Theor Appl Genet (1986) 72:602–605



# Free and bound indole-acetic acid is low in the endosperm of the maize mutant *defective endosperm-B18*

**G. Torti [1], L. Manzocchi [1] and F. Salamini [2]**

[1] Istituto Biosintesi Vegetali, Via Bassini 15, Milano, Italy
[2] Istituto Sperimentale per la Cerealicoltura, Via Stezzano 24, Bergamo, Italy

Received February 24, 1986; Accepted March 10, 1986
Communicated by H. F. Linskens

**Summary.** The maize mutant defective endosperm-B18 (*de*-B18*), which is recessive to its wildtype, accumulates substantially less dry matter in the endosperm than its normal counterpart. Both free and bound indole-acetic acid (IAA) content has been measured at 5 different developmental stages. In endosperm tissue, the level of IAA is at least 15 times lower in the mutant *de*-B18* than in the wildtype. The situation found in the diploid tissues is somewhat different: in the mature embryo the level of total IAA is lower in the mutant than in the wildtype, while in 4-day old seedlings the level of total IAA is, to some degree, similar in both genotypes. Naphthalene-acetic acid (NAA), a stable synthetic auxin which mimics IAA in its biochemical effects, is able to normalize the seed weight of the mutant when applied to developing grains. The results favor the conclusion that in maize endosperm the mutation *de*-B18* is involved in IAA metabolism.

**Key words:** Indole-acetic acid – Auxin – Defective endosperm mutants – Hormonal mutants – Seed development

## Introduction

Mutants affecting hormone levels have been, to some extent, useful for studying the physiology of hormone action in plants. In particular, the use of gibberellins and abscisic acid related mutants helped to clarify the physiology of dwarfism, and of stomatal behaviour and vivapary in several plants like maize and tomato (reviewed in Marx 1983; Walton 1980).

The recognition and isolation of mutants with altered levels of auxin or with abnormal responses to auxin application have been more difficult. Only dominant lethal strains of tobacco resistant to naphthalene-acetic acid (Mueller et al. 1985) and mutants of *Arabidopsis thaliana* resistant to the synthetic growth regulator 2,4 dichloro-phenoxy-acetic acid (2,4-D) have been described (Maher and Martindale 1980; Mirza et al. 1984).

In this paper we report the discovery of a maize mutant which contains very low levels of free and bound IAA in the endosperm.

## Materials and methods

Plants of the inbred line B37+ (carrying a wildtype allele at the locus *De*-B18*) and of its isogenic version for the mutant allele *defective endosperm-B18* were grown in the field in 1983. B37+ and B37 *de*-B18* plants were selfed; F1 seeds between the two strains were also produced pollinating silks of B37 *de*-B18* plants with B37+ pollen. Selfed and F1 kernels were harvested at 12, 15, 20, 30 and 40 days after pollination (DAP). Immature seeds were frozen and before lyophilization endosperms were isolated from embryos and teguments.

Seedlings of the wildtype and mutant strains were grown in the dark at 27 °C for four days under sterile conditions, harvested under dim light, and frozen. When needed, mature embryos were dissected from dry seeds after 15 h of imbibition in ice-cold water.

All materials used for the analyses were freeze-dried and stored at −30 °C until use.

For the in vivo treatments with NAA, plants of the lines B37+ and B37 *de*-B18* were grown in the field in 1985. Pollinated ears between 13 and 16 DAP were treated as described by Britten (1947, 1950). The husks and silks were removed and two opposite vertical sectors were marked with pins. One sector was treated with a lanolin paste containing NAA and the second sector only with lanolin paste. Treatments were repeated every fourth day. NAA treated and untreated seeds were excised from the ear at 29 DAP and 32 DAP for B37+ and B37 *de*-B18,* respectively, and processed.

IAA analyses were performed as previously described (Torti et al. 1984). Lyophilized material was extracted with

70% acetone, and after evaporation under reduced pressure, the residue was made to 1M with NaOH and incubated at 30°C for one hour to hydrolize the bound IAA. The IAA was purified by repeated extractions with ethyl ether in both basic and acidic conditions. Analysis was performed by HPLC on a RP18 column using a spectrofluorimetric detection procedure.

## Results

### *IAA content in normal and mutant seeds*

The accumulation of dry matter (d.m.) by the developing seeds of the B37+, *de*-B18* and of their F1 B37 *de*-B18*×B37+ from 16 to 40 DAP is reported in Fig. 1. At all stages investigated the *de*-B18* mutant accumulated significantly less dry matter than its norml counterpart. The figure also shows that the F1 kernels originating from B37 *de*-B18* plants when silks were fertilized by B37+ pollen, were similar to the wildtype in their capacity to accumulate dry matter. This indicates that the mutant allele is completely recessive to the wildtype.

The content of total indole acetic acid (free + the fraction hydrolized by 1M NaOH) in the endosperm of the three examined genotypes is reported in Table 1. Five developmental stages covering the most important part of the grain filling period were considered, namely 12, 15, 20, 30, 40 DAP: at all stages the content of total IAA in B37 *de*-B18* was at least 15 times lower than in B37+. The values found for the hybrid endosperms B37 *de*-B18*×B37+ were very similar to those of B37+; it can therefore be stated that one single dose of the wildtype allele is capable of normalizing the low IAA content induced in the endosperm by the *de*-B18* mutation. When the total IAA present in B37+ and in B37 *de*-B18* endosperms was separated into fractions of free and bound IAA, it was observed that B37 *de*-B18*, while still having a far lower than normal content of free IAA, was almost deprived of IAA in the ester forms. Such analyses, done on mature endosperms showed contents of $1.13 \pm 0.35$ and $0.13 \pm 0.03$ mg free IAA/kg d.m. for B37+ and B37 *de*-B18*, respectively; the corresponding figures for the ester forms were $6.72 \pm 0.50$ versus $0.03 \pm 0.01$ (data not tabulated). The same situation was found during endosperm development; here, for instance, the values found at 20 DAP were $2.29 \pm 0.35$ and $2.45 \pm 0.06$ mg free IAA/kg d.m., respectively for B37+ and B37 *de*-B18*, while the bound forms were $17.48 \pm 4.7$ versus $0.14 \pm 0.04$.

In addition, the effect of the *de*-B18* mutation was studied in diploid tissues: Table 2 shows data for total IAA content in mature embryos and in 4-day old seedlings germinated in the dark. While in the embryo the level of total IAA was by far much lower in the mutant than in the wildtype, this was not true for etiolated seedlings. Here the levels of total IAA were much more similar in both wildtype and mutant. In the embryo, a separation of total IAA into the free and bound fractions gave values of $0.9 \pm 0.1$ mg/kg d.m. in B37+ and $0.08 \pm 0.003$ in B37 *de*-B18*. The purification of extracts from 4-day old seedlings was more difficult; under the conditions adopted, the free IAA content found in B37 *de*-B18* was 70% of the value found for B37+.

**Table 1.** Total IAA content in developing endosperms of genotypes B37+, B37 *de*-B18* and B37 *de*-B18*×B37+ (mg IAA/kg d.m. ±SE in brackets)

| Genotype | Stage of development (DAP) | | | | |
|---|---|---|---|---|---|
| | 12 | 15 | 20 | 30 | 40 |
| B37+ | 88 (±4) | 48 (±9) | 52 (±6) | 31 (±6) | 15 (±6) |
| B37 *de±-B18* | 6 (±0.2) | 0.6 (±0.1) | 0.4 (±0.1) | 0.3 (±0.07) | 0.3 (±0.04) |
| B37 *de*-B18* ×B37+ | 75 (±12) | 81 (±13) | 51 (±2) | 21 (±2) | 21 (±3) |

### *Normalizing effect of NAA treatments on de*-B18 seeds*

Experiments were performed to induce a normalization of the seed weight in *de*-B18*. The assumption was made that the defect in the seed weight was the direct effect of the limited capacity of *de*-B18* to accumulate IAA. To verify this point, NAA, a synthetic auxin which mimics IAA in its biochemical function and which is not degraded to such an high extent as IAA, was applied to developing seeds. Three sets of preliminary

**Table 2.** Total IAA content (mg IAA/kg d.m. ±SE in brackets) in mature embryo and in germinating seedlings[a] of gentoypes B37+ and B37 *de*-B18*

| Genotype | Dry weight (mg) | | Total IAA content (mg/kg d.m.) | |
|---|---|---|---|---|
| | Embryo | Seedlings | Embryo | Seedlings |
| B37+ | 20.7 | 18.4 | 1.4 (±0.1) | 0.94 (±0.3) |
| B37 *de*-B18* | 11.1 | 13.2 | 0.16 (±0.003) | 0.48 (±0.3) |

[a] Germination was for 4 days at 27°C in the dark

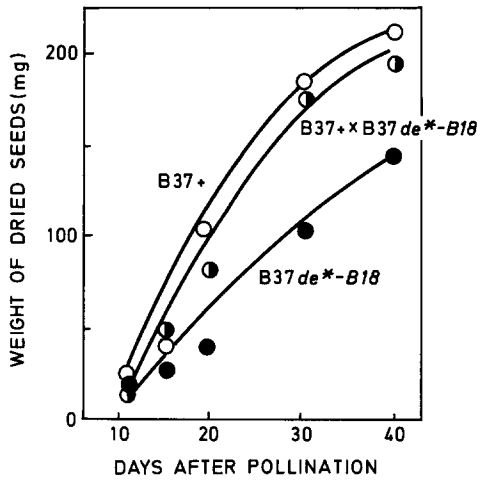


**Fig. 1.** Accumulation of dry matter in the seeds of B37+, B37 *de*-B18* and B37 *de*-B18*×B37+. Data shown are the mean of three samples of 20 seeds obtained from the central part of different ears.

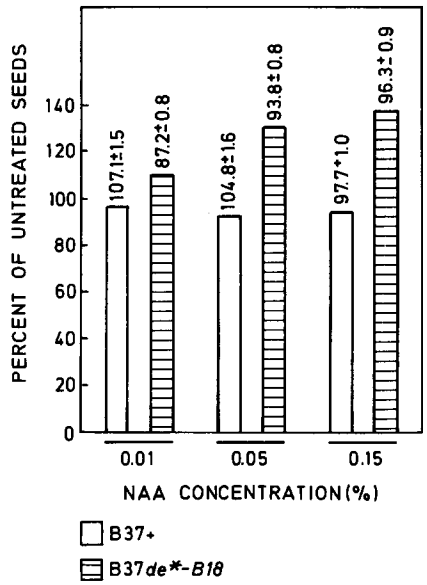


**Fig. 2.** Normalizing effects of NAA treatments on B37 *de*-B18* developing seeds. Data are expressed as % of untreated seeds made equal to 100. Absolute endosperm weight of treated kernels (mg) are also reported at the top of the histograms. Data have been obtained from mean values of ten endosperms from 3 ears. For the line B37+ ears were treated at 13, 17, 20 and 24 DAP and harvested at 29 DAP; for B37 *de*-B18*, at 16, 20, 23 and 27 DAP and harvested at 32 DAP

experiments were performed: one in a growth chamber and two in a greenhouse. Ears were treated with 0.05% NAA diluted in lanolin paste as described under methods. In these three experiments the endosperm weight of the treated *de*-B18* seeds increased by 11.5%, 22.0% and 21.0% compared to untreated seeds. Under the same conditions the treated wildtype seeds showed a decrease in seed weight by 3.0, 3.0 and 2.5%.

In summer 1985 the normalizing effect of NAA was further tested in field experiments with three concentrations (0.01, 0.05 and 0.15%) of NAA. The results reported in Fig. 2 show that NAA was capable of inducing a substantial repair of the endosperm weight of B37 *de*-B18*. The larger increase in seed weight observed in the mutant corresponded to the highest concentration of NAA tested. As in the preliminary experiments, the weight of the wildtype endosperms treated with NAA resulted in a slight loss compared with the untreated ones. It is interesting to note that at 0.15% NAA, the absolute weights of NAA treated wildtype and mutant endosperms were almost identical.

## Discussion

The search for viable mutations interfering with the development of the maize endosperm lead to the isolation of a series of mutants which exhibit a modified capacity of the endosperm to accumulate dry matter (Manzocchi et al. 1980a, b). The same mutations were also considered when searching for genes inducing disturbances in the level of the phytohormone indole-3-acetic acid (Torti et al. 1984). In Torti et al. 1984 the mutation *de*-B18* was suggested to be a putative IAA mutant because of its low level of free and bound IAA in mature endosperm.

The data presented here add to the evidence that *de*-B18* is indeed a mutation interfering with IAA metabolism. In the mutant endosperm tissue a low level of this phytohormone in its free or bound form is in fact evident at stages which cover the most important part of seed development. Moreover, we have found that a synthetic auxin can compensate the growth capacity of the mutant seed. This provides direct evidence that it is the low level of endogenous IAA that leads to an abnormal seed development in the *de*-B18* line.

It is interesting to note that apparently the effect of the *de*-B18* mutation is restricted to the developmental phases of seed formation. In this respect both mutant endosperm and embryo are low in free and bound IAA. However, as soon as *de*-B18* seeds germinate into seedlings, the IAA increases to levels which are more similar to those of the normal genotype. It may be suggested that the low level of IAA in *de*-B18* developing embryos is due to the lower than normal synthesis of bound IAA in the endosperm tissue. As a matter of fact, it has been suggested that IAA migrates in its bound form between different compartments of

the maize seed (Pangelly and Bandurski 1981; Cohen and Bandurski 1982).

Although the experiments described in this paper were originally not designed to reveal specific aspects of the physiology of IAA in the maize seed, they nevertheless can offer some insight into this matter. The hypothesis of Skoog (1937), that during germination auxin precursors migrate from the caryopsis to the coleoptiles where free auxin, the hormonally active compound (Bialek et al. 1983), is liberated, has never been fully accepted. The group of Bandurski produced data which favour the hypothesis that bound auxins are at least partly involved in the supply of active free IAA necessary for the growing of young seedlings (Bandurski 1982; Cohen and Bandurski 1982; Momonoki et al. 1983; Momonoki and Bandurski 1984). Other reports, however, suggest that active IAA in germinating seedlings of maize is directly synthesized from other sources than bound IAA (Ino and Carr 1982; Weiler and Wischniewki 1984), a situation similar to the one found for *Avena* coleoptiles (Jackson and McWha 1983).

Our data indicate that a very low level of free and bound IAA in the seed, as in the case of *de*-B18,* do not prevent the germination and the vitality of the seedling to a dramatic extent. This favours the view that during germination the young seedling is an autonomous synthesizing system of active IAA. The last part of our discussion illustrates the kind of problems that mutations affecting IAA metabolism can help clarify. In particular, this mutant should be very useful as an experimental tool in the "in vivo" studies of the effect of IAA on gene expression.

*Acknowledgement.* We thank Prof. Dr. C. Soave for the criticism and suggestions given in the course of our work.

## References

Bandurski RS (1983) Mobilisation of seed indole-3-acetic acid reserves during germination. In: Nozzolillo C, Lea PI, Loewus FA (eds) Mobilization of reserves in germination. Plenum Press, New York, pp 213–228

Bialek K, Mendt WJ, Cohen JD (1983) Indole-3-acetic acid (IAA) conjugates applied to bean stem sections. IAA content and the growth response. Plant Physiol 73: 130–134

Britten EJ (1947) The effect of naphthalene-acetic acid on the developing maize caryopsis. Am J Bot 34:211–217

Britten EJ (1950) Natural and induced parthenocarpy in maize and its relation to hormone production by developing seeds. Am J Bot 37:345–352

Cohen JD, Bandurski RS (1982) Chemistry and physiology of the bound auxins. Annu Rev Plant Physiol 33:403–430

Ino M, Carr DJ (1982) Sources of free IAA in the mesocotyl of etiolated maize seedlings. Plant Physiol 69:1109–1112

Jackson DL, McWha JA (1983) Translocation and metabolism of endosperm applied 〈2–14 C〉 indole-3-acetic acid in etiolated *Avena sativa* L. seedlings. Plant Physiol 73: 316–323

Maher EP, Martindale SJB (1980) Mutants of *Arabidopsis thaliana* with altered responses to auxins and gravity. Biochem Genet 18:1041–1053

Manzocchi LA, Daminati MG, Gentinetta E, Salamini F (1980) Viable defective endosperm mutants in maize. 1. Kernel weight, protein fraction and zein subunits in mature endosperms. Maydica 25:105–116

Manzocchi LA, Daminati MG, Gentinetta E (1980) Viable defective endosperm mutants in maize. 2. Kernel weight, nitrogen and zein accumulation during endosperm development. Maydica 25:199–210

Marx GA (1983) Developmental mutants in some annual seed plant. Annu Rev Plant Physiol 34:384–417

Mirza IT, Olsen GM, Iversen TH, Mahor FP (1984) The growth and gravitropic response of wildtype and auxin resistant mutants of *Arabidopsis thaliana.* Physiol Plant 60: 516–592

Momonoki YS, Schulze A, Bandurski RS (1983) Effect of deseedling on the indole-3-acetic acid content of shoots and roots of *Zea mays* seedlings. Plant Physiol 71:526–529

Momonoki YS, Bandurski RS (1984) Effect of endosperm removal on 7 normal NaOH-labile indole-3-acetic acid conjugates in shoots and roots of *Zea mays* seedlings. Plant Physiol 75:67–69

Mueller JF, Goujod J, Caboche M (1985) Isolation in vitro of naphthalene-acetic acid tolerant mutants of *Nicotiana tabacum,* which are impaired in root morphogenesis. Mol Gen Genet 199:194–200

Novacki J, Bandurski RS (1980) Myo-inositol esters of indole-3-acetic acid as seed auxin precursor of *Zea mays.* Plant Physiol 65:421–427

Pengelly WL, Bandurski RS (1981) Longitudinal distribution of indole-3-acetic acid in the cortex and vascular stele of the dark-grown *Zea mays* mesocotyl. Plant Physiol (Suppl) 67:2

Pengelly WL, Bandurski RS (1983) Analysis of indole-3-acetic acid metabolism in *Zea mays* using deuterium oxide as a tracer. Plant Physiol 73:445–449

Torti G, Lombardi L, Manzocchi LA, Salamini F (1984) Indole-3-acetic acid content in viable defective endosperm mutants of maize. Maydica 29:335–343

Walton DC (1980) Biochemistry and physiology of abscisic acid. Annu Rev Plant Physiol 31:453–484

Weiler EW, Wischniewki S (1984) The relationship between diffusible, extractable and conjugated (base-labile) forms of indole-3-acetic acid in isolated coleoptile tips of *Zea mays* L. Planta 161:30–32