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Changes in germination of the aged rice seeds after priming with rice bran, husks, brassinosteroids and chitosan

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Abstract This study described rice bran and paddy husk that are by-products from rice. By immersing seeds, the researchers sought to increase seed germination efficiency by using rice bran and husk extracts from milled rice using three varieties of organic Thai rice seeds: KDML 105, Chai Nat 1, and Hom Nin, stored for 2 years at room temperature. Hom Nin rice seeds showed normal to high seedling compared to KDML105 and Chai Nat1. KDML 105 was shown the normal seedling responses to water-soaked rice bran and centrifuged rice bran, which was the same effect as soaked in water. It was found that the germination index of KDML 105 and Chai Nat1 responded to soaking in brassinosteroids, water-soaked rice bran, centrifuged rice bran, and centrifuged rice husks. For Hom Nin rice seeds had affected to high germination rates for all experimentals process. The use of brassinosteroid hormones in the three varieties of rice had affected on the increase of root lengths and root numbers, especially in Hom Nin. The maximum shoot dry weight was found in Chai Nat 1 which soaked in water. However, abnormal seeds, hard seeds, shoot heights, root dry weights, and root shoots did not display statistically significant differences.

Keywords: Seed priming, Germination, Vigour, Seedling

Introduction

Rice (*Oryza sativa*) is the main food of the Thai people and is also produced for export (Tulyathan and Leeharatanaluk, 2007). The areas where rice can be grown easily were planted first because Thai people eat rice as their principal food. The waste straws from the rice threshing in paddy fields after the harvest becomes cattle and buffalo feed. The harvested grain is polished to the grain, while the rice bran, broken-milled rice and paddy husks are the by-products. Rice bran and broken-milled rice can be fed to animals such as chickens, pigs, and ducks, among others (David J, 1994). At present, food security is at risk because of the changing world climate which often results in sudden flooding. In some years, rice farmers lose their entire crop to such disasters. Farmers therefore practice collecting grains

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for two years for food security, and that can be used as alternative seeds for cultivation. Rice seeds have a high percentage of germination, and the rapid germination period is crucial to the cultivation of rice. The germ seeds are prepared by soaking in water until it penetrates the seed. The gibberellin hormone produced in the embryo diffuses to the aleurone layer, where enzymes break down food stores into assimilable smaller molecules (Okamoto and Akazawa, 1979; Kaneko, 2002). These molecules provide nourishment to allow root germination and leaf development.

However, a comprehensive analysis of the germinated Thai rice by using the different forms of waste from rice products to improve germination has yet to be undertaken. In the current work, we focused on Thai indica rice since it is widely consumed in and exported from Thailand and other countries in South East Asia (Dawe, 2002). Physiological role of brassinosteroids in the plant is similar to auxin in some cases. It is also similar to cytokinin and gibberellin hormones affecting both cell division and the elongation of cells in the stem and leaf sections (Clouse, 1996; Hong *et al*., 2002). Therefore, the actual physiological role of brassinosteroids in the plant is complex. Brassinosteroids have demonstrated several physiological roles in promoting the growth and development process of seed germination. Kartal *et al*. (2009) Studies on the effects of the use of brassinosteroids with a concentration of 1 μM have shown effects upon the germination of the roots of barley, stimulating increased germination. Root lengths increased within 48 hours, with long roots of 2.49 ± 0.8 cm, which is almost twice as long compared to a concentration of 0 μM, which generated lengths of 1.62 ± 0.6 cm. Using brassinosteroids can also enhance the germination and maturation of rice seedlings even under stress, as would occur in salty soil or at low temperatures (Anuradha and Seeta Ram Rao, 2001; Fujii and Saka, 2001). Brassinosteroids are related to the synthesis of mRNAs and protein in seeds (Nomura *et al*., 2007; Han *et al*., 2014), and to the synthesis of hormone gibberellic acid (Ullah *et al*., 2002). In addition, enzymes can digest the nutrients accumulated within the seeds for the embryo, which causes the development of the roots and leaves of the seedlings (Reinhold *et al*., 2011).

Chitosan is a substance derived from chitin, which is found in a variety of species of crustaceans and insects as well as some species of fungi. Chitosan consists of glucosamine (Hirano *et al*., 2014). By using chitosan-coated seeds, the seed is cultivated with the chitinase enzyme and soluble protein within the seed, resulting in more viable grains during the seed germination process. Grain coatings such as chitosan increase the activity of amylase enzymes, thereby causing biodegradable starches to change to sugars, enhancing seed germination (Songlin and Qingzhonh, 2002; Thananun and Wongrueang, 2009).When the effects of chitosan upon the germination and growth of the rice seedlings of the San Pa Tong variety were studied by soaking the grains in chitosan solutions of 0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, and 10.0 g/l, the result showed that the use of the 8.0 g/l chitosan solution resulted in the maximum germination rate at 6.94%, while the chitosan concentration level that resulted in the longest roots and the greatest stem length was 10.0 g/l.

Rice grain, when polished to white rice, produces rice bran and husks as the waste products. The bran and paddy husks include part of the aleurone layer and grain encapsulation, which may contain organic compounds, hormones, enzymes, nutrients, or phenolic compounds (Batsut and Siriamornpun, 2010) that are beneficial to the germination of grains. The use of the paddy husk has no effect on inhibiting the growth of rice (Mitsuaki *et al*., 1977), but the amounts of phenolic compounds in the bran and husk also have an effect on the seed germination and vigour of the rice seeds. Seed germination is the first success factor in the rice planting process in both transplanting and direct sowing methods. The use of agricultural waste, including rice bran and paddy husk, could improve the germination and vigour of seedlings, as could the technique of using the effects of brassinosteroid hormones and chitosan at different concentrations applied to various rice varieties in Thailand.

Therefore, the aim of this study was to investigate and compare the rice seed germination of the three varieties by the use of materials from milled rice (rice bran and husk) compared with un-soaked grains, soaking in water, soaking in brassinosteroids, and chitosan.

Materials and methods

Plant materials

Three varieties of organic rice seeds, namely KDML 105, Chai Nat 1, and Hom Nin, were harvested from rice fields at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University. All rice seeds were stored for two years at room temperature, in dark conditions, and with approximately 14% moisture content before conducting the experiment.

Experimental design

Fifty seeds were used as a sample group for each treatment. The experimental methodology was designed as two factors factorial in completely randomised design (CRD) with four replications. Factor A (3 rice varieties) and factor B (priming treatments: Tr1-grains not soaked, Tr2-soaked in water, Tr3-soaked in 1% acetic acid, Tr4-soaked in 1% chitosan, Tr5-soaked in 0.04 ppm brassinosteroid, Tr6-soaked in 10% rice bran, Tr7-soaked in 10% centrifuged rice bran (5,000 rpm for 15 min; Eppendorf 5804 R, Canada), Tr8-soaked in 10% rice husk solution, and Tr9-soaked in 10% centrifuged rice husk solution (Eppendoft 5804 R, Canada). The rice seeds were soaked in each treatment for 2 hours. The treated seeds were incubated for 14 days in sterilized Petri dishes at 25 °C fitted with paper towels, and 10 ml distilled water was added to each Petri dish.

Germination

To test seed germination, four replicates of 100 seeds were estimated in accordance with the International Rule of Seed Testing used by the International Seed Testing Association (ISTA, 1995). Seeds were germinated in 12 cm diameter Petri dishes. Germinated seeds were counted after two periods of germination at 7 days and 14 days, respectively. For the final count, the germinated seeds were harvested to check the effects of priming treatments on Normal Seedlings (NS), Abnormal Seedlings (AS), and Hard Seeds (HS) at day 14.

Germination Index (GI)

The numbers of seedlings emerging daily were counted from day 7 until day 14 after planting. Thereafter a Germination Index (GI) was calculated by using the following formula as proposed by Gupta (1993).

$$\mathrm{G.I.} = \frac{\text{number of germinated seeds}}{\text{day of first count: 7 th day}} + \frac{\text{number of germinated seeds}}{\text{day of final count: 14 th day}}$$

Growth response

Fourteen-day-old seedlings were harvested to check the effects of priming treatments on shoot height (SH), root length (RL), number of roots (RN), and root/shoot ratio (RSR).

SH and RL were measured by selecting five seeds randomly for each treatment. SH was measured from the base of the primary leaf to the base of the hypocotyls, and RL was measured from the tip of the primary root to the base of the hypocotyls, with all measurements expressed in centimeters. Shoot dry weight (SDW) and root dry weight (RDW) were examined using digital weight scales and expressed in grams. All measurements were carried out according to the method previously used (Dash, 2012).

Statistical analysis

Data were analysed using the two-way Analysis of Variance method (ANOVA) and Duncan's Multiple Range Test (DMRT) at a significance level of 0.05, using Significance Analysis System (SAS, version 9.1). The average values are presented.

Results

Effects of seed priming on normal seedlings (NS), abnormal seedlings (AS), hard seeds (HS) and germination index (GI) of rice seedlings after germination for 14 days. The result show that normal seedlings (NS) of Hom Nin and Chai Nat 1 rice seeds were high germination compared to KDML 105. NS of KDML 105 responded to soaked rice bran and centrifuged rice bran, which had the same germination effect as soaking in water. There was a significant response to unsoaked grains, rice husk solution, and centrifuged rice husks (Table 1).

Abnormal seedlings (AS) and Hard seeds (HS) of all seed varieties with various priming methods had no statistical differences (Table 1). Meanwhile, the non soaked seeds of the KDML 105 had the lowest germination index (GI) value. The GI of Chai Nat 1 slightly responded in germination when soaked in chitosan, acetic acid, water, and not soaking. However, the GI had a high statistical difference in germination when soaked in brassinosteroid, rice bran, centrifuged rice bran, and centrifuged rice husk. The Hom Nin rice seeds had high GI value for all experimentals process.

Effects of seed priming on shoot height, root length, root number, shoot dry weights, root dry weights and root shoot ratio rice seedlings 14 days after germination. The use of brassinosteroid hormones in the three varieties of rice had variable effects on root lengths compared to other treatments (Figure 1). In particular, Chai Nat 1 and Hom Nin had root lengths (RL) of 8.33 cm and 8.03 cm respectively (Table 2).The highest root number (RN) showed in Chai Nat 1 unsoaked seeds (6.33) but had not statistically different from seeds soaked in rice husk solution, brassinosteroid, centrifuged rice bran, centrifuged rice husk and water had RN of 5.83 5.75, 5.33, 5.16 and 5.08 respectively, and Hom Nin seeds soaked in brassinosteroid had RN of 5.66 (Table 2). The higest shoot dry weights (SDW) showed in Chai Nat 1 seeds soaked in water. In this trial, shoot heights (SH), root dry weights (RW), and root shoots ratio (RSR) had no statistically significant differences (Table 2).

Table 1. Effects of seed priming on the normal seedlings, abnormal seedlings, hard seeds, and germination index of rice seedlings 14 days after germination

Variety	**Treatment**	**normal seedlings (NS) (%)**	**abnormal seedlings (AS) (%)**	**hard seeds (HS)(%)**	**germination index (GI)**
KDML 105	Unsoaked grains	90.00 ^{F}	4.00	6.00	6.25 ^{J}
	Soaked in water	93.50 $^{A–D}$	2.50	4.00	6.85 ^{DEF}
	Soaked in acetic acid	91.00 ^{DEF}	3.00	6.00	6.64 ^{GHI}
	Soaked in chitosan	93.00 $^{B–E}$	2.00	5.00	6.68 ^{FGH}
	Soaked in brassinosteroid	91.50 $^{C–F}$	3.00	5.50	6.64 ^{GHI}
	Soakedin rice bran solution	94.50 ^{AB}	2.00	3.50	6.89 ^{CDE}
	Soaked in centrifuged rice bran	94.00 ^{ABC}	2.00	4.00	6.82 ^{EFG}
	Soaked in rice husk solution	90.50 ^{EF}	4.00	5.50	6.46 ^{I}
	Soaked in centrifuged rice husk	89.75 ^{F}	3.00	7.25	6.53 ^{HI}
Chai Nat 1	Unsoaked grains	94.50 ^{AB}	2.50	3.00	6.89 ^{CDE}
	Soaked in water	94.50 ^{AB}	2.00	3.50	6.89 ^{CDE}
	Soaked in acetic acid	94.00 ^{ABC}	3.25	2.75	6.89 ^{CDE}
	Soaked in chitosan	93.00 $^{B–E}$	2.50	4.50	6.85 ^{DEF}
	Soaked in brassinosteroid	95.00 ^{AB}	2.00	3.00	6.96 $^{A–E}$
	Soaked in rice bran solution	94.00 ^{ABC}	3.00	3.00	6.96 $^{A–E}$
	Soaked in centrifuged rice bran	96.00 ^{A}	2.00	2.00	7.03 $^{A–D}$
	Soaked in rice husk solution	93.50 $^{A–D}$	3.00	3.50	6.92 $^{B–E}$
	Soaked in centrifuged rice husk	95.00 ^{AB}	2.25	2.75	7.00 $^{A–E}$
Hom Nin	Unsoaked grains	95.25 ^{AB}	2.50	2.25	7.14 ^{A}
	Soaked in water	93.75 ^{ABC}	3.00	3.25	7.14 ^{A}
	Soaked in acetic acid	95.25 ^{AB}	2.75	2.00	7.10 ^{AB}
	Soaked in chitosan	95.00 ^{AB}	2.50	2.50	7.14 ^{A}
	Soaked in brassinosteroid	94.00 ^{ABC}	3.00	3.00	7.14 ^{A}
	Soaked in rice bran solution	95.25 ^{AB}	2.50	2.25	7.14 ^{A}
	Soaked in centrifuged rice bran	93.75 ^{ABC}	3.00	3.25	7.07 ^{ABC}
	Soaked in rice husk solution	94.50 ^{AB}	2.50	3.00	7.00 $^{A–E}$
	Soaked in centrifuged rice husk	94.75 ^{AB}	2.75	2.50	7.07 ^{ABC}
F-test		*	Ns	ns	*
CV(%)		2.01	17.47	14.34	2.13

ns = do not differ significantly; superscript letters shared between treatments indicate similarity between treatments.
*, = **were significant at 0.01, 0.05 probability levels, respectively.

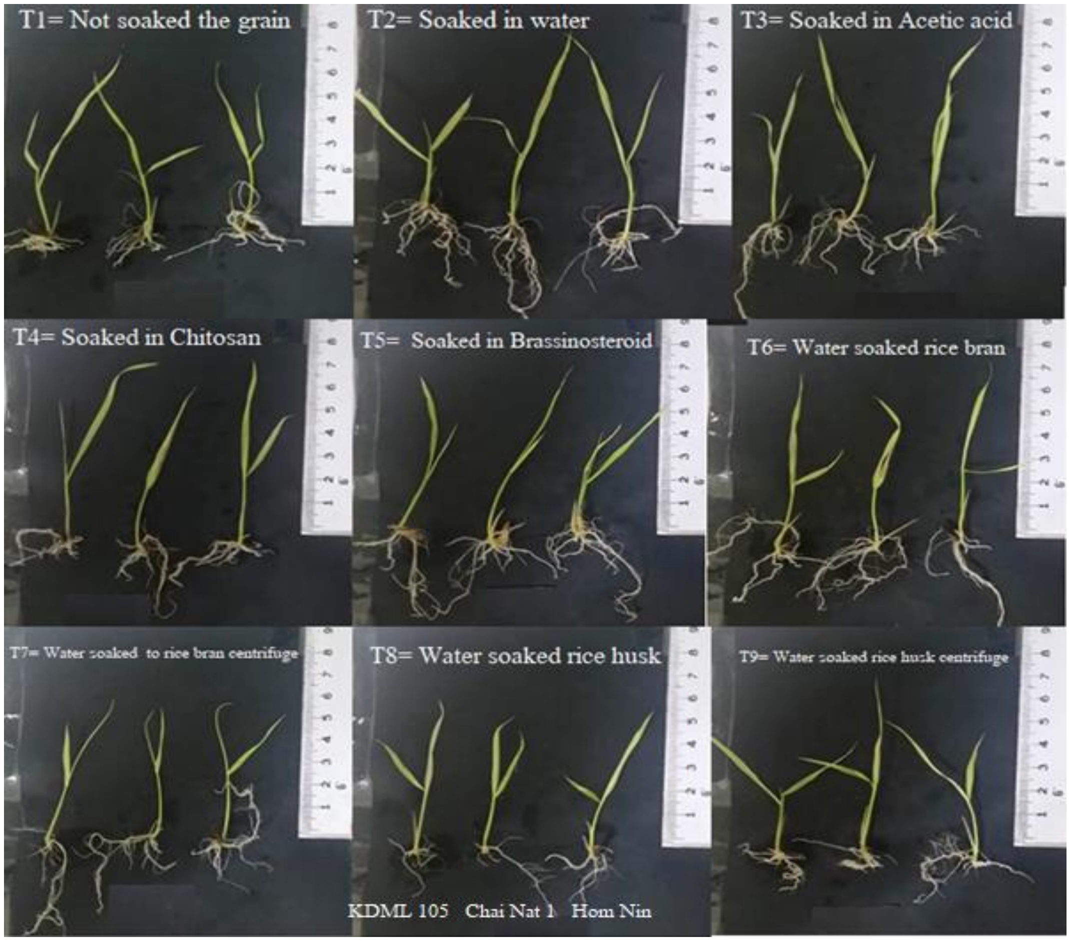

Figure 1. Seedlings of three varieties of organic rice seeds, namely KDML 105, Chai Nat 1, and Hom Nin, 14 days after treatments 1-9 (photo top row T1-T3, middle row T4-T6, and bottom row T7-T9): Tr1-grains not soaked, Tr2-soaked in water, Tr3-soaked in 1% acetic acid, Tr4-soaked in 1% chitosan, Tr5-soaked in brassinosteroid, Tr 6-soaked in rice bran, Tr7-soaked in centrifuged rice bran, Tr8-soaked in rice husk solution, and Tr9-soaked in centrifuged rice husk solution

Table 2. Effects of seed priming on shoot height, root length, root number, shoot dry weights, root dry weights and root shoot ratio of rice seedlings 14 days after germination

Variety	Treatment	shoot height (SH) (cm)	root length (RL) (cm)	root number (RN)	shoot dry weights (SDW) (mg/shoot)	root dry weights (RDW) (mg/root)	root shoot ratio (RSR)
KDML 105	Unsoaked grains	4.04	4.83^{BCD}	3.83$^{E–I}$	1.90$^{C–H}$	1.82	0.99
	Soaked in water	4.15	3.47^{EF}	3.58$^{F–J}$	1.93$^{C–H}$	2.17	1.13
	Soaked in acetic acid	4.57	4.09^{DEF}	2.50^{JK}	2.00$^{C–G}$	2.10	1.04
	Soaked in chitosan	4.57	4.70^{BCD}	4.75$^{B–F}$	1.90$^{C–H}$	2.17	1.22
	Soaked in brassinosteroid	4.32	5.43^{BC}	4.91$^{B–E}$	1.40^{GH}	1.68	1.20
	Soaked in rice bran solution	4.18	4.44^{CDE}	3.58$^{F–J}$	1.85$^{C–H}$	1.93	1.04
	Soaked in centrifuged rice bran	4.14	5.02^{BCD}	2.75$^{J–K}$	1.85$^{C–H}$	2.17	1.28
	Soaked in rice husk solution	4.29	3.16^{F}	3.91$^{D–I}$	1.90$^{C–H}$	1.83	0.98
	Soaked in centrifuged rice husk	4.61	4.06^{DEF}	3.58$^{F–J}$	1.85$^{C–H}$	1.50	0.80
Chai Nat 1	Unsoaked grains	4.71	5.27^{BC}	6.33^{A}	2.32^{BC}	2.67	1.26
	Soaked in water	4.58	4.50$^{B–E}$	5.08$^{A–E}$	3.58^{A}	4.00	1.10
	Soaked in acetic acid	4.29	4.79^{BCD}	4.83$^{B–F}$	2.30^{BC}	2.68	1.14
	Soaked in chitosan	4.72	4.61$^{B–E}$	4.08$^{C–H}$	2.17$^{B–E}$	2.25	1.07
	Soaked in brassinosteroid	5.03	8.33^{A}	5.75^{AB}	2.67^{B}	2.58	0.98
	Soaked in rice bran solution	4.41	4.66^{BCD}	4.33$^{C–G}$	2.08$^{B–F}$	2.93	1.41
	Soaked in centrifuged rice bran	4.16	5.21^{BCD}	5.33^{ABC}	1.90$^{C–H}$	1.82	1.04
	Soaked in rice husk solution	4.37	4.83^{BCD}	5.83^{AB}	2.35^{BC}	2.25	1.00
	Soaked in centrifuged rice husk	4.12	4.58$^{B–E}$	5.16$^{A–D}$	1.98$^{C–G}$	2.08	1.10
Hom Nin	Unsoaked grains	4.41	5.04^{BCD}	3.99$^{D–I}$	2.08$^{B–F}$	2.25	1.08
	Soaked in water	5.08	4.66^{BCD}	3.33$^{G–K}$	2.08$^{B–F}$	2.22	1.09
	Soaked in acetic acid	4.79	5.09^{BCD}	2.25^{K}	1.93$^{C–H}$	2.08	1.09
	Soaked in chitosan	4.95	5.62^{B}	4.58$^{B–G}$	2.25$^{B–D}$	2.10	0.94
	Soaked in brassinosteroid	4.75	8.03^{A}	5.66^{AB}	2.10$^{B–F}$	2.00	0.98
	Soaked in rice bran solution	3.70	4.83^{BCD}	3.58$^{F–J}$	1.32^{H}	1.90	1.50
	Soaked in centrifuged rice bran	4.12	4.66^{BCD}	3.00$^{H–K}$	1.57$^{E–H}$	1.60	1.00
	Soaked in rice husk solution	3.37	1.62^{G}	3.91$^{D–I}$	1.65$^{D–H}$	1.93	1.20
	Soaked in centrifuged rice husk	3.96	5.04^{BCD}	3.33$^{G–K}$	1.50^{FGH}	1.42	0.95
F-test		ns	**	*	*	ns	ns
CV(%)		11.76	10.88	12.36	9.87	12.04	11.44

ns = no statistically significant difference; superscript letters shared between treatments indicate similarity between treatments
*, = ** were significant at 0.01, 0.05 probability levels, respectively

Discussion

Different soaking techniques resulted in variation of germination rate and germination index at 14 days after cultivation. For the Hom Nin rice seeds had high normal seedling and germination index values for all experimentals process. Withthis, Patcha (2016) found that Hom Nin is a local non-glutinous rice breed whose seeds have a high resistance to environmental conditions. KDML 105 using rice bran and centrifuged rice bran solution had high normal seedlins because rice bran is part of the aleurone layer, which may contain organic compounds, hormones, enzymes and antioxidants (Lloyd *et al*., 2000; Batsut and Siriamornpun, 2010) that are beneficial to germination (Eisvand *et al*., 2010).

As it is soaked in water, the paddy husk has a small amount of these compounds, thus not affecting the germination of KDML 105 which Ramarathnam *et al*. (1986) reported higher levels in longlife rice seeds in comparison with the shortlife rice seeds. These values seemed to agree with the germination data of the corresponding rice variety. The investigation revealed a strong influence of the level of phenolic constituents in the rice hull on the storability of rice seeds. Despite the previous research findings that the use of chitosan-primed rice seeds had enhanced seed germination (Songlin and Qingzhonh, 2002; Thananun and Wongrueang, 2009). There was no effect on the seed germination of KDML 105 aged rice seeds in this trial, which matched the results of Divya (2019) who found chitosan to be effective upon seed germination for seven months when stored at room temperature. The use of brassinosteroid hormones in the three varieties of rice had variable effects on root lengths compared to other treatments. Because brassinosteroid has a rolein promoting the growth and development process of seed germination, related to cell elongation (Yokota, 1997; Fridman and Sigal, 2013). There were no significant effects on heights, but longer roots as well as greater numbers of roots were found when aged rice seeds were soaked in brassinosteroid. These findings were consistent with the works that stated that different concentrations of brassinosteroid significantly affected the root tips and that the lengths of taproots increased (Kartal *et al.*, 2009). The highest number of normal seedlings (96) was found in the seeds of the varieties Chai Nat 1 and Hom Nin; this may be influenced by brassinosteroid and the level of antioxidants in the rice hull (Ramarathnam *et al.*, 1986).

Summary Hom Nin and Chai Nat 1 rice seeds were high germination compared to KDML 105. The use of brassinosteroid hormones in the three varieties of rice had variable effects on root lengths compared to other treatments.The higest shoot dry weights showed in Chai Nat 1 seeds soaked in water. In this trial, abnormal seeds, hard seeds, shoot heights, root dry weights, and root shoots ratio had no statistically significant differences. Suggesting to study in other grain varieties as well as further studying growth and productivity.

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