Effect of seed priming on germination, emergence and seedling growth of spring rice (*Oryza sativa* L.) cv. Hardinath-1

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Article Info

ABSTRACT

Accepted:
11 Jan. 2019

Keywords:

Emergence,
Fertilizers,
Germination, Seed
priming, Spring rice

The study was conducted from December 2017 to May 2018 to depict reliable and effective technique of seed priming in spring rice variety. The laboratory analysis was performed in the Regional Seed Testing Laboratory, Sundarpur, Kanchanpur under controlled conditions. The completely randomized design with 6 treatments- without priming, water/ hydro-priming, 2% urea priming, 2% DAP priming, 2% MOP priming and 2% ZnSO₄ priming with 4 replications was used. Two different experimental setups were made for germination and emergence test using germination paper roll and sand tray method. Meanwhile, the parameters like germination percentage, germination energy, vigor index, root length and shoot length at 7 DAS and emergence percentage were remarkably affected. However, the parameters like the speed of germination and shoot length, fresh weight and dry weight at 10th, 15th and 20th DAS were not affected significantly by the priming treatments. The priming treatment with 2% MOP solution gave the best results for germination percentage (93.5%), germination energy (92.5%), vigor index (138.1) and emergence percentage (93.0%). 2% urea priming treatment resulted in longest root length (12.04 cm) and shoot length (7.97 cm) at 7 DAS. This study suggests seed priming with 2% MOP solution as an easy and effective technique for improving the germination, emergence and seedling parameters of spring rice, variety Hardinath-1.

INTRODUCTION

Rice (*Oryza sativa* L.) or “Dhan” in Nepali is an annual, self-pollinated and semi-aquatic plant of Poaceae family. In terms of area under cultivation and production, rice comes first among the cereals in Nepal occupying about 41.22% of the total area under cereal crops and contributing about 49.9% of total edible cereal production (MoAD 2016).

Rapid germination and emergence are essential for successful crop establishment, for which seed priming could play an important role. Seed priming is a controlled hydration technique that activates the normal metabolic processes

during the early phase of germination before the germination is observed (Hussain et al. 2015; Silva and Silva 2016). The major cause behind the fact that spring rice is cultivated in less area in Nepal is the poor seedling establishment resulting in the lowered vigor, crop stand and final yield. Mostly marginalized families are involving in crop production, which has low purchasing power thus they can't afford investments in irrigation and improved nursery management in spring rice, they need low-cost technology for better crop production. Although the findings from previous studies suggests seed priming as an effective technique to enhance crop vigor and stand establishment (Nawaz et. al 2013; Singh et. al 2015; Koirala 2017), very few research has been conducted in Nepal about seed priming in Nepalese rice varieties.

Seed priming, by increasing speed and uniformity of germination, could help to obtain elite seedling along with better crop stand (Ghiyasi et al. 2008; Islam, Mukherjee and Hossin 2012). The variety used in the present study (Hardinath 1)

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is the most popular spring rice variety, suitable for both direct seeding and transplanting method of cultivation. The priming agents used in this study are easily available in the Nepalese market hence; it would be easy for the extension personnel to recommend the priming techniques to the farmers. This study aims to assess easy and effective technique for improving the germination, emergence and seedling quality of spring rice variety.

MATERIALS AND METHODS

General experimental details

To assess the effects of priming on germination and emergence of spring rice variety, the seeds were primed using various priming solutions and studied under controlled conditions. The study was conducted in the laboratory of Regional Seed Testing Laboratory, Sundarpur, Kanchanpur. The seed sample for conducting research work was obtained from the International Rice Research Institute, Kailali, Nepal. The sample was taken from commercial seed lot of certified seeds of variety "Hardinath-1" and the sample size and sampling techniques were based on the ISTA rules for seed sampling.

The study consisted of six treatments: control (no priming), hydro-priming, priming with 2% solutions of Urea, Diammonium Phosphate (DAP), Murate of Potash (MOP) and Zinc Sulphate ($ZnSO_4$). The priming of seed was done in the dark at $25 \pm 2^\circ C$ for 24 hours. The solution was changed after 12 hours to facilitate aeration in seeds. The ratio of seed weight to solution volume (w/v) was 1:5. After 24 hours, the primed seeds were washed with water for 3-4 times and dried in shade at $25 \pm 2^\circ C$ until the moisture content was decreased to initial moisture content.

Experimental design

There were 6 treatments in the research experiment with 4 replication of each. The experiment was conducted on a Completely Randomized Design (CRD). Each of the 6 treatments was replicated 4 times, thus there were altogether 24 experimental setups for each germination test and emergence test.

Germination test

Four replications of 100 seeds for each treatment were placed in germination test paper rolls, moistened with water. Then the rolls containing the seeds were transferred to a germination chamber at $25 \pm 2^\circ C$, where they were kept for 7 days. The observation was made every day for evaluating germination. During the final count at 7th day, 10 normal seeds were chosen randomly and their root length and shoot length was measured using the graduated scale.

Seedling emergence test

Four replications of 50 seeds per treatment, were sown in trays containing sand, screened and washed, 2 cm deep. Daily irrigation was performed. The emergence was counted at every two days interval until the count became constant. Randomly 10 seedlings were selected on 10th, 15th and 20th days and were uprooted with minimum damage to the roots, washed with water to remove sand and dried with paper towels then the shoot length was measured using the graduated scale. After measuring shoot length, the 10 seedlings were kept in small aluminum containers and were weighted to measure fresh weight using an electronic balance with 10-4g precision. Then the seedlings in aluminum containers were kept in a hot air oven at $165^\circ C$ for 2 hours for drying and dry weight was taken using electronic balance after cooling down the containers using desiccators.

Data and data types

Germination test data

The data recorded or derived from germination test are as follows

- Germination percentage
- Speed of germination
- Germination energy
- Vigor index
- Root length 7 Days after sowing (DAS)
- Shoot length 7 Days after sowing (DAS)

The germination percentage was calculated using the following formula-

$$\text{Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Total number of seeds set for test}} \times 100\%$$

The speed of germination (%) was calculated using the following formula (Krishnasamy and Seshu 1990).

$$\text{Speed of germination (\%)} = \frac{\text{Number of seed germinated at 72h}}{\text{Number of seed germinated at 168h}} \times 100\%$$

Germination energy = Percentage of seeds germinated at 72 h (Bam *et al.*, 2006; Islam, Mukherjee and Hossin 2012).

Vigor index = No. of germinated seeds / days of first count + + No. of germinated seeds/days at final count.

Emergence test data

The data obtained from the emergence test experiment are as follows:

- Emergence Percentage (similar formula as for germination percentage)
- Shoot length (at 10th, 15th and 20th DAS)
- Fresh weight of seedling (at 10th, 15th and 20th DAS)
- Dry weight of seedling (at 10th, 15th and 20th DAS)

Data analysis techniques

All the data were entered through Microsoft excel and data analysis was done using statistical package- GenStat, 15th edition. The hypothesis was tested using f-test at 5 percent level of significance and the means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The analysis of data provided various results which are presented and discussed below.

Germination test

[Table 1: Effect of different priming treatments on germination percentage, germination energy, speed of germination, vigor/germination index and root length and shoot length of seedlings at 7 DAS]

Germination Percentage:

Germination percentage was affected significantly by the type of priming treatment. The germination percentage was found to be highest in MOP primed seeds (93.5%). Germination percentages in priming treatments with 2% DAP solution (92.0%), 2% ZnSO₄ solution (92.0%) and water/hydro-priming (90.25%) were statistically similar and were superior to control or no priming treatment (87.5%). The priming treatment with 2%

urea solution resulted in the least germination percentage and was 79.5 percent.

Commercial Murate of Potash fertilizer is the 95:5 mixtures of Pottasium Chloride (KCl) and Sodium Chloride (NaCl). KCl being the major constituent in MOP, it has similar effects as that of KCl in various other studies. In a study conducted by Islam, Mukherjee and Hossin (2012), priming with 20.74g/l KCl solution enhanced germination than hydropriming. Prasad, Prasad and Singh (2012) reported that hydro-priming for 24 hours enhanced germination percentage than unprimed rice seeds. Silva and Silva (2016) reported that hydro-priming and priming with zinc sulfate had a similar effect on germination percentage in "*Puita*" cultivar and was superior to control/no priming treatment. These studies are in agreement with the findings of present study.

In a study by Farooq et al. (2005) complete failure of seed germination was observed in 5% urea priming treatment and 5% DAP priming treatment reduced the germination percentage than control in fine rice which is in contradiction with the findings of our study. This difference could be due to the difference in concentration of solutes.

Germination energy, speed of germination and vigor index:

Different type of priming treatment significantly affected the germination energy and vigor index while the speed of germination was not affected significantly.

Priming treatment with 2% MOP solution gave the highest value of germination energy percentage (92.5%) and vigor index (138.1). Priming with 2% solutions of DAP and ZnSO₄ and water showed statistically similar results in the

Table 1. Effect of different priming treatments on germination percentage, germination energy, speed of germination, vigor/germination index and root length and shoot length of seedlings at 7 DAS

Treatment	Germination%	Germination energy %	Speed of germination %	Vigor Index	Root length (cm)	Shoot length (cm)
No priming (control)	87.5 ^b	86.0 ^b	98.01	120.2 ^{cd}	10.83 ^{bcd}	7.75 ^a
Water (Hydro-priming)	90.25 ^{ab}	87.5 ^{ab}	97.00	122.4 ^c	11.55 ^{ab}	7.69 ^a
2% Urea solution	79.5 ^c	76.25 ^c	96.03	112.9 ^d	12.04 ^a	7.97 ^a
2% DAP solution	92.0 ^{ab}	90.5 ^{ab}	98.39	132.1 ^{ab}	9.98 ^d	7.01 ^b
2% MOP solution	93.5 ^a	92.5 ^a	98.94	138.1 ^a	11.15 ^{abc}	7.77 ^a
2% ZnSO ₄ solution	92.0 ^{ab}	89.5 ^{ab}	97.24	123.6 ^{bc}	10.35 ^{cd}	6.96 ^b
f-probability	<0.001	<0.001	0.424 ^{ns}	<0.001	0.004	0.022
CV%	4.1	3.6	2.1	4.4	6.0	6.1

Note: CV- Coefficient of variation, 'ns' represent the values are insignificant, letters a, b, c, d. represent the ranking of treatments according to DMRT at 0.05 level of significance.

context of germination energy percentage which was inferior to priming with 2% MOP solution and superior to control and urea priming. Control treatment had lower germination energy percentage (86.0%) than all other treatments except priming treatment with 2% urea solution (76.25%). All the 6 treatments showed statistically dissimilar results for vigor index. The highest value of vigor index was shown by priming with 2% MOP solution (138.1) followed in decreasing order by priming with 2% DAP solution (132.1), priming with 2% ZnSO₄ solution (123.6), priming with water/hydro-priming (122.4), no priming/control (120.2) and lastly by priming with 2% urea solution (112.9).

In a study conducted by Islam, Mukherjee and Hossin (2012), priming with 20.74g/l KCl solution showed better results than hydropriming for germination energy and vigor index. Prasad et al. (2012) reported that hydro-priming for 24 hours enhanced germination energy and vigor index significantly than unprimed rice seeds. These studies also have similar findings as of our study. Farooq et al. (2005) studied the effect of commercial fertilizers urea, nitrophos, DAP and SOP as priming agents at 5% concentration in coarse and fine rice. All the treatments resulted in lowering the vigor compared with that of control. In the recent study, urea priming has lowered the vigor, which is in line with this study but it has a contradiction in an enhancement of vigor by DAP priming which may be due to the difference in concentrations of solutes used.

Root length and shoot length of seedlings at 7 DAS:

Root length and shoot length of seedlings were affected significantly by the type of priming

treatments.

Root length varied significantly in all six treatments. Root length was found highest in 2% urea solution priming treatment (12.04 cm) followed in decreasing order by water/ hydro-priming (11.55 cm), 2% MOP solution priming (11.15 cm), no priming/control (10.83 cm), 2% ZnSO₄ solution priming (10.35 cm) and lastly by 2% DAP solution priming (9.98 cm). Shoot length was found to be highest on priming treatment with 2% urea solution (7.97 cm) which was statistically similar to the priming treatments of 2% MOP solution (7.77 cm), no priming/control (7.75 cm) and water/hydro-priming (7.69 cm). Shoot length was lowest on priming treatment with 2% ZnSO₄ solution (6.96 cm) which was statistically similar to priming treatment with 2% DAP solution (7.01 cm).

Silva and Silva (2016) reported that hydro-priming and priming with zinc sulfate enhanced seedling length than control treatment. In a recent study, hydro-priming has increased the seedling length which is in line with the study but it has a contradiction in a reduction of seedling length by ZnSO₄ priming which may be due to the difference in concentrations of solutes and variety used.

Emergence test

[Table 2: Effect of different priming treatments on emergence percentage and shoot length, fresh weight and dry weight of rice seedlings at 10, 15 and 20 DAS]

Emergence percentage:

Emergence percentage was affected significantly by the types of priming treatments. The emergence percentage was found to be highest in priming treatment with 2% MOP solution

Table 2. Effect of different priming treatments on emergence percentage and shoot length, fresh weight and dry weight of rice seedlings at 10, 15 and 20 DAS

Treatment	Emergence %	10 DAS			15 DAS			20 DAS		
		Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
No priming (control)	88.00 ^{ab}	12.87	0.4899	0.1414	14.66	0.5723	0.1337	15.56	0.5754	0.1361
Water (Hydropriming)	92.50 ^a	12.84	0.5375	0.1434	14.88	0.5900	0.1388	14.64	0.5880	0.1335
2% Urea solution	85.00 ^b	13.01	0.5032	0.1491	15.35	0.5766	0.1370	15.34	0.5669	0.1325
2% DAP solution	89.00 ^{ab}	13.12	0.5252	0.1448	15.23	0.5801	0.1368	15.09	0.5816	0.1331
2% MOP solution	93.00 ^a	12.88	0.5018	0.1417	15.16	0.5912	0.1379	15.19	0.5662	0.1345
2% ZnSO ₄ solution	92.00 ^a	12.84	0.5616	0.1391	14.38	0.5771	0.1380	14.91	0.6259	0.1386
f-probability	0.045	0.999 ^{ns}	0.966 ^{ns}	0.657 ^{ns}	0.883 ^{ns}	0.994 ^{ns}	0.709 ^{ns}	0.952 ^{ns}	0.375 ^{ns}	0.650 ^{ns}
CV%	4.1	8.8	24.2	6.0	8.6	9.2	3.3	9.3	7.1	4.1

Note: CV- Coefficient of variation, 'ns' represent the values are insignificant, letters a, b, c, d. represent the ranking of treatments according to DMRT at 0.05 level of significance.

(93.00%) which was statistically similar to priming treatment with water/hydro-priming (92.50%) and 2% ZnSO₄ solution (92.00%). Emergence percentage in priming treatments with 2% DAP solution (89.00%) and no priming/control (88.00%) were found to be statistically similar and was superior to priming with 2% urea solution which had least value of emergence percentage and was 85.00%.

Farooq et al. (2006) reported that KCl and hydropriming had a similar effect on emergence percentage of fine rice which was superior to control. Silva and Silva (2016) reported that hydro-priming and priming with zinc sulfate had similar effects on emergence percentage of “*Puita*” cultivar. These studies also have similar findings as of our study.

Shoot length, Fresh weight and dry weight of seedlings at 10, 15 and 20 DAS:

The effect of different types of priming treatments on shoot length, Fresh weight and dry weight of 10, 15 and 20 days old seedlings were statistically non-significant.

Silva and Silva (2016) reported that there was no significant difference in seedling dry mass of rice seedlings in zinc sulfate priming, hydro-priming and control treatments. This result is in line with the findings of our study.

Seed priming with various priming agents affected the germination, emergence and growth characteristic of spring rice variety “Hardinath-1” significantly.

In the germination test experiment, priming treatment with 2% MOP solution performed best in terms of germination percentage (93.5%), germination energy percentage (92.5%) and vigor index (138.1) and in emergence test experiment, it showed the highest value of emergence percentage (93.0%). Similarly, in the germination test experiment, 2% MOP priming treatment had second longest average shoot length (7.77cm) which was statistically similar to the treatment with longest average shoot length i.e. 2% urea solution priming (7.97cm). Also, it had the third longest root length (11.15cm) only after 2% urea priming (12.04cm) and hydro-priming (11.55cm) treatments. Hence we can say that 2% MOP solution priming was the best among all the treatments. Although 2% urea solution priming had longest root length and shoot length in germination test experiment, it had the least values for germination percentage (79.5%), germination energy percentage (76.25%) and vigor index (112.9). Also, 2% urea solution priming treatment had least emergence percentage (85.0%) in emergence test experiment. So, we can say that 2%

urea priming treatment would be the least preferable among all treatments.

Priming allows the seed to imbibe water and go through the first phase of germination in which pre-germination metabolic activities are started while the latter two phases of germination are inhibited (Pill and Necker 2001). Osmo-priming may also contribute to rapid seed germination by reducing the mechanical restraint of endosperm on developing embryo (Mayer and Poljakoff-Mayber 1989) this was due to the increased endo-beta mannanase activity (Toorop, van Aelst and Hilhorst 1998). The improvements in seed germination and seedling emergence were due to an enhanced supply of soluble carbohydrates to the growing embryo, which was caused by an increase in α -amylase activity (Roy and Srivastava 1999). The better germination and vigorous growth of primed-rice seedlings was associated with (1) higher starch metabolism, (2) enhanced respiration rate, (3) better membrane integrity (4) higher metabolite synthesis, and (5) increased activities of antioxidants in these seedlings (Hussain, Khan, Hussain and Nie 2016). These studies show the possible reasons for better performance of primed seeds to the unprimed seeds.

Farooq et al. (2005) reported that the reduction in germination and seedling vigor in urea-based osmo-priming treatment might be the result of toxicity of the solute. Rapid hydration may cause leakage of essential nutrients out of the seed, resulting in seed damage (Pill and Necker 2001). Higher seed leachates from the urea primed seeds compared with other fertilizers including control might be due to its higher molecular weight than other fertilizers used, that may have damaged the membranes to much greater extent (Farooq et al. 2005). These findings provide the possible reasons for the lowering of physiological characteristics of seedlings by urea priming treatment. The better performance by other priming agents over control and hydro-priming might be due to the controlled hydration as a result of increased osmotic potential and maintained membrane integrity.

CONCLUSIONS

Priming with 2% MOP solution resulted in the most preferable physiological characteristics of seedlings and priming with 2% urea solution resulted in the least preferable physiological characteristics of seedlings. All other priming treatments except priming with 2% urea solution performed better than control/no priming. This enhancement in the vigor of seedling at an early stage of seedling growth could help to increase final crop yield by ensuring better stand establishment. This study suggests seed priming with 2% MOP solution as an easy and effective technique for improving the germination,

emergence and seedling quality of spring rice variety Hardinath-1. Further studies may be required for suggesting the best possible method of seed priming in rice by considering other priming agents as well as assessing all the growth parameters and final yield. Farmers are suggested not to prioritize the use of urea as seed priming agent. Seed priming with 2% MOP solution for 24 hours could be beneficial for farmers willing to grow spring rice, especially variety Hardinath-1.

ACKNOWLEDGMENTS

We would like to express our deep gratitude to Pawan Giri, Senior Crop Development Officer, Regional Seed Testing Laboratory, Sundarpur, Kanchanpur; Aashif Iqbal Khan, International Rice Research Institute (IRRI), Kailali, Nepal; Prof. Dr. Shrawan Kumar Sah, Agriculture and Forestry University, Rampur, Chitwan, Nepal and Prime Minister Agriculture Modernization Project, Kanchanpur. We are thankful to the lab assistants who co-operated us during our study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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