

# ENDOGENOUS GROWTH REGULATOR DETECTION IN GUINEA GRASS SEEDS<sup>1</sup>

ROBERTO USBERTI<sup>2</sup> and IVANY FERRAZ MARQUES VALIO<sup>3</sup>

**ABSTRACT** - Endogenous growth regulator activity/level was detected in guinea grass (*Panicum maximum* Jacq.) seeds, a species widely used as a forage crop in the tropics, aiming at explaining its high seed dormancy level. Seeds were previously scarified or not with sulphuric acid and osmoconditioned on PEG-6000. Endogenous growth regulators were detected as follow: gibberellin-like activity (growth of lettuce hypocotyl bioassay); cytokinins (increase of fresh mass of radish cotyledon bioassay); ABA (spectrophotometer at 230 nm). Exogenous application of GA<sub>3</sub> showed a germination increasing effect while ABA had a complete effectiveness to prevent it. High-dormancy seed samples had higher gibberellin-like activity than low-dormancy ones and intact seeds showed higher gibberellin-like activity than scarified seeds; however, after osmoconditioning, opposite results were recorded. No significant activity of neutral promoters and cytokinins was detected. Average levels of ABA for untreated, osmoconditioned after zero- and two-month-storage seed samples were 0.51, 0.39 and 0.21 mg.100 kg<sup>-1</sup>, respectively. Chemical scarification did not alter either ABA levels in high-dormancy seed samples (0.66 mg.100 kg<sup>-1</sup>) or those of low-dormancy (0.23 mg.100 kg<sup>-1</sup>), the former being significantly higher than the latter. Finally, the results show that a gibberellin-ABA interaction appears to be the main factor accounting for dormancy, germination and osmoconditioning control in guinea grass seeds.

**Index terms:** germination, dormancy, osmoconditioning, *Panicum maximum*.

## DETECÇÃO DE REGULADORES ENDÓGENOS DE CRESCIMENTO EM SEMENTES DE CAPIM-COLONIÃO

**RESUMO** - Foi detectada a atividade/nível endógeno de reguladores de crescimento em sementes de capim-colonião (*Panicum maximum* Jacq.), espécie muito usada para formação de pastagens nos trópicos, visando explicar o seu alto nível de dormência. As sementes foram previamente escarificadas, ou não, com ácido sulfúrico, e osmocondicionadas em PEG-6000. Os reguladores de crescimento foram detectados como segue: atividade giberélnica (bioteste do hipocótilo de alface), citocininas (bioteste do aumento do peso fresco de cotilédones de rabanete), e ABA (espectrofotometria a 230 nm). A aplicação exógena de GA<sub>3</sub> aumentou a germinação, enquanto ABA inibiu-a completamente. Amostras com alta dormência apresentaram maior atividade giberélnica que as de baixa dormência, bem como maior atividade em sementes intactas do que em escarificadas; mas após o osmocondicionamento, essa situação se inverteu. Não foram detectadas diferenças em atividade de promotores neutros e de citocininas. Os valores médios de ABA foram 0,51, 0,39 e 0,21 mg.100 kg<sup>-1</sup> nas sementes normais, imediatamente após o seu osmocondicionamento, e dois meses após, respectivamente. O nível endógeno de ABA nas sementes com alta dormência (0,66 mg.100 kg<sup>-1</sup>) foi maior que nas sementes com baixa dormência (0,23 mg.100 kg<sup>-1</sup>), mas esses valores não foram alterados pela escarificação química. Uma possível interação entre giberelinas e ABA parece ser a causa do controle da dormência, germinação e osmocondicionamento nessas sementes.

**Termos para indexação:** germinação, dormência, osmocondicionamento, *Panicum maximum*.

## INTRODUCTION

Growth regulators are considered as primary agents of seed germination and dormancy processes (Jan & Amen, 1977). Abscisic acid and gibberellins are the growth regulators most frequently suggested

<sup>1</sup> Accepted for publication on March 18, 1997.

<sup>2</sup> Eng. Agr., Dr., Dep. de Sementes, Mudanças e Matrizes - CATI, Caixa Postal 1291, CEP 13073-001 Campinas, SP.

<sup>3</sup> Biólogo, Ph.D. Prof. Titular, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Caixa Postal 6109, CEP 13081-970 Campinas, SP. Bolsista do CNPq.

to control seed dormancy and germination. Abscisic acid plays a main role during the development of primary dormancy while gibberellins are involved in the induction of germination. Changes in sensitivity to these growth regulators occur with changes in dormancy (Hilhorst & Karssen, 1992).

Exogenous gibberellins enhance germination in seeds in which dormancy (or quiescence) is imposed by several mechanisms such as immature embryos, hard seed coats and inhibitors. The hypothesis on the mode of action of gibberellins is based on the mobilization of endosperm reserves of barley seeds. Thus, it is possible that for seeds where germination is stimulated by gibberellins, the first stage of this process is via enzymes acting in the breakdown of starch and other substrates (Jones & Stoddart, 1977).

Cytokinin activity is high in developing fruits and seeds but the levels drop with the ripening of the tissues. Research on the endogenous levels and exogenously applied cytokinins suggest that they are involved in the control of bud and seed dormancy (Thomas, 1977).

Inhibitors may act during seed embryogenesis and ripening mainly by preventing precocious germination. They can be responsible for the beginning and maintenance of dormancy, but this effect can be overcome by promoters (Black, 1980/81). ABA (abscisic acid) concentration in acid lime seeds and fruits has been suggested not to determine the rate of growth and development, but low ABA concentrations during the final phase of seed development may be of significance for seed germination (Murti, 1993).

It is well known that seed osmoconditioning on PEG (polyethylene glycol) solutions has improved seed germination rate and uniformity of a wide spectrum of plant species, including grass seeds (Hardegree, 1994).

Guinea grass was chosen for this study in view of its broad application to establish forage crops in the tropics. However, the freshly harvested seeds of almost all of its cultivars probably show a low percentage of germination and a high level of dormancy, due probably to uneven maturation (Harty et al., 1983).

The main objective of this paper was the detection of endogenous growth regulator in dormant and quiescent seeds of *Panicum maximum*, before and

after osmoconditioning with PEG-6000, aiming at explaining its high seed dormancy level.

## MATERIAL AND METHODS

Six samples of seeds (spikelets) of *Panicum maximum* Jacq. from two regions of São Paulo State, Brazil, previously grouped according to the degree of dormancy detected by scarification in H<sub>2</sub>SO<sub>4</sub> for five minutes (high dormancy - samples 1 and 6; low dormancy - samples 2, 3, 4 and 5), were used (Table 1).

The optimum conditions for osmoconditioning guinea grass seeds were detected in preliminary tests. Seed samples (1.5 g each) were kept during seven days in gerboxes containing 25 mL of PEG-6000 solution (288 g.kg water<sup>-1</sup>), at 25°C (-0.95 MPa) and 15°C (-1.1 MPa), for intact and scarified seeds, respectively. After imbibition in PEG solution the seeds were rinsed in distilled water for one minute, placed in a 5% sodium hypochloride solution for 20 minutes, rinsed again for one minute and surface dried with filter papers. After a week storage at 15% relative humidity (drying cabinet), half of the samples was immediately extracted for growth regulators detection while the others were stored at 10°C for two months before analysis.

The following treatments were applied on both intact and scarified seeds: a) control seeds (no imbibition in PEG); b) seeds immediately after imbibition in PEG; c) seeds stored for two months at 10°C, after imbibition in PEG.

Seed samples (1.5 g) were then extracted in 80% methanol (Valio & Schwabe, 1970) to detect the activity of growth regulators, also the possible changes caused by the osmoconditioning. The fractionated extracts were chromatographed on glass plates coated with silicagel and developed with ethyl acetate : chloroform : acetic acid (15:5:1). The plates were coated with fluorescent silicagel to detect ABA through fluorescence and spectroscopy.

TABLE 1. Quality parameters of guinea grass seed samples used for endogenous growth regulators detection.

Seed sample	Physical purity (%)	Germination (%)	
		Control	H <sub>2</sub> SO <sub>4</sub>
1	23.9	7	42
2	62.7	18	25
3	51.6	16	28
4	57.7	14	22
5	70.2	9	19
6	58.2	4	43

For bioassays the chromatograms were divided in 10 horizontal strips corresponding to Rf 0.1 - 1.0. Each strip was also divided in three replicates per Rf region. Silicagel from plates without application of extracts was used as control. Bioassays were processed only in areas of the chromatograms that showed biological activity in previous experiments such as Rf 0.1 - 0.6 for acid and neutral fractions, and Rf 0.1 - 0.5 for basic fraction.

The growth of lettuce hypocotyl bioassay was used to detect gibberellin-like activity on acid and neutral fractions (Frankland & Wareing, 1960). Cytokinins were detected chemically by the Wood reagent (Wood, 1955) and biologically through the increase of fresh mass using the radish cotyledon bioassay (Letham et al., 1964). Four lettuce seedlings or radish cotyledons were used in these bioassays for each Rf strip replicate.

ABA was detected through fluorescence under an ultraviolet lamp (230 nm). Extracts of 1.0 g of seeds also standard solution of ABA ( $10^{-3}$  M) were chromatographed, identified under ultraviolet lamp, scraped off the plates and analysed by a spectrophotometer at 230 nm, according to Kefeli (1978).

Preliminary experiments of normal seed germination tests, using different batches of seed samples, were carried out to detect the effects of application of growth regulators in high- and low-dormancy seeds ( $GA_3$  - gibberellic acid  $10^{-3}$  M and  $10^{-4}$  M; 6-BA - benzyl adenine  $10^{-4}$  M and  $10^{-5}$  M; Ethrel 60  $\mu\text{g.kg}^{-1}$  and 6  $\mu\text{g.kg}^{-1}$ ; ABA  $10^{-3}$  M,  $10^{-4}$  M and  $10^{-5}$  M).

The statistical design was completely randomized (Sokal & Rohlf, 1969). ABA standard curve was plotted by linear regression. Comparisons between treatment means in relation to ABA were carried out through Tukey's test at the 5% level. Analysis of variance and F test were also used for the same Rf of different treatments.

## RESULTS AND DISCUSSION

The application of  $GA_3$   $10^{-3}$  M and  $GA_3$   $10^{-4}$  M had similar effects on increasing final germination percentages while the other growth regulators showed no effect (Table 2). This stimulation of germination by  $GA_3$  has been also shown for lettuce (Toyomasu et al., 1994) and for *Panicum maximum* seeds (Basra et al., 1989). ABA application showed a complete effectiveness to prevent seed germination so its results are not shown at Table 2.

High-dormancy control seeds (not imbibed in PEG) showed a gibberellin-like activity at 0.4 - 0.6 Rf region with higher activity in intact seeds

TABLE 2. Effects of exogenous growth regulators in guinea grass intact seed germination.

Treatment	Mean germination <sup>1</sup>
$GA_3$ $10^{-3}$ M	41.49 a
$GA_3$ $10^{-4}$ M	39.83 a
Control	33.76 b
6 - BA $10^{-5}$ M	33.47 b
Ethrel 6 $\mu\text{g.kg}^{-1}$	33.25 b
6 - BA $10^{-4}$ M	31.66 b
Ethrel 60 $\mu\text{g.kg}^{-1}$	31.08 b

<sup>1</sup> Means followed by different letters are statistically different, according to Tukey's test, at 5% probability level.

at Rf 0.4 while seeds imbibed in PEG showed it at Rf 0.6, with higher levels for scarified seeds (Fig. 1). In seeds stored for two months after imbibition in PEG, gibberellin-like activity was also detected but higher differences were found for scarified seeds at Rf 0.2 and at Rf 0.6. Low-dormancy intact and scarified seeds did not show gibberellin-like activity.

Intact and scarified seeds with high dormancy presented higher gibberellin-like activity at Rf 0.4, though scarified seeds showed one additional higher gibberellin-like activity at Rf 0.6 (Fig. 2).

The presence of higher gibberellin-like activity in control seeds with high dormancy over scarified ones and the reversed situation after imbibition in PEG could be explained by an increased permeability of the tissues by  $H_2SO_4$  treatment, followed by a faster activation of biochemical processes related to GA production. A new approach states that the gibberellins are indispensable for germination and that dormancy-release treatments can induce either their synthesis or a change in their compartmentation or a change in sensitivity of the tissue (Le Page-Degivry, 1990).

Neutral and basic fractions obtained from intact and scarified seeds with high and low dormancy did not show promoting activity, suggesting that neutral and cytokinin-like promoters appear not to be involved in seed dormancy and osmoconditioning of this species, according to the model proposed by Khan (1975), in which gibberellins, abscisic acid and cytokinins have special role in these processes. On the other hand, application of kinetin in *Panicum*

*miliaceum* significantly increased seed germination; low ABA concentrations however slightly increased germination but inhibited seedling growth (Dhir & Sharma, 1991).

The mean values detected for ABA were  $1.939 \cdot 10^{-5}$  M ( $0.51 \text{ mg} \cdot 100 \text{ kg}^{-1}$ ),  $1.48 \cdot 10^{-5}$  M ( $0.39 \text{ mg} \cdot 100 \text{ kg}^{-1}$ ) and  $1.822 \cdot 10^{-6}$  M ( $0.21 \text{ mg} \cdot 100 \text{ kg}^{-1}$ ) for seeds before imbibition, immediately after imbibition in PEG solution and two-month-storage later, respectively. These results agree with other data showing a minimum limit of  $10^{-5}$  M for germination inhibition, for example in tomato seeds (Finch-Savage & McQuistan, 1991), which presented an increase on emergence percentage and a reduction on both time and spread of emergence after exogenous application of ABA  $10^{-5}$  M. In other species, higher values were necessary for seed inhibition such as for lettuce,  $12 \text{ mg} \cdot 100 \text{ kg}^{-1}$  (Braun & Khan, 1975); *Fraxinus americana*,  $45 \text{ mg} \cdot 100 \text{ kg}^{-1}$  (Sondheimer et al., 1968); *Acer saccharum*,  $82.6 \text{ mg} \cdot 100 \text{ kg}^{-1}$  (Webb et al., 1973).

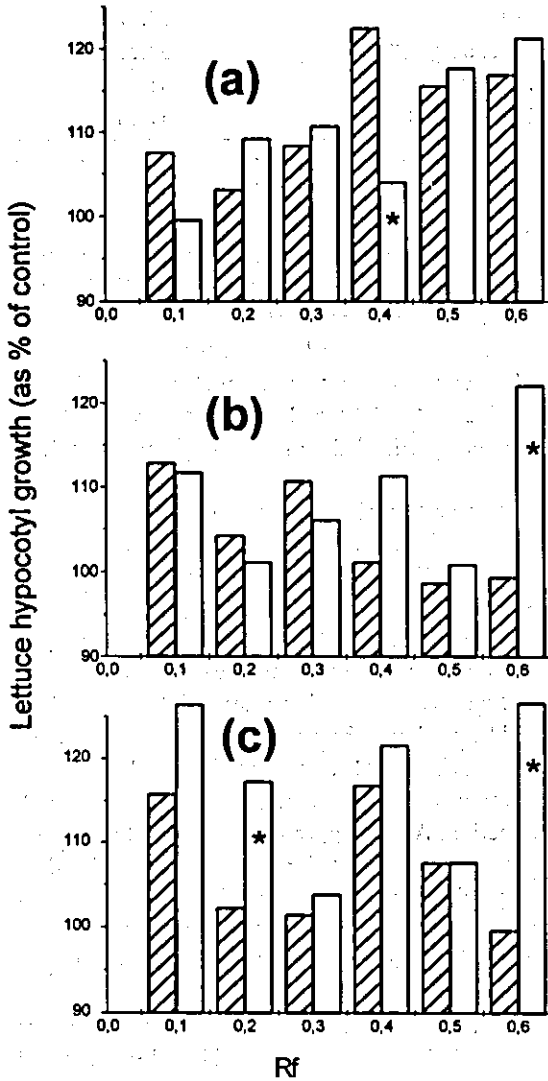


FIG. 1. Gibberellin-like activity in high-dormancy intact (hatched) and scarified (open) seeds for control (a), immediately after imbibition on PEG (b) and two months after imbibition in PEG (c). Signal \* shows statistical differences at 5% level between values at the same Rf.

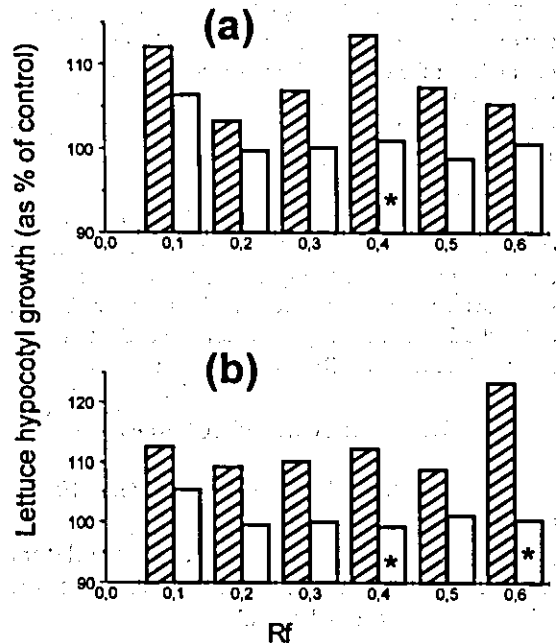


FIG. 2. Gibberellin-like activity in high-(hatched) low-dormancy (open) seed samples for intact (a) and scarified seeds (b). Signal \* shows statistical differences at 5% level between values at the same Rf.

The amounts of ABA detected in intact and scarified seeds, before and after osmoconditioning, were similar. However, high-dormancy seeds showed higher values than low-dormancy ones ( $2.49.10^{-5}$  M : 0.66 mg.100 kg<sup>-1</sup>;  $0.87.10^{-5}$  M : 0.23 mg.100 kg<sup>-1</sup>, respectively).

The localization of ABA in the seeds is important to explain the dormancy. In hazelnut, ABA was found mainly in the pericarp and coat and its removal releases the seed from dormancy (Ross, 1984). Nevertheless, in guinea grass the chemical scarification did not reduce the levels of ABA. It is suggested that the positive effect of H<sub>2</sub>SO<sub>4</sub> in breaking the dormancy of this species could be due to its action on dehydration of the tissues thus breaking the teguments and allowing more oxygen to the embryo (Durán & Tortosa, 1985).

High-dormancy seed samples showed higher values for both gibberellin-like activity and ABA levels than low-dormancy ones. It is suggested that dormancy and germination control in guinea grass seeds depends on these growth regulators, with gibberellins playing a main role in the process. The possible effect of osmoconditioning on high-dormancy seed samples could be explained by an increase in the levels of acid promoters after imbibition in PEG solution as occurred on scarified seeds.

### CONCLUSIONS

1. No statistical differences of neutral promoters and cytokinin-like activity are detected.
2. Scarification with concentrated sulphuric acid does not affect endogenous ABA levels.
3. High-dormancy seed samples show higher gibberellin-like activity and ABA levels than low-dormancy ones.

### REFERENCES

- BASRA, A.S.; SARLACH, R.S.; MALIK, C.P. Seed germination of *Panicum maximum* Jacq. in relation to respiratory inhibitors and gibberellic acid treatments. *Indian Journal of Plant Physiology*, v.32, p.139-143, 1989.
- BLACK, M. The role of endogenous hormones in germination and dormancy. *Israel Journal of Botany*, v.29, p.181-192, 1980/81.
- BRAUN, J.W.; KHAN, A.A. Endogenous abscisic acid levels in germinating and nongerminating lettuce seeds. *Plant Physiology*, v.56, p.731-733, 1975.
- DHIR, K.K.; SHARMA, R. ABA-caused inhibition of seedling growth and its removal by kinetin. In: DHIR, K.K.; DUA, I.S.; CHARK, K.S. (Eds.). *New trends in plant physiology*. New Delhi, India: Today and Tomorrow's Printers & Pub., 1991. p.71-73. Proceedings of the National Symposium on Growth and Differentiation in Plants.
- DURÁN, J.M.; TORTOSA, M.E. The effect of mechanical and chemical scarification on germination of charlock (*Sinapsis arvensis* L.) seeds. *Seed Science & Technology*, v.13, p.155-163, 1985.
- FINCH-SAVAGE, W.E.; McQUISTAN, C.I. Abscisic acid: an agent to advance and synchronise germination for tomato (*Lycopersicon esculentum* Mill.) seeds. *Seed Science & Technology*, v.19, p.537-544, 1991.
- FRANKLAND, B.; WAREING, P.F. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. *Nature*, London, v.185, p.255-256, 1960.
- HARDEGREE, S.P. Drying and storage effects on germination of primed grass seeds. *Journal of Range Management*, v.47, n.3, p.196-199, 1994.
- HARTY, R.L.; HOPKINSON, J.M.; ENGLISH, B.H.; ALDER, J. Germination, dormancy and longevity in stored seeds of *Panicum maximum*. *Seed Science & Technology*, v.11, p.341-351, 1983.
- HILHORST, H.W.M.; KARSSSEN, C.M. Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. *Plant Growth Regulation*, v.11, p.225-238, 1992.
- JAN, R.C.; AMEN, R.D. What is germination? In: KHAN, A.A. (Ed.). *The physiology and biochemistry of seed dormancy and germination*. Amsterdam: North-Holland Pub., 1977. p.7-28.
- JONES, R.L.; STODDART, J.L. Gibberellins and seed germination. In: KHAN, A.A. (Ed.). *The physiology and biochemistry of seed dormancy and germination*. Amsterdam: North-Holland Pub., 1977. p.77-110.
- KEFELI, V.I. *Natural plant growth inhibitors and phytohormones*. Boston: W. Junk Pub., 1978. 277p.

- KHAN, A.A. Primary, preventive and permissive roles of hormones in plant systems. *Botanical Review*, v.41, p.391-420, 1975.
- LE-PAGE-DEGIVRY, M.T. Rôle des gibbérellines et de l'acide abscissique dans la germination et la dormance des sementes: Pour une approche dynamique. *Seed Science & Technology*, v.18, p.345-356, 1990.
- LETHAM, D.S.; SHANNON, J.S.; McDONALD, I.R. The structure of zeatin, factor inducing cell division. *Proceedings of the Chemical Society*, p.230-231, 1968.
- MURTI, G.S.R. Endogenous abscisic acid in seed in relation to seed and fruit growth in acid lime. *Indian Journal of Plant Physiology*, v.36, p.9-11, 1993.
- ROSS, J.D. Metabolic aspects of dormancy. In: MURRAY, D.R. (Ed.). *Germination and reserve mobilization: seed physiology*. Sidney: Academic Press, 1984. v.2, p.45-75.
- SOKAL, R.R.; ROHLF, F.J. *Biometry*. San Francisco: Freeman and Co., 1969. 776p.
- SONDHEIMER, R.; TZOU, D.S.; GALSON, E.D. Abscisic acid levels and seed germination. *Plant Physiology*, v.43, p.1443-1447, 1968.
- THOMAS, T.H. Cytokinins, cytokinins-active compounds and seed germination. In: KHAN, A.A. (Ed.). *The physiology and biochemistry of seed dormancy and germination*. Amsterdam: North-Holland Pub., 1977. p.111-144.
- TOYOMASU, T.; YAMANE, H.; MUROFUSHI, N.; INOUE, Y. Effects of exogenously applied gibberellin and red light on the endogenous levels of abscisic acid in photoblastic lettuce seeds. *Plant and Cell Physiology*, v.35, p.127-129, 1994.
- VALIO, I.F.M.; SCHWABE, W.W. Promotion and inhibition of growth in *Lunularia cruciata* (L.) DUM.VII - The isolation and bioassay of lunularic acid. *Journal of Experimental Botany*, v.21, p.138-150, 1970.
- WEBB, D.P.; VAN STADEN, J.; WAREING, P.F. Seed dormancy in *Acer*: Changes in endogenous cytokinins, gibberellins and germination inhibitors during the breaking of dormancy of *Acer saccharum* March. *Journal of Experimental Botany*, v.24, p.105-106, 1973.
- WOOD, T. A reagent for the detection of chloride and certain purines and pyrimidines on paper chromatograms. *Nature*, London, v.176, p.175-176, 1955.