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PLANT PHYSIOLOGY

Impact of nitrate salt hardened seeds and sowing dates on seedling stand, growth, yield attributes, nitrogen and stress metabolism of rice

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Abstract

Impact of seed hardening (hydration followed by dehydration-a kind of seed priming) with water and nitrate salts were evaluated in the present piece of work in timely and late sown rice variety HUBR 2-1. Crops raised from distilled water(hydro)/$Mg(NO_3)_2$ and KNO_3(osmo)hardened seeds showed an increment in fresh and dry weights, RWC(%),plant height, no of leaves, LA,LAI,CGR,RGR,NAR, effective tillers, nitrogen, proline and chlorophyll contents, nitrate reductase and super oxide dismutase activities in leaves over control set(representing non-hardened seeds). The hardening treatments resulted a reduction in membrane leakage, shortened the days from sowing to 50% flowering , increased days to plant maturity after sowing and flowering and improved the yield attributes. Best performance was resulted from Mg $(NO_3)_2$ hardened set and that was followed by KNO_3 and distilled water hardened sets but control was always found inferior in timely and late sown crops.

Highlights

- Nitrate (nitro-hardened)salts or with distilled water (hydro hardened) enhanced various parameters of vegetative growth Nitrogen content and NR activity of nitrate salt hardened plants are highest in respect to other sets in both sowing dates.

Keywords: Seed hardening, nitrate salts, different sowing time, growth, nitrogen and stress metabolism

Rice, the dominant staple crop in India, requires large amount of water for its proper growth. In India monsoon onsets generally in first fortnight of June but sometimes it delays 2-4 weeks, resulted in late sowing which finally limits the yield in transplanted varieties of rice. Seed, the most importent system of plant faces the adverse conditions of late sowing during the process of germination. Further, the seed also contains some specific internal conditions which make it to stay and tackle the adverse situations of its surroundings. Even then also seed losses its homeostasis with ambient condition and called under stress. Late sowing of crop seeds also suffer from abiotic /biotic stresses. However, on the other hand seed invigoration/ priming/ hardening techniques are being successfully used to favor the synchronized germination, with uniform seedling stand followed by better plant Phenology (changes in growth of plant parts over time) in many horticultural and field crops (Bose and Mishra 1999, Bose and Mishra 2001, Ashraf and Foolad 2005, Farooq *et al*. 2005). These techniques are able to ameliorate the adverse effects of various abiotic like high and low soil moisture availability (Du and Tuong 2002), low temperature (Anaytullah and Bose 2007) and biotic(Nakaune *et al*. 2012, Mondal and

Bose 2014) stresses. In hardening process, a single cycle of wetting and drying of seeds in water is adopted previously but in recent years, different salts were added in water during hydration of seeds ,found more efficient in improving the plant processes (Sharma and Bose 2006, Farooq *et al*. 2006, Anaytullah and Bose, 2007, Mondal *et al*. 2011) ,named as osmohardening (Farooq *et al*. 2006). The chemicals frequently used for osmohardening are namely NaCl, $CaCl_2$, KH_2PO_4, K_3PO_4, KCl, KNO_3, $MgSO_4$, $Mg(NO_3)_2$ etc. These chemical solutions of low water potential enhance the performance of various crops via inducing different metabolic events or by enhancing the synthesis/activity of α-amylase during germination (Lee and Kim 2000, Anaytullah and Bose 2007, Mondal and Bose 2012), enhance protease activity and increase solubilization of nitrogen in endosperm and its mobilization towards embryo (Bose *et al*. 1982), improve seedling vigor, seedling establishment i.e. plant density, fertile tillers, vegetative growth and yield attributes like test weight, no. of grains per panicle (in rice), ear head characteristics and grains per plant (in wheat) as compared to the crop raised from non-primed seeds (Du and Tuong 2002, Sharma and Bose 2006, Bose *et al.* 2008a, Sharma *et al*. 2009). Application of $Mg(NO_3)_2$ and KNO_3in form of osmohardening has resulted an improved performance of late sown wheat (Anaytullah and Bose 2012). In present piece of work distilled water and nitrate salts ($Mg(NO_3)_2$ and KNO_3) were used for hydro and osmo hardening respectively in rice seeds before sowing in the field at timely and late of its cropping season to find out the performance of crop in respect to allometry, yield potential, nitrogen and stress metabolisms and they were compared with control (used non hardened seeds) plants.

Materials and Methods

The present experiment was conducted in the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India during the year 2006-2007, 2007-2008 and 2009-10 in field condition. The seeds of a popular cultivar of Varanasi region HUBR 2-1 were obtained from the seed production section of Department of Genetic and Plant Breeding of same Institute. The experiment was conducted in randomized block design with three replications. Each plot was made of 6 m^2 and every hill contained 3 rice plants with 20 cm spacing between rows and 10 cm spacing between plants. Young seedlings were transplanted at 25 days after sowing (DAS). Sowing of seeds were made on 18th June and 8th July and referred as timely (D_1) and late sowing (D_2) crops respectively. For hydro hardening and nitrate seed hardening healthy rice seeds were taken and the surface sterilization was done by keeping them in 0.1% of $HgCl_2$ solution for 2-3 minutes followed by 3-4 times washing with distilled water. Thereafter the seeds were kept in beakers (1000 ml) by using distilled water (T_2) and solutions of 3.75 mM of $Mg(NO_3)_2$ (T_3) and 7.5mM of KNO_3 (T_4) respectively for 18 h only at room temperature i.e. 30±2 ^{0}C. After that the seeds were dried back to their initial weight under forced air in room temp. The air-dried seeds were referred as hydro-hardened (T_2) and nitro-hardened (T_3&T_4) one, these were used for field experiments with in one month. Non-hardened directly field sown seeds were served as control (T_1).

Growth and yield parameters: All the growth parameters were measured at 25, 50, 75 and 100 DAS (days after sowing) of plant age. Plant height, no. of green leaves, fresh weight (FW) and dry weight (DW) were determined by conventional method. Leaf area (LA) was measured by using leaf area meter (model-211, Systronics, New Delhi) and leaf area index (LAI), relative growth rate (RGR), crop growth rate (CGR), and net assimilation rate (NAR) were calculated with the help of following formula as described by Watson (1952), Hughes (1962) and Gomez (1962)

$$\text{RGR} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \quad \text{g g}^{-1}\text{day}^{-1}$$

$$\text{CGR} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{A} \quad \text{mg cm}^{-2}\text{ day}^{-1}$$

$$\text{NAR} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{\log LA_2 - \log LA_1}{LA_2 - LA_1} \quad \text{mg cm}^{-2}\text{ day}^{-1}$$

$$\text{LAI} = \frac{\text{Leaf area}}{\text{Ground area}}$$

Relative water content (RWC) of the leaf samples were determined by using the method and the formula introduced by Weatherly(1962)

$$RWC\,\% = \frac{FW - DW}{TW - DW} \times 100$$

At maturity, plants per hill of each plot were harvested for determining yield and yield components. Maturity was determined when the second spikelet on the secondary rachis branch located on the lower primary rachis branch change to yellow. Then various yield attributes i.e. number of effective tillers, panicle length, number of fertile spikelets panicle^{-1}, percentage of fertile spikelets panicle^{-1}, test weight and yield hill^{-1} were recorded on the basis of five randomly selected plants. Days to 50% flowering, days to maturity, dry matter accumulation and harvest index were also recorded by using conventional method. Yield ha^{-1} was recorded on the basis of grain obtained per plot.

Super oxide dismutase activity, membrane stability index (membrane injury), proline content, nitrate reductase activity and nitrogen content: Super oxide dismutase activity (EC 1.15.1.1), proline content, nitrate reductase activity (EC 1.6.6.1), chlorophyll and nitrogen contents in different plant parts were measured as per the procedure described by Yu and Rengel (1999), Shanahan *et al*. (1990), Bates *et al*. (1973), Hageman and Flesher (1960) and Lang (1958) respectively. Chlorophyll content was determined by using chlorophyll meter (SPAD-602, Jay Instruments, Mumbai, Maharashtra).

Membrane injury (MI), determined by measuring the amount of electrolyte leakage from plant tissue in response to heat treatment (45^0 C) relative to the total amount of electrolyte released from the same plant tissue upon autoclaving, (Sullivan, 1972)

Statistical analysis was introduced as per the requirement of experiment by adopting the method described by Chandel, (1984).

Results

Growth parameters and yield attributes: Values of all derived growth parameters were found to increase with increasing age of plants irrespective of various treatments in both dates of sowing i.e at timely(D1) and late(D2) ones. Nitro hardened seeds (T_3 and T_4) showed higher values in respect to hydro hardened (T_2) and control (T_1). However Figure-1(a) and 1(b) showed a slow rate of increment in fresh and dry weights up to 50 DAS thereafter the rate was increased up to 100 DAS. Among the treatments T_4 exhibited both, the maximum percent and amount of fresh and dry matter accumulation. However, RWC (fig.-1 (c)) showed continuous decline with increasing rate till last date of observation (85.47 to 72.04 in D_1 and 85.66 to 72.98 in D_2). Maximum RWC was found in T_4 treatment in both sowing dates. Plant grew in a continuous manner till harvest but rate of increment in plant height was varying among treatments (fig.-1 (d)). Highest enhancement was occurred in T_4 nitro hardened seeds followed by T_3, T_2 and T_1 sets. Marked effects of treatment on number of leaves (fig.-1 (e)), leaf area (LA) (fig.-1 (f)) and leaf area index (LAI) (fig.-1 (g)) were recorded in each date of observation and both dates of sowing. Leaf area increased at a faster rate till 50 DAS and then the rate of increment became slow at 75DAS then started to decline at a slower rate, when measured at 100 DAS. The same trend was also observed in case of LAI. In all the three parameters, the peak was noted at 75 DAS and then declined whereas the rate of increment was the maximum between 25 to 50 DAS. Among the treatments maximum values were recorded in T_4 hardened ones followed by T_3, T_2 and T_1 sets. Likewise, in both the dates of sowing, nitro hardened sets represented maximum improvement in CGR_(fig.-1 (h) but derived less difference in case of RGR (fig.-1(i) and no significant difference in NAR (fig.-1(j) in all or most of the study periods. Data regarding number of tillers hill^{-1} (fig.-2), depicted significant effect in seed hardening treatments in both the dates of sowing. Tiller no. was increased up to 75 DAS then declined. Least number of fertile tillers was recorded in control plants and it was maximum in nitrate hardened sets (fig.-2). Data regarding days from sowing to 50% flowering and days from sowing to maturity were not found to show a notable difference in 1st date of sowing but represented a remarkable difference in 2nd date of sowing where nitrate salts had taken lesser time to flower while more to harvest, resulted an increment in the time for grain filling and had a positive correlation to yield and ultimately to harvest index (H.I.) (table 1). Biological weight, straw yield and harvest index in D_2 were significantly improved but represented less difference in case of H.I. in both the dates of sowing with nitro-hardened and hydro-hardened sets in respect to non-primed control seeds (table 1).

Panicles and spikelets characteristics: Panicle characteristics such as panicle weight, total no. of

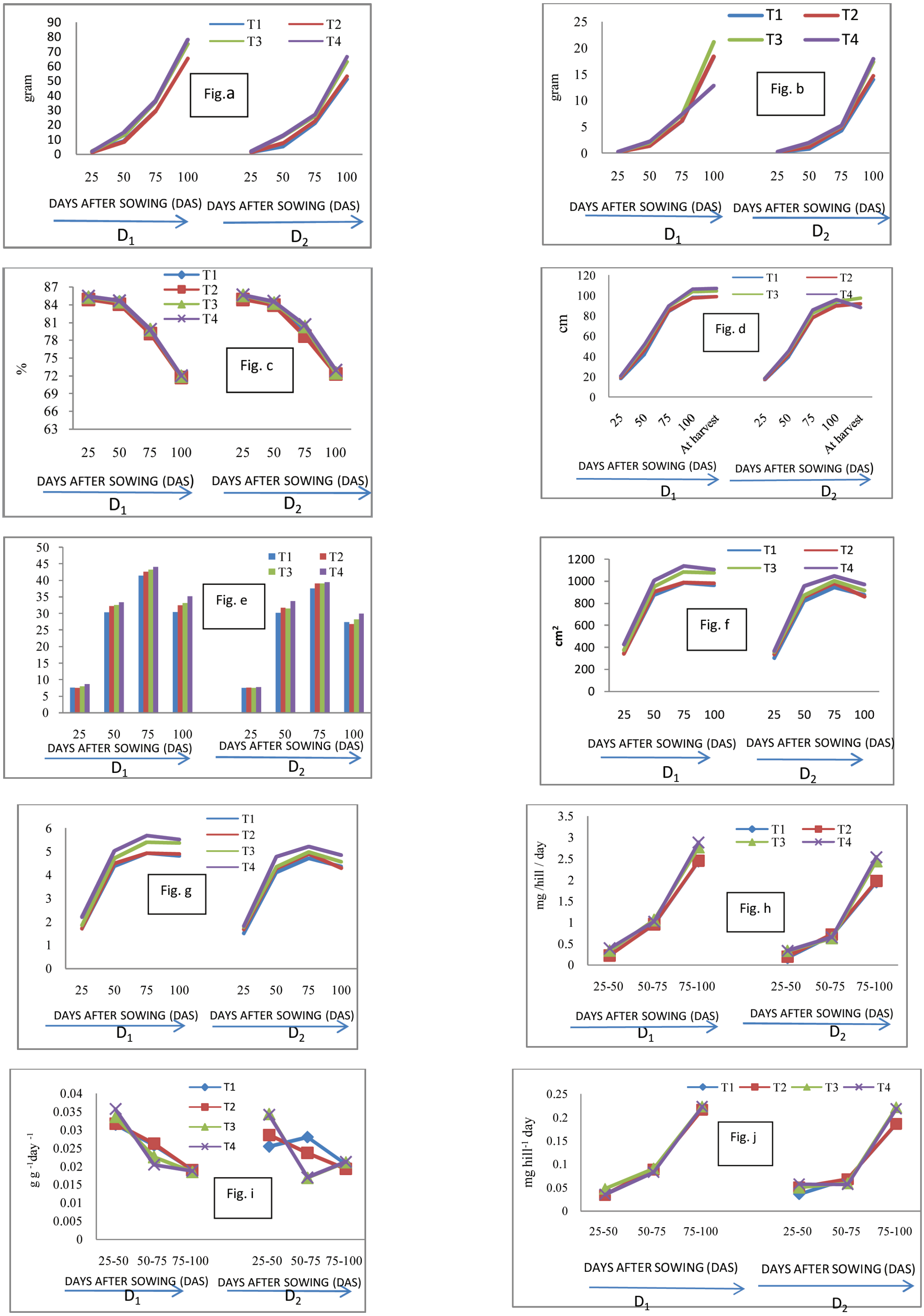

Fig. 1: Effect of seed priming with nitrate salts and date of sowing on fresh weight (a), dry weight (b), RWC (c), plant height (d), no. of leaves (e), leaf area (f), LAI (g), CGR (h), RGR (i), and NAR (j) of rice variety HUBR 2-1; (D_1: 1st date of sowing and D_2: 2nd date of sowing; T_1: Control; T_2: Priming with DW; T_3: Priming with KNO_3; T_4: Priming with $Mg(NO_3)_2$).

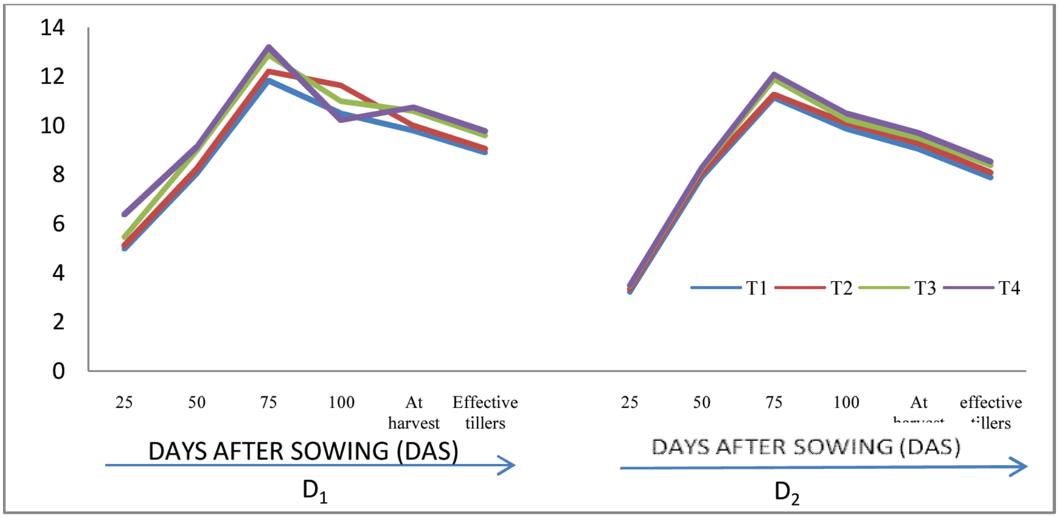

Fig. 2: Effect of seed priming with nitrate salts and date of sowing on number of tillers in rice variety HUBR 2-1 (D_1: 1st date of sowing and D_2: 2nd date of sowing; T_1: Control; T_2: Priming with DW; T_3: Priming with KNO_3; T_4: Priming with $g(NO_3)_2$).

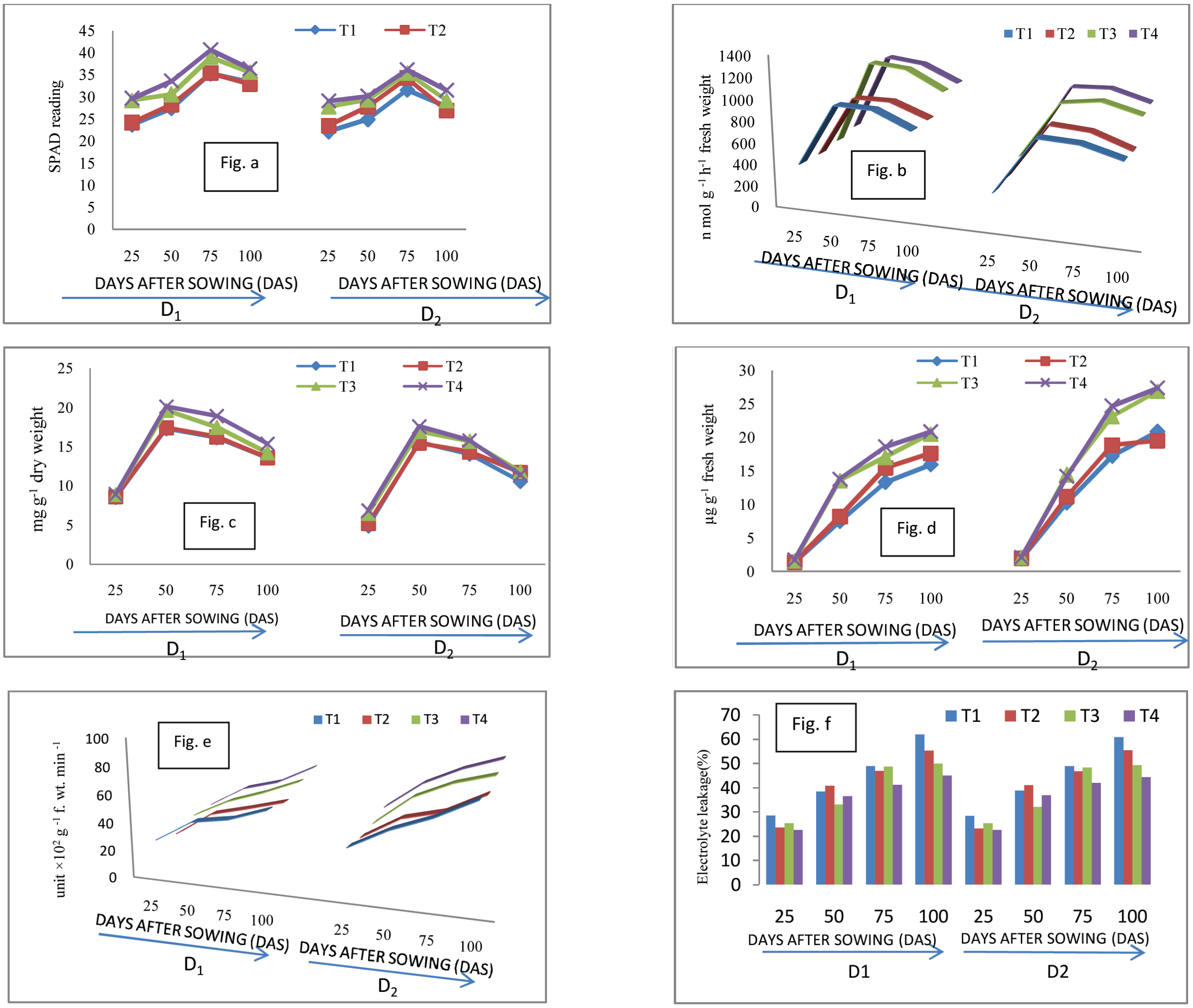

Fig. 3: Effect of seed priming with nitrate salts and date of sowing chlorophyll content (a), nitrate reductase activity (b), nitrogen content (c), proline content (d), super oxide dismutase activity (e) and electrolyte leakage (f) in leaves of rice variety HUBR 2-1(D_1: 1st date of sowing and D_2: 2nd date of sowing; T_1: Control; T_2: Priming with DW; T_3: Priming with KNO_3; T_4: Priming with $Mg(NO_3)_2$).

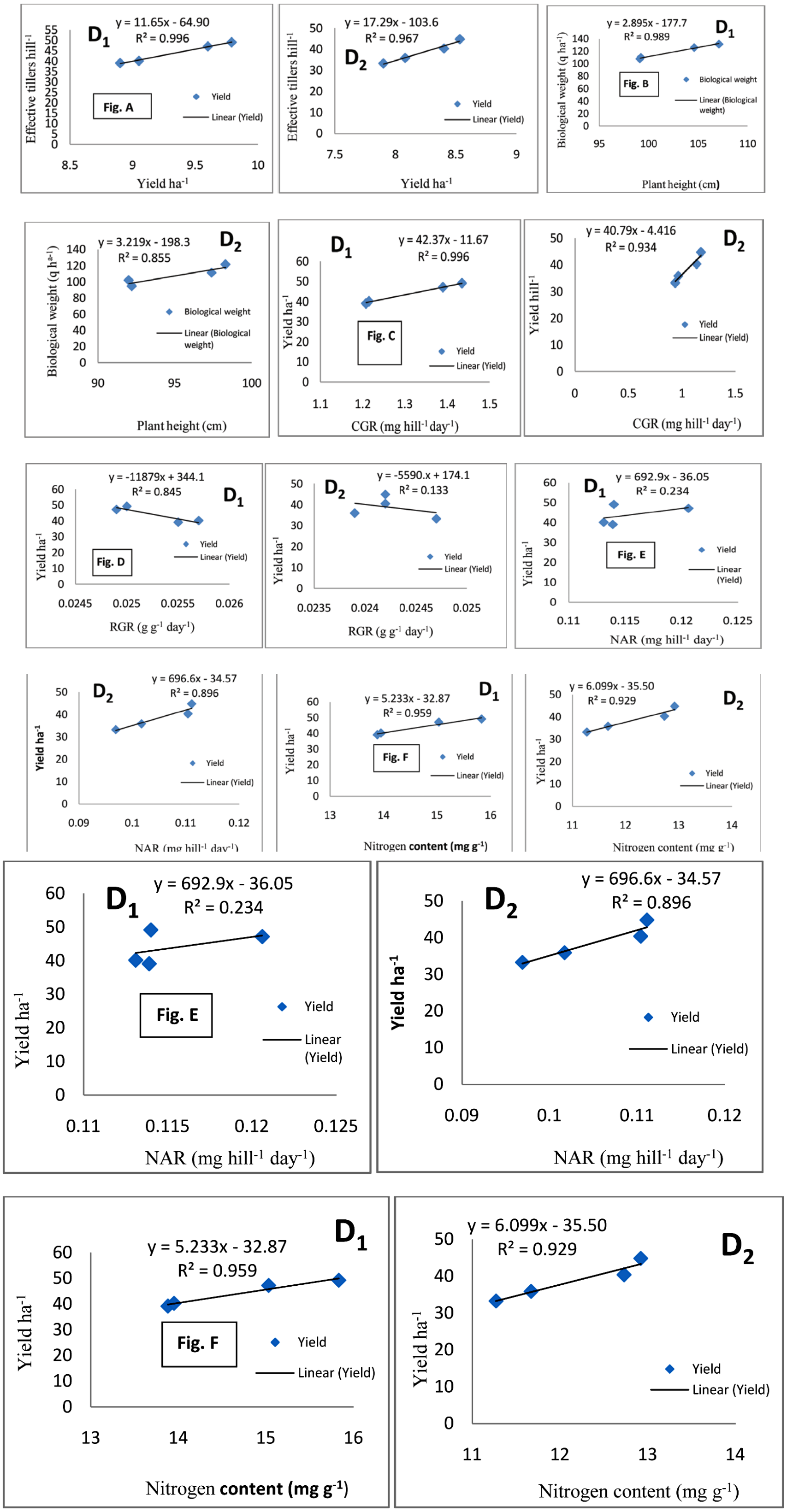

Fig. 4: Effect of seed priming with nitrate salts and date of sowing on relationship between yield and effective tillers (A), plant height and biological weight (B), CGR and yield ha^{-1} (C), RGR and yield ha^{-1} (D), NAR and yield ha^{-1} (E) and nitrogen content yield ha^{-1} (F) of rice variety HUBR 2-1 (D_1: 1st date of sowing and D_2: 2nd date of sowing; T_1: Control; T_2: Priming with DW; T_3: Priming with KNO_3; T_4: Priming with $Mg(NO_3)_2$).

Table 1: Effect of seed priming with nitrate salts and date of sowing on various phenological and yield parameters of rice variety HUBR 2-1. (D_1: 1st date of sowing and D_2: 2nd date of sowing; T_1: Control; T_2: Priming with DW; T_3: Priming with KNO_3; T_4: Priming with $Mg(NO_3)_2$).

Treatments	Days from sowing to 50% flowering		Days from sowing to maturity		Days from flowering to maturity		Biological weight (q ha^{-1})		Straw yield (q ha^{-1})		Harvest index (%)	
	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2
T_1	87.00	84.00	127.00	124	39.67	40.00	107.92	94.71	68.79	61.43	36.26	35.14
T_2	86.67	82.00	127.00	125	40.33	43.00	110.41	101.96	70.21	66.07	36.41	35.20
T_3	86.33	81.00	129.33	126	43.00	45.00	126.19	111.27	79.02	70.89	37.38	36.29
T_4	85.67	80.00	130.00	126.33	44.33	46.33	131.59	121.54	82.44	76.73	37.35	36.87
C.D. at 5%	3.420008		3.578226		2.685639		12.3804693		5.82457695		1.128544942	

grain panicle^{-1}, weight and number of filled and half filled spikelet panicle^{-1}, and test weight were improved by the different hardening treatments in rice but data was found insignificant; however the improvement was found more with nitrate hardened seeds (table 2). Seed hardening strategies remarkably reduced the number and weight of sterile spikelets panicle^{-1}, in contrast a significant enhancement was recorded in panicle weight, yield hill^{-1} and yield ha^{-1} although number of branches panicle^{-1} was insignificant (table 2). T_4 was more effective in enhancing the number of filled spikelet panicle^{-1} among treatments followed by T_3 and T_2 whereas T_1 performed poor. Number of sterile spikelet panicle^{-1} reduced much in T_4 and the pattern was $t_1 > t_2 > t_3 > t_4$ in this regard (table 2). Among yield enhancing parameters, osmohardening with T_4 ($Mg(NO_3)_2$) was found best followed by T_3, T_2 and T_1. It was observed that nitrate hardened sets (T_3 and T_4) presented higher yield hill^{-1} and yield ha^{-1}.

Chlorophyll and nitrogen contents, nitrate reductase and super oxide dismutase activities, proline content and Membrane Injury: Figure 3 (a) showed an uninterrupted increment in chlorophyll content till 75 DAS then reduced at 100 DAS in both dates of sowing. Superiority was observed in T_4 among treatments in all dates of observation and followed by T_3, T_2 and T_1. Peaks in NR activity (fig.-3 b) among treatments were measured in leaves at different observation dates. However, the maximum activity was presented by T_4 and minimum activity was recorded in T_1 although it was slightly more in some stages to T_2 in 1st date of sowing but cumulative effect was inferior (figure 3b). Change in nitrogen content (in leaves) with the progress of developmental stage in rice genotype showed in figure 3 (c), where an increment was noticed till 50 DAS after that it diminished up to last date of observation (100DAS); further it has been realized that D_1 had higher nitrogen content at each and every date than D_2 and peak was observed at 50 DAS in all cases where T_4 performed best. Proline accumulation in leaves (figure 3d) was significantly higher in hardening treatments during all studied dates. A sudden increment was found in all cases (treated and untreated one) at 50 DAS however attained the greatest amount at late reproductive period (100 DAS) (figure 3d). Another important parameter of stress metabolism, representing the activity of the enzyme Super oxide dismutase (SOD) has shown a linear and significant increment in each treatment including T_1 where T_4 expressed the highest activity during progressive growth period in both sowing dates followed by T_3 (figure 3e). It has been noticed that T_2 represented inferior activity of SOD. A significant treatment variation was noted in membrane injury at each observation date as well as in both sowing dates (figure 3f). In each observation date the highest leakage of electrolytes was noted in T_1 plant's leaf with progressive age. Further, it showed a close relation between plant T_1 and T_2 as well as T_3 and T_4. Sudden increment in leakage was observed at 50 DAS then advanced in decreasing rate till 100 DAS. Thus, peak was noted at last date of sowing where T_4 showed lower leakage than other treatments.

Discussion

The present study has shown that different hardening (a type of priming) treatments either with

Table 2: Effect of seed priming with nitrate salts and date of sowing on panicle characteristics and yield attributes of rice variety HUBR 2-1. (D_1: 1st date of sowing and D_2: 2nd date of sowing; T_1: Control; T_2: Priming with DW; T_3: Priming with KNO_3; T_4: Priming with $Mg(NO_3)_2$).

Treat-ments	Panicle weight(g)		Total no. of grain panicle^{-1}		No. of filled spikelet panicle^{-1}		Weight of filled spikelet panicle^{-1} (g)		No. of half filled spikelet panicle^{-1}		Weight of half filled spikelet-panicle^{-1}(g)		No.of sterile spikelet panicle^{-1}		Weight of sterile spikelet-panicle^{-1} (g)		Test weight(g)		Yield hill^{-1}		Yield(q) ha^{-1}		Number of branches panicle^{-1}	
	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2	D_1	D_2
T_1	1.397	.364	92.03	93.07	62.31	59.81	1.281	1.214	2.61	3.20	.026	.032	27.11	30.06	.082	.083	20.56	20.30	39.13	33.28	49.13	41.28	11	10.8
T_2	1.415	1.408	93.37	93.49	65.67	62.74	1.301	1.263	2.67	3.25	.029	.033	25.03	27.50	0.076	0.078	19.81	20.13	40.2	35.89	50.20	43.89	10.8	10.9
T_3	1.493	1.474	93.65	91.78	66.52	65.63	1.373	1.335	2.56	2.50	.028	0.024	24.56	23.75	0.066	0.069	20.64	20.37	47.17	40.38	57.17	48.38	11.4	11.2
T_4	1.529	.558	92.46	95.95	67.26	70.35	1.397	1.433	2.53	2.50	.022	.025	22.67	23.10	.063	.068	20.77	20.37	49.15	44.81	59.15	52.81	12.0	11.6
C.D. at 5%	0.42244096		4.42343421		6.45485545		0.36920302		0.64729683		0.00853923		2.74254954		0.01752291		1.18372374		2.18257832		4.09521981		1.76359028	

nitrate (nitro-hardened) salts or with distilled water (hydro hardened) enhanced various parameters of vegetative growth, number of tillers hill^{-1}, number of fertile tillers, biological weight, straw yield and harvest index with a significant difference in 1st date of sowing (timely sown) and also represented a remarkable difference in 2nd date of sowing(late sown) where nitrate salt treated sets had taken lesser time to flower and more to harvest, resulted an increment in the time for grain filling and showed a positive correlation to yield and ultimately to harvest index (H.I.) (Fig.1 &2 and table 1). Mondal *et al.* (2011) elucidated that seed hardening improves seedling growth may be due to a modulation in seed vigor which helps in improving the successive crop growth in rice variety MTU-7029. Further, seed hardening strategies also improved assimilated area (LA) which resulted in enhancing appropriate partitioning of photosynthate towards tillers of plant. Du and Toung (2002) and Srivastava and Bose (2012) reported that improved number of fertile tillers, test weight, kernel yield and harvest index owing to seed priming with KCl and nitrate salts of K and Mg in rice. Enhanced performance of direct seeded rice by sand priming is also reported by Hu *et al.* (2005). Thakuria and Choudhary (1995) reported that improved nutrient and moisture supply from primed seeds might have resulted in enhanced fertilization, which ended in the lower number of sterile spikelets in direct seeded rice primed with salts of potassium. Further, it is well established that sowing time of any crop specifically rice suffers from reduced vegetative growth followed by low yield (Nadeem *et al.* (2010). However, in present study improved LAI, CGR, RGR and NAR from hardened rice seed, even in late sown condition (D2), might be resulted from proper utilization /channelization of photosynthates towards developing reproductive parts. Improved CGR (figure 1 (h)) was possibly due to strong and energetic start (Mondal *et al.* (2011), which resulted in improved number of leaves, LA and LAI that ended in improved RGR and NAR. Basra *et al.* (2004) and Farooq *et al.* (2006) also reached on the conclusion that osmohardening is more effective in improving yield than osmopriming or hydrohardening alone, which supports the present study. Marked effect of hardening treatment in both sowing dates on rice were expressed in fresh and dry weights and RWC of plants revealed that T_3 and T_4 improved water holding capacity over hydro-primed and non-primed plants that might help during stress condition in physical and physiological drought for maintaining proper water status into them to carry on metabolic activities (Fig. 1c). This is also to be noted that plant, raised from hardened seeds had higher water-use-efficiency expressed in increment in dry weight per unit of water absorbed. Number of branches per panicle was also affected but this could not affect much the number of kernels per panicle by seed hardening (table 2). Increased chlorophyll content (SPAD reading) (figure 3a), occurred due to the absorption of more soil nitrogen in leaves by plants raised from hardened seeds ,representing more nitrate reductase activity and nitrogen content (figure 3b and c), which in turn helped in synthesizing more chlorophyll leading to higher leaf growth (photosynthetic area). However, chlorophyll and nitrogen contents increased up to heading stage (75 DAS) then reduced at 100 DAS might be due to nitrogen mobilization towards reproductive part. Seed hardening with nitrate salts had consistently higher SPAD reading for all stages measured, possibly because of significantly more nitrate reductase activity (NRA) and nitrogen content (figure 3b and 3c) in leaves.

One of the most important findings in this study was that in nitrate salt hardened plants the nitrogen content and NR activity (figure 3 (b&c) both are highest in respect to other sets in both sowing dates. It might be possible that higher NR activity converts more amount of NO_3^- to NO_2^- which further converts to ammonia in plant's leaves and roots which led to change into more protein. Activity of NR increased till 75 DAS (heading stage) then decline owing to the partitioning of nitrogen towards sink side. This was probably due to less substrate (NO_3^-) available for reduction. Similar trend and positive correlation among chlorophyll content (figure 3 a), NR activity (figure 3 b) and nitrogen content (figure 3c) were found in this work. Proline, a compatible solute, takes part in osmoregulation and increases its content into cell, makes the plant more stress tolerant towards many adverse conditions of atmosphere (Sarkar *et al.* 1999, Yan-Chung and Lee 1999, Claussen 2005). It is also a nitrogenous compound (amino acid), the accumulation of which advances with enhancing nitrogen proportion in plants. In present piece of study T_4 that had higher nitrogen content and also greater proline; the later increased even after 75 DAS when nitrogen content started to decline (figure 3d). Membrane injury (figure 3f) was less in nitrate hardened sets reflected that this treatment has the capacity to protect the plant from adverse condition (late sowing stress to crop) via avoiding the membrane disruption, as a result enzyme's activity and their molecular integrity may sustain even in stressed situation. The result supports the finding of Farhoudi (2014), who established that KNO_3 primed *Helianthus annus* seeds showed lower cell membrane damage in salt stressed condition.

Nitrogen, an essential nutrient in higher plants and a constituent of most organic molecules, also plays an important role in osmotic adjustment to balance the osmotic pressure in cytoplasm during stress in plants when used in NO_3^- form (Mott and Steward 1972). The finding of Munns (2002) suggested that ATP requirement for the synthesis or accumulation of proline is 41 when NO_3^- is the source of N. During seed osmohardening, when seeds are subjected to nitrate salts they enhance accumulation of nitrate/ anions in vacuole to absorb more water and hold it till life span. In the present case, increasing nitrogen content in rice leaves also helped in improving grain yield (figure F). The same has been suggested by Bose and Mishra (1999) and Bose and Pandey (2003) during their studies with mustard and okra respectively in context to pre-sowing-soaking of seeds with nitrate salts. They observed more Mg and NO_3^- content in cotyledonus of mustard and okra respectively resulted from nitrate soaking seeds. SOD is the primary scavenger in the detoxification of active oxygen species in plants, generate during various types of stress conditions. In general, its activity elevates with plant age and stress severity. It was negatively correlated with M.I., responsible for damaging lipid bi-layer resulted a reduced de-novo synthesis of protein, an important part of all enzymes. In present experiment, osmohardening as well as hydrohardening has the capacity to enhance SOD activity and reduce MI in leaves significantly (fig-3c and 3f), consequently, ameliorates the stress tolerating characters, hence plant grows better.

However, a positive correlation has also been observed in no. of effective tillers in respect yield and plant height vis-à-vis biological yield (Figure 4A and B) in this respect. This might be due to the presence of Mg and $(NO_3)_2$ ions ,played their beneficial role from the first phase i.e. germination followed by seedling stand ,consequently changed the phenological events (table 1) of rice crop. Growth analysis showed that CGR during the late reproductive period was crucially associated with final grain yield (Figure 4-C). Improved grain yield influenced by seed hardening positively correlated with RGR, NAR and nitrogen content in leaf (figure 4D, E and F). Improved grain yield from hardening seeds might be a result of improved yield contributing factors i.e. number of panicle bearing tillers and test weight (table 2). Improved harvest index by seed priming was related with enhanced dry matter partitioning towards the panicle that resulted in improved grain yield. Among the treatments, osmohardening with $Mg(NO_3)_2$ (T_4) greatly improved plant height, reduced the days from sowing to 50% anthesis, in contrast, took more days for 50% anthesis to maturity. This might be happened from enhanced rate of nutrient mobilization and more assimilate deposition towards the panicles resulted in lower opaque (half filled) and chalky (sterile) kernels, reduce spikelet sterility and increased filled kernels, higher test weight in rice (table 2) because of uniform distribution of photo assimilates within them; more number of filled panicle might be the result of improved NAR that resulted in photo assimilation and its translocation and partitioning towards the sink. Further it has been noted that seed priming with DW (T_2) could not improve test weight over control (table 2) may be due to non involvement of any other ion as happened in case of salt priming.

Conclusion

In conclusion it can be suggested that nitrate seed hardening (a kind of seed priming) is a pragmatic strategy in producing higher yield in rice. This technique speedup plant growth, yield attributes, kernel quality, photo assimilate synthesis and partitioning resulted reduction in spikelet sterility. It also proved fruitful in increasing enzyme activity (NRA and SOD), nitrogen and proline contents and reduces membrane injury to create stress tolerance capacity in late sown rice. The finding showed a greater efficiency of osmohardening with nitrate salts ($Mg(NO_3)_2$ and KNO_3) in improving yield and amelioration of stress tolerance in rice. Osmohardening with $Mg(NO_3)_2$ perform better than all other treatments, followed by KNO_3 and hardening with DW over control. These results may have implication for growing rice in stressed area. Further, it would be added that Bose research group has used the nitrate seed hardening technology with Mg $(NO_3)_2$ for the first time in their works with different field crops like wheat (Sharma and Bose 2006, Anaytullah and Bose 2007), mustard (Bose *et al.* Bose *et al.* 2008; Bose and Kuril, 2008c) and rice (Bose *et al.* 2008a, Bose and Srivastava, 2008b), hence now the group claimed to name seed hardening with Mg $(NO_3)_2$ as BHU- 1-Seed Hardening Technology.

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