ACTION OF GIBBERELLIC ACID (GA$_3$) ON DORMANCY AND ACTIVITY OF α-AMYLASE IN RICE SEEDS[1]

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RESUMO - Para avaliar a eficiência do ácido giberélico (GA$_3$) na superação da dormência de sementes de arroz, bem como a atividade da enzima α-amilase como indicador do grau dessa dormência, foram utilizadas sementes da cultivar irrigada Urucuia, que apresentam alta intensidade de dormência pós-colheita. Para tanto, as sementes foram submetidas à pré-secagem em estufa de circulação forçada de ar a 40ºC por 7 dias e à submersão em 30 ml de soluções de GA$_3$ nas concentrações de 0, 10, 30 e 60 mg/litro de H$_2$O, nos tempos de 2, 24 e 36 horas. Após os tratamentos, foi determinada a atividade da α-amilase através da eletroforese em gel de poliacrilamida e da espectrofotometria. Simultaneamente, foi realizado o teste de germinação. Pelos resultados, observa-se que houve ganho na germinação e na atividade da α-amilase em maiores concentrações e tempos de embebição das sementes em GA$_3$. A embebição das sementes em 60 mg GA$_3$/litro H$_2$O por 36 horas apresenta-se eficiente como um tratamento rápido na superação da dormência de sementes de arroz, sendo equivalente a estufa de circulação forçada de ar a 40ºC por 7 dias. A atividade da enzima α-amilase apresentou-se como um eficiente marcador do grau de dormência das sementes.

Termos para indexação: Sementes de arroz, dormência, germinação, ácido giberélico (GA$_3$), α-amilase.

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ABSTRACT - To evaluate the effectiveness of gibberellic acid (GA$_3$) in breaking rice seed dormancy and the use of a-amylase enzyme activity as an indicator of the dormancy level, seed from the intensively dormant irrigated cultivar Urucuia were used. The seeds were submitted to a pre-drying process in a forced air circulation chamber under 40ºC during 7 days and submersed in 30 mL of GA$_3$ solution under 0, 10, 30 and 60 mg/L H$_2$O concentrations, during 2, 24 and 36 hours. After the treatments, the a-amylase activity was determined by using the polyacrilamide electrophoresis and spectrophotometry. At the same time, the germination test was made. The results indicated a gain in germination and in a-amylase activity in higher concentrations and soaking time of seeds in GA$_3$. These observations support the conclusion that soaking seed in 60 mg GA$_3$/L during 36 hours can be used as a quick and efficient treatment in breaking rice seed dormancy and is equivalent to the forced air circulation chamber at 40ºC during 7 days. The a-amylase enzyme activity proved to be as an efficient marker of the seeds dormancy level.

Index terms: Rice seeds, dormancy, germination, gibberellic acid (GA$_3$), a-amylase.

INTRODUCTION

Seed quality evaluation soon after harvest is important in any seed production program. The information must be quickly and accurately obtained to help the producer decide whether to process or to discard the seeds.

Within the rice seed production process, the long dormancy period of irrigated cultivars has hindered the implementation of quick seed quality assessment tests.

The methods used to overcome dormancy in rice seeds, recommended by the Rules for Seed Analysis, Brazil (1992) have not provided the precision and speed required by the seed industry. On the other hand, the pre-drying tests to overcome rice seed dormancy reported by Vieira et al. (1994), although efficient, have not been as quick when used for irrigated cultivars that have a greater dormancy.

Inhibiting substances and the impermeability to oxygen caused by the husk (glumelas) and pericarp complex and are the most important dormancy inducers in rice seeds germination. Oxidation of phenolic compounds, along with the high respiratory activity in the cover tissues, restrict the entry of oxygen to the seed, limiting its availability to the embryo (Edwards, 1973; Vieira, 1992; Amaral, 1992; Bewley and Black, 1994). Besides these possible causes of dormancy, which directly or indirectly affect the seed metabolism of carbohydrate, protein and other reserves during the germination process, dormancy can also be credited to an equilibrium among growth regulating hormones, which play a fundamental role in the seed germination process (Amn, 1968; Barbosa, 1978).

The abscisic acid (ABA) hormone is a growth regulator that has received considerable attention due to its important role in inducing and maintaining seed dormancy (Walker-Simmons and Sessing, 1990; Wang et al., 1994; Wang, Heimovaara-Dijkstra, 1995). Bewley and Black (1994) reported that the difference between cultivars showing dormant and non-dormant seeds results from the ability of the dormant seed cultivars to retain ABA during maturity. However, Carvalho and Nakagawa (1988) reported that dormancy was affected by other factors besides a seed germination inhibitor. The authors hypothesis considered the existence of a complex balance among the concentrations of growth inhibitors and stimulators responsible for dormancy, as well as some mechanism that alters the sensitivity of the seeds to the promoter/inhibitor.

Growth regulators, such as auxins, cytokinins, gibberellins and others, have been applied with positive results for breaking seed dormancy (Koller et al., 1962). Among these regulators, the gibberellins have a primordial function, as their exogenous application counter balance the inhibition imposed by the abscisic acid and also causes an endogenous increase of gibberellic acid, which plays a key role in seed germination. The gibberellic acid is involved both in overcoming dormancy and in controlling the hydrolysis of reserves. The presence of adequate levels of this acid in the seeds stimulates the synthesis, activation and secretion of hydrolytic enzymes, mainly α-amylase, releasing reducing sugars and aminoacids which are essential for embryo growth (Khan, 1971; Metivier, 1979; Mayer and Poljakoff-Mayber, 1989).

The present study was carried out to assess the efficiency of gibberellic acid (GA$_3$) to quickly overcome dormancy in irrigated cultivar rice seeds and to investigate the α-amylase enzyme activity as an indicator of dormancy interruption, using spectrophotometric and electrophoretic techniques.

MATERIAL AND METHODS

Seeds were harvested and dried on a cement patio to approximately 13% moisture, processed and sampled. The collected sample was homogenized and assessed for dormancy by the germination test prescribed in the Rules for Seed Analysis (Brazil, 1992) before and after submission to a dormancy breaking treatment in a chamber with forced air at 40ºC for 7 days (Vieira et al., 1994).

After detecting seed dormancy, new samples were submitted to pre-germination treatments with GA$_3$ at different concentrations. The seeds were immersed in 30 ml of GA$_3$ solution at concentrations of 0, 10, 30, and 60 mg/liter of distilled water (corresponding approximately to 0, 25, 75 and 150 mM of GA$_3$) at 30ºC for periods of 2, 24 and 36 hours. The experimental doses and immersion times were those of Barbosa (1978) with modifications to the methodology.

Additional treatments were included, involving seeds not submitted to dormancy breaking treatments (control) and seeds submitted to forced air chamber at 40ºC for 7 days.

To assess the efficiency of the dormancy breaking treatments, seeds were submitted to germination tests, to determinations of the α-amylase enzymatic activity (E.C.3.2.1.1) and the protein quantification.

Germination tests - Four replications of 50 seeds per treatment were sown on paper towel in the roll system. The paper towels were moistened using a water quantity equivalent to 2.5 times their weight and placed in a germination chamber previously set at 30ºC. Assessments of normal seedlings were made 7 and 14 days after sowing, according the Rules for Seed Analysis (Brazil, 1992).

At the end of the test, the seeds that did not germinate were assessed for viability and dormancy by the tetrazolium test. these seeds were cut longitudinally through the embryo and submerged for coloring in a solution of 2, 3, 5 tri-phenyl

tetrazolium at 0.1% for a period of 4 hours at 30ºC in the dark. After this period, they were washed in running water and assessed for viability according to criteria established by the Rules for Seed Analysis (Brasil, 1992) and the results expressed in percentage of dormant seeds.

α-amylase and protein activity - The evaluation was carried out by electrophoresis and spectrophotometry. One hundred seeds from each treatment were placed to germinate in paper rolls in a germination chamber at 30ºC, until one of the treatments had 50% or more of root protrusion, which occurred in approximately 60 hours. In sequence, the seed palea, integument and caryopsis were hand removed from seed starting root protrusion and cold ground in a refrigerated mill and stored in a freezer at -84ºC.

The determination of the α-amylase enzymatic activity by electrophoresis involved 100 mg of ground seed samples treated with 200 ml of extraction buffer (0.2M Tris - HCl; 1% bmercapto-ethanol; 0.4% PVP; 0.4% PEG + 1 mM EDTA; 8.0 pH). The Eppendorf tubes containing the homogenized samples were kept on ice for 24 hours, after which the extracts from the different treatments were re-suspended and centrifuged at 16,000 x/g for 60 minutes at 4ºC and the supernatants collected.

To determine the a-amylase activity and the quantity of soluble protein by spectrophotometry, extractions were performed as described above, altering the volume of the extraction buffer to 1 ml.

Electrophoresis - 40 ml of each obtained supernatant were applied to polyacrylamide gels at 4.5% (concentrating gel) and 7.5% (separator gel containing 5% soluble starch). The gel/electrode system used was Tris-glycerin 8.9 pH. The electrophoresis runs were made at 12 mA in the concentrating gel and at 24 mA in the separator gel.

After electrophoresis, the gels were developed for α-amylase enzymatic activity at room temperature. By incubatingin 100 ml of sodium acetate buffer solution 50 mM + 200 mg of $CaCl_2$ (10%) at 50ºC for 60 minutes. The gels were then washed in distilled water and placed in 100 ml of a 10 mM I_2 solution containing 14 mM of KI until achromatic bands (pale) appeared in a bluish background (Alfenas et al., 1998).

Spectrophotometry - starch at 0.5% concentration was added to part of the supernatants obtained for this analysis. The determination of the reducing groups was carried out according to Noelting and Bernfeld (1948). In each determination, the reaction mixture was incubated for 5 different periods. Controls without enzyme (substrate blank) and

without substrate (enzyme blank) were incubated in the same way as the experiment tubes. The α-amylase enzyme activity was calculated and expressed in m mols of hydrolyzed substrate per minute (mileunit - mU).

The determination of the quantity of soluble proteins was carried out on the other part of the supernatant, following the methodology described by Bradford (1976) and using bovine albumin as control. The results were expressed in milligrams of soluble proteins per 100 mg of seed powder (mg/100mg).

The specific activity was later calculated dividing the a-amylase activity by the quantity of soluble proteins, and the results were expressed in mileunit per milligram (mU/mg).

Statistics - A completely randomized design with four replications and treatments arranged in a 4 x 3 factorial scheme (four concentrations and three immersion times) was used. The experiment also included, as two additional treatments, seeds submitted and not-submitted to the germination treatment in forced air chamber at 40ºC for 7 days.

Analyses of variance of the percentage values transformed to arc sin $\sqrt{(x/100)}$ were used to compare the results. Regression analyses were performed for the variables significant by the F test. The means of the additional treatments were contrasted with the best treatment of the factorial.

RESULTS AND DISCUSSION

Assessment of the pre-germination treatment efficiency

Regression equations and the adjusted germinated (Fig.1) and dormant (Fig.2) rice seed mean values obtained in the treatments with GA_3 were calculated.

Using the equation 1 (y= 49,14 + 0,669003 C - 0,008738 C^2 + 0,184167 T + 0,002716 CT) there was a quadratic effect of GA_3 concentration, a linear effect of imbibition time and an interaction effect of the two main factors (Fig.1). In general, greater GA_3 concentration and longer seed imbibition time increased the seed germination percentage. Similar results were obtained by Ramos (1994) when working with tangerine seeds treated with GA_3 at doses of 0, 25, 50 and 100 mg/liter of water and imbibition times of 0, 6, 12 and 24 hours. The author observed that as the GA_3 dose was increased the percentage of tangerine seeds germinated also increased at 0 and 6 hours of immersion time. Using the equation of Figure 1, a maximum germination of 90% can be found (from transformation reversed data) occurring for the 57.80 mg of GA_3/liter of H_2O concentration and the longest imbibition time (36 hours). Such observation reinforced reports by Paleg, Sparrow and Jennings (1962), Chrispeels and Varner (1967),
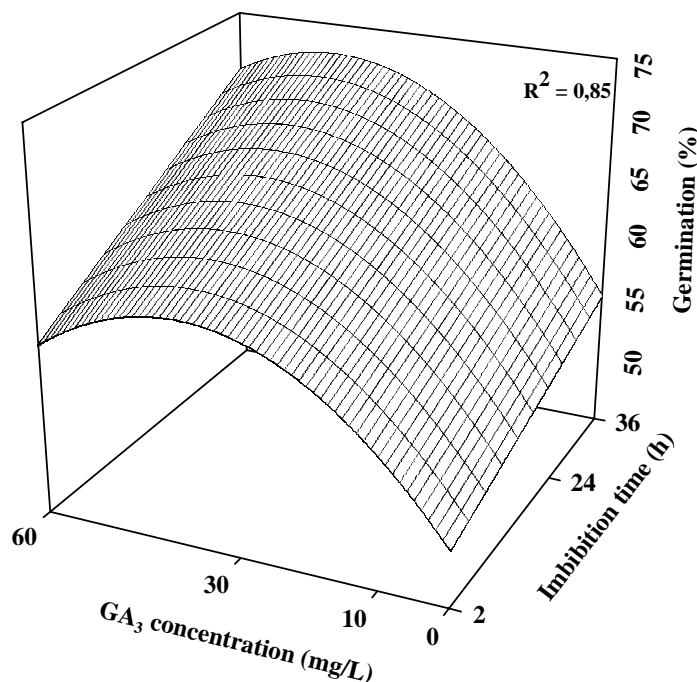
FIG. 1. Estimate of the percentage of germination of rice seeds after submission to pre-germination treatments with different GA$_3$ concentrations (mg/l) and imbibition times (h).
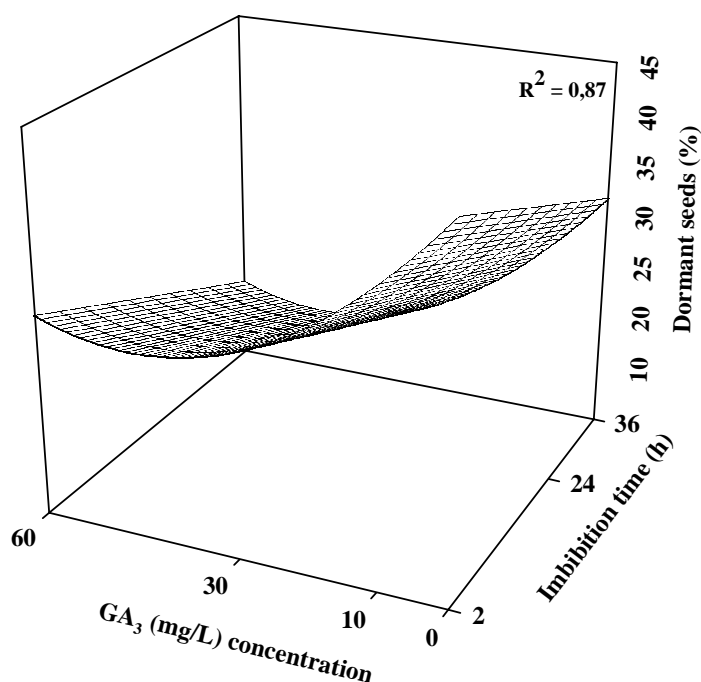

FIG. 2. Estimate of the percentage of dormant rice seed after submission to pre-germination treatments with different GA$_3$ concentrations (mg/l) and imbibition times (h).

Tanaka, Ito and Akazawa (1970) and Steinbach, Bech-Arnold and Sánches (1997) who emphasized that the presence of gibberellic acid (GA$_3$) at adequate endogenous levels in cereal seeds seemed to be an essential requirement for germination, and that the GA$_3$ hormone is continuously required throughout the germination process. The percentage of dormant seeds was reduced as the in the GA$_3$ concentration and imbibition time of the seeds increased (Fig. 2). As expected there was an inverse relationship between the germinated and the dormant seed percentages (Figs.1,2). The lowest dormant seed percentage values were found for treatment in the forced air chamber at 40ºC for 7 days and 60 mg/GA$_3$ liter H$_2$0 for 36 hours and both treatments had high germination. These results are in line with those of Khan (1971), Metivier (1979) and Mayer and Poljakoff-Mayber (1989), who demonstrated that gibberellin, more than any other plant hormone in the test, plays a key role in seed germination and is involved both in the dormancy breaking process and in the control of seed reserve hydrolysis, which is essential for embryo growth.

The treatment with forced air chamber at 40ºC for 7 days did not differ significantly from the treatment with 60 mg of GA$_3$/liter H$_2$O for 36 hours, by the Tukey test at the 5% level of probability (Table 1). Barbosa (1978) obtained similar

results, while Seshu and Dadlani (1991) observed that moistening the germination substrate with GA$_3$ solution was also an efficient way to break dormancy in rice seed. Our results reinforced reports by Lecat, Corbineau an Comê (1992) who found that GA$_3$ has the property of breaking dormancy in cereal seeds providing significant gains in germination.

It can be concluded that GA$_3$ used at 60mg/liter H$_2$0 concentration for 36 hours of seed imbibition is an alternative to the forced air circulation chamber at 40ºC for 7 days for breaking dormancy of rice seeds. The chamber method was found by Vieira et al., (1994) to be the best in their studies

TABLE 1. Contrasts comparing the mean germination values (%) of the best factorial treatment (60 mg/GA$_3$ liter H$_2$O for 36 hours - GA) and of the additional treatments, forced air circulation chamber at 40ºC for 7 days - EF and dry seeds (control - TE). UFLA-Lavras-MG, 2000.

y = EF - GA = 1,3125 - 1,2498 = 0,0627 [ns]
y = EF - TE = 1,3125 - 0,7303 = 0,5822*
y = GA - TE = 1,2498 - 0,7303 = 0,5195*

ns - Not significant at the 5% level of probability by the Tukey test.
* - significant at 5% level of probability by the Tukey test.

with dormant rice seeds. GA_3 use, however, has the advantages of higher speed and easy laboratory application.

α-amylase activity

In general there was an increase in amylase activity with the increase of GA_3 concentration and in imbibition time (Fig. 3, Table 2).
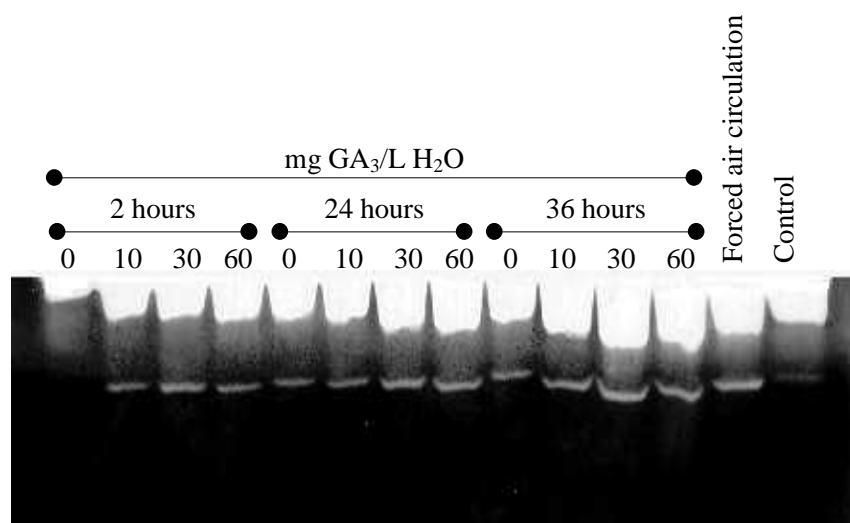

FIG. 3. **Electrophoretic profiles of α-amylase enzymatic activity in recently germinated rice seeds after pre-germination treatments with GA_3.**

TABLE 2. **Mean values of α-amylase activities (mU), quantity of soluble protein (mg/100mg) and specific activity (mU/mg) in rice seed extracts, submitted to dormancy breaking treatments.**

Treatments	α-amylase activity	Protein quantity	Specific activity
Dry seeds (control)	77,75	0,52	148,38
0 mg GA_3/L H_2O/ 2h	103,66	0,47	221,50
0 mg GA_3/L H_2O/ 24h	123,07	0,49	252,71
0 mg GA_3/L H_2O/ 36h	181,41	0,50	360,65
10 mg GA_3/L H_2O/ 2h	161,97	0,52	314,51
10 mg GA_3/L H_2O/ 24h	184,65	0,51	364,91
10 mg GA_3/L H_2O/ 36h	259,15	0,53	485,30
30 mg GA_3/L H_2O/ 2h	239,72	0,56	431,92
30 mg GA_3/L H_2O/ 24h	323,94	0,52	627,79
30 mg GA_3/L H_2O/ 36h	366,05	0,58	628,80
60 mg GA_3/L H_2O/ 2h	161,97	0,58	278,78
60 mg GA_3/L H_2O/ 24h	333,66	0,54	615,60
60 mg GA_3/L H_2O/ 36h	421,12	0,59	708,96
Forced air circulation 40ºC/7 days	647,88	0,53	1.227,04

These treatments were not efficient in promoting a balance among the regulatory growth hormones, which play a fundamental role in the seed germination response (Amen, 1968; Barbosa, 1978).

The greatest enzymatic activities occurred when the GA_3 concentrations and the seed imbibition times were greatest. This result was similar to but not as high as that obtained with the treatment with forced air circulation chamber at 40ºC for 7 days, Das and Sem-Mandi (1992), working with wheat seeds observed a considerable increase in α-amylase activity during imbibition and an association between the start of germination with the increase in a-amylase activity. It is worth pointing out, however, that this activity was not observed soon after the start of imbibition.

The results obtained in this study were confirmed by the quantification of the enzymatic activity and of the specific α-amylase activity through spectrophotometry (Table 2). Although the seed treatment in the forced air circulation chamber at 40ºC for 7 days produced higher activity values in the spectrophotometric analyses, this superiority was not detected in terms of a-amylase activity in the electrophoretic gel nor in the physiological assessments. Probably, from a certain level of activity, there is no corresponding increase in starch degradation. The results suggest, however, that the α-amylase enzyme activity is an important indicator of breaking dormancy in rice seeds.

CONCLUSIONS

The results obtained in the present study allow the following conclusions to be drawn:

- immersion of the seeds in 60 mg GA_3/litter H_2O for 36 hours was a quick and efficient method for breaking dormancy in rice seeds;

- the α-amylase enzyme activity is an efficient indicator of the degree of dormancy in the rice seeds.

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