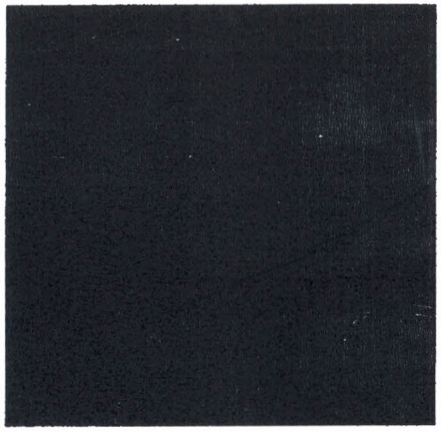
V.D
086

ORGANIZAN

Facultad de Agronomía

Facultad de Ciencias

Sociedad Argentina de Fisiología Vegetal



23 al 25 de Octubre de 2002

XI RLFV

Hotel Las Dun
Punta del Est
Manantiales
Uruguay

CON EL APOYO DE:

FUNDACION ANTORCHAS

Http://www.fvegetal.edu.uy/

XI REUNION LATINOAMERICANA DE FISIOLOGIA VEGETAL

XXIV REUNION ARGENTINA DE FISIOLOGIA VEGETAL

I CONGRESO URUGUAYO DE FISIOLOGIA VEGETAL

Calcium and gibberellic acid effect on starch degradation during germination of flooded maize seeds

Bárbara França DANTAS[1], Carlos Alberto ARAGÃO[2], Ana Catarina CATANEO[3], João NAKAGAWA[4], Cláudio CAVARIANI[4], João Domingos RODRIGUES[3]

[1]Embrapa Semi-Árido BR428, km152, Zona Rural, Caixa Postal 23, 56302-970, Petrolina-PE. Fone (87)3861-1711 Fax (87)3861-1744 , [2] DTCS/UNEB, Juazeiro-BA, [3]IBB/ UNESP, Botucatu-SP, [4]FCA/ UNESP, Botucatu-SP

Introduction

The reserve degradation hydrolytic pathway in cereal seeds begins with α-amylase (EC 3.2.1.1) activity (Dunn, 1974; Sun & Henson, 1991). Among the active starch degradation enzymes during germination, α-amylase has a major role due to its ability to degrade insoluble native starch grains (Beck & Zeigler, 1989) and its high activity at the beginning of the germination process (Mayer & Poljakoff-Mayber, 1985). However, according to Sun & Henson (1991), only the coordinated activity of all hydrolytic enzymes results on complete starch degradation.

The role of gibberellic acid (GA) in seed germination has been elucidated in mid 90's when Lenton et al. (1994) observed that the embryo produces and secretes GA_1 to the endosperm. Thus, the embryo regulates seed reserve mobilization by a GA-induced transduction pathway at aleurone and scutellum cells leading to α-amylase messenger RNA (mRNA) transcription (Lenton et al., 1994). The signal transduction pathway begins when GA binds to the GA receptor site at the aleurone plasma membrane. This signals calcium ions (Ca^{2+}), that act as secondary messengers (Bush,1990). These messengers will then initiate a response from an unknown tertiary messenger which induces α-amylase mRNA transcription. However, the signal transduction events leading from the receptor to the coordination of the complex events that make up and regulate the secretory activity of these cells are still poorly understood.

Subbaiah et al. (1994) and He et al. (1996) observed that oxygen (O_2) deficit stimulates the Ca^{2+} influx to the citosol, activating important genes for anaerobic metabolism Akazaua & Hara-Nishimura (1985) verified that an increase on intracellular Ca^{2+} controlled by bioregulators induces the production and secretion of α-amylase in rice and barley seeds. Al-Ani et al. (1985), observed that starch reserves and their efficient mobilization by α-amylase exert important roles on hypoxic maize (*Zea mays* L.) seed metabolism.

This work aimed to observe the effect of GA and calcium on germination and amylase activity of hypoxic maize seed.

Materials and Methods

Maize seeds cv. CATI AL34 were submerged in 50 mL distilled water and incubated at 27°C, in the dark during 0, 1, 2, 3, 4 and 5 days. After that the seeds were germinated on germitest paper imbibed in distilled water and 100 mg GA /L, 0,375% $CaCl_2$ or 100 mg GA /L + 0,375% $CaCl_2$ solutions and assessed for germination and vigor tests and amylolitic activity. The vigor tests were evaluated 4 days after sowing and consisted in first counting and seedlings root and shoot growth.

For the amylolitic activity assay the seeds subjected to hypoxia were germinated during 7 days. The enzymes were extracted by the homogenization of seeds reserve tissues in TRIS-HCl 0,1mol.L^{-1}, pH 7, buffer with NaCl 0,1mol.L^{-1} and $CaCl_2$ 10mmol.L^{-1} and centrifuged at 12.000*g*, 4°C during 10 minutes. The pellet was discarded. The amylolitic activity was measured in a reaction buffer containing 50mmol.L^{-1}sodium acetate, pH 5.2, 10mmol.L^{-1} $CaCl_2$ and 2,5% soluble potato starch as substrate, incubated at 35°C during 15minutes. To inactivate other amylases, and purify α-amylase, the crude extract was incubated at 70°C, during 15 minutes. The amylase activity was expressed in reducing sugars produced by starch degradation (mmol)/ protein (mg)/ minute.

Results and Discussion

Although the Ca^{2+} and GA treatments did not result in higher germination of stressed seeds, those germinated in Ca^{2+} (0,375%) maintained high germination even after 5 days of hypoxia. The other treatments improved germination only in no- or low-stressed seeds, which were subjected up to three days of hypoxia (Figure 1a and b).

The treatments had little effect on the seedlings root and shoot growth, but the hypoxic seeds germinated in a Ca^{2+}(0,375%)+ GA (100ppm) solution had increased shoot and root growth than those germinated in distilled water up to 4 days of hypoxia (Figure 1c and d). This result can be explained by the high α-amylase activity caused Ca^{2+} and GA synergism, as shown in Figure 1e. GA and Ca^{2+} are the signal and the second messenger, respectively, of an important transduction pathway that leads to α-amylase production and activation, along with an efficient reserve hydrolysis and mobilization.

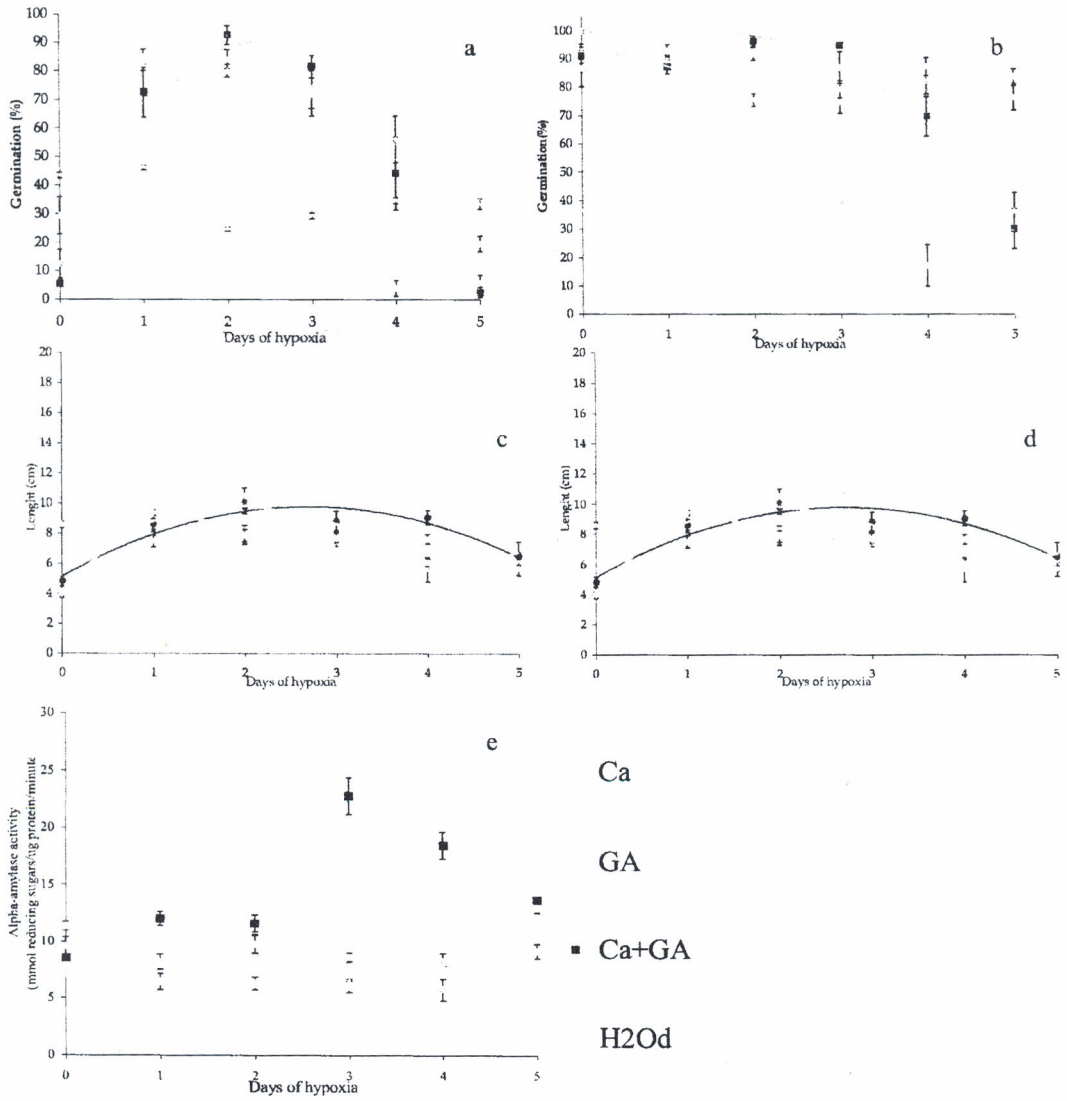


Figure 1. Effect of Ca^{2+} and GA on maize seeds cv. CATI AL34.
(a)germination first counting, (b) final germination, (c) seedling shoot length, (d)seedling root length and (e) α-amylase activity.

Conclusion

Calcium and gibberellic acid alone have no effect on hypoxic maize seeds but whenassociated they have a synergistic effect improving reserve degradation and mobilization, which leads to a stress aleviation.

Literature cited

AKAZAWA, T & HARA-NISHIMURA, I. Topographic aspects of biosynthesis, extracellular secretion, and intercellular storage of proteins in plan cells. **Annual Review of Plant Physiology and Plant Molecular Biology**. v. 36, p.441-472, 1985.

AL-ANI, A., BRUZAU, F., RAYMOND, P., SAINT-GES, V., LEBLANC, J. M., PRADET, A. Germination, respiration, adenylete energy charge of seeds at various oxygen pressures. **Plant Physiology**. v. 79, p.885-980, 1985.

BECK, E. & ZEIGLER, P. Biossynthesis and degradation of starch in higher plants. **Annual Review of Plant Physiology and Plant Molecular Biology**. v. 40, p. 95-117, 1989.

BUSH, D.S.; STICHER, L.; JONES, R.L. Gibberellin A_3-regulated α-amylase synthesis and calcium transport in the endoplasmic reticulum of barley aleurone cells. In: **Gibberellins.** TAKASHI, N.; PHINNEY, B.O.; MACMILLAN, J. (eds.). Tokyo: Springer-Verlag, 1990. 426p.

DUNN, G. A model for starch breakdown in higher plants. **Phytochemistry**, v.13, n.2, p.1341-6, 1974.

HE, C.J.; MORGAN, P.W.; DREW, M.C.. Transduction of an ethylene signal required for cell death and lysis in the root cortex of maize during aerenchyma formation during hipoxia. **Plant Physiology** n.112 p.463-472. 1996.

LENTON, J.R.; APPELFORD, N.E.J.; CROKER, S.J. Gibberellins and α-amylase gene expression in germinatin wheat grains. **Plant Growth Regulators**. v.15, p.261-270. 1994.

MAYER, A.M. & POLJAKOFF-MAYBER, A. **The germination of plants.** Oxford: Pergamon Press, 4ed, 1978. 270p.

SUBAIAH, C.C.; BUSH, D.S. & SACHS, M.M. Elevation of cytosolic calcium precedes anoxic gene expression in maize suspension cultured cells. **The Plant Cell,** v.6, p.1747-1762, 1994.

SUN, Z. & HENSON, C.A. Degradation of native starch granules by barley α-glucosidases. **Plant Physiology**, v.94, p.320-327. 1990.