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# Endogenous Abscisic Acid and Indole-3-Acetic Acid and Somatic Embryogenesis in Cultured Leaf Explants of *Pennisetum purpureum* Schum.[1]

## EFFECTS *IN VIVO* AND *IN VITRO* OF GLYPHOSATE, FLURIDONE, AND PACLOBUTRAZOL

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### ABSTRACT

**Effects of application *in vivo* of glyphosate, fluridone, and paclobutrazol to glasshouse-grown donor plants of *Pennisetum purpureum* Schum. on endogenous levels of abscisic acid (ABA) and indole-3-acetic acid (IAA) in young leaves and on somatic embryogenesis in cultured leaf explants were studied. Treatment of plants with glyphosate (100 milligrams per liter) resulted in elevated levels of endogenous ABA and IAA in young leaves. In contrast, paclobutrazol (50% active ingredient; 200 milligrams per liter) did not alter the endogenous levels of ABA and IAA. Fluridone (100 milligrams per liter) markedly inhibited synthesis of ABA and leaf explants from fluridone-treated plants lost the capacity for somatic embryogenesis. Explants from glyphosate- or paclobutrazol-treated plants did not show any reduction in embryogenic capacity when compared with untreated control plants. Glyphosate and fluridone were also incorporated into the culture media at various concentrations (0 to 20 milligrams per liter) to study their effects *in vitro* on somatic embryogenesis in leaf explants from untreated, field-grown plants. Glyphosate was inhibitory to somatic embryogenesis but only at concentrations above 5 milligrams per liter. Fluridone inhibited somatic embryogenesis at all concentrations tested. Inhibition of somatic embryogenesis by fluridone, by either *in vivo* or *in vitro* application, could be overcome partially by (±)-ABA added to the culture medium. Exogenous application of (±)-ABA enhanced somatic embryogenesis and reduced the formation of nonembryogenic callus. Application of IAA or gibberellic acid ($GA_3$; >5 milligrams per liter) was inhibitory to somatic embryogenesis. These results indicate that endogenous ABA is one of the important factors controlling the embryogenic capacity of leaf explants in Napier grass.**

It has been shown recently that the embryogenic capacity of leaf explants of *Pennisetum purpureum* Schum. (Napier or Elephant grass) that is closely related to the spatial and temporal gradients in young leaves is associated also with high levels of endogenous IAA and (+)-ABA (21). These relationships can be investigated further by experimental manipulation of endogenous levels of IAA and ABA using inhibitors of their biosynthesis. Although no specific inhibitors of IAA and ABA biosynthesis are known, it was recently shown that fluridone (1-methyl-3-phenyl-5-(3-[trifluoromethyl]phenyl)-4-(1*H*)pyridinone), an inhibitor of carotenoid biosynthesis (3, 6, 7), completely inhibited ABA synthesis in maize seedlings (18). Also, glyphosate (*N*[phosphonomethyl]glycine) treatment has been reported to promote IAA oxidation, thereby reducing endogenous IAA levels in tobacco callus and in soybean and pea seedlings (14, 15). Paclobutrazol (PP333; [2RS, 3RS]-1-[4-chlorophenyl]-4,4-dimethyl-2-[1,2,4-triazol-1-yl]pentan-3-ol), a triazole GA biosynthesis inhibitor (4, 20), was used to investigate the effect of reduced GA levels on embryogenic capacity.

Donor plants and isolated leaf explants of Napier grass were treated with glyphosate, fluridone, and paclobutrazol *in vivo* and *in vitro*. The effects of these treatments on endogenous levels of ABA and IAA in young leaves and the embryogenic capacity of leaf explants are presented in this report.

## MATERIALS AND METHODS

**Callus Cultures.** Callus cultures of *Pennisetum purpureum* Schum. (accession No. 15; a selection from a Brazilian cv Merkeron Menlo) were initiated from young, tightly rolled, innermost 3 to 4 leaves according to the procedures of Haydu and Vasil (11). The basal portion of young leaves (30 mm from the shoot meristem) was used in all the experiments for the preparation of leaf explants and for the analyses of endogenous growth regulators (21). The culture medium (PP medium) consisted of inorganic and organic constituents of Murashige and Skoog (19) medium supplemented with 0.5 mg/L 2,4-D, 0.5 mg/L BA, 1.0 mg/L naphthalene-1-acetic acid (NAA) and 5% (v/v) coconut milk. Growth regulators including coconut milk were added before autoclaving. Culture conditions were as described before (21). Evaluations of cultures were carried out under a stereomicroscope 30 d after initiation.

**Application of Glyphosate, Fluridone, and Paclobutrazol.** *Treatment in Vivo.* Young tillers of Napier grass, 5 to 7 d old, were transferred from the field to plastic pots (250 mm diameter) containing soil (GroMix 300, Grace Horticultural Products, Cambridge, MA). The potted plants were grown in a glasshouse (26 ± 2°C, photon flux density 350 $\mu E\ m^{-2}\ s^{-1}$) during August to October 1985.

One-month-old glasshouse grown plants with 12 to 15 leaves were irrigated with water containing glyphosate (100 mg/L) or fluridone (100 mg/L). Paclobutrazol (50% active ingredient, 200 mg/L) was suspended in a minimum volume of DMSO, 0.01% (v/v), and then made to the required volume with water before irrigation. Control plants were irrigated with water or water

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containing DMSO. Twelve plants were used for each treatment. Plants were irrigated daily to field capacity (approximately 500 ml/pot) for 5 d. Young leaves were harvested on the 6th d and were placed in culture on PP medium as described above. Explants from fluridone-treated plants were cultured on PP medium with or without exogenous ABA (5 mg/L). Half of the leaf material collected was stored at −80°C until required for analysis of endogenous growth regulators. Samples of explants and callus were removed from the culture dishes, rinsed in cold water, and stored at −80°C until required.

After growth measurements were taken from treated plants, they were cut back to 50 mm above the soil level. Growth measurements were also conducted on tillers from regrowth as described previously (22).

*Treatment in Vitro.* Leaf explants collected from untreated field-grown plants were used to study the effect of added growth regulators *in vitro*. Glyphosate and fluridone were dissolved in water and filter-sterilized (0.22 μm, Millipore Corp., Bedford, MA) prior to incorporation into the sterile culture medium. Due to solubility problems, effects of paclobutrazol *in vitro* were not studied; (±)-ABA, IAA, and $GA_3$ were first dissolved in a minimum volume of 70% (v/v) ethanol, brought to required volume with water, and filter-sterilized before adding to the culture medium. The following concentrations of (±)-ABA, IAA, $GA_3$, glyphosate, fluridone, or paclobutrazol were used in all the studies of *in vitro* treatment: 0, 0.02, 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 mg/L. A minimum of 100 explants were used at each concentration and the experiments were repeated once.

**Extraction and Analysis of Endogenous IAA and ABA.** The procedures of extraction and analysis of endogenous IAA and ABA were as described before (21). A minimum of three replicate analyses were carried out for each sample and the average sample size was 1 g fresh weight. The samples were spiked with [$^{14}$C] IAA (10.9 ng; 12 Bq/ng) and [$^{3}$H]ABA (0.07 ng; 5433 Bq/ng) to monitor the loss during extraction and analysis. Quantification of IAA was by HPLC using combined fluorimetry and amperometry (12). Identification of IAA in HPLC peaks from representative samples (young leaf explants, callus) was accomplished by GC-MS as described previously (21). ABA was quantified by radioimmunoassay using monoclonal antibody (obtained from Dr. E. W. Weiler) which has been demonstrated to be specific to (+)-ABA (17). Recovery of IAA and ABA were 57.8 ± 6.6% and 81.3 ± 7.3%, respectively.

**Chemicals.** All the solvents used were of HPLC or analar grade. (±)-Abscisic acid, IAA, and gibberellic acid were purchased from Sigma Chemical Co. Technical grade fluridone (98.1% pure) was a gift from Eli Lilly Research Laboratories (Greenfield, IN). Analytical grade glyphosate (97% pure) was obtained from Monsanto Co. Paclobutrazol (PP333; 50% active ingredient formulation) was obtained through the courtesy of Dr. Kevin Crosby (Monsanto Co.).

## RESULTS

**Effects of Fluridone, Glyphosate, and Paclobutrazol Application *in Vivo* to Donor Plants of Napier Grass.** *Morphological Characters.* Morphological characters of the glasshouse-grown donor plants were recorded on the 6th d after the initiation of treatment. All plants were of similar height except those treated with paclobutrazol. A marked reduction (about 70%) in growth occurred in paclobutrazol-treated plants. The young leaves of fluridone-treated plants were pale green and their basal portions were totally devoid of Chl. No differences were observed with regard to tiller number among the potted plants.

An examination of the morphological characters of the tillers from 15-d-old regrowth indicated the residual effect of all the three chemicals. Glyphosate-treated plants were similar to the controls. Treatment with paclobutrazol enhanced axillary bud growth and as a result there was a greater number of tillers. Leaf size was drastically reduced in these plants. Very few tillers arose from stubbles of fluridone-treated plants and they died within 10 to 15 d of regrowth due to lack of Chl (Table I).

*Endogenous Levels of IAA and ABA in Young Leaves.* Free IAA levels in basal portions of the young leaves were not affected by treatments with fluridone or paclobutrazol. However, glyphosate-treated plants contained 1.7 times more IAA in the basal portion of young leaves than controls. Endogenous levels of both IAA and ABA were similar in control plants which were irrigated with water only or with water containing 0.01% DMSO indicating that the DMSO required for dissolution of the hormone had no effect on endogenous hormone levels. Glyphosate-treated plants contained almost twice the amount of ABA as did control plants. There was no difference between control and paclobutrazol treatment with regard to endogenous ABA levels, but a significant reduction (50-fold) in endogenous ABA levels was observed in fluridone-treated plants (Table II).

*Somatic Embryogenesis in Cultured Leaf Explants.* Leaf explants from glyphosate-treated plants were as embryogenic (35%) as control plants (30%). Embryogenic capacity of leaf explants from paclobutrazol-treated plants (27%) was also similar to explants from controls. Embryogenic callus formation was completely inhibited in explants obtained from fluridone-treated plants and formation of nonembryogenic callus was also reduced (Table II).

**Effects of Glyphosate and Fluridone on Somatic Embryogenesis *in Vitro*.** Leaf explants collected from field-grown plants were used to study the effects of glyphosate and fluridone on somatic embryogenesis when included in the culture medium. Fluridone at all concentrations (0.02–20 mg/L) resulted in a marked reduction in the number of explants forming embryogenic callus (Table III). Glyphosate, at less than 5 mg/L, did not affect embryogenic callus formation; however, at concentrations of 5 mg/L and higher, the formation of embryogenic callus was inhibited (Table IV). In a parallel experiment, embryogenic callus pieces were transferred to PP medium containing fluridone (0.02 to 20 mg/L). After 10 to 15 d of culture almost all the callus pieces turned black and became nonembryogenic (Table V).

Endogenous levels of ABA and IAA were measured only in those explants grown in the presence of fluridone for 5 d. ABA levels decreased with increasing concentrations of fluridone (Fig. 1). Endogenous IAA levels were unaffected except for a reduction in IAA levels observed at 10 mg/L fluridone (Fig. 1).

**Restoration of Embryogenic Capacity by Exogenous ABA in Fluridone-Treated Leaf Explants and Embryogenic Callus.** Leaf explants collected from fluridone-treated, glasshouse-grown plants were cultured on PP medium or on PP medium supplemented with 5 mg/L (±)-ABA. Somatic embryogenesis was observed in 18% of the leaf explants cultured in the presence of (±)-ABA as compared to none on PP medium without (±)-ABA (Table V). In another experiment, explants from field-grown, untreated plants were first cultured for 5 d on PP medium containing fluridone (5 mg/L). After this culture period, explants were collected, rinsed in sterile $H_2O$, and divided into two equal groups. One group of explants was transferred to fresh PP medium and the other to PP medium containing 5 mg/L (±)-ABA. A significant proportion of the leaf explants (28%) produced embryogenic callus 15 to 20 d after transfer to PP medium containing (±)-ABA. Formation of embryogenic callus was minimal (5%) from the leaf explants in the absence of (±)-ABA. Similarly, 40% of the embryogenic callus pieces which were first grown on fluridone containing medium for 5 d, continued to produce embryogenic callus on transfer to PP medium containing (±)-ABA (5 mg/L) compared to 32% in the absence of (±)-ABA (Table V). All the embryogenic callus pieces continued to

Table I. *Effect of Treatment in Vivo with Glyphosate, Fluridone, and Paclobutrazol on Morphological Characters of Napier Grass*

| Treatment[a] | First Growth | Regrowth[c] | | |
|---|---|---|---|---|
| | Height of plants[b] | No. of tillers | Height of plants | Leaf size[d] |
| | *cm* | | *cm* | *l* × *b cm* |
| Control (water) | 72 ± 2.4[e] | 4.0 ± 0.6 | 14 ± 4.1 | 35 × 2.2 |
| Control (water + DMSO) | 74 ± 1.6 | —[f] | — | — |
| Fluridone (100 mg/L) | 70 ± 2.2 | 0.8 ± 0.2[g] | 11 ± 3.0 | 28 × 1.6 |
| Glyphosate (100 mg/L) | 75 ± 0.8 | 5.0 ± 1.2 | 10 ± 8.0 | 34 × 2.1 |
| PP333 (50% a.i; 200 mg/L) | 67 ± 0.8 | 9.0 ± 4.1 | 3 ± 2.8 | 12 × 2.4 |

[a] Potted plants were treated daily by drenching the soil for 5 d. [b] Measured on the 6th day; the mean height of all the plants was 64 ± 1.8 cm before treatment. [c] 15 d after regrowth. [d] Length × breadth of leaf blades. [e] Mean of three replicates ± SE. [f] Not measured. [g] Regrowth from fluridone-treated plants was devoid of Chl and died within 15 d.

Table II. *Effect of Treatment in Vivo with Glyphosate, Fluridone, and Paclobutrazol on Endogenous Levels of ABA and IAA in Young Leaves of Napier Grass*

| Treatment[a] | Endogenous Levels | | Explants Producing[b] | | |
|---|---|---|---|---|---|
| | IAA | ABA | Embryogenic callus | Nonembryogenic callus | No callus |
| | *ng/g fresh wt* | | | % | |
| Control (water) | 8.3 ± 0.6[c] | 10.0 ± 2.2 | 30 | 25 | 45 |
| Control (water + DMSO) | 8.5 ± 1.2 | 11.2 ± 1.8 | —[d] | — | — |
| Fluridone (100 mg/L) | 10.2 ± 3.0 | 0.2 ± 0.3 | 0 | 13 | 87 |
| Glyphosate (100 mg/L) | 13.9 ± 1.8 | 19.7 ± 3.2 | 35 | 29 | 36 |
| PP333 (50% a.i; 200 mg/L) | 8.9 ± 2.9 | 11.9 ± 2.4 | 27 | 18 | 59 |

[a] Potted plants were treated daily by drenching the soil for 5 d. [b] 180 explants were cultured in each treatment except for Fluridone (60 explants). [c] Mean of three replicates ± SE. [d] Treatment not tested.

Table III. *Effect of Exogenous Application of Fluridone on Somatic Embryogenesis in Vitro from Cultured Leaf Explants of Napier Grass*

| Concentration of Fluridone | Explants Producing | | |
|---|---|---|---|
| | Embryogenic callus | Nonembryogenic callus | No callus |
| *mg/L* | | % | |
| 0.02 | 13 | 47 | 40 |
| 0.1 | 9 | 46 | 45 |
| 0.5 | 4 | 60 | 36 |
| 1.0 | 0 | 46 | 54 |
| 5.0 | 0 | 10 | 90 |
| 10.0 | 2 | 10 | 88 |
| 20.0 | 0 | 18 | 82 |
| PP medium[a] | 44 | 46 | 10 |

[a] MS medium containing 0.5 mg/L each of BA and 2,4-D, 1 mg/L NAA and 5% coconut milk.

Table IV. *Effect of Exogenous Application of Glyphosate on Somatic Embryogenesis in Vitro from Cultured Leaf Explants of Napier Grass*

| Concentration of Glyphosate | Explants Producing | | |
|---|---|---|---|
| | Embryogenic callus | Nonembryogenic callus | No callus |
| *mg/L* | | % | |
| 0.02 | 38 | 37 | 25 |
| 0.1 | 25 | 35 | 40 |
| 0.5 | 44 | 39 | 17 |
| 1.0 | 40 | 23 | 37 |
| 5.0 | 5 | 24 | 71 |
| 10.0 | 0 | 18 | 82 |
| 20.0 | 0 | 23 | 77 |
| PP medium[a] | 42 | 38 | 20 |

[a] MS medium containing 0.5 mg/L each of BA and 2,4-D, 1 mg/L NAA and 5% coconut milk.

be embryogenic when cultured on PP medium, but those which were continuously grown on fluridone containing medium turned black and became nonembryogenic (Table V). Leaf explants from field-grown, untreated plants did not form embryogenic callus when grown continuously on a medium containing fluridone (5 mg/L).

**Effects of Exogenous IAA, (±)-ABA and $GA_3$ on Somatic Embryogenesis.** Use of IAA as the sole auxin source did not produce any morphogenic response in leaf explants except occasional formation of roots. When added to the PP medium in addition to 2,4-D and NAA, IAA promoted only nonembryogenic callus formation (Table VI). Gibberellic acid at concentrations lower than 5 mg/L did not inhibit embryogenesis but there was a marked inhibition of callus formation at concentrations more than 5 mg/L (Table VII). (±)-ABA, at all concentrations tested (0.02–20 mg/L), did not inhibit somatic embryogenesis from young leaf explants. On the contrary, an increase in the percentage of explants producing embryogenic callus was observed at 1.0 and 5.0 mg/L (Table VIII). Abscisic acid promoted embryogenic callus formation at all concentrations as indicated by the increase in the ratio of embryogenic to nonembryogenic callus with increasing concentrations of (±)-ABA (Fig. 2).

## DISCUSSION

In a previous study we showed that morphogenically competent leaf tissues and embryogenic callus contained high endogenous levels of IAA and ABA (21). Attempts to alter the endogenous levels of IAA by application of exogenous IAA (0.02–20

Table V. *Effect of Exogenous (±)-ABA on Fluridone (FL) Inhibition of Somatic Embryogenesis in Vitro in Leaf Explants and Callus of Napier Grass*

| Source of Explants | Details of Treatment with Fluridone | Culture Medium/Transfer Medium | No. of Explants Used | Explants Producing | | |
|---|---|---|---|---|---|---|
| | | | | Embryogenic callus | Nonembryogenic callus | No callus |
| | | | | % | | |
| Glasshouse | None | PP[a] | 60 | 30 | 25 | 45 |
| | *in vivo*[b] | PP | 60 | 0 | 13 | 87 |
| | *in vivo* | PP+FL 5 mg/L | 60 | 0 | 0 | 100 |
| | *in vivo* | PP+ABA 5 mg/L | 60 | 18 | 23 | 59 |
| Field | None | PP | 150 | 44 | 46 | 10 |
| | *in vitro*[c] | PP | 120 | 5 | 16 | 79 |
| | *in vitro* | PP+FL 5 mg/L | 120 | 0 | 0 | 100 |
| | *in vitro* | PP+ABA 5 mg/L | 120 | 28 | 18 | 54 |
| Embryogenic callus | None | PP | 200 | 100 | 0 | |
| | *in vitro*[c] | PP | 60 | 32 | 68 | |
| | *in vitro* | PP+FL 5 mg/L | 60 | 0 | 100 | |
| | *in vitro* | PP+ABA 5 mg/L | 60 | 40 | 60 | |

[a] MS medium containing 0.5 mg/L each of BA and 2,4-D, 1 mg/L NAA, and 5% coconut milk. [b] Potted plants were irrigated with water containing 100 mg/L fluridone for 5 d. [c] Explants were grown on PP medium containing 5 mg/L fluridone for 5 d.

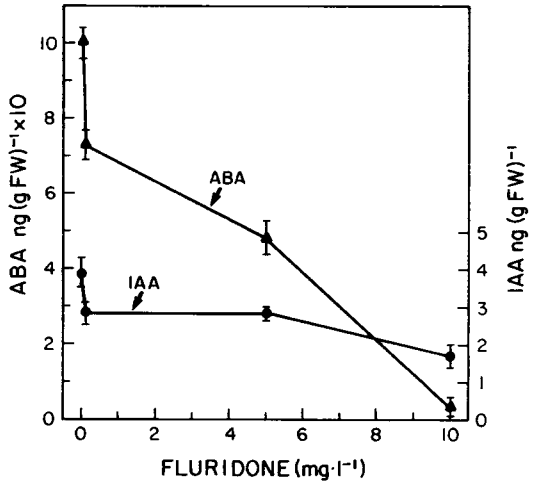


FIG. 1. Effect of fluridone on endogenous levels of IAA and ABA in young leaf explants cultured for 5 d. Vertical bars indicate SE ($n = 3$).

Table VI. *Effect of Exogenous Application of IAA on Somatic Embryogenesis in Vitro from Cultured Leaf Explants of Napier Grass*

| Concentration of IAA[a] | Explants Producing | | |
|---|---|---|---|
| | Embryogenic callus | Nonembryogenic callus | No callus |
| *mg/L* | | % | |
| 0.02 | 11 | 81 | 8 |
| 0.1 | 1 | 92 | 7 |
| 1.0 | 6 | 88 | 6 |
| 5.0 | 0 | 97 | 3 |
| 10.0 | 0 | 93 | 7 |
| 20.0 | 5 | 83 | 12 |
| PP medium[b] | 40 | 54 | 6 |

[a] In addition to 2,4-D and NAA in the PP medium; IAA alone, as an auxin source does not elicit embryogenic response. [b] MS medium containing 0.5 mg/L each of BA and 2,4-D, 1 mg/L NAA, and 5% coconut milk.

Table VII. *Effect of Exogenous Application of $GA_3$ on Somatic Embryogenesis in Vitro from Cultured Leaf Explants of Napier Grass*

| Concentration of $GA_3$ | Explants Producing | | |
|---|---|---|---|
| | Embryogenic callus | Nonembryogenic callus | No callus |
| *mg/L* | | % | |
| 0.02 | 44 | 22 | 34 |
| 0.5 | 24 | 34 | 42 |
| 1.0 | 18 | 26 | 54 |
| 5.0 | 6 | 35 | 59 |
| 10.0 | 0 | 15 | 85 |
| 20.0 | 0 | 3 | 97 |
| PP medium[a] | 47 | 34 | 19 |

[a] MS medium containing 0.5 mg/L each of BA and 2,4-D, 1 mg/L NAA, and 5% coconut milk.

Table VIII. *Effect of Exogenous Application of (±)-ABA on Somatic Embryogenesis in Vitro from Cultured Leaf Explants of Napier Grass*

| Concentration of ABA | Explants Producing | | |
|---|---|---|---|
| | Embryogenic callus | Nonembryogenic callus | No callus |
| *mg/L* | | % | |
| 0.02 | 45 | 34 | 21 |
| 0.1 | 49 | 41 | 10 |
| 0.5 | 44 | 25 | 31 |
| 1.0 | 59 | 28 | 23 |
| 5.0 | 55 | 30 | 15 |
| 10.0 | 47 | 17 | 36 |
| 20.0 | 31 | 29 | 40 |
| PP medium[a] | 41 | 40 | 19 |

[a] MS medium containing 0.5 mg/L each of BA and 2,4-D, 1 mg/L NAA, and 5% coconut milk.

mg/L) either produced no response or resulted in the formation of nonembryogenic callus. The leaf explants were also not responsive to IAA-aminoacid conjugates (K Rajasekaran, IK Vasil, unpublished observations), which are considered to be more stable sources of auxin than the labile free IAA or the persistent synthetic IAA analogs (10). Our attempts to lower the endogenous levels of IAA both *in vivo* and *in vitro* by treatment with

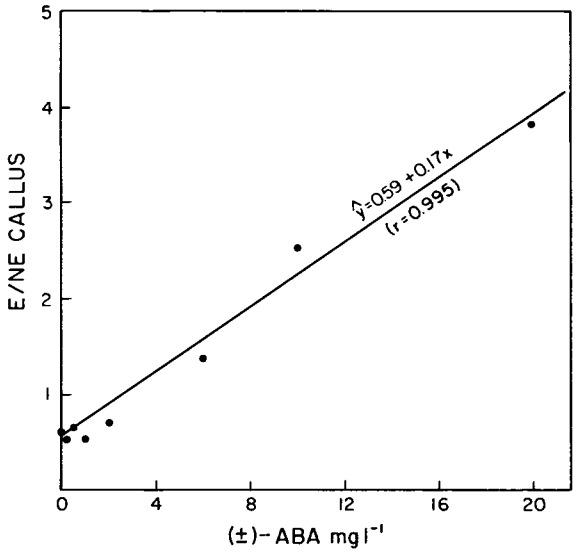


FIG. 2. Effect of exogenous (±)-ABA on the dry weight ratio of embryogenic to nonembryogenic callus. Sixty explants were used at each concentration and were cultured for 30 d.

glyphosate (14, 15) were not successful. It is not clear why glyphosate-treated plants contained higher levels of IAA and ABA when compared with controls. Lee and Dumas (16) compared the effect of 2 mM glyphosate on different species and concluded that plants with high natural rates of IAA metabolism were probably less dependent on IAA and thus less susceptible to glyphosate. It is possible that *Pennisetum* is either less susceptible to glyphosate or that the concentration of glyphosate used in this study (0.6 mM) was too low to bring about the desired results. Glyphosate was also ineffective in inhibiting 2,4-D induced somatic embryogenesis *in vitro* at lower concentrations (<5 mg/L).

Gibberellic acid at concentrations more than 5 mg/L inhibited somatic embryogenesis in *P. purpureum* and has been shown to inhibit somatic embryogenesis in other species (8, 9). Paclobutrazol, a biosynthesis inhibitor of gibberellins (20), had no effect on the embryogenic nature of explants in this experiment. Neither the paclobutrazol nor the reduced levels of gibberellins which may have resulted from its application altered embryogenic character of the explants. The effect of paclobutrazol on other hormones in higher plants is not clearly understood, but treatment of plants with paclobutrazol did not alter the endogenous levels of ABA and IAA in the present study.

Although the treatments designed to alter the IAA and GA levels in explants indicated no definitive role(s) for these molecules in the regulation of somatic embryogenesis, several interesting findings have emerged from this study regarding the role of endogenous ABA in somatic embryogenesis from leaf explants of Napier grass: (a) Incorporation of (±)-ABA in culture medium enhances embryogenic callus formation and somatic embryogenesis (Table VIII; Fig. 2). These results are consistent with those of several studies with other plants (1, 2, 24). (b) Treatment *in vivo* of plants or *in vitro* of leaf explants with fluridone resulted in a significant reduction of endogenous ABA level and inhibition of somatic embryogenesis (Tables II, III; Fig. 1). (c) Embryogenic capacity of fluridone-treated explants or callus can be restored, albeit partially, by subsequent application of (±)-ABA (Table V).

These results, along with our earlier findings that embryogenically competent young leaf tissues and callus contain higher levels of endogenous ABA than noncompetent mature leaves and nonembryogenic callus (21), indicate that endogenous ABA is causally related to somatic embryogenesis from leaf explants of Napier grass. However, the mode of action of ABA is not known. Since accumulation of starch is one of the characteristic features of embryogenic cells of *Pennisetum* (25) and other grasses, ABA may be involved in regulation of carbohydrate metabolism. ABA may enhance embryogenesis by promoting sucrose uptake from the medium (23) or starch synthesis by controlling $\alpha$-amylase activity (13). Abou-Mandour and Hartung (1) have suggested that promotive effects of osmotic stress on callus growth and differentiation in *Zea mays* could possibly be mediated by ABA. In addition, interaction between ABA and other growth regulators (5) may play an important role in determining the course of differentiation *in vitro*.

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