

Plant hormones like auxins, gibberellins, cytokinins, abscisic acid, ethylene and polyamines affect plant development from seed germination to seed formation (Bagni, 1986; Galstone & Kaur-Sawhney, 1990; Mengoli *et al.*, 1992; Van Loon & Bruinsma, 1992). An understanding of the hormonal action at the subcellular level is of fundamental importance in developmental plant physiology. However, despite a very great deal of effort, the biosynthetic pathways of plant hormones are poorly understood and their precise physiological roles and mechanisms of action are not clear (Scott, 1990; Venis & Napier, 1991; Reid, 1993). The characterization of mutants blocked in some step of hormone biosynthesis or action is a promising genetic approach to the study of these hormones (Koornneef *et al.*, 1984; Scott, 1990; Van Loon & Bruinsma, 1992; Reid, 1993).

Although a number of single gene mutants have been reported in higher plants that are affected either in biosynthesis or response to auxins, gibberellins, abscisic acid or ethylene (King, 1988; Scott, 1990; Reid, 1993), but there is no existing mutant of cytokinins or polyamines among hihger plants. Studies were therefore carried out to induce and isolate mutants of *Arabidopsis thaliana* (a small cruciferous weed) affected in their response to polyamines, auxins or cytokinins.

Materials and Methods

Plant hormone mutants were induced and isolated in *Arabidopsis thaliana* L., ecotype Landsberg (*erecta*). The parent plant is designated here as wild-type. About 7,500 wild-type seeds were mutagenized using the chemical mutagen ethylmethane sulphonate (40 mM, 4h). These M_1 seeds were raised and M_2 seeds harvested in groups of 7-8 plants per seed bag which were numbered 1 to 1000. The isolation number of mutants refers to the M_2 seed bag number.

ed as resistant mutants. Response of the mutant lines to various growth regulators was determined by dose-response experiments. For genetic characterization, the mutants were crossed with wild-type plants and the resulting F_1 and F_2 generations scored for their phenotype.

Results

The screening criteria used for the isolation of hormone mutants of *A. thaliana* proved successful. Consequently, a number of mutant lines resistant to spermine, NAA or BA have been isolated. Each of these mutants is also characterised by some developmental abnormality.

The response of the mutants to increasing concentrations of growth regulator was studied by dose-response experiments. Fig.1 shows an increased resistance of the mutant line N527-1 to NAA. At each auxin concentration, the mutant root growth was increased compared to the wild-type. Both the seed germination and root growth of Spm 96 was resistant to spermine compared to that of wild-type (Fig.2). At 0.4 mM spermine concentration, mutant seed germination was 80% whereas none of the wild-type seeds germinated.

Each hormone mutant showed a distinct developmental phenotype such as dwarf or semi-dwarf stature, yellow seed colour, precocious germination, agravitropic roots, absence or abundance of root hairs, long hypocotyls, twisted shoot, reduced apical

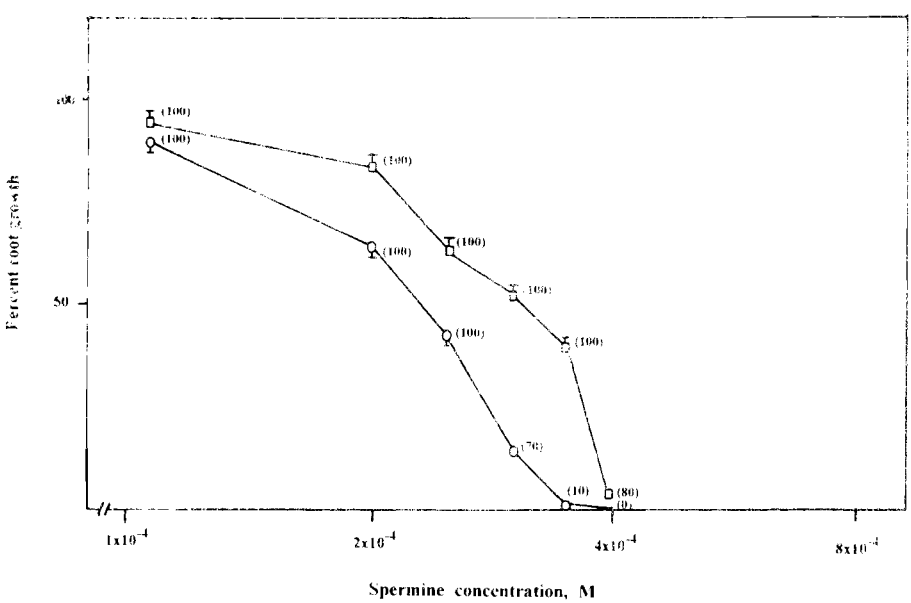

Fig. 2. Effect of spermine on seed germination and root growth of wild-type (O) and mutant line Spm 96 (□). Each value represents mean root length ± SEM of 30 seedlings expressed as percent of control (without spermine) mean. Control means (mm ± SEM) were, wild-type = 5.02 ± 0.18; Spm 96 = 3.42 ± 0.15. Percent seed germination at each concentration is given in parentheses.

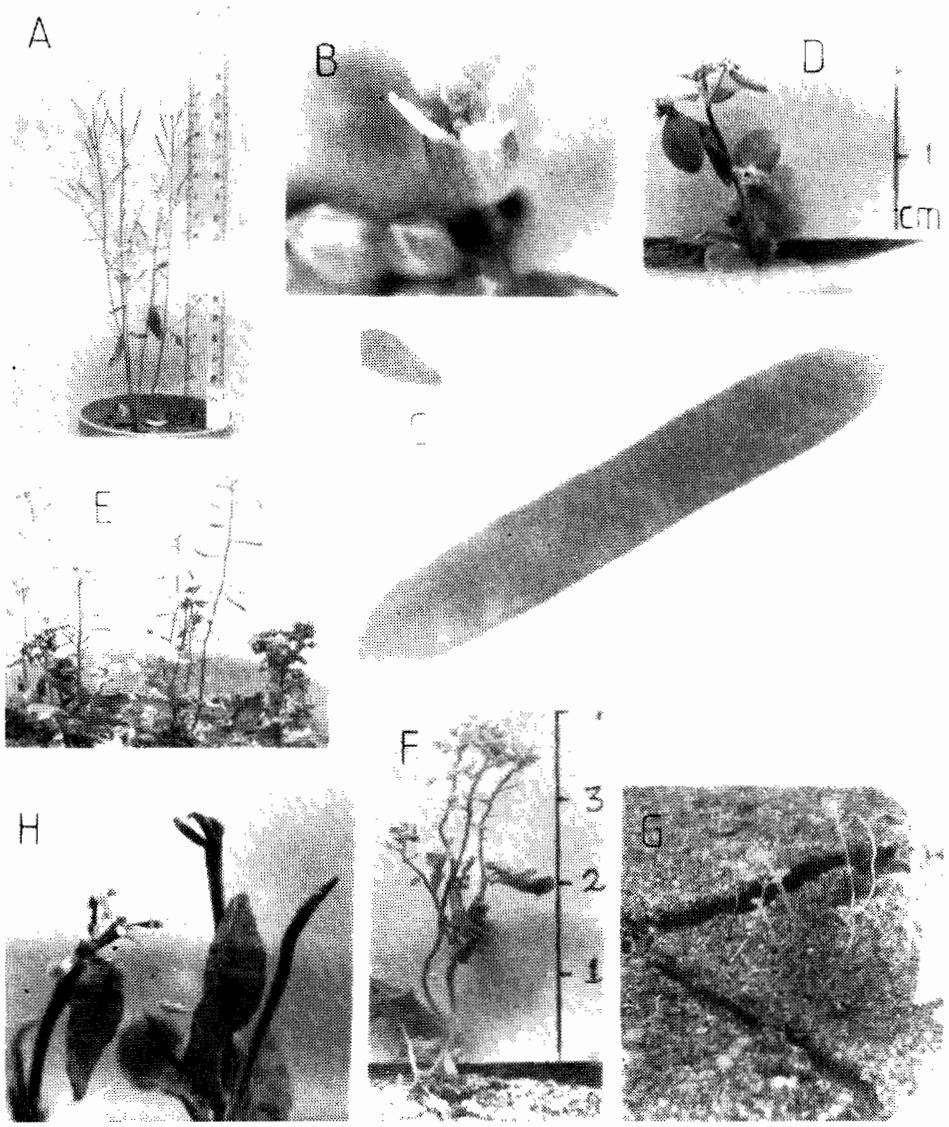

Fig.3 (A-H). Phenotype of wild-type and hormone resistant mutant lines of *Arabidopsis thaliana*: (A) A wild-type plant. (B) A wild-type inflorescence, (C) A wild-type pod, (D) Spm 515 plants showing dwarf stature, (E) Spm 171 plants showing semi-dwarf stature, (F) Spm 935-3 plant showing twisted stems and leaves, (G) Spm 918-7 seedligns showing long hypocotyls, (H) P 722-1 showing large, thick leaves and malformed flowers.

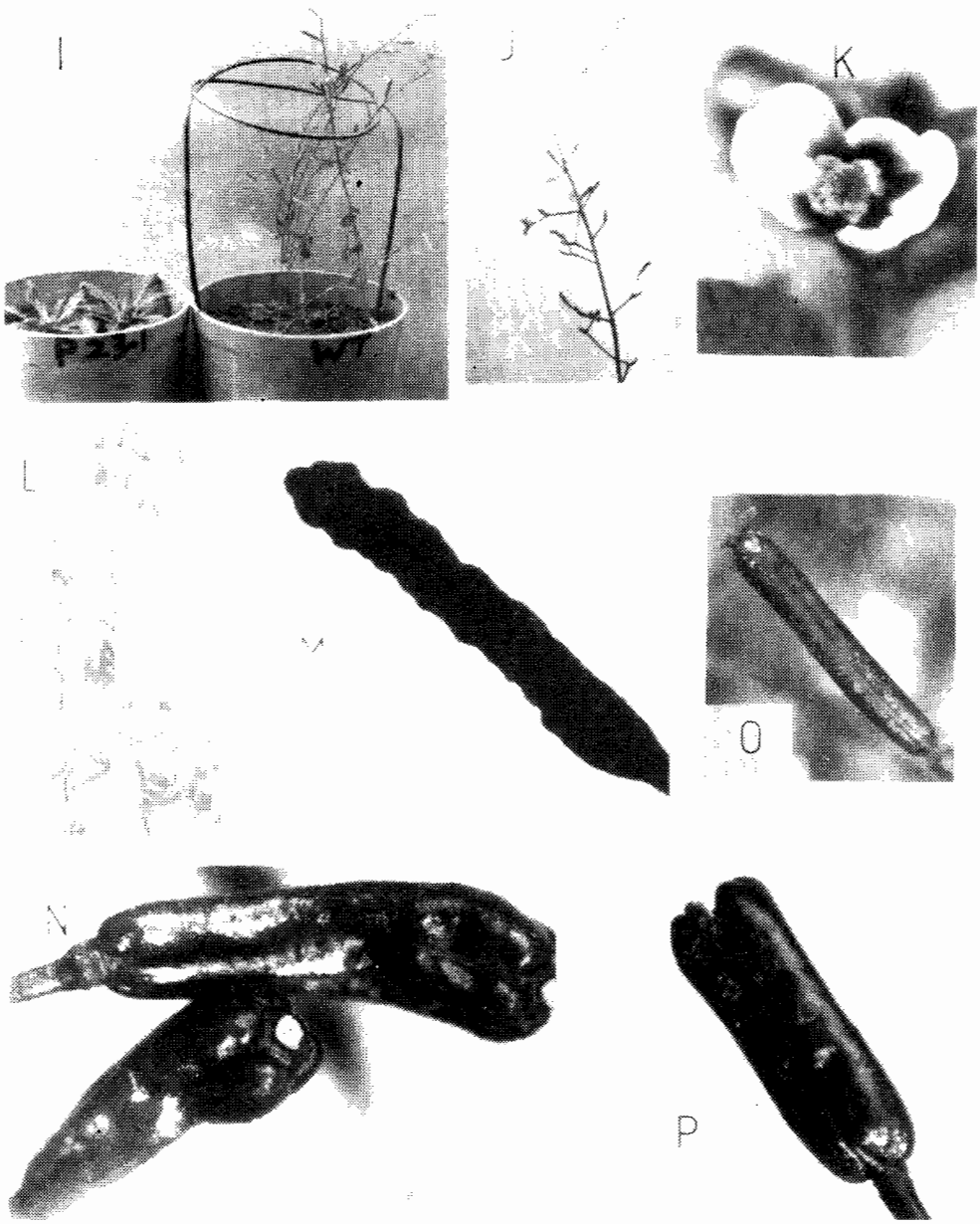

Fig.3. (I-P). Phenotype of wild-type and hormone resistant mutant lines of *Arabidopsis thaliana*: (I) wild type and P 23 1 plants of same age, (J) Spm 849 inflorescence branch showing multiple pod formation. (K) Spm 507 flower with seven petals, (L) BA 253 plants showing pendulant pods. (M) Spm 916-2 crinkled pod. (N) Spm 898-1 club-shaped tetralocular pods (O) Spm 251 pod with bifid stigmatic remains. (P) Spm 916-3 pod with sunken stigmatic remains.

dominance, frequent branching with numerous cauline leaves, variation in leaf size and shape, absence of trichomes, late flowering, deformed flowers, variation in the number of floral organs, homeotic conversions of floral organs, inconspicuous or conspicuous petals, male or female sterility, bifid or sunken stigma, crinkled or club-shaped pods, poor seed set, etc. Some of these developmental abnormalities are compared with wild-type (Fig.3A-P).

A number of hormone mutants were affected in fertility (Table 1). They showed a range of reproductive abnormalities and are categorized either as male or female sterile. Some hormone mutants were affected in photomorphogenesis (Table 2). Wild-type seedlings grown in darkness are etiolated and have long hypocotyls, whereas those grown in continuous light have short hypocotyls. The inhibitory effect of light on hypocotyl growth is reduced or absent in photomorphogenetic mutants which have long hypocotyls even when grown in continuous light. The mutant Spm 918-7 hypocotyls are about four times longer than wild-type hypocotyls when grown in continuous light.

Mutant lines N 882-1 and N 740-1 showed short root hairs about half in length compared to that of wild-type (Table 3). On the other hand, mutant line N 154 was altogether lacking in root hair.

The genetic basis of hormone resistance was studied by crossing each mutant line with wild-type and scoring both phenotypes in the F_1 and F_2 generations. Segregation data for one of the mutant line N 527-1 is presented in Table 4. All the F_1 plants were susceptible to NAA and segregation of F_2 into susceptible and resistant phenotypes fits the expected raio of 3 to 1. This suggests that the NAA resistance of mutant line N 527-1 is due to a single recessive mutation. Segregation data for 18 other mutant lines (data not shown) indicate single recessive mutations.

Table 1. Hormone mutants of *Arabidopsis thaliana* characterized by sterility.

Muant line	Phenotype
N 882-2	Stamens absent-male sterile
Spm 164	Stamens rare, remain smaller than carpels, need manual selfing-male sterile and self-sterile.
N 154	Anthers withering earlier-male sterile.
N 201	Stamens remain smaller than carpels and not maturing-male sterile
Spm 979-2	Stamens fertile but diverged, need manual selfing-self sterile.
Spm 151-3	Stamens fertile but diverged, stigma bifid and hairy, manual selfing or pollination does not result in seed set-female sterile.
Spm 251	Stamens fertile and normal, stigma bifid and non-hairy, manual selfing or pollination does not result in seed set-female sterile.

Table 2. Hypocotyl lengths of *Arabidopsis* seedlings germinated and grown during four days incubation in light or darkness.

Plant line	Mean hypocotyl length (mm ± SEM) Light	Darkness
Wild-type	1.12 ± 0.04	7.48 ± 0.26
N 909	2.00 ± 0.17	7.15 ± 0.37
N 854	3.72 ± 0.21	6.74 ± 0.29
Spm 918-7	4.38 ± 0.24	6.58 ± 0.32

Discussion

Arabidopsis seed germination is very sensitive to spermine (Mirza & Bagni, 1991) whereas its primary root growth is sensitive to NAA (Maher & Martindale, 1980) and BA (Bourguin & Pilet, 1990). These criteria were therefore used to screen mutants resistant to seed germination inhibitory concentration of spermine or root growth inhibitory concentrations of NAA or BA. The resistance of mutant lines to different growth regulators was studied by dose-response experiments. The mutant phenotype in 19 lines is due to single recessive mutations.

Hormone resistance could be due to either reduced uptake, reduced transport, low level of endogenous PGR, or low level of PGR receptors. In view of the other developmental abnormalities and the method of assessing PGR resistance, these mutants do not appear to be uptake or transport mutants. More likely they could either be synthesis mutants or response mutants. In case of response mutants, one could speculate that the recessive mutations described here probably result in a loss of function, such as a reduction in the level of the PGR receptor, a reduction in the affinity of the PGR receptor or an alteration of subsequent steps in the response pathway.

In addition to the growth regulator resistance, each mutant line is also affected in some aspect/s of development. Some of these developmental abnormalities are comparable to those of other hormone mutants. For example, ABA insensitive mutants *abi1* and *abi2* of *Arabidopsis* (Koornneef *et al.*, 1984; Finkelstein, 1994), and *vpl* of maize (Robichaud *et al.*, 1980) exhibit precocious germination. One of the spermine-resistant mutant lines, Spm 213 also showed precocious germination. This suggests that like ABA, spermine may be involved in seed dormancy. Aberrant root hair development in the NAA-resistant mutants (Table 3) can be compared with complete lack of root hair in the auxin resistant mutants *dwf* (Mirza & Maher, 1987) and *axr2* (Wilson *et al.*, 1990). These mutants suggest auxin involvement in root hair development. A number of photomorphogenetic mutants of *Arabidopsis* are known that show reduced inhibition of hypocotyl growth by light (Koornneef *et al.*, 1980; Parks & Quail, 1993). These

Table 3. Maximum root hair lengths of *Arabidopsis* seedlings grown on the surface of agar during four days incubation in light.

Plant line	Maximum root hair length (mm ± SEM)
Wild- type	0.65 ± 0.06
N 882-1	0.30 ± 0.04
N 740-1	0.26 ± 0.04
N 154	0

mutants have altered phytochrome levels or responses. The long hypocotyl mutants given in Table 2 have modified responses to NAA or spermine and could provide useful information on the interaction of phytochrome and phytohormones. Male sterile mutants are known in many plants (Kaul, 1988; Dawson *et al.*, 1992). Cytokinins, gibberellins, auxins, abscisic acid and polyamines have been implicated in the regulation of male sterility (Shukla & Sawhney, 1992). Aarts *et al.*, (1993) have succeeded in isolating a male sterility gene, *MS2*, from a nuclear male sterile *Arabidopsis* mutant. The male sterile mutant lines shown in Table 1 would increase the spectrum of male sterility loci. Characterization of such mutants will increase our knowledge on stamen development and provide methods for the production of male sterile crop plants that will be useful in hybrid seed production.

Plant hormone mutants provide one of the most promising tools to study hormone physiology in higher plants (Reid, 1993). Present hormone mutants are affected in a number of developmental processes (including those given in Fig.3 and Table 1-3). Further characterization of these mutants would help us understand the developmental processes affected.

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Table 4. Genetic segregation of NAA resistance in mutant line N 527-1 of *Arabidopsis thaliana*.

Cross	No. of plants		
	Resistant	Susceptible	X^2
N 527-1 x Wild-type F_1	0	16	-
N 527-1 x Wild-type F_2	22	79	0.557[a,b]

[a] X^2 was calculated based on an expected ratio of 3 susceptible to 1 resistant.

[b] $P > 0.05$

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