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Evaluation of techniques on breaking seed dormancy on four philippine inbred rice varieties

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Abstract

This study evaluated the efficiency of six dormancy - breaking techniques on the germination of the four inbred rice varieties (PSB Rc82, NSIC Rc160, NSIC Rc218, NSIC Rc222) through the measurement of root and shoot length. This provides information to researchers, rice farmers and breeders in identifying the most efficient breaking dormancy techniques for the four inbred rice varieties to address the challenge of long seed dormancy period. The techniques used were soaking with water, sun drying, dry heat method or oven drying and chemical treatments, potassium nitrate (KNO_3) and gibberellic acid (GA_3). Results from the treatments showed that at the seventh day of germination, soaking in water, sun drying, oven drying, and chemical treatments have achieved more than 90% of germination rate. Amongst the treatments, oven drying at 50°C for 5 days and the use of 100ppm gibberellic acid (GA_3) are the most effective, while seeds germinated on control treatment had a comparatively low effect. Furthermore, the effects of these dormancy - breaking techniques on shoot and root length showed potassium nitrate could induce both root and shoot length while GA_3 produced the least length on root. Soaking with water had the shortest shoot measured amongst the treatments. Germination rate is not correlated with the length of shoot and roots. KNO_3 induced both root and shoot length. GA_3 had the highest germination rate and shoot length, and performed least on root length.

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Introduction

Dormancy is when growth and development are temporarily stopped and enables the plant to minimize metabolic activity to help in conservation of energy. Seed dormancy is advantageous in maintaining seed quality and is an important varietal trait in tropical rice where rain fall and high humidity are of frequent occurrence during the maturity and harvest periods (Tung, *et al.*, 2011), as observed in Philippine climate conditions. It is determined by genetics with a substantial environmental influence which is mediated, at least in part, by the plant hormones abscisic acid and gibberellins (Baskin, 2014). The dormancy status is further influenced by the seed maturation environment and is continuously changing with time following shedding in a manner determined by the ambient environment (Savage *et al.*, 2006).

Seed dormancy is an important aspect in rice production. Seed dormancy is caused by endogenous characteristics of the embryo that prevent germination (Bewly *et al.*, 1994). Endogenous dormancy is the most common form of dormancy. This is when a seed has an excess of a germination inhibitor such as abscisic acid (ABA). This happens when the seed is not physiologically mature and has high moisture content. Endogenous dormancy may also be due to presence of other germination inhibitors. Application of low level of growth regulators such as gibberellins and kinetics may break the seed dormancy. Presoaking seed treatment with GA_3 at the concentration of 100 ppm have been used for breaking seed dormancy and among other chemicals, potassium nitrate (0.2%) and thio – urea (0.5 to 3%) are widely used for breaking seed dormancy in oat (*Avena sativa*), barley (*Hordeum vulgare*), tomato (*Lycopersicon spp*) (Agri Info, 2015).

Subjecting the seed to very cold conditions will relieve this type of dormancy naturally. Other techniques are also employed for breaking dormancy and enhance seed germination by using growth regulators such as Gibberellic acid or Potassium nitrate. Giberrellic Acid (GA_3) promotes seed germination and growth, and its biosynthesis and responses are highly coordinated during seed germination (Ogawa *et al.*, 2003). Complex regulatory events in the giberellic acid signaling pathway include cross talk with other hormones and the regulation of genes involved in promoting cell elongation and division (Ogawa *et al.*, 2003). Accumulation of gibberellic acid during seed germination is accompanied by a reduction in abscisic acid (ABA) levels, suggesting that giberellic acid and abscisic acid ABA play antagonistic roles in this process (Olszewski *et al.*, 2002).

Variation in seed dormancy has been reported in different varieties of rice and several studies have been undertaken to break seed dormancy of cultivated rice. Some study reported than seeds soaked in tap water for 24 hours proved the best treatment for early emergence and seed breaking dormancy in tea seed after sun drying for 24 hours (Waheed, *et al.*, 2012). The most common method is by pre-chilling the seed at 7°C for a number of days, usually 3 days, before moving the seed to the germination chamber which is operating at 20°C. Other Simple and widely used methods are Mechanical scarification and acid scarification treatments (soaking of seeds in H_2SO_4 for 60 and 80 min and in HCl for 12 and 15 h) were very efficient in breaking dormancy and promoting germination. Seed soaking in HNO_3 for 1 to 5 days in various concentrations of thiourea and KNO_3 were also used in breaking seed dormancy (Ali *et al.*, 2011).

In line with this, this study evaluated six (6) breaking-dormancy techniques such as chemical treatments using potassium nitrate (KNO_3) and gibberellic acid (GA_3), dry heating (oven drying), sun drying and 24 hours soaking in water on four inbred rice varieties: PSB Rc82, NSIC Rc160, NSIC Rc218, NSIC Rc222.

Materials and methods

Four inbred breeder seeds variety cultivated in irrigated lowland in Tarlac City, Tarlac, Philippines were used in the study. Initial test was done immediately after threshing, to ensure the seeds'

dormancy, wherein 100 seeds per replicate of four were wrapped in paper towels moistened with water for 5 days. Seeds that germinated within the 5 days tests were considered not dormant.

After the initial test to determine the seed's dormancy, the following techniques from AgriInfo.in (2017) was adapted. This study used four hundred seeds per treatment, with 100 seeds per replicate.

Control treatment (Ct)

Seeds were stored in Petri plates at room temperature and were individually wrapped per replicate in moistened paper towels.

Soaking in water (W)

Seeds were soaked in 15 ml water on petri plates for twenty four hours and were individually wrapped per replicate in moistened paper towels after soaking.

Sun-drying(SD)

Seeds were placed in petri plates and sun dried from 8am to 4pm for 5 days. At night, petri plates were sealed and covered with aluminum foil to prevent accumulation of moisture.

Dry heat treatment (DHt)

Seeds were placed in Petri plates and incubated at 50°C for 5 days in oven.

Chemical treatment (Cht)

Seeds were soaked in 0.2% potassium nitrate (KNO_3) and 100ppm Giberellic Acid (GA_3) for 24 hours and were individually wrapped per replicate in paper towel.

Seed germination rate under dormancy-breaking treatments

After the above dormancy breaking techniques, germination test was done using the International Seed Testing Association (ISTA) procedure. Seeds subjected from the four breaking -dormancy techniques were germinated in moistened paper towels, rolled and placed in standing position which were placed in trays covered with plastic and placed in germination room. First counting was made 7 days after seeding. Ungerminated seeds after the first counting were further incubated for 7 days and then counted again. Germination results were expressed as the percentage of the average normal seedlings. Shoot growth and roots were measured to observe the difference on the response of the four inbred seeds variety. Treatments were observed until more than 90% of the seeds have germinated.

Statistical analysis

Data gathered was analyzed using completely randomized design and treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% level.

Results and discussion

Initial test

To establish the dormancy of the seeds, initial test was conducted. One hundred (100) seeds per replicate of four were wrapped in paper towels soaked in distilled water for 5 days. This was done immediately after threshing. As a result, no germination was observed within 5 days, therefore, the seeds are still dormant and that it was safe to proceed with the dormancy -breaking techniques.

Table 1. Effect of the 6 breaking dormancy techniques on the seed germination of the four Philippine inbred rice varieties.

Treatment	Variety				Treatment Mean
	NSIC RC160	NSIC RC218	NSIC RC222	PSB Rc82	
Ct	85.25	91.75	91.50	76.00	86.13^{d}
W	98.25	98.25	98.75	74.00	92.31^{c}
SD	94.75	88.25	99.50	97.50	95.00^{b}
DHt	100.00	96.75	99.50	99.25	98.88^{a}
Cht - KNO_3	94.25	95.00	93.00	92.25	93.63^{bc}
Cht - GA_3	97.00	98.25	100.00	94.00	97.31^{a}
Variety Mean	94.92^{b}	94.71^{b}	97.04^{a}	88.83^{c}	

Control treatment (Dry, unaltered seeds)

Unaltered seeds, or seeds that were not subjected to any of the dormancy techniques was used as Control treatment (Ct). Seeds were stored in petri plates at room temperature without prior soaking and were individually wrapped per replicate in moistened paper towels. On seed germination, results of this treatment showed the lowest treatment mean of 86.13% compared with all the treatment means performed by all the other dormancy - breaking techniques across all rice varieties (Table 1).

Table 2. Effect of the 6 breaking dormancy techniques on root length of the four seed varieties.

Treatment	Variety				Treatment Mean
	NSIC RC160	NSIC RC218	NSIC RC222	PSB RC82	
Ct	88.95	97.95	101.50	69.33	89.43[b]
W	84.68	114.58	98.70	69.85	91.95[b]
SD	80.28	87.25	97.55	84.25	87.33[b]
DHt	81.80	78.73	97.68	95.85	88.51[b]
Cht - KNO_3	87.23	110.65	121.55	84.95	101.09[a]
Cht - GA_3	68.98	89.73	73.58	62.68	73.74[c]
Variety Mean	81.98[b]	96.48[a]	98.42[a]	77.82[b]	

Soaking in water

Results in this treatment showed that prior soaking in water resulted to 92.31% treatment mean in germination. All three varieties, NSIC Rc160, NSIC Rc218, and NSIC Rc222 had 98.25%, 98.25% and 98.75% germination respectively (Table1). Similarly, this result was observed in the study of Tilahun-Tadesse *et al.*, (2013) on the Effect of hydro-priming and pre-germinating rice seed on the yield and terminal moisture stress mitigation of rain-fed lowland rice, wherein their study revealed that planting pre-germinated seeds as well as seeds soaked and dried for 24 hrs at the local (farmers') sowing time resulted in significantly earlier seedling emergence, heading, and maturity. The study concluded that planting pre-germinated seeds or hydro-primed seeds soaked and dried for 24 hrs could be practiced as the first and second best alternatives for rice production on Fogera plains in northwestern Ethiopia.

Sun drying

Sun dried seeds germinated at 95.00% treatment mean after 7 days of seeding compared with 86.13% germination of the seeds that were unaltered (Control treatment) (Table1). This study confirms the results of Banful *et al.,* (2011) on the effect of seed drying on germination behavior and seedling growth of *Annona squamosa* wherein sun-drying of seeds for three consecutive days each resulted in significantly early germination than the use of fresh seeds. This technique also resulted to the consistency in obtaining a significantly higher number of germinated seeds to be used in planting. Local farmers in the Philippines do the same practice of sun drying the seeds in combination with pre-germination in water, in preparation to sowing. This is done 2-3 days before the seeds are soaked for 24 hours.

Dry Heat Treatment

This study used 50°C temperature for 5 days on all the rice varieties. As a result, germination rate was at 98.88%, performing the highest amongst all the treatments (Table1). Oven or dry heat is not often recommended since the temperatures required are more suitable to an incubator than a kitchen oven. For this seed coat treatment, it was advised that the seeds should be placed in shallow containers in a preheated incubator or oven. After the treatment, the seeds should be cooled immediately and sown.

Chemical treatment (KNO_3 and GA_3)

Chemicals that have proven very helpful in breaking certain types of dormancy in this study are gibberellic acid (GA_3) and potassium nitrate (KNO_3). In a study conducted by Naredo, *et. al.,* (1998), response of rice

species (*Oryza* sp.) to different seed dormancy-breaking treatments showed that soaking at 1000 ppm GA_3 for 48 hours are the recommended breaking procedures for different *Oryza* species. In addition, gibberellic acid (GA_3) has been successful in alleviating the chemical constraints that prevent radical emergence and increasing embryonic growth in a number of physically dormant species (Koornneef *et al*, 2002; Leubner-Metzger, 2003).

Table 3. Effect of the 6 breaking dormancy techniques on shoot length of the four seed varieties.

Treatment	Variety				Treatment Mean
	NSIC RC160	NSIC RC218	NSIC RC222	NSIC RC82	
CONTROL	148.53	167.43	154.48	55.98	131.60[b]
H_2O	98.25	140.93	118.38	69.40	106.74[c]
SUNDRIED	137.18	148.35	159.03	57.58	125.53[b]
OVENDRIED	145.20	168.53	157.83	81.73	138.32[b]
KNO_3	169.68	227.85	160.29	119.25	169.27[a]
GA_3	198.45	174.83	170.48	137.35	170.28[a]
Variety Mean	149.55[b]	171.32[a]	153.41[b]	86.88[c]	

Seed germination rate under breaking dormancy treatments

Table 1 presents the data on the germination of seeds subjected to the different dormancy techniques. Results showed that oven drying (98.88%) and gibberellic acid (97.31%) had the highest treatment mean. It could also be observed that there are variations in dormancy intensity between different rice varieties wherein PSB Rc82 variety reflected the lowest response, at 88.83% variety mean, to all the dormancy treatment compared with all the other rice varieties with >94% variety mean. On the other hand, NSIC Rc222 was observed to have the highest variety mean at 97.04% (Table1). Moreover, among all the techniques, the use of 100ppm of GA_3 induced 100% germination rate versus 91.50% germination rate from the control treatment. Therefore, the overall result on the seed germination rate agrees with the result of the study done by Karimmojeni *et al.*, (2011), however, significantly higher rate of seed germination were obtained in this experiment using KNO_3 alone, compared with their use of GA_3, in combination with potassium nitrate (KNO_3) on pepper weed germination with result of 61% optimum germination rate only.

Evaluation of root and shoot length

Although all the techniques used produced >90% dormancy- breaking response 7 days after seeding, their effect on the length of roots and shoot are not correlated with the rate of germination of the seed varieties. The root length of the seeds in the control treatment, soaking in water, sun drying and oven drying treatment had no significant difference from each other wherein the treatment with potassium nitrate had the greatest (101.09cm) and gibberellic acid (73.74cm) having the least mean (Table 2). In addition, shoot length results of KNO_3 and GA_3 treatments had no significant difference at 5% level using the Duncan Multiple Range Test (DMRT) and performed the highest treatment mean at 169.27 cm and 170.28 cm respectively while the control, sun-dried, and oven dried treatments have no significant differences with each other at 125.53cm, 138.32cm and 131.60cm respectively. Observable differences on the shoot length were seen on treatment soaked in water, which had 106.74cm length (Table 3). It could therefore be established from the results shown in both Table 2 and Table 3 that Potassium Nitrate (KNO_3) is an effective primer to obtain good seed germination and development. This result concedes with recent studies using KNO_3 as primer that shows influences in the morphological development and seed germination even when subjected to different environmental conditions. Priming with KNO was also done by Ahymadvand *et al.,* (2012) which

obtained the same significantly positive result on the increase in germination and emergence percentages, radicle and plumule length, seedling dry weight, plant height, plant leaf area and plant dry weight on two soybean varieties studied. On cotton plants, application of potassium nitrate as seed pre-treatment influenced the morphological and structural development and was marked with prime reference to cotton growth and productivity under water-limited environment (Shafiq *et al.,* 2015).

The results suggests that among the six techniques in breaking dormancy, the use of 100ppm of gibberellic acid (GA_3) and oven drying at 50°C for five(5) days could be considered the best option among the treatments in seed germination. Although, consideration should be made in using these techniques. This is because though having the highest germination rate along with oven drying, the effect of 100ppm of gibberellic acid on root development produced the lowest mean versus control. Among the six techniques observed for root length, potassium nitrate had the highest mean, while soaking in water, sun drying and oven drying had no significant difference with the performance of control on root length. Meanwhile, for the shoot length, both gibberellic acid and potassium nitrate produced the highest result which is relatively higher versus control. Furthermore, soaking with water, which is the most commonly used breaking dormancy method by farmers in the Philippines could be considered economical, but would not have the best result for seed germination as well as in root and shoot development for the four varieties used in this study. In the Philippines, sun drying for two to three days before storage and soaking rice seeds in tap water for 24 hours are the most common practiced breaking dormancy and germination techniques. However, with this practice, farmers still have to wait for one month after harvest, dry and store their seeds and wait for the seed's dormancy to break to ensure better germination which gives additional costs on manpower during drying and storage. Thus, too long seed dormancy period can be a problem in breeding when seeds are used in research, to seed producers as well as to farmers in areas with limited water supply and available only in a certain period time.

In a study conducted by Naredo, *et al.*, 1998, response of rice species (*Oryza* L.) to different seed dormancy-breaking treatments showed that hull removal, heat treatment at 50°C for 7 to 14 days, soaking at 0.1 M Nitric acid (HNO_3) for 48 hours and soaking at 1000 ppm GA_3 for 48 hours are the recommended breaking procedures for different *Oryza* species. Seed hull removal is very effective in breaking dormancy but it is labor-intensive and may also have the risk of damaging embryos. Alternating temperature of 45/30°C are generally effective in species of Sativa complex but seedling growth is adversely affected. Dry heat treatment, although promoting moderate germination, provides an easy method when handling a large number of samples. Likewise, chemical treatment markedly promotes germination and it is also an easily applied method. In Pakistan, dry heat treatment at 50°C both for 7 and 14 days substantially increased germination of intact seeds for both wild species, *O. longistaminata* and *O. rufipogon*, and on cultivated rice varieties namely Swat-1, F. Malakand and JP-5 at the optimum temperature regimes, compared with the germination responses of intact seeds under their optimum temperature regimes without heat treatments (Waheed *et al.*, 2012). In a study by Karimmojeni *et al.*, (2011) on the germination of pepper weed seed using filter paper soaked with 3ml Potassium nitrate (KNO_3) prepared on a petri plate, potassium nitrate induced germination by 61% at 0.02% concentration. Thus, results in the study showed that at 0.02% of KNO_3 is the optimum amount needed for seed germination. Also, the study showed that seed dormancy for pepper weed seed can be broken most effectively by scarification, flooding and after-ripening.

A challenge in dormancy and germination research is to identify the nature of the crucial regulators that prevents the onset of germination or dormancy, which triggers the germination process and their mutual interaction. Furthermore, it is important to know how the environmental factors such as light and

cold affect the endogenous factors that control germination.

It is then recommended that seeds are transferred to soil, immediately after radicle emergence to observe maximum seedling growth. And that, dry heat treatment, although promoting moderate germination, provides an easy method when handling a large number of samples. Like chemical treatment promotes germination for the four rive varieties, and at the same time, it is also an easily applied method.

Therefore, the choice of an appropriate dormancy-breaking treatment depends to a considerable extent on the varieties, the amount of seeds, and the available conditions.

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