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Storage and hydro-priming treatments to improve the seed quality of two traditional rice varieties; Batapola-el and Suwendal, from Sri Lanka

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ABSTRACT

Although the demand for traditional rice varieties has increased, low initial germination and poor storability of these varieties impede its popularization. Thus, the aim of the study was to improve the seed quality using priming treatments and to suggest an effective storage practice of two of the traditional rice varieties; Suwendal and Batapola-el, in Sri Lanka. Seeds were subjected to hydro-priming under various time periods (0, 24, 48 and 72 hours). Seeds were stored in gunny, polysac and polythene bags at room temperature, 25 and 8 °C. Seed quality of primed and stored seeds was evaluated using seed germination, seed vigour (seedling growth and conductivity) and seedling emergence. Hydro-priming for 72 hours has increased the seed germination of the two varieties significantly compared to other priming methods. Further, seeds primed for 72 hours had the highest vigour and highest seedling emergence in both varieties. Storing Suwendal and Batapola-el seeds in polythene bags at 8 °C retained the highest viability and highest vigour for more than six months. Thus, hydro-priming for 72 hours could be recommended to enhancethe seed quality of these two tested varieties and storage at 8 °C in sealed polythene bags could be recommended as an effective storage practice to maintain the seed viability till the next sowing season.

Key words: *Emergence, germination, priming, storage material, storage temperature, vigour*

Abbreviations: *Dw: Distilled water; F: F value; Hrs: Hours; P: P-value; SD: Standard deviation; Temp: Temperature*

INTRODUCTION

Rice cultivation in Sri Lanka has a long history. Further, rice is the staple food of Sri Lanka and it plays a significant role in the food industry since ancient time. The total land area of the rice cultivation at present is 780,000 ha, which is approximately 12 % of the total land area of the country (Bambaradeniya et al., 2004).

During the early periods, traditional rice varieties have been used by Sri Lankan farmers (Rajapakse et al., 2000). However, there were several drawbacks in those verities including most of them are being low yielding. From the beginning of the 20th century, rice improvement programs in Sri Lanka have attempted to develop new rice varieties with higher yields. The yield of most of the newly developed varieties seems to be heavily dependent on fertilizers and other agrochemicals (Rajapakse et al., 2000). Further, the heavy use of agrochemicals in cultivating these new varieties thought to be linked with immerging noninfectious diseases including chronic kidney disease of unknown etiology (CKDu) (Jayasumana et al., 2014) in the rice cultivating areas. However, with the introduction of these improved varieties, about thousands of traditional rice varieties have been ignored by farmers and being extinct from the field (Rajapakse et al., 2000; Kennedy and Burlingame, 2003).

Many of the traditional rice varieties are claimed to have several important medicinal properties (Dharmasena, 2010; Abeysekera and Premakumara, 2016). Especially, these rice varieties have antioxidant properties and seem to be preventing non-infective diseases (Abeysekera and Premakumara, 2016; Samaranayake et al., 2017). Thus, currently, there is a developing demand for traditional rice, especially as the form of organic rice. However, poor germinability and storability of the seeds of these traditional rice varieties is a drawback in popularizing these varieties (Personal communication with the organic farming community). Thus, we tried to improve the germinability and storability of seeds of two traditional rice varieties; Suwendal and Batapola-el. Seed priming was used to improve the seed quality, while various storage treatments have been employed to improve the storability.

Heretofore, no research has been conducted to improve the seed quality of traditional rice varieties of Sri Lanka. Few studies have been conducted to reveal the medicinal (Samaranayake et al., 2017) and physical (Ranawake, 2013; Rebeira et al., 2014) properties of these rice varieties.

Seed priming is controlled hydration of seeds to initiate the molecular level process towards germination and cease prior to radicle emergence (Bradford and Bewley, 2002). It especially improves seed performance under stress conditions. Priming generally induces the establishment of rapid and vigorous seedlings and it promotes earlier maturity of the crop (Tilahun et al., 2013). However, the achievement of seed priming is influenced by the period of priming treatment. For every crop species, there is a `safe limit', (the maximum length of time) if exceeded, could lead to the deterioration of the seed. The optimum priming time and the best priming method could vary from species to species as well sometimes from variety to variety. Harris et al. (2002) suggested that 24-hr as the best time period for priming of rice seeds. Effect of priming on seed quality of different rice varieties as well as on seed quality of other crop varieties has been studied in various other countries (Masuda et al., 2005; Andoh and Kobata, 2001; Matsushima and Sakagami, 2013) revealed that seed priming enhanced the rate of emergence and seedling growth of some rice varieties even under low soil moisture levels. Dey et al. (2013) have reported that the lowest mean germination time of rice seeds was observed from hydro-priming for 30-hours at 35°C. Goswami et al. (2013) studied the effects of hydro-priming, dehydration priming and osmo-priming (by NaCl and KH_2PO_4) on germination of rice seeds. They showed that germination percentage of hydro-primed seeds was the highest compared to the seeds subjected to other types of priming treatments.

Only a little information available in the scientific literature on rice seed storage, although there is much information on storing rice grain prior to consumption as a food. Roberts (1961) has studied the effect of temperature and moisture on the viability of stored rice and developed an allometric equation to describe the effect of temperature and moisture on rice seed viability of five tropical rice varieties. However, there is no information on the storage of seeds of the two studied rice varieties.

MATERIALS AND METHODS

Materials

Suwendal is recommended to grow under the rain-fed rice cultivation system in the low country wet zone. The yield can be harvested within 3½ months period. Its cooked rice appearance and the taste are comparatively higher than most of the other traditional varieties due to its milky taste (Personal comments from farmers). Batapola-el is another 3½ months-aged variety having relatively long sized grain. Farmers in wet zone prefer to cultivate this variety (Personal comments from farmers). According to the farmers, these two traditional rice varieties have very low germination rates in the field and low storability.

Seeds of the studied rice varieties were obtained from Hela Sahal Ayurvedic Seed Suppliers Pvt. Ltd, Kottawa, Sri Lanka on 4th of January 2017 and were stored in polysac bags (710 x 480 mm^2 with 60 GSM thicknesses). Seeds were from Maha cultivation season (cultivation season from September to March) 2016 and were stored for only < 2 weeks by the supplier under ambient temperature conditions in gunny bags. Visually healthy seeds were selected for the field and laboratory experiments. Experiments were initiated at least within a week from the date of purchase. All laboratory and field experiments were conducted in the Department of Botany, University of Peradeniya. These experiments were conducted from 2016 to 2017. The temperature of the experimental site fluctuates between 18 - 30 °C with a mean annual temperature of 27 °C.

Germination of untreated seeds

The purpose of this experiment was to evaluate the germinability of seeds of the study varieties at different temperature conditions. Three samples containing three replicates of twenty seeds from each rice variety was taken randomly and incubated on tissue papers moistened with distilled water in 9 cm diameter Petri dish. Two samples of each variety were incubated at 25 and 35 °C in light/dark (12/12 hr) conditions in temperature and light controlled incubators (Model: MGC- 250P). The other sample was incubated at the ambient laboratory temperature (~27 ^{o}C) and light conditions (white fluorescent light and diffused sunlight during the day time). Samples were watered every day with distilled water and germinated seeds were counted for two weeks at daily intervals. Germination rate and the percentage were calculated.

Moisture test

The purpose of this experiment was to determine whether the seeds have moisture content appropriate for storage or not. Ten replicates of 10 seeds were taken randomly from each variety and the seed samples were crushed separately using motor and pistol. Then fresh weight was measured using an analytical balance to the nearest 0.0001 g. The samples were oven-dried at 121 °C for 3 hours. Seed samples were reweighed and moisture content was calculated in fresh mass basis (Fischer, 2007).

Imbibition of seeds

The purpose of this experiment was to determine the time taken to achieve the lag phase during imbibition of each variety to determine the suitable priming time for each variety. Four replicates of 20 seeds from each variety were weighed using a digital chemical balance to nearest 0.001 g. These seed samples were immersed in 30 ml of distilled water in beakers and closed with an aluminium foil lid. These beakers were incubated at 25 °C. Seed samples were retrieved after 2, 4, 6, 8, 24, 48 and 72 hours, surface blotted, reweighed and returned to the beakers. Imbibition curves were prepared.

Hydro-priming

Four replicates of 100 seeds from each variety were used for each priming treatment. Seed samples were weighed and were immersed in 100 ml of distilled water in a 250 ml beaker for 24, 48 or 72 hours. Seed samples were retrieved and reweighed. Then the seed samples were air-dried until they came to their initial weight.

Seed germination under laboratory conditions

The aim of this experiment was to determine the effect of priming on seed germination. Hydro-primed seed sample from each treatment and none-primed seed sample from each species (Four replicates of 100 seeds from each variety were used) were incubated on manila papers (made from semi-bleached wood fibers, 100 GSM thickness) moistened with distilled water. Manila papers with seeds were rolled and kept for germination under room temperature. Seeds were observed for germination after 7 days.

Seed vigour test

Seed vigour of the primed seed and non-primed seed samples (germinated in the manila paper) was determined using the seedling growth criteria (Hampton and TeKrony, 1995). Twenty-five seedlings from each replicate of each sample mentioned above were selected randomly and were placed on a black coloured cloth and photographed. Root length and shoot length of the seedlings were measured using the images with the aid of ImageJ software.

Germination test under the glasshouse conditions

The experiment was conducted to determine the effect of priming on seedling establishment under simulated field conditions. Four replicates containing 100 primed and pre-soaked seeds of each species were incubated in separate 30 × 45 cm^2 plastic trays filled with moistened paddy field soil in an uncontrolled glasshouse (at ~ 28 ^{o}C and diffused sunlight). Trays were watered every day for 7 days. Emerged seedlings were counted after 7 days. Vigour, germination and seedling emergence tests were conducted with primed and non-primed seeds of each species.

Storability test

The experiment was conducted to determine the effect of different storage conditions on seed vigour and germinability of the studied varieties. Three replicates of 100 seeds from each variety were stored in three different types of storage bags; polythene bags (30 x 30 cm^2, 100 GSM thickness), jute gunny bags (30 x 30 cm^2) and polysac bags (30 x 30 cm^2, 60 GSM thicknesses) and sealed. These bags were stored at three temperature regimes separately; ambient room temperature, 8 and 25 °C. Two seed samples from each variety were retrieved after 2, 4 and 6 months and germinability of the seed samples was tested as mentioned above. Further, seed vigour of the fresh and stored samples were measured using the conductivity test according to the following criteria.

Conductivity test

Three replicates of 5 g of seeds were taken from each stored sample and washed with 1% clorox solution for 1 minute (Hampton & TeKrony, 1995) and rinsed three times with distilled water. Seed samples were immersed in 100 ml of de-ionized water at room temperature and kept for 24 hours. Then conductance of de-ionized water and solutions of seed samples were measured using a conductivity meter (CON 400 and CON 410, Company - EUTECH, Singapore).

Analysis of data

Arc-sine transformed germination percentage data, shoot and root length data and conductivity data were analyzed using one-way, two way and general linear model ANOVA procedures. Data analysis was conducted with Minitab 17 statistical software. All the graphs were prepared with Sigma plot 10.0 software.

RESULTS AND DISCUSSION

Germination of untreated seeds

Seeds of Suwendal and Batapola-el germinated to > 90% at 25 °C and at ambient laboratory temperature (Fig. 1). However, at 35 °C germination of both varieties were < 40 %.

Initial information for seed priming

Seed moisture

The dry mass of 100 Batapola-el and Suwendal seeds were 1.163 g and 2.081 g respectively. There seed moisture contents were 14.8   1.6 and 13.4   1.1 %, respectively.

Imbibition of seeds

Seeds of two rice varieties increased in mass during the imbibition (Fig. 2). Even after 72 hours of imbibition, the mass of Batapola-el seeds has not come to the constant, while the mass of Suwendal seeds almost reached to a constant i.e. came to the lag phase of germination.

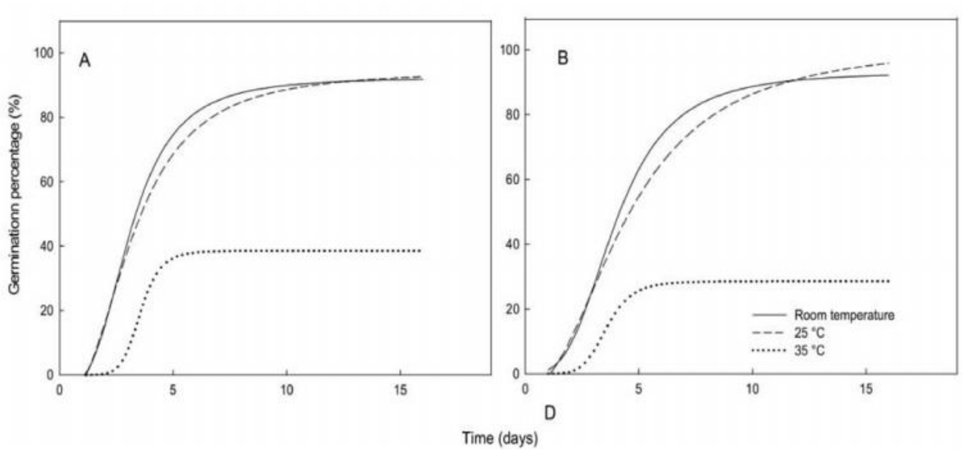

Fig. 1. Cumulative germination of Suwendal (A) and Batapola-el (B) seeds at different temperature regimes; Solid line, at ambient laboratory temperature; dashed line, at 25 °C and dotted line, at 35 °C. Logistic 4-parameter curves were fitted to the germination percentage data to prepare the cumulative germination curves.

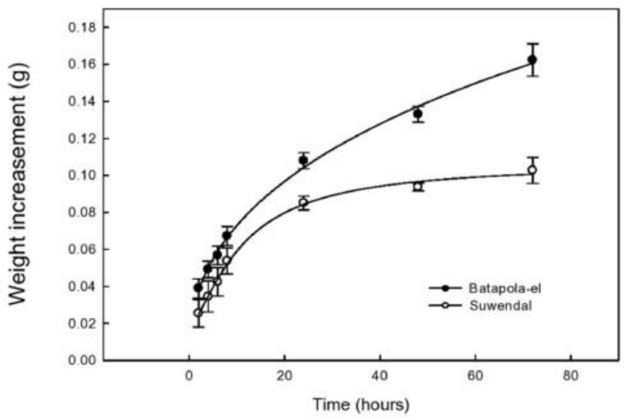

Fig. 2. Mass increase of seeds of Batapola-el and Suwendal during imbibition of distilled water at ambient laboratory temperature and light conditions. Error bars are ±SD.

Effect of priming on the quality of seeds of the study varieties

Seed germination at laboratory conditions

Germination percentage of Batapola-el seeds increased after priming (Fig. 3A). Seeds primed using distilled water for 72 hours showed significantly higher germination than other treatments (98 %, F =14.4, P < 0.01). The same trend was observed in Suwendal as germination percentage of non-primed seeds (89.5 %) significantly increased after hydro priming for 72 hours (98 %, F = 14.3, P < 0.01, Fig. 3B).

Seed vigour of primed seeds

Both root(F = 33.2, P < 0.001) and shoot (F = 135.4, P < 0.001) lengths were significantly higher in Batapola-el seedlings developed from 72 hours primed seeds (121.1 and 80.71 mm, respectively) than those developed from non-primed seeds (82.8 mm and 37.5 mm, respectively; Fig. 4A, C). Both root (F = 15.93, P < 0.001) and shoot (F = 16.97, P < 0.001) lengths were significantly higher in Suwendal seedlings developed from 72 hours primed seeds (109.09 and 20.88 mm, respectively) than those developed from non-primed seeds (79.63 mm and 14.63 mm, respectively; Fig. 4B, D).

Effect of priming on seedling emergence under plant house conditions

Seventy-six percent of non-primed Batapola-el seeds emerged seedlings in rice field soil at plant house conditions. Seedling emergence was significantly increased (F = 72.51, P < 0.01) to 94.3 % when the seeds were primed with distilled water for 72 hours. About 80 % of non-primed Suwendal seeds emerged seedlings under plant house conditions. The seedling emergence percentage has significantly (F = 238.6, P < 0.01) increased to 95 % after seeds were primed with distilled water for 72 hours.

Effect of different storage procedures on seed germination and vigour

Germination percentage of Suwendal seeds reduce gradually with time, in all tested storage temperatures (Fig. 5A). However, the reduction of germination percentage was greatly reduced when the seeds were stored at 8 ^{o}C (P < 0.01). Especially after 6 months of storage, the germination is significantly higher in seeds stored at 8 ^{o}C (P < 0.01) compared to other storage

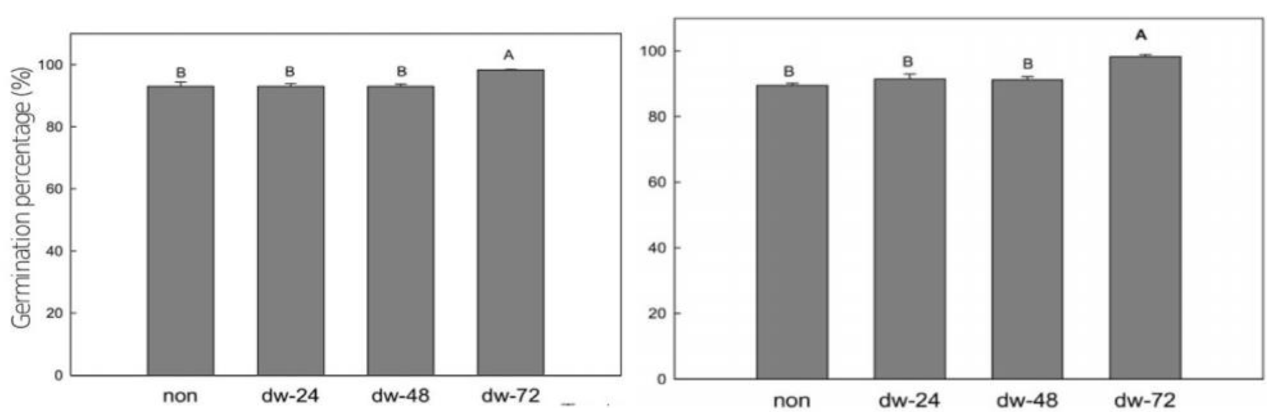

Fig. 3. Effect of hydro-priming treatments on germination percentage of seeds of (A) Batapola-el and (B) Suwendal. Different uppercase letters indicate significant differences between treatments. Error bars + SD.

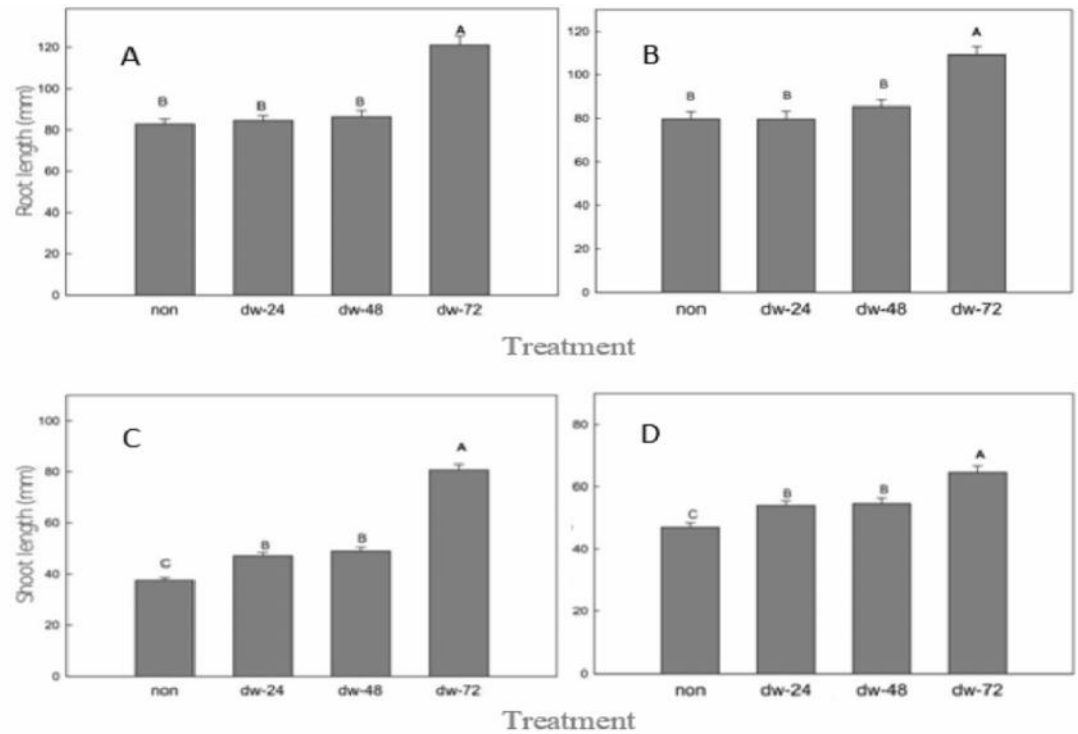

Fig. 4. Root lengths of seedlings developed from seeds of (A) Batapola-el, (B) Suwendal and shoot lengths of seedlings developed from seeds of (C) Batapola-el and (D) Suwendal subjected to different priming treatments. Different uppercase letters indicate significant differences between treatments. Error bars are + SD.

temperatures. Similarly, the reduction of germination percentage was significantly reduced when they were stored in sealed polythene bags ($P < 0.01$).

After six months of storage, the highest germination percentage of Suwendal seeds were observed in seeds stored at 8 °C in sealed polythene bags or in polysac bags (Fig. 5A). After six months of storage, germination percentage was significantly affected by the interaction effect of storage temperature and the storage materials ($P < 0.01$). The highestgermination percentage of Suwendal seeds was shown by seeds stored in polythene bags or polysac bags at 8 °C.

According to the conductivity test, the least conductivity (highest vigour) was shown by seeds stored in polythene bags at 8 °C (Fig. 6A). The vigour of seeds stored at 8 °C in polythene bags was significantly different from those stored in other bags in other temperature conditions after 2 ($P < 0.001$), 4 ($P < 0.001$) and 6 months ($P < 0.001$) of storage.

Germination percentage of Batapola-el seeds reduced gradually with storage at all the temperatures tested in all the types of bags tested (Fig. 5B). However, the reduction of germination percentage was significantly reduced when seeds were stored at 8 °C ($P < 0.01$) and when they were stored in polythene bags or in polysac bags ($P < 0.01$). After 6 months of storage, the highest germination percentage was observed in seeds stored at 8 °C in sealed polythene bags and polysac bags (Fig. 5B). The germination percentage was significantly different from seeds stored in other conditions ($P < 0.001$).

According to conductivity test results, the least conductivity (highest vigour) was shown by seeds stored in polythene bags at 8 °C (Fig. 6B). Germination percentage of seeds stored at 8 °C in polythene bags was significantly different from germination percentage of seeds stored in other conditions for 2 ($P < 0.001$), 4 ($P < 0.001$) and 6 ($P < 0.001$) months.

Fresh seeds of Suwendal and Batapola-el rice varieties had > 90 % germination at 25 °C and at laboratory temperature conditions. However, at 35 °C,

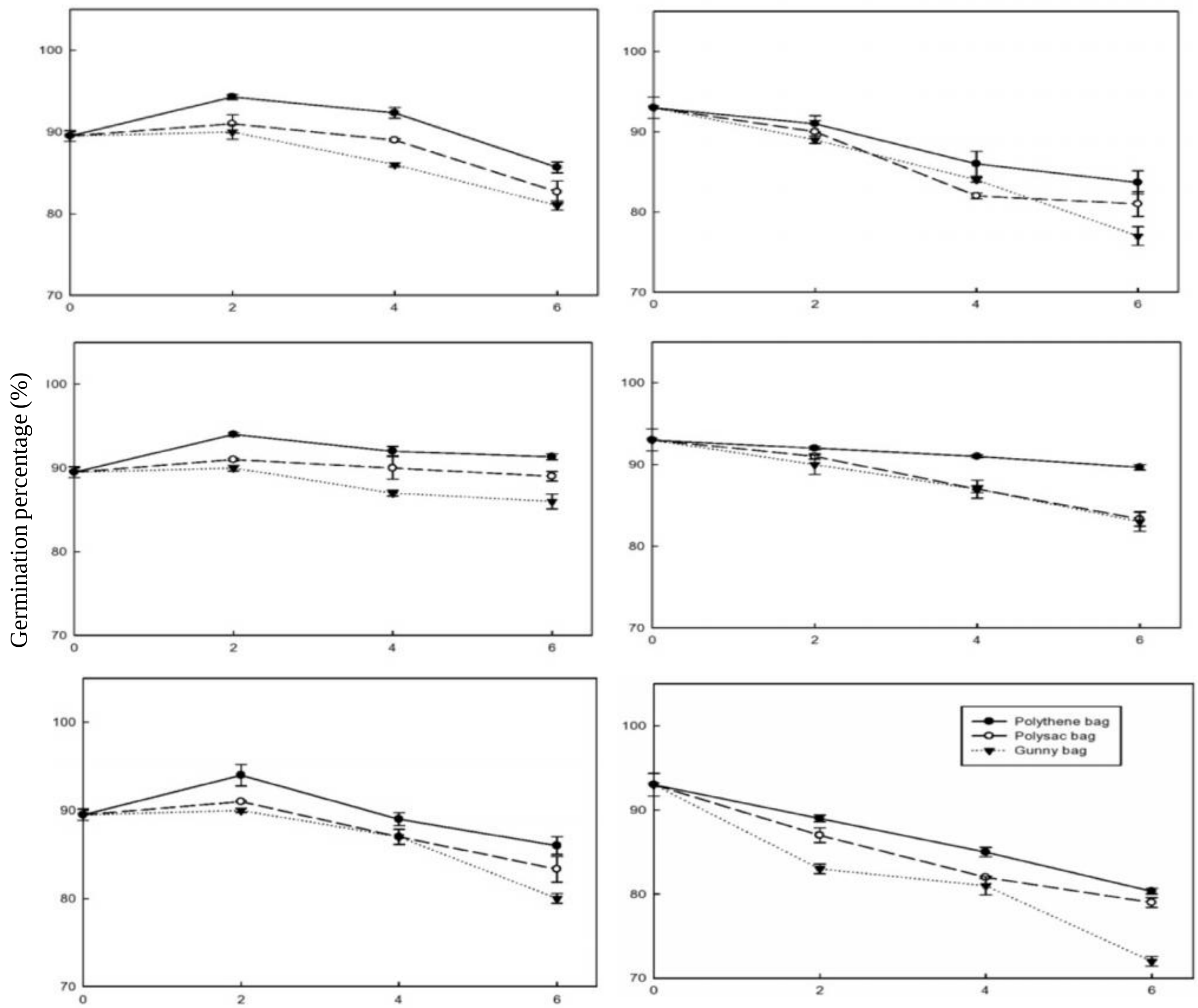

Fig. 5. Germination percentage of (A) Suwendal and (B) Batapola-el seeds stored in different storage bags (Polythene, polysac, Gunny) and temperature conditions; (1) room temperature, (2) 8 ºC, (3) 25 ºC for 2, 4 and 6 months. Error bars ± SD.

germination of seeds of both varieties was significantly low. It may be because 35 ºC inhibit the metabolic and physiological process of seeds required for germination in these varieties. However, thermos inhibition of rice germination at 35 ºC was unexpected as rice is a crop mainly developed in the tropical-subtropical regions of the world (Krishnan et al., 2011). However, several researchers have shown that the temperature requirement of rice seeds for germination depends on the rice variety (Nishyama, 1976; Tanida, 1996; Krishnan et al., 2011).

The seed moisture content of two rice varieties was < 15%, indicating that seed moisture content is suitable for seed storage (Harrington & Douglas, 1970). Seed deterioration rate is high when the seed moisture content of seeds is high (Vertucci et al., 1994; Strelecet al., 2010; Gladys et al., 2013). If the moisture content is very low, then the cracks are developed in the seed coat which impeds the seed germination (Vertucci et al., 1994). Preliminary imbibition experiments showed that seeds of both rice verities do not attain the lag phase of seed germination (See Bewley, 1997 for

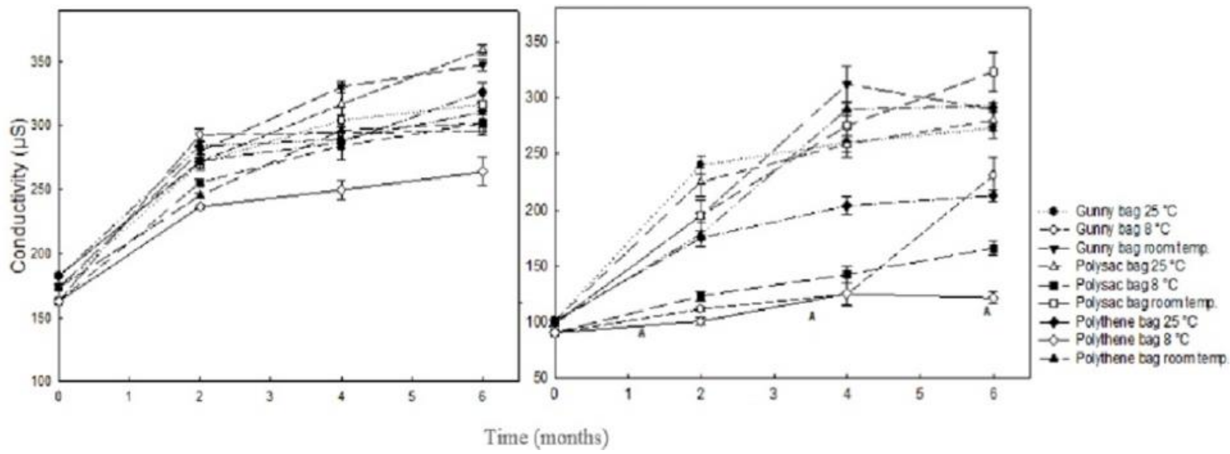

Fig. 6. Conductivity of A) Suwendal and B) Batapola-el seeds stored at different temperatures in different storage bags for 2, 4 and 6 months. Different uppercase letters indicate significant differences between treatments. Error bars are ± SD.

information about phases of germination), even they were allowed to imbibe water at ambient laboratory temperature for 72 hours. Thus, we used 24, 48 and 72-hour-duration seed priming treatments in all the priming experiments conducted in the current research.

Various priming methods have been used to improve the seed quality of crop varieties (Harris et al., 2002). However, hydro-priming was used during the current research as it is a less complex method. Thus, even small-scale farmers can perform hydro-priming without any difficulty. Hydro-priming treatments we used have increased the germinability of seeds of both verities. Priming with distilled water for 72 hours was the best treatment for seeds of both varieties, as after 72 hours distilled water priming, germinability of both varieties increased compared to all the other priming treatments and to the unprimed seeds. Further, seed vigour test too revealed that the distilled water priming for 72 hours was the best treatment to improve the seed quality of these two rice varieties. Thus, we could recommend 72 hours of hydro priming as the best priming treatment for these two varieties. Seedling emergence of primed (72 hours) seeds of both varieties under simulated natural conditions was dramatically higher than that of non-primed seeds, showing the true impact of priming.

It was not surprising to see an improvement of seed quality of two varieties after priming as seed priming is one of the best methods that was suggested to enhance the seed quality of many crop species. Seed priming is controlled hydration of seeds to initiate pre-germinative metabolic activity, but cease the imbibition prior to actual radicle emergence (Parera & Cantlifee, 1994; Bradford & Bewley, 2002). Thus, it has many advantages over other seed improvement techniques. Seed priming is a simple method that could be conducted without any sophisticated equipment (Bradford & Bewley, 2002). Nevertheless, it improves the overall germination rate, growth and also enhances the speed of emergence, uniformity of germination and further increases the shelf life of seed (Bradford & Bewley, 2002). Ultimately it contributes to the enhancement of crop production and provides higher yields.

There are three main reasons to reduce seed quality. Those are imbibition stress (Paparella et al., 2015), pathogenic activity (Islam & Borthakur, 2012) and antioxidant stress (Liu et al., 2007). During our experiments, hydro-priming treatment has increased the seed quality (both germination and vigour) of Batapola-el and Suwendal. It may be due to the reduction of imbibition stress or antioxidant stress. Hydro-priming controls the imbibition process and induce pre-germinative metabolic process as ATP production, accumulation of sterols and phospholipids and activate DNA repair an antioxidant mechanisms. However, care has to be taken to cease the priming prior to the loss of desiccation tolerance of the embryo (Paparella et al., 2015).

In Sri Lanka, Illangakoon et al. (2016) have studied the effect of priming on seeds of several rice varieties including Bg 300, Bg 310 and At 308. They found that hydro-priming improved the survival of rice seeds to about 40%. They showed that the hydro-priming has enhanced the anaerobic germination tolerance in seeds of those rice varieties. They further reported that the synthesis of soluble sugars, starch degradation and α-amylase activity was higher in hydro-primed seeds compared to non-primed seeds especially when they were germinated under submerged conditions.

Another significant problem faced by organic farmers growing traditional rice varieties in Sri Lanka was the low storability of rice seeds for the next growing season. Normally farmers use gunny bags and polysac bags for storage of rice seeds. In commercial scale, they use polythene bags as well. Therefore, in the current research, the effect of storage of rice in these three types of bags was evaluated. Further, three temperature regimes; ambient laboratory temperature, 25 and 8 °C were used as storage temperatures. As seeds stored at 8 °C, had a significantly higher seed germination percentage and vigour, 8 °C could be considered as the best temperature to store seeds of Suwendal and Batapola-el. Although, the long term storage has reduced the seed germinability and vigour of the two study varieties, even after six months it had significantly higher germination percentage of seeds stored at 8 °C compared to those stored at other temperatures. Further, the highest germinability was shown in Suwendal and Batapola-el seeds stored in polythene bags.

When considered both the temperature and the type of bag, there were two treatment combinations with significantly higher germinability retained after six months of storage. Seeds stored in sealed polythene bags or in polysac bags at 8 °C retained significantly higher germinability compared to other storage treatments. However, seeds of Suwendal and Batapola-el varieties stored in sealed polythene bags at 8 °C had a higher vigour (least conductivity in seed leachate) than seeds stored in polysac bags at the same temperature. Therefore, we could recommend storage in polythene bags at 8 °C as the best way to store Suwendal and Batapola-el seeds.

Temperature and RH (or seed moisture content) are two important factors that affect the longevity of seeds during storage (Ellis et al., 1990). Our experiments revealed that storage at 8 °C retained the seed viability of both Suwendal and Batapola-el seeds longer than at other temperatures. This may be because the low temperatures reduce microbial growth as well as it reduces the metabolic activities of seeds. Storage in polythene bags is better than storing them in bags produced with other materials may be due to the moisture retention ability of the polythene bags. Moreover, polythene bags prevent insects from reaching the seeds and also it could prevent oxygen loss.

CONCLUSION

According to the results obtained from our experiments, it revealed that hydro-priming is a promising method to enhance seed quality of both tested varieties. Priming treatments increased the seed germinability, seed vigouras well as the seedling emergence. Thus, for both Suwendal and Batapola-el seeds, we could recommend hydro-priming for 72 hrs to enhance the seed quality. Further, seeds of these two varieties could be stored in polythene bags at 8° C (normal refrigerator temperature) as it can maintain seed viability at 85 % up to 6 months.

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