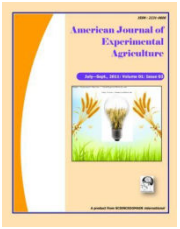

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Improving the Malting Qualities of Rice Grain Using Gibberellic Acid (GA_3)

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Research Article

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ABSTRACT

Application of exogenous gibberellic acid stimulates high production of hydrolytic enzymes in malts. To investigate this effect on rice malt, different concentrations of the GA_3 hormone were applied with the aim of establishing the optimum level that significantly affects rice malting qualities. Parameters evaluated included germination energy, shoot length, and enzymatic activity (diastatic and alpha amylase) of the rice malt. Shoot length, and enzyme activity were significantly affected by the GA_3 hormone at concentrations of 0.001, 0.1, 10, and 100 mg/L. 10 mg/L of GA_3 solution stimulated the highest production of diastase (1305 U/g dry malt), and shoot length of 2.93 cm after 60 hours of germination. Alpha amylase activity was increased by twofold. The maximum diastatic activity of GA_3 treated rice grains was found on the 8th day of germination, occurring earlier than the untreated which peaked at the 10th day. GA_3 treatment at a concentration of 10mg/L is adequate to stimulate higher production of diastase in rice malt.

Keywords: Gibberellic acid – 3 (GA_3), enzyme activity, germination energy;

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1. INTRODUCTION

Gibberellic acid, a plant growth regulator, is known to stimulate the growth of intact plant seeds (Arteca, 1995; Salisbury and Ross, 1992). It was originally used for breaking seed dormancy, and to enhance germination, especially under sub-optimal conditions (Bevilaqua et al., 1995; Dunand, 1992). With these same objectives, it was later employed in barley malting during the 1940s in Japan (Briggs, 1998). However, the significant effects of this acid on malting were not widely recognised until 1958 and 1959 when Sandegeron and Beling published a paper on the stimulatory effect of this hormone on enzyme activities of malt (Briggs, 1998). Thereafter, application of gibberellic acid (GA), especially GA_3 and GA_1, became a common practice in malting. Gibberellic acid (GA_3) stimulates higher production of enzymes during malting (Wahyuni et al., 2003). It also enhances growth and development of shoot (Asborno et al., 1999), and exhibits synergistic effect on endogenous GA_3 in stimulating production of hydrolytic enzymes especially α-amylase (Kaur et al., 1998). An increase in amount of reducing sugars in germinating grain is a direct reflection from the effect of this hormone (Vieira et al., 2002). Reducing sugars, which are osmotically active substances, reduce the water potential of germinating grains resulting in the entry of water into cells thereby causing elongation (growth of the shoot) (Arteca, 1995).

Application of GA_3 in rice malting is however not a common practice especially in Ghana. Present studies however show that amylolytic enzyme development is more significant in rice malts than in other cereals (Dziedzoave, 2004). In order to explore the enzyme development potential of rice malt, GA_3 was applied in this study with the aim of establishing the optimum GA_3 concentration that could further enhance the malting qualities of rice malt and improve upon the economics of its industrial applications.

2. MATERIALS AND METHODS

A randomised complete block design with three replicates was used for all the experimental runs. Gibberellic acid concentrations of 0.001, 0.1, 10, and 100 mg/L were used. The acid concentrations and values of the other experimental parameters including temperature (28, 60, and 80^{o}C and pH (5.5) used were selected based on preliminary works and literature values (Ayernor et al., 2002; Wahyuni et al., 2003).

2.1 Materials

Paddy rice (Jasmine 85 variety) was obtained from Crop Research Institute (CRI), Fumesua, Kumasi. Gibberellic acid (GA_3) and other materials were obtained from Biochemistry and Chemistry Departments, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, and Department of Botany, University of Ghana, Legon, Ghana.

Data obtained were subjected to statistical analysis to determine significant differences or otherwise between the treatment means at $p = 0.05$ with Microsoft Excel Programme. The interactions were tested using Statistical Package for Social Sciences (SPSS) software (version 13).

2.2 Malting and Gibberellic Acid (GA_3) Treatment of Rice Grains

Three replicates of 1.5g Jasmine 85 rice grains were soaked in 200mL distilled water for 48 h at a temperature of 28±1^{o}C. The steeping was carried out in a micro malting chamber, and

the steep water was changed every 12 h. During the steeping process, the grains were subjected to 6 h air-rest treatment. At air-rest, the steep liquor was drained off during which the grain underwent aerobic respiration. GA_3 solution at concentrations of 0.001, 0.1, 10, and 100 mg/L of distilled water were applied during the air-rest period and immediately after steeping where most of the grains have chitted. The soaked grains were germinated for 12 days at a temperature of 28 + 1^{o}C. Kilning was carried out at a temperature range of 42- 45 ^{o}C for 5 h. Germination energy, shoot length and the diastatic activities of the malts were measured (Bam et al., 2006; Hammond and Ayernor, 2001; Osman, 2002). In addition, the alpha amylase activity of the malt was evaluated in accordance with Osman (2002) and Canizares-Macias et al., (2001).

2.3 Germination Energy Determination

Three replicates of 1.5g rice grains were steeped in the different GA_3 solutions for 48 h and germinated in the malting chamber at a temperature of 28$\pm$1^{o}C. The steeped grains were placed in petri dishes lined with two Whatman's filter papers. 5-10mL of distilled water was sprinkled to the grains daily. The germination was carried out in the dark. To assess the rate of the grain emergence, emergence counts were made in 48 hours. The ratio of germinated grains to the total grains was calculated and recorded as the germination energy of the grain in according with Bam et al. (2006).

2.4 Extraction of Enzymes from Rice Malt

Methods described by Osman (2002), and Ayernor et al. (2002) were employed. Some modifications were however made. Na-phosphate buffer (pH 8) was used and the final extract was diluted by a factor of 50 using 0.1M malate buffer (pH 5.5). Rice Malt Extracts (RME) were prepared from both the soaked rice grains and the germinated grains.

2.5 Alpha Amylase Assay using DNSA Method

To evaluate α-amylase activity, β-amylase was inactivated by heating the Rice Malt Extract (RME) for 7 minutes at 70^{o}C in the presence of $CaCl_2$. To pre-equilibrated soluble starch solution (0.25 mL, 50 mg/mL in 0.1 M malate buffer of pH 5.5, containing 40 mM $CaCl_2$), 0.05 mL heated RME was added while mixing. The reaction was continued for 10 minutes and terminated by adding 2mL of 0.1 M NaOH solution. 1.0 mL freshly prepared DNSA reagent was added, mixed and heated for 5 minutes at 80^{o}C. After cooling, the absorbance of the mixture as well as the control and the standard (maltose) were read at 480nm from spectrophotometer (Heλious UV spectrophotometer). The standard and control were treated similarly except that the RME was added to the control after NaOH. The α-amylase activity of the rice malt extracts was determined and expressed in U/g (Osman, 2002; Canizares-Macias et al., 2001)

2.6 Diastase Assay Using DNSA Method

To 0.25mL pre-equilibrated soluble starch solution, 0.05mL of appropriated diluted (dilution factor; 50) RME was added while mixing and incubated for 10 minutes at 60^{o}C. The reaction was terminated by adding 2mL of 0.1M NaOH solution. 1.0mL freshly prepared DNSA reagent was added, mixed and heated for 5 minutes at 80^{o}C. After cooling, the absorbance of the mixture as well as the control and the standard were read at 480nm from spectrophotometer. The diastase activity was also calculated according to Osman (2002).

3. RESULTS AND DISCUSSION

3.1 Germination Energy and Diastatic Activity of GA_3 Treated Rice Grain

Germination energy and diastatic activity of 60 h germinated GA_3-treated rice grains are presented in Table 1. Significant differences ($p< 0.05$) were observed between the diastatic activities of the malts. However, the germination energy was not significantly affected by the GA_3 treatment. In general, increasing GA_3 concentration increases the synthesis of diastase. The increase was however observed to a point (10 mg/L) where the effect of the hormone was at its peak. Beyond this level, it exhibited some inhibitory action on the biochemical activities of the grains causing the decrease in the malt quality (diastase activity). High concentration of hormones tends to have a negative effect on the physiological development of a tissue (Salisbury and Ross, 1992). The results imply that GA_3 application at a concentration of 10 mg/mL is adequate to cause a significant increase in diastatic activity of rice malt. This concentration was hence used for the rest of the germination process.

Table 1. Germination energy and diastatic activity of GA_3 treated rice malts

GA_3 concentration (mg/L)	Germination energy (%)	Diastase activity (U/g dry malt)
0	91.52 (0.27)^a	974.12 (0.52)^a
10^{-3}	91.20 (0.15)^a	1027.90 (0.47)^b
10^{-1}	91.30 (0.10)^a	1112.10 (0.37)^c
10	91.64 (0.11)^a	1305.27 (0.68)^d
10^2	91.56 (0.14)^a	1045.87 (0.48)^e

Standard error of means are presented in parenthesis
Mean values in each column with the same superscript are not significantly different (p = 0.05) based on LSD test.

3.2 Shoot Length Development of GA_3 Treated Rice Seedlings

Figure 1 shows the effect of the GA_3 treatment on shoot length development of rice grains. The rate of shoot growth increases with increasing germination period with a correlation factor of $r = 0.99$ at $p< 0.05$. A significant difference was also observed between GA_3 (10mg/ml) treated seedlings and the control ($p = 0.0096$). In general, shoot growth was improved by GA_3 treatment. The differences in the shoot lengths were noticeable from the 4th day of germination. The average increase in the shoot length under GA_3 treatment was about 2-fold compared to that of the control. This finding agrees with other studies in which GA_3 improved development of rice seedlings (Wahyuni et al., 2003; Dunand, 1992). Wahyuni *et al.* (2003) further reported that GA_3 can increase rice shoot length by 1.3 – 2.3 folds. Application of GA_3, as seed treatment, significantly improves germination, seedling emergence, and height of rice grain (Asborno et al., 1999; Bevilaqua et al., 1995).

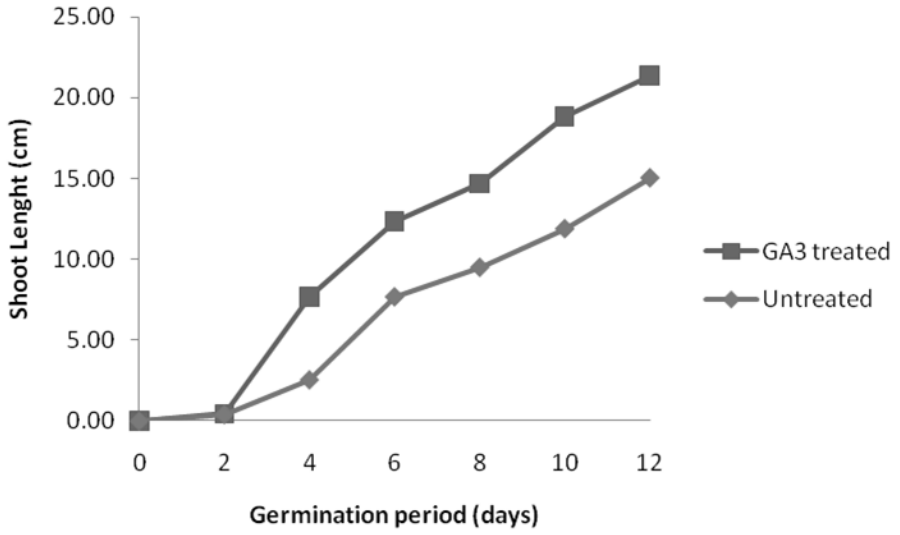

Fig. 1. Shoot length development of GA_3 treated rice seedlings

The increasing effect of GA_3 on the shoot length development could be attributed to its stimulatory ability on the production of hydrolytic enzymes which catalyses the chemical breakdown of food reserves, making them available for development (Briggs, 1998). The degradation processes increase the osmotically active substances (reducing sugars) thereby increasing the water potential gradient between the cells of the rice seedlings and the water available. Hence water moves into the cells causing shoot elongation. Gibberellins promote shoot elongation by increasing plasticity of cell walls (Arteca, 1995). Kaur et al., (1998) also reported that exogenous application of GA_3 enhances the ability of endogenous GA_3 to stimulate higher synthesis of hydrolytic enzymes in germinating seed thereby increasing the formation of glucose from the stored starch and the growth of seedling. Gibberellic acid application and air-rest treatment generally promote shoot growth (Briggs, 1998).

3.3 GA_3 and Diastatic Activity of Rice Malt

In general, the diastatic activity of the rice malt increased alongside germination period to a point (Figure 2). Agbale et al. (2007) and Hammond and Ayernor (2001) observed a similar trend in rice malting with diastatic activity increasing with increased malting period. GA_3 treated malt produced more diastase (1545.94 U/g) than the control (1456.33 U/g), averagely (Fig. 2). Moreover, malts resulting from GA_3 treatment had their optimum diastatic activity on the 8^{th} day after germination, occurring earlier than the control which peaked at day 10. The diastatic activity of the GA_3 treated malt remained fairly constant after the 10^{th} day while that of the control declined. The decrease in enzymes production in general could be attributed to the physiological feedback from the embryo switching off enzyme production. During germination, the embryo takes simple sugar produced from the enzymatic hydrolysis, putting a check on enzyme production through regulatory feedback loop (Briggs, 1998).

Gibberellic acid is known to stimulate enzyme production for mobilization of food reserves in germinating grains (Arteca 1995; Salisbury and Ross, 1992). GA_3 application in addition to other treatments such as aeration accelerates malting (Briggs, 1998). These effects

accounted for the increase in diastatic activity in the GA_3 treated malt compared to the untreated. Application of GA_3 during rice malting thus improves the final quality of the malt.

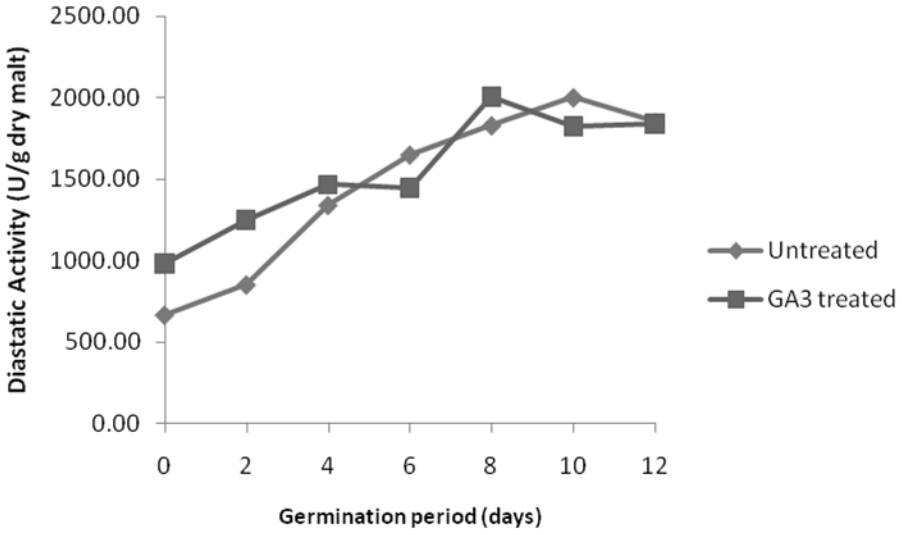

Fig. 2. Diastatic activities of GA_3 treated rice seedlings

3.4 GA_3 and Alpha Amylase Activity of Rice Malt

Figure 3 shows the activity of α-amylase in GA_3 treated rice malts. The increase in activity was only significant on the fourth day in the control rice seedlings; reaching its maximum at the 6th day.

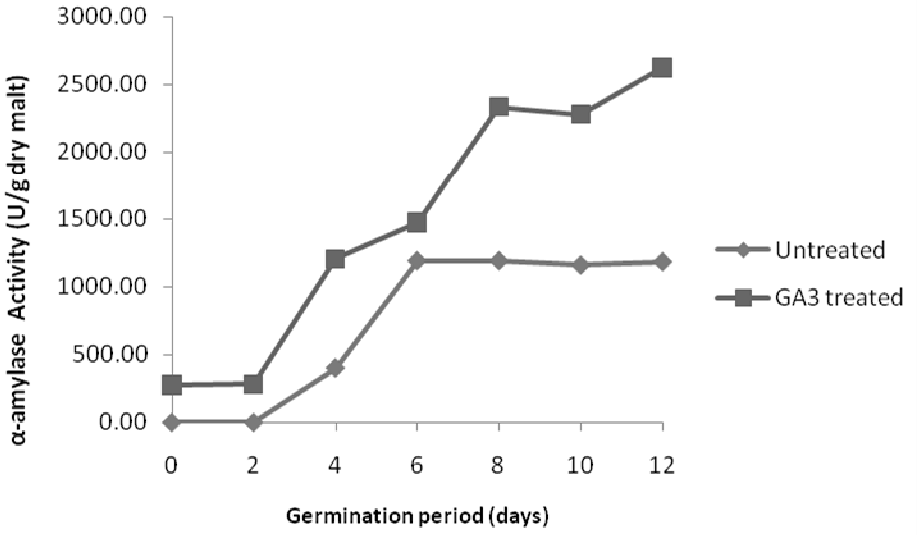

Fig. 3. Alpha amylase activity of GA_3 rice seedlings

Enzyme activity remained fairly constant thereafter whilst that of the GA_3 treated increased constantly throughout the malting period, producing the highest mean α-amylase activity of 1494.89 U/g. The alpha amylase activity in the treated malt was about 2 fold compared to that in the control.

Statistical analysis showed a positive correlation ($r = +0.97$, $p < 0.05$) between α-amylase activity of GA_3 treatment seedlings and malting periods. Generally, α-amylase activities and its ratio to other starch-degrading enzymes tend to increase with time under most germination conditions (Briggs, 1998). External application of GA_3 can increase α-amylase levels in malts by as much as fourfold, demonstrating that endogenous hormone levels are insufficient to trigger maximum enzyme formation (Briggs, 1998). The current findings do support the early assertions that exogenous application of GA_3, at optimum level, synergistically enhances enzymes production with more effect on alpha amylase synthesis (Vieira et al., 2002; Kaur et al., 1998).

4. CONCLUSION

It is clear from the present study that Gibberellic acid-3 (GA_3) has a significant effect on malting qualities of rice malt. Concentration of 10 mg/L is adequate to stimulate higher production of diastase. It also exhibited an exponential effect on alpha amylases production in rice malt. Application of 10 mg/L of GA_3 during rice malting will significantly improves amylases production for efficient conversion of more soluble starch to simple sugars. Optimising the total amylase activity of rice malt implies that lesser quantity of the malt would be utilised during hydrolysis of starches. This would constitute a significant cost savings and enhance the utilization of local rice in food industries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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