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Effect of hydropriming and osmopriming with magnesium nitrate in the early vegetative growth phase of rice variety Swarna

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Abstract
In the present piece of work, rice seeds were primed with distilled water (T_2) and different concentrations of magnesium nitrate (2 to 50 mM) T_3 to T_{13}, whereas; seeds without any treatment referred as control (non-primed) (T_1). Various physio-morphological (shoot and root lengths, root number, fresh and dry weights) and biochemical (proline content, total chlorophyll content and superoxide dismutase activity (SOD at 20 days after sowing)) parameters were studied in the seedlings, obtained from 10, 15 and 20 DAS old primed and non-primed plants. Primed seeds were found to perform better as compared to non primed control one (T_1). However, treatment T_2 and T_4 performed best among all the treatments.

Keywords: Rice, osmopriming, magnesium nitrate

Introduction
Seed priming is a technique which controls the hydration level within seeds induce/ promote the metabolic activities for germination but radical protrusion is arrested. Various literatures have revealed that rice seed priming can enhance seed germination, vigour index and germination energy. Osmopriming (‘osmotic priming’, ‘osmotic conditioning’) is one of them, which is a pre-sowing procedure that involves treatment with osmotic solutions at low water potential facilitating the control of water uptake. During imbibition, water entry into the seed associates with progressive ROS accumulation and oxidative damage of cellular components (lipid membranes, proteins, nucleic acids). The main goal of osmopriming is to limit the ROS-mediated oxidative injury by delaying water entry. Thus, the water potential of the osmotic agent is a crucial parameter [1, 2]. The osmotic agent mainly used is PEG, inorganic salts of sodium, potassium and magnesium (most commonly NaCl, $NaNO_3$, $MnSO_4$, $MgCl_2$, K_3PO_4 and KNO_3) and organic molecules (glycerol and mannitol) etc. Now a day it is known to us that ‘Priming’ is a well-established seed treatment technology which enhances the quality of seed. Seeds subjected to priming show increased germination rates which result in high levels of biotic/abiotic stress resistance and crop yields. With all these prospects kept in mind in our present experiment rice variety Swarna was taken to explore the potentiality of the osmopriming agent magnesium nitrate [$Mg(NO_3)_2$] at different concentrations on the early vegetative growth phase of rice.

Materials and Methods
In the present investigation, before sowing the healthy and bold rice (*Oryza sativa* L. var. Swarna) seeds were surface sterilized by keeping them in 0.1% $HgCl_2$ (Mercuric chloride) solution for 2 minutes and then thoroughly washed with distilled water for 5-6 times. For priming, the sterilized seeds were soaked either in distilled water or in different concentrations of magnesium nitrate ($Mg(NO_3)_2$) (ranging from 0 to 50 ppm) for 20h (total 11 treatments T_1-T_{13}). After that the seeds were taken out and gently washed with distilled water once and then dried back to its initial weight at the room temperature by placing them forced air under room temperature (32 ± 2ºC). Dried seeds were then packed in paper bags separately for each treatment and were used as per requirement but within one month of priming. The seeds without any treatment referred as control (non-primed) and both the primed and non-primed seeds were sown in small pots (3.5 x 5 inch) in kharif season 2011 in a complete randomized design (CRD) with three replications according to recommended practices. The physio-morphological (shoot and root lengths, root number, fresh and dry weights) and biochemical (proline content, total chlorophyll content and superoxide dismutase activity (SOD at 20 days after sowing)) observations were measured at 10, 15 and 20 days after sowing (DAS).

The length of the shoot was taken by using centimetre- scale by placing it on the surface of the soil of the pot and upto the top of the plant leaf. To get the length and number of roots upto 20 days, the seedlings were first watered vigorously and then uprooted very gently to avoid any type of injury to root system of the seedlings. These were kept in a beaker of 1 L capacity filled with distilled water. The soil present on root surface was washed thoroughly for its proper cleaning by using small brush. The centimetre scale was placed at the base of shoot to the tip of the longest root to measure the root length. The number of roots of plants was also counted by placing the root part on a glass plate and by using a needle.

The dry weight of seedlings was obtained by keeping the sample for an hour in an oven pre-set at 100 – 110 ºC. Thereafter it was placed in another oven, which was set at 60 to 70 ºC till to get the constant weight (5 seedlings were taken into consideration for each treatment and per replication).

Proline and total chlorophyll content of leaves and SOD activity were measured by using the method of Bates *et al.* [3] Witham *et al*. [4] and Dhindsa [5] respectively. However, the treatment details were as follows: (i) Non primed seeds (control) (T_1), (ii) Seeds primed with distilled water (T_2), (iii) 2 mM $Mg(NO_3)_2$ (T_3), (iv) 4 mM $Mg(NO_3)_2$ (T_4), (v) 6 mM $Mg(NO_3)_2$ (T_5), (vi) 8 mM $Mg(NO_3)_2$ (T_6), (vii) 10 mM $Mg(NO_3)_2$ (T_7), (viii) 12.5 mM $Mg(NO_3)_2$ (T_8), (ix) 15 mM $Mg(NO_3)_2$ (T_9), (x) 20 mM $Mg(NO_3)_2$ (T_{10}), (xi) 30 mM $Mg(NO_3)_2$ (T_{11}), (xii) 40 mM $Mg(NO_3)_2$ (T_{12}), (xiii) 50 mM $Mg(NO_3)_2$ (T_{13}).

Results and Discussion

In the present piece of work magnesium nitrate primed seeds were taken to study various morpho-physiological and biochemical parameters of growing seedlings. While studying the shoot length of the rice seedlings at 10, 15 and 20 DAS it was observed that T_2 (16.5, 19 and 20.82 cm) showing higher value as compared to the other treatment and statistically significant also. When the root length of primed and non-primed seedlings were estimated then it was observed that the best performer was T_2 (7.58 cm) followed by T_4 (7.77 cm), T_5 (7.62 cm) at 10 DAS, T_7 (12.18 cm) followed by T_5, T_6 and T_8 (11.47, 11.47 and 12.08 cm) at 15 DAS and T_2 (14.72 cm) followed by T_4 (15.08 cm), T_5 (15.2 cm), T_7 (15.07 cm), T_8 (15.22 cm) at 20 DAS respectively. Among the primed and non-primed rice seedlings T_4 (4.7 and 7) showed highest number of roots at 10 and 15 DAS and at 20 DAS the highest result showed by T_5 (11.2) and statistically significant from other treatments. Whereas, T_2 (0.33g), T_6 and T_7 (0.43g) and T_5 (0.65g) respectively showed highest fresh weight and statistically significant in all the 3 days. While estimating the dry weight of seedlings the highest performer was T_2, T_3 to T_8 (0.09g) at 10 DAS, T_2, T_4 to T_7 (0.10 and 0.11g respectively) at 15 and 20 DAS.

Seeds are excellent dispersal units which have emerged in the course of plant evolution. The chemical energy produced during photosynthesis accumulates a seed reserves in multiple forms including carbohydrates, lipids and proteins. Mobilization of these reserves following germination is essential for the embryo to complete seedling establishment and, also signals the start of a new life cycle, an important aspect of agricultural production [6].

Priming essentially involves partial imbibition (as opposed to full hydration) of seeds by various strategies, such as shortening imbibition duration (hydropriming), or exposing seeds to relatively low external water potential (osmopriming). In osmopriming if the osmotic agent is not properly chosen in relation to permeability of the treated seeds, ions released from salts in the priming solution can easily penetrate the seed, disrupting the endogenous osmotic equilibrium. Bradford [7] reported that accumulation of uncontrolled ions within the seed system results into cytotoxic effects and nutritional imbalance. Primed seeds thus reach a 'germinating state' but without radicle protrusion. Due to this 'head-start', they typically exhibit improved germination rate and uniformity. The priming-led enhanced germination performance under both optimal and adverse environments has been reported in diverse species, such as maize, soybean, spinach, pepper, wheat and rice etc [8- 12].

The same has been observed in the present investigation where in respect to control all primed seedlings enhanced the shoot and root lengths, fresh and dry weights of the growing seedlings. The priming technology offered the synchronized germination and improved seedling vigor which has been represented by a number of workers time to time [13, 14, 12]. Root number and root length both are very important criteria for a growing crop. It gives the proper anchorage to the plant as well as supply the water and minerals also. The critical scrutinization of the result for the all above mentioned parameters showed that the application of $Mg(NO_3)_2$ salt with an optimum concentration where the maximum attainments of the data were observed. This shows that the system has the capacity to act as per the concentration of the chemicals, which are beneficial for their growth, otherwise the level more than that may be either toxic or inhibitory [15].

The treatment number T_{11}, T_{12} and T_{13} (0.36 and 0.72) at 10 and 15 DAS and at 20 DAS T_4 (0.81), T_5, T_6 and T_{13} (0.83) showed statistically at par result and presented higher proline contents (mgg^{-1}dry weight of leaf) on the 3 respective days. The treatment number T_9 to T_{13} (0.27) at 10 DAS, in the day 15th T_4 to T_6 and at 20 DAS T_6 (0.432) showed the highest chlorophyll content (mgg^{-1}fresh weight of leaf) followed by the other treatments of this set. Superoxide dismutase activity of rice seedling was studied at 20 DAS and T_{13} (16.63 unit x 10^2 g^{-1} min^{-1} fresh weight of leaf) showed the highest enzyme activity (Fig 1).

As reported by Bose and her group and also from the present experiment it can be concluded that plants raised from nitrate primed seeds showed a significant increase in proline content in comparison to control (non primed seeds) seeds. It was recorded that when rice seeds were primed with $Mg(NO_3)_2$, showed the maximum effectiveness in accelerating the proline content in each and every study period than the non-primed seeds. The result suggested that the increment in the proline may improve the stress ameliorating capacity in the growing crops being an important molecule for osmo-regulation in plants in adverse condition during growth. This has been supported by a number of scientists time to time working on different plant systems under various stresses like salinity, water deficit, drought, heat etc. [16-22]. Therefore, in the present study it has been shown very clearly that the nitrate has more ability to improve the proline content in leaves at each and every study period. However, the total chlorophyll content was more while seeds were primed with $Mg(NO_3)_2$. This is well correlated with the findings of Sharma and Bose [23], and Anaytullah *et al*. [24] they reported that when the seeds of wheat were hardened with nitrate salts, the $Mg(NO_3)_2$ was found to have higher capacity to improve total chlorophyll content in respect to KNO_3, this might be possible due to presence of Mg which is the central atom in chlorophyll molecule and also act as cofactor for a number of important enzymes of plant in which Rubisco is one of them [25].

Superoxide dismutase (SOD) is a key enzyme in cell which plays a vital role in cell against oxidative damage and severe environmental conditions. In the present case, the activity of SOD enzyme was more in primed seeds presented in the Fig 1. For this parameter the rice seeds primed with higher concentration of salt or PGR showed higher values. These findings are in line with previous reports in which seed priming also increased the activity of SOD in rice seedlings [14]. Similar type of results was also observed by Anaytullah *et al*. [24] in wheat while primed with the nitrate salts (KNO_3 and $Mg(NO_3)_2$). Abiotic as well as biotic stresses have the capacity to degenerate and modulate the metabolic activities in living system [26, 27] as a result reactive oxygen species (ROS) are formed and SOD is an enzyme which constitutes the first line of defence against reactive oxygen species (ROS). These ROS are produced in both non stressed and stressed cells but later produces more which can be mitigated by SOD enzyme. However, the increased value of SOD always reflects the defence capacity of plants toward various stresses.

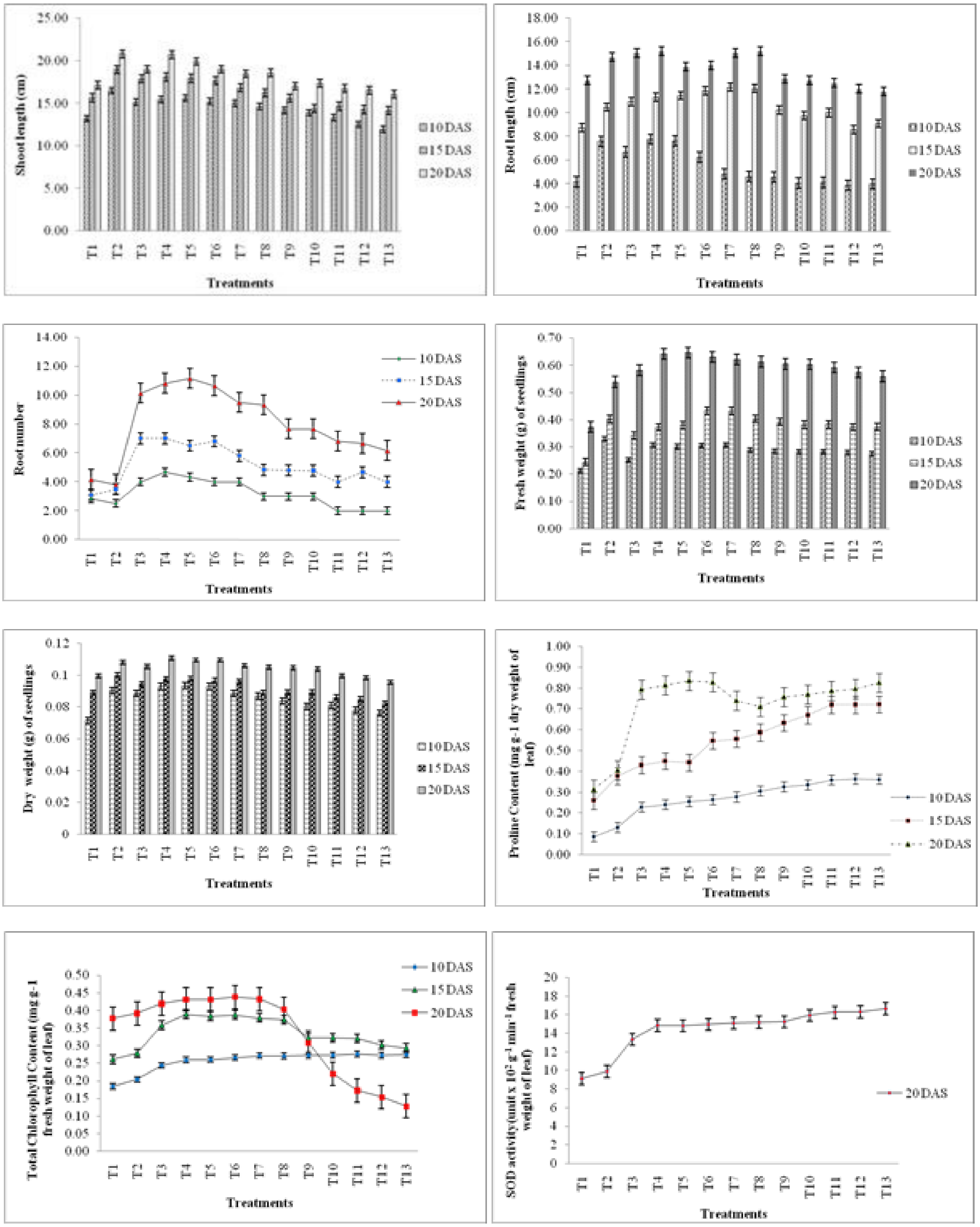

Fig 1: Effect of hydro and osmo-priming on shoot and root lengths, root number, fresh and dry weights, proline content, total chlorophyll content and super oxide dismutase activity of rice var. Swarna

Conclusion

Literature suggests that there are lots of priming agent now a day's used by various scientist in different experiment but magnesium nitrate as priming agent is used by very few scientists in rice or in other crops. It can be concluded from our present piece of experiment the amount used for seed priming for obtaining best result in rice variety Swarna is very low i.e. 4mM magnesium nitrate. From the farmers point of view it is a very cost effective and gives a positive result in terms of growth, development and productivity.

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