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Effect of Water Seed Priming on Establishment of Direct Seeded Rice in Well Watered Conditions and Aerenchyma Formation under Varying Water Regimes

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ABSTRACT

The application of seed hydro-priming as a tool for managing the adverse effect of flooding on seed germination and emergence in rice was studied, along with aerenchyma formation in sections of roots for comparisons between rice subjected to flooded and non-flooded conditions during establishment. Three replicates of three hydro-priming times (0, 24 and 48 h) were used on three cultivars. Seeds were germinated in a growth chamber at 25 °C, 65 % RH, or sown in pots in a controlled temperature glasshouse for the emergence and aerenchyma studies. Aerenchyma formation in root cortical tissues was determined from microscopic images of hand-cut sections at 5 and 50 mm behind the tips of 60-70 mm long nodal roots. The results showed that hydro-priming for both 24 and 48 h significantly hastened germination and emergence. Water seed-priming increased plant height from 20 mm to 40 mm 4 days after sowing. Flooding for 7 days significantly enhanced aerenchyma formation at 50 mm behind the root tips. The results showed that hydro-priming for 48 h was the best seed invigoration treatment for shortening the mean emergence time. It hopefully provides seeds an opportunity to escape from the negative effect of flooding on seedling establishment.

INTRODUCTION

Rice establishment by transplanting in flooded conditions associated with lowlands is often demanding in terms of labor required for the puddling of soil and transplanting of seedlings (Singh, R., Singh, V., & Singh, C., 1994; Kumar & Ladha, 2011). Even though the manual labor requirement may be greatly reduced by mechanization, the additional water resources required to accomplish the process can increase over all water use for the production of the crop. The alternative to transplanting, i.e. direct seeding as practiced in upland cultures, can eliminate the need for puddling, thereby saving costs for labor and water supply (Naresh, Singh, & Kumar, 2013). Studies show that a direct seeded rice matures earlier than transplanted rice, and this may increase the turn-around period for subsequent

crops (Balasubramanian & Hill, 2002) and provide high yields in excess of 10 t ha⁻¹ (Stevens, Vories, Heiser, & Rhine, 2012), accompanied by improved water use. Although the advantage of direct seeding can be exploited to increase production, the possible gain of this practice in wetland ecosystems may be seriously undermined by flooding, particularly if this occurs and persists before the crop has fully emerged. Yield of direct seeded rice is often limited by poor germination, emergence, and uneven crop stands (Balasubramanian & Hill, 2002). Therefore, a rapid, even and vigorous germination of seeds and establishment of seedlings will be required to minimize the incidence of poor stand in direct seeded rice, as emerged seedlings are less prone to the negative effects of flooding. Although various seed priming techniques are known to improve seed germination and emergence (Basra, Farooq,

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& Khaliq, 2003; Mahajan, Sarlach, Japinder, & Gill, 2011), there are limited reports regarding the use of hydro-priming as a cheap, farmer-friendly seed invigoration technique to overcome the problem of poor stand establishment in direct seeded lowland rice (Abdallah, Musa, Mustafa, Sjahril, & Riadi, 2016), potentially subjected to flooding at any stage of crop growth.

Following germination, roots of most crop plants normally obtain oxygen needed for aerobic respiration directly from the pore spaces in the soil. However, excess water due to flooding can displace gases in the soil, thereby undermining the exchange of gases between the root and its environment, and eventually limiting the survival of the plant (Nishiuchi, Yamauchi, Takahashi, Kotula, & Nakazono, 2012). Rice plants subjected to flooding reportedly develop a special anatomical feature (aerenchyma) that helps them survive by facilitating the movement of gases into their submerged tissues (Drew, He, & Morgan, 2000; Nishiuchi, Yamauchi, Takahashi, Kotula, & Nakazono, 2012). Studies on aerenchyma formation at the seedling stage is limited. A study of such gas filled spaces at this stage is important for understanding its genesis and whether the direct seeded crop can rely on it at the establishment stages to grow well in flooded conditions.

The objectives of this study were therefore: 1) to determine the effect of hydro-priming on the germination and emergence of rice, with the view of enhancing early and uniform establishment when crops are direct-seeded in well-watered conditions, and 2) to examine gas-filled space formation in the cortical tissues of flooded and non-flooded rice roots during the early growth stage, as a vehicle for enhanced seedling establishment, especially in flooded conditions.

MATERIALS AND METHODS

The study was conducted through both laboratory and greenhouse experiments in the research facilities of the University of KwaZulu-Natal in South Africa from July to September of 2010.

Plant Material

Three rice cultivars, NERICA-L-19 (N-19) and FKR-19, acquired from the Africa Rice Centre (formerly WARDA) and Golden Mountain # 1 (GM-1), were used. To ensure the use of good quality

seeds, each seed lot was submerged in two volumes of 0.2 % NaCl solution and the floating seeds were discarded. Seeds that sank to the bottom of the container were collected and washed three times with distilled water.

Seed Priming

Seeds were hydro-primed for 0, 24 and 48 h (P_0 , P_{24} , and P_{48}). The P_{24} seeds were soaked in distilled water at ambient temperature for 12 h and incubated for another 12 h. For the P_{48} treatment, seeds were soaked in distilled water for 24 h and incubated for another 24 h. Seeds were spread thinly between moist double layered paper towels, sealed in plastic bags and incubated in a growth chamber (Labex, Ltd.) at 25 °C. After incubation, the seeds were surface dried at ambient temperature for 24 h and sown. The P_0 seeds were neither soaked, nor incubated.

Germination

Three replicates, each containing 100 seeds of every combination of cultivar and priming treatment were lined between double layers of moist paper towels, arranged in complete randomized blocks and placed in a growth chamber set at 25 °C. Germination was monitored at 24 hour intervals for 5 days and the final germination percentage recorded. Germination velocity index, calculated according to Woodstock (1976), was used to determine the speed of germination:

$$\text{Germination velocity index (GVI)} = G_1/N_1 + G_2/N_2 + G_3/N_3 + G_4/N_4 + G_5/N_5$$

where G_1 , G_2 , G_3 , G_4 and G_5 = number of seeds germinated on each day, and N_1 , N_2 , N_3 , N_4 , and N_5 = number of days from sowing to the first and last count.

Emergence

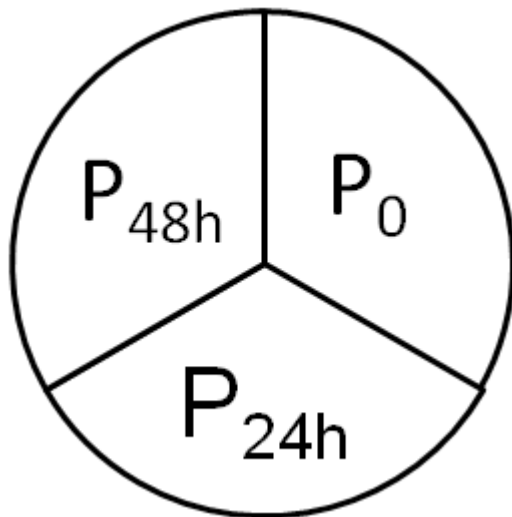
The plant emergence test was carried out in a controlled temperature glasshouse with day / night temperatures 30 °C / 20 °C and consisted of three replications in a split-plot combination of the three hydro-priming treatments and three cultivars in a randomized complete block design. The cultivars were the main plots while the priming treatments constituted the sub-plots. Pots (4.8 L) of 30 cm diameter were used for the main plots, and each pot was divided into three 235.5 cm² sections (Fig. 1).

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Twenty-five seeds of each priming treatment were sown, each 2.0 cm deep and 3.0 cm x 3.0 cm apart, to one of three 235.5 cm² sections per pot. Pots were well watered and kept at field capacity throughout the emergence experiment. Final emergence and the time to 50 % emergence were determined by daily seedling count over a period of 7 days. Mean emergence time (MET) was calculated as:

$$\text{MET} = \frac{\sum(\text{Ni} \times \text{Di})}{\sum \text{Ni}}$$

where: Ni = number of newly emerged seedlings at day Di.



Remarks: P₀ = seeds were hydro-primed for 0 h; P₂₄ = seeds were hydro-primed for 24 h; P₄₈ = seeds were hydro-primed for 48 h

Fig. 1. Sub plot division of 30 cm diameter pots used for plant emergence using primed seeds of three rice cultivars. Each sub plot measured 235.5 cm². Twenty-five seeds were sown 2.0 cm deep to each 235.5 cm² section, with spacing of 3.0 cm x 3.0 cm.

Seedling height was measured at 50 % emergence and at 28 DAS. Seedling above ground dry mass was determined at 28 DAS. Ten seedlings from each treatment combination were selected randomly for seedling height and above ground dry mass measurements.

Root Aerenchyma

The root anatomy study involved a split-plot combination of three rice cultivars (GM-1, FKR-19 and N-19) and two water regimes (flooded and non-

flooded) established in 4.8 L (30 cm diameter) pots. The water regimes were the main plot, and cultivars the sub-plot arranged in a randomized complete block design. Ten plants of each cultivar were grown in 235.5 cm² sections per pot for a period of 28 days. Flooding was established and maintained at 5 cm above the surface for 7 days beginning from seedling emergence. The non-flooded plants were watered daily, ensuring that the soil moisture tension (monitored with tensiometers, Irrometer Co., California) at the bottom of the pots was kept below 15 kPa. At 28 DAS, pots were submerged in a basin of water and the soil carefully washed off the roots.

Three seedlings of similar height per cultivar were selected for aerenchyma measurements. Hand cut sections (approximately 0.2 mm thick) were taken at 5 and 50 mm behind the tips of 60 – 70 mm long nodal roots and examined under a Nikon light microscope. Microscopic images of the transverse root sections were photographed using a digital camera attached to the microscope. The total areas of each hand-cut section, the outer layers, cortex, gas spaces and inner layers were measured from the digital images using Image J digital imaging software for windows. The measurements were used to calculate the proportion of root cortex occupied by aerenchyma.

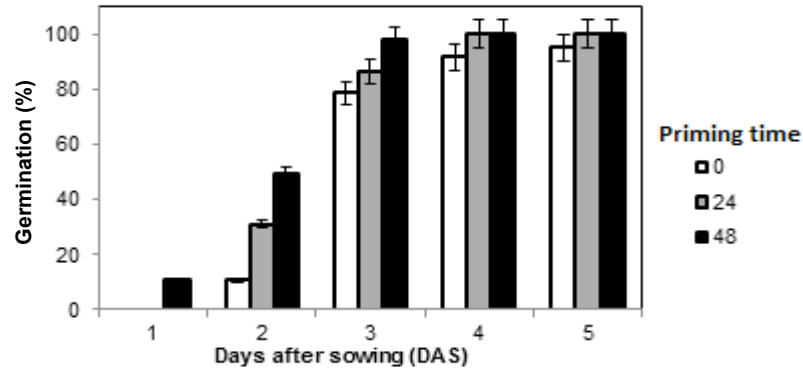
Data Analysis

Data on all measurements from the germination, emergence and aerenchyma aspects of the current study were subjected to analysis of variance, using the statistical software package Genstat version 12 (VSN International). The means of factors showing significant differences were separated using Fisher's Least Significant Difference (LSD) test at the five percent level of significance (P = 0.05).

RESULTS AND DISCUSSION

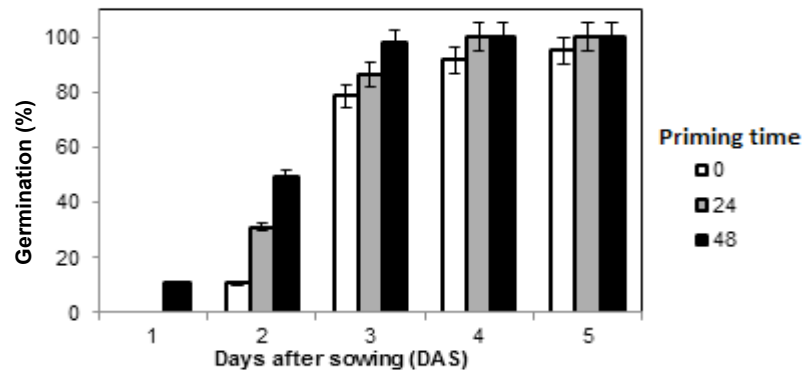
Germination and Seedling Emergence

The speed of germination measured by the germination velocity index increased significantly (P < 0.001) with priming time in all cultivars; however, in GM-1 the difference in germination speed between the 24 and 48 h hydro-priming times was negligible (Fig. 2). Seed priming for both 24 and 48 h led to significant increases in the percentage of germination by 5 DAS (Fig. 3).



Remarks: FKR-19, GM-1 and N-19 = rice cultivars; the white, gray and black coloured bars represent GVIs for seeds primed for 0, 24 and 48 hours, respectively.

Fig. 2. Effect of seed priming on seed germination speed. Bars indicate priming time (h). Error bars indicate 95 % CI of means.



Remarks: The white, gray and black coloured bars represent rate of germination for seeds primed for 0, 24 and 48 hours, respectively.

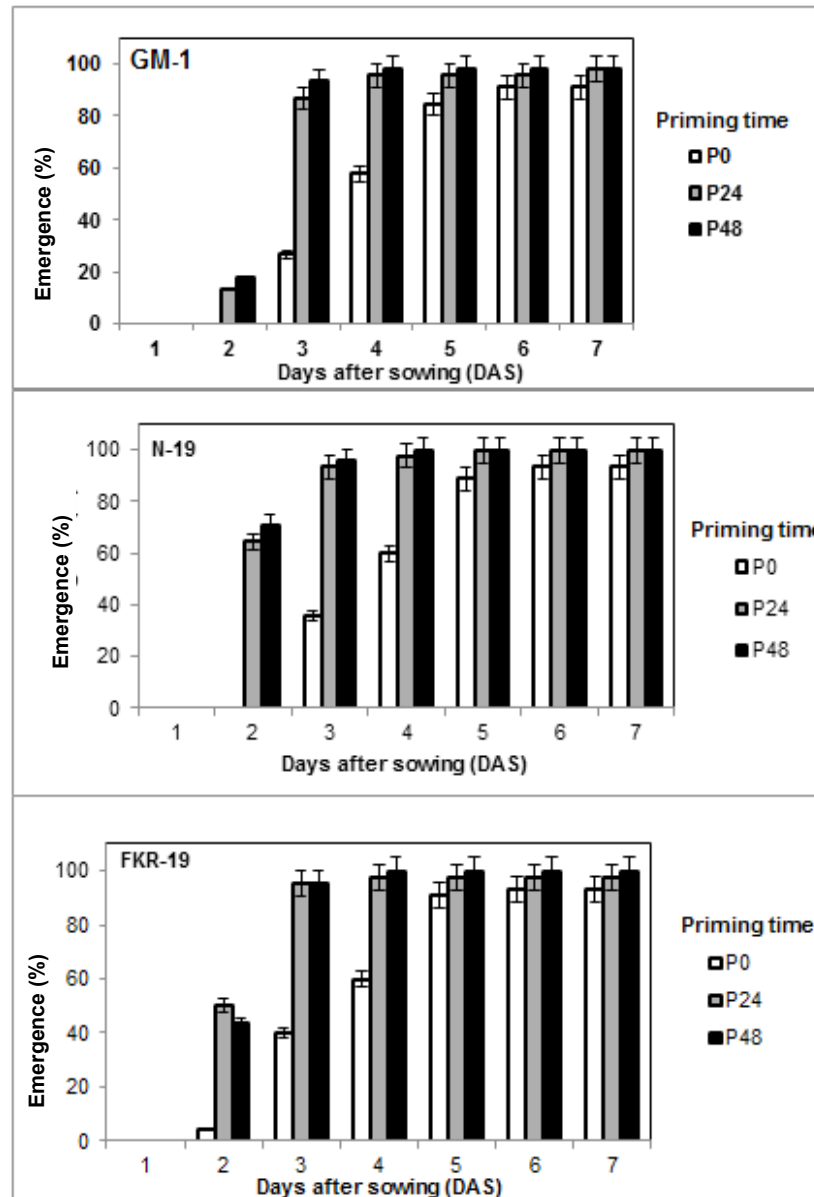
Fig. 3. Rate of seed germination as affected by hydro-priming time. Bars show priming time (h). Error bars indicate 95 % CI of means.

Seed priming for both 24 and 48 h significantly ($P < 0.001$) shortened the days to 50 % emergence (Table 1). Similarly, primed seeds emerged faster ($P < 0.001$) than the non-primed seeds (Table 1). However, there was no cultivar difference in emergence times. Compared to the non-primed controls, seed priming for both 24 and 48 h led to a significant ($P < 0.001$) increase in final emergence (at 7 DAS) for all the cultivars; however, the difference between the primed treatments was negligible (Fig. 4). These results suggest that water seed priming can be successfully applied to improve crop establishment in rice. Similar positive effects of water seed priming on germination and emergence of rice have been reported from several studies (Mahajan, Sarlach, Japinder, & Gill, 2011; Prasad, S., Prasad, B., & Singh, 2012; Matsushima & Sakagami, 2013).

Plant height at both 50 % emergence (i.e. at 4 DAS) and at 28 DAS differed significantly ($P < 0.001$) with hydro-priming time (Fig. 5). At 50 % emergence, seedlings produced from seeds primed for 48 h were at least two times taller than those of the control. They were also taller than those produced by the 24 h priming treatment. Although seedling height varied significantly ($P < 0.001$) with the cultivar, there was no significant ($P > 0.05$) interaction between seed priming and cultivar at both 50 % emergence and at 28 DAS. Seed priming significantly ($P < 0.001$) increased dry matter accumulation at 28 DAS (Table 1). Like plant height, above ground dry matter accumulation (shoot dry mass) at 28 DAS varied significantly with cultivar; but the variation was not due to the priming treatment (Table 1).

Table 1. Effect of hydro-priming time on the emergence time and shoot dry mass of rice seedlings

Parameters	GM-1			FKR-19			N-19			LSD _(0.05)
	P ₀	P ₂₄	P ₄₈	P ₀	P ₂₄	P ₄₈	P ₀	P ₂₄	P ₄₈	
Mean emergence time (days)	4.2	3.0	2.9	3.9	2.5	2.6	4.0	2.5	2.3	1.1
Days to 50 % emergence	4.0	3.0	3.0	4.0	2.0	3.0	4.0	2.0	2.0	1.4
Shoot dry mass at 28 DAS (g plant ⁻¹)	0.20	0.22	0.24	0.17	0.22	0.22	0.19	0.20	0.22	0.02



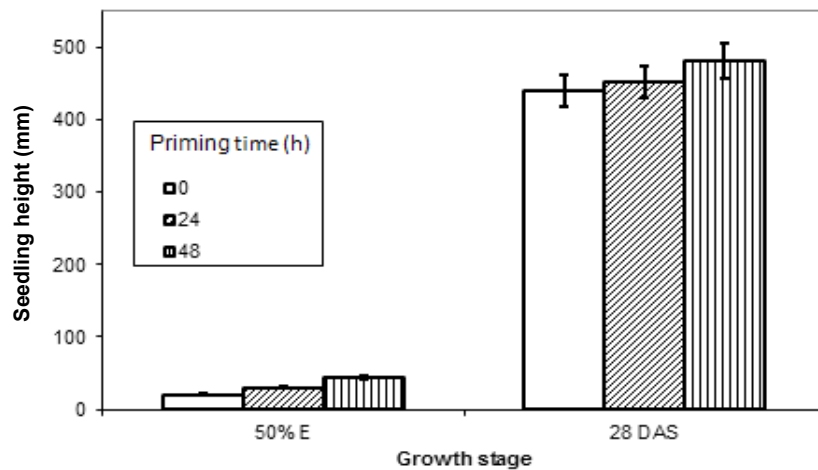
Remarks: FKR-19, GM-1 and N-19 = rice cultivars; the white, gray and black coloured bars represent P0, P24 and P48, which are GVIs of seeds primed for 0, 24 and 48 hours, respectively.

Fig. 4. Effect of hydro-priming time on seedling emergence. Error bars show 95% CI of means.

Root Aerenchyma

The extent of aerenchyma formation in nodal roots varied according to the positions examined behind the root tip. At 5 mm behind the root tip, only GM-1 showed a minimum (9 %) air space in the root cortex when flooding was imposed for 7 days. Apart from this, no aerenchyma was seen irrespective of flooding regime or cultivar (Fig. 6). However, at 50 mm behind the root tip, aerenchyma spaces were more prominent in all the cultivars as a result of flooding, covering up to 92 % of the root cortex (Fig. 7). Thus, compared to the 5 mm position behind the root tips, significant aerenchyma developments occurred in the root cortex at 50 mm behind the root tips of all the cultivars (Fig. 7 and Fig. 8). Aerenchyma

were present by 7 days from commencement of flooding when root sections were examined. Studies have shown that aerenchyma formation takes place specifically in the root cortex, rather than in the epidermis, endodermis, hypodermis or steel of the roots (Yamauchi, Shimamura, Nakazono, & Mochizuki, 2013). Shiono et al. (2011) observed the enhancement of aerenchyma formation in the roots of rice seedlings within 12 h of transferring in stagnant deoxygenated nutrient solution, and that the level of aerenchyma formation increased with the distance behind the root tip. The volume of aerenchyma was also found to increase the time (Malik, Colmer, Lambers, & Schortemeyer, 2003; Shiono et al., 2011).



Remarks: E = emergence; DAS = days after sowing

Fig. 5. Effect of hydro-priming time on seedling height. Error bars show 95 % CI of means.

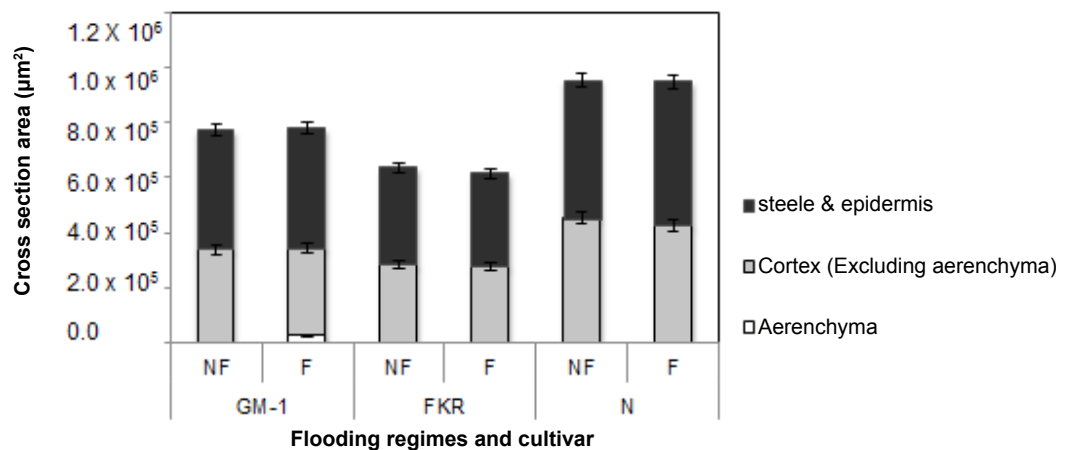


Fig. 6. Effect of flooding regime (NF = non-flooded, F= flooded) on aerenchyma development at 5 mm behind the root tip observed at 7 days after submergence

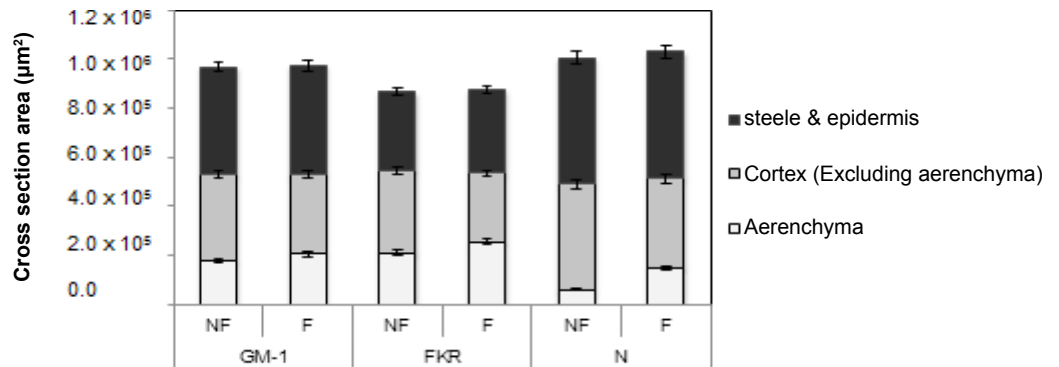


Fig. 7. Effect of flooding regime (NF = non-flooded, F= flooded) on aerenchyma development at 50 mm behind the root tip observed at 7 days after submergence.

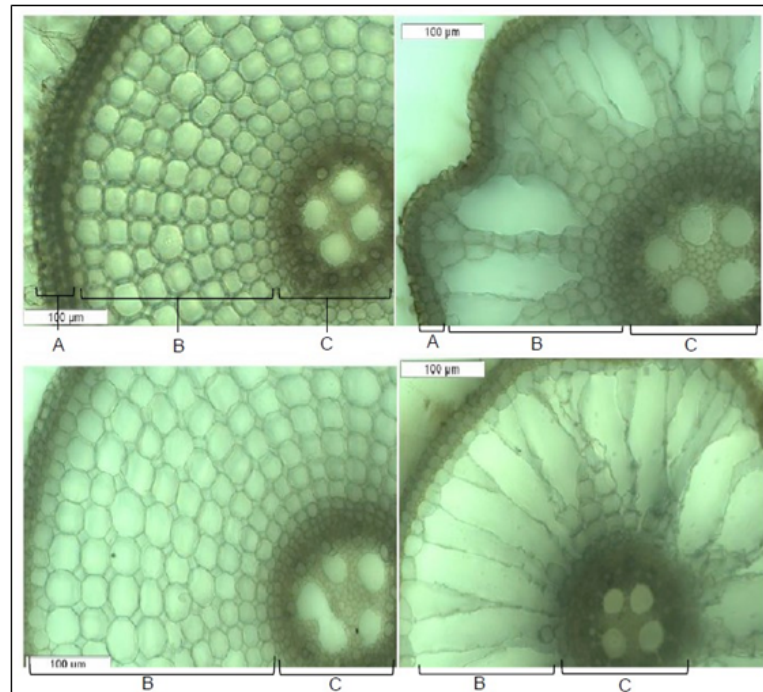


Fig. 8. Effect of water regime on aerenchyma formation in roots of rice plants grown in flooded (below) and non-flooded (above) conditions. Hand-cut sections were taken at 5 mm (left) and 50 mm (right) behind the root tips. Cross sections labelled A, B, and C are the outer cell layers, cortex and stele, respectively. Cortical cells in both top and bottom plates on the left hand portion of the figure are normal, while lysigenous aerenchyma are shown in both top and bottom plates on the right hand side of the figure.

Rice establishment by direct seeding in sufficient water areas is a suitable alternative to transplant because it often demands labor and water use (Farooq et al., 2011; Liu et al., 2015). Flooded conditions during germination and emergence can have detrimental effects on seedling establishment, and in extreme cases, cause crop failure. The current results showed that hydro-priming is an important seed invigoration treatment which increases the

speed as well as percentage of germination of all the cultivars studied; hence, providing an opportunity for direct seeded rice to develop into seedlings quickly enough to possibly escape the adverse effects of flooding. Seed invigoration by hydro-priming is believed to result from the metabolic repair of deterioration sustained by the seed during the hydration-dehydration process prior to actual sowing (Thornton & Powell, 1995).

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The timing of seedling emergence is known to be a major determinant for crop establishment and yield (Ghassemi-Golezani, Chadordooz-Jeddi, Nasrollahzadeh, & Moghaddam, 2010). Priming seeds for both 24 and 48 h in this study hastened emergence by at least one day and was important for the early establishment of all the cultivars. About 90 % emergence was achieved earlier i.e. by 3 DAS as a result of water seed priming, compared to at most 40 % for the non-primed seeds. In lowland rice culture where flooding is unpredictable, such a speedy establishment could be beneficial when rice is direct-seeded because the seedlings can be able to more easily withstand flooded conditions compared to the germinating or non-germinated seeds (Angaji, Septiningsih, Mackill, & Ismail, 2010; Mackill, Ismail, Singh, Labios, & Paris, 2012). This early germination/emergence provides an important window of opportunity for the seeds to escape the detrimental effects of flooding that can ultimately lead to crop failure. Improved germination and establishment by seed priming has been reported by Farooq, Basra, & ur-Rehman (2006) and Farooq et al. (2011) who suggested that seed priming did not only increase the rate of seedling emergence, but also increased the subsequent yield of direct seeded rice.

Aerenchyma is a space continuum which facilitates the movement of gases between submerged plant parts and the atmosphere, and therefore constitutes an essential anatomical feature for survival in wetland plants exposed to flooding (Nishiuchi, Yamauchi, Takahashi, Kotula, & Nakazono, 2012). This study showed that aerenchyma formation began quickly with the onset of flooding and were present within 7 days of flooding. Hence, once seeds have turned into seedlings, the survival of the rice crop was probably assured in flooded conditions due to the fast formation of aerenchyma, with no other constraining factors. The complete absence, or poor development of aerenchyma at 5 mm behind the root tip might be due to its close proximity to the apical meristematic region, where most of the cells were still in their early stages of development and therefore it was not easily prone to the lysis processes that led to aerenchyma formation. The response observed at 5 cm from the root tip is consistent with results by Malik, Colmer, Lambers, & Schortemeyer (2003) who reported that aerenchyma commonly forms over most parts of the rice plant body, with the exception of meristems and other tissues such as

vascular bundles, sclerenchyma, and the epidermis. Compared to the non-flooded plants, aerenchyma formation, particularly at the 50 mm position behind the root tip, was more prominent in the plants subjected to flooding and this would increase root porosity and ultimately, the ability of the plants to maintain much needed oxygen in the submerged roots. Such seedlings would have the ability to thrive in flooded conditions due to the rapid development of aerenchyma in the root cortex in response to even short episodes of flooding. The observation of aerenchyma formation in the roots of the non-flooded plants in this study suggests that the process is an integral part of normal root development in rice, since it also occurs speedily, in well aerated media (Jackson, Fenning, & Jenkins, 1985). It is, however, exacerbated by flooding.

CONCLUSION

The results of this study showed that hydro-priming of seed could be used as a tool for improving stand establishment in direct seeded rice production since it shortened the times of germination and emergence, increased seedling height and dry mass, and improved the uniformity of emergence. Most importantly, it provided an opportunity to escape the potentially detrimental effects of early flooding at the germination and emergence stages. The prominence and early formation of aerenchyma in the cortical tissues of rice subjected to flooding represented an important anatomical adaption that makes it possible for seedlings of direct seeded rice to survive and flourish in wetland habitats by facilitating root aeration.

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