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Seed soaking treatment with nitrate salts *vis a vis* physiology of germination and seedling vigor in rice (*Oryza sativa* L. var. Ratna)

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ABSTRACT

In a short term experiment seeds of rice were subjected to pre-sowing soaking treatment with various salts containing 15 mM nitrate for 72 h. An improvement in seed germination, seedling vigor (length of plumule and radicle) and á-amylase activity were found in nitrate treated sets in comparison to distilled water treated sets. In an other experiment (long term) seeds were sown in pots after providing 30 h soaking treatment with above mentioned salts and distilled water and observations were made for seedling emergence at 5, 7 and 9 days after sowing (DAS) and seedling vigor at 13 and 26 DAS and these were compared with non-soaked seeds (control) which directly sown to pots. The results showed an increase in seedling emergence and vigor in nitrate salt treated sets by facilitating a number of parameters like plant height, number and length of root, fresh and dry weight of shoots and roots, number of leaves and tillers as compared to plants raised from DW soaked and control seeds. Electrical conductivity and nitrate reductase activity in leaves also increased with the use of nitrate salts as seed treatment. However, KNO_3 was found more effective during germination rather than Mg $(NO_3)_2$; but reverse was noted in studies related to seedling vigor. This type of nitrate salt treatment to rice seeds prior to sowing improves seedling vigor including root number and its mass than distilled water treated one, therefore, in system of rice intensification (SRI) cultivation which has now been practiced in a number of states of India, this type of nitrate salt treatment to the seeds can be added before seed sowing.

Key words : Rice seeds, Nitrate salt, Soaking treatment, Germination and seedling vigor, SRI cultivation

INTRODUCTION

Production of any crop has significant impact with the formation of robust and healthy seedlings which comprises the foundation stone for high yield. In case of rice, yield has direct relation with sowing of seeds in well prepared nursery beds and there after the transplanting of seedlings/plant lets to properly cultivated fields. Therefore, the aim lies in the formation of healthy seedlings which have good initial vigor, certainty to utilize the input effectively and efficiently and will able to transform the nutrients towards the yield performance of crop with efficiency.

There are various reports on pre-sowing soaking seed treatment with various salts by which seed germination and seedling vigor is found to improve. Kiss (1979) reported that 1% $MgSO_4$ for 2-24 h enhances the germination percentage in sugar beet and maize. Bose *et al.* (1982) observed that $Ca(NO_3)_2$ in respect to NH_4NO_3 and $(NH_4)_2SO_4$ enhanced rate of germination and protease activity of maize seeds which also resulted more solubilization of nitrogen in its mobilization towards embryo. Hilhorst (1990) noticed that NO_3^- while used as soaking treatments acts as dormancy breaking agents via stimulating the pentose phosphate path way while producing NADP, which is one of the essential criteria for the relieve of dormancy in seeds of many plant species. Soaking of rice seeds with urea (450 ppm) found to increase seedling height, dry weight of shoot and culms diameter except number of green leaves as reported by Guoxing and Wang (1998).

In present investigation an effort has been made to get an improvement in per cent germination including the physiology of seeds, primed with nitrate salts and distilled water before sowing and to get a good seedling vigor which can be used in system of rice intensification (SRI) cultivation of rice (var. RATNA).

MATERIALS AND METHODS

Treatments description:

Surface sterilized (0.1 % $HgCl_2$) rice seeds were given pre-sowing soaking treatment with distilled water (DW) and nitrate (15 mM conc.) salts [$Mg(NO_3)_2$ and KNO_3] either for 72 or for 30 h (this includes 6 h imbibitions in DW and nitrate salts) for short and long term experiments, respectively whereas one lot of seeds kept untreated which was used as control.

Short term experiments:

In these experiments the seeds were kept in Petri dishes (diameter 7.5 cm) in normal light condition for upto 72 h at a temperature of 30 ± 2^0C and following observations were made;

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Germination % (GP):

On the basis of number of seeds germinated in each Petri dish GP was calculated using following formula at 40, 48, 64 and 72 h.

$$GP = \frac{X}{N} x\, 100$$

where;

X = Number of seeds germinated

N = Number of seeds placed in Petri dishes

Plumule / radicle length:

It was measured with the help of graph paper and calculated per seed basis by using following formula at 48 and 72 h.

$$\text{Plumule / Redicile length (cm) per seed} = \frac{l_1 + l_2 + l_3 + ... + l_n}{N}$$

where;

$(l_1+l_2+l_3.....+l_n)$ represent plumule / radicle length of each seedling

N = Number of seeds used for calculating length

(III) α-amylase activity: This was determined by using method of Bernfeld, (1955) in endosperm at 24 and 48 h.

Long term experiment:

For long term experiment the DW and nitrate treated seed after 30 h soaking, respectively sown to pots (20 seeds pot^{-1}) having diameter (40 cm), non-soaked seeds were sown directly (control) and seedlings were compared with DW and nitrate treated sets for following parameters.

Seedling emergence percentage (SEP):

The number of seeds emerged at 5, 7 and 9 DAS was counted for calculation of SEP pot^{-1}

$$SEP = \frac{\text{Number of seeds emerged pot}^{-1}}{\text{Number of seeds sown}} \times 100$$

Number of leaves and tillers:

These were counted per plant at 13 and 26 DAS.

Plant height, root length and number of roots:

These parameters were also measured at 13 and 26 DAS. For root length and root number counting pots were well irrigated before uprooting the plants.

Fresh and dry weights:

These were also measured at 13 and 26 DAS by using electronic balance.

Electrical conductivity {EC (m Mho)} and nitrate reductase activity (m mol $h^{-1}g^{-1}$) in leaves of 26 DAS old plantlets were determined by using conductivity meter and a method introduced by Srivastava (1974), respectively.

Total nitrogen content (mg g^{-1}) of dry leaves at 26 days old seedlings was determined by employing modified micro kjeldhal method (Lang, 1958).

Statistical analyses:

All the experiments were repeated twice with three replications and the data were statistically analyzed by analysis of variance as described by Cochran and Cox (1963) with Randomized Block Design.

RESULTS AND DISCUSSION

When the rice seeds provided with a soaking treatment of nitrate salts, the per cent germination was found to improve significantly in KNO_3 soaked seeds that was followed by $Mg(NO_3)_2$ at 40 and 48 h in comparison to DW soaked seeds. However, at 64 and 72 h there was no significant difference in between treatments. Same trend was noted for radical and plumule length. Amylase activity was also significantly higher in nitrate treated sets at both the study hours *i.e.* 24 and 48 h in respect to DW treatment (Table 1). Singh and Chatterjee (1980) reported that pretreatment of seeds with various salts in rice increases germination, plant growth and grain production. However Taegsu and Woo (2000) suggested that seeds of rice, when soaked in GA_3 in addition to various inorganic salts including $MgCl_2$ and KNO_3 showed no significant difference in respect to germination and coleoptile elongation. In present investigation also same type of results were observed and no statistical differences were found with KNO_3 and $Mg\ (NO_3)_2$ treated sets at late hours of germination *i.e.* 64 and 72 h.

To find out the carry over effect of nitrate seed treatment over DW soaked and direct sown seeds (control) the rice seeds were soaked with respective salt/ DW for 30 h and then sown in pots and compared with control set for various parameters (Table 2). Seedling emergence percentage (SEP) at 5, 7 and 9 DAS was significantly improved in $Mg(NO_3)_2$ treated sets over other treatments. Highest SEP noted in $Mg\ (NO_3)_2$ *i.e.* 94.00 % followed by KNO_3, DW and Control, respectively (92.66, 87.00, and 84.33 %) at 9 DAS. Same trend also noticed for shoot and root fresh weights where $Mg\ (NO_3)_2$ showed significantly higher fresh weight over rest of the treatments at 13 and 26 DAS. Both the nitrate

Table 1 : Effect of seed soaking treatment with DW and nitrate salt on some parameters related to germination physiology of the rice (Short term experiment)

Parameters	Time of observation (h)	Soaking treatment DW	KNO_3	$Mg(NO_3)_2$	SEM ±	C.D. (P=0.05)
Germination percentage	40	33.33	41.66	40.00	1.924	5.341
	48	76.66	85.00	90.00	1.924	5.341
	64	86.66	91.66	90.00	1.924	5.341
	72	93.33	95.00	95.00	1.360	3.770
Radicle length (cm)	48	0.688	0.718	0.713	.004	0.013
	72	2.787	2.823	2.830	0.006	0.017
Plumule length (cm)	48	0.471	0.518	0.514	0.007	0.019
	72	2.265	2.393	2.341	0.017	0.049
α-amylase activity (mg	24	80.92	98.5	95.5	0.49	1.360
maltose h^{-1} g^{-1} fresh weight)	48	98.25	152.33	124.30	0.722	2.006

salts found equally effective for parameters like number of leaves and tillers at early stage of growth (13 DAS) than latter stage (26 DAS).However nitrate treatments showed significant differences to DW soaked and control sets. More or less same trend recorded for parameters like plant height, root number and length, fresh and dry weights of root and shoot at 13 and 26 DAS (Table 2). Electrical conductivity and Nitrate reductase activity in

Table 2 : Effect of seed soaking treatment with DW and nitrate salts on morpho-physiological parameters of seedlings of rice (Long term experiment)

Parameters	Period of Observation (DAS)	Soaking Treatments Control	DW	KNO_3	$Mg(NO_3)_2$	S.E.±	C.D. (P=0.05)
Seedling emergence (%)	5	58.25	61.08	67.16	70.00	0.830	2.031
	7	71.00	72.00	81.00	84.33	0.983	2.405
	9	84.33	87.00	92.66	94.00	0.561	1.375
No. of tiller $plant^{-1}$	13	1.00	1.00	1.33	1.66	0.333	0.768
	26	2.00	2.00	3.00	3.66	0.235	0.548
No. of leaves $plant^{-1}$	13	3.00	3.00	3.33	4.00	0.682	1.586
	26	9.33	9.33	12.33	13.00	0.577	1.331
Plant height (cm)	13	15.20	15.27	16.43	17.76	0.529	1.221
	26	25.90	26.13	28.03	28.86	0.374	0.862
Root length (cm)	13	7.50	7.76	8.46	8.56	0.167	0.385
	26	11.60	12.20	13.06	13.10	0.221	0.509
No. of roots	13	9.00	9.66	11.33	13.33	0.816	1.883
	26	15.00	16.66	20.66	20.33	0.913	2.106
Fresh wt. of shoot (mg $plant^{-1}$)	13	108.00	109.00	117.66	120.00	1.322	3.050
	26	148.00	150.33	158.33	162.00	1.472	3.394
Fresh wt. of root (mg $plant^{-1}$)	13	76.33	77.66	81.00	82.66	0.707	1.630
	26	99.33	100.66	103.33	105.33	0.624	1.438
Dry wt. of root (mg $plant^{-1}$)	13	35.33	35.67	41.00	43.00	0.882	2.033
	26	47.00	47.33	52.33	54.00	1.105	2.549
Dry wt. of shoot (mg $plant^{-1}$)	13	56.00	56.67	61.00	62.33	0.782	1.802
	26	73.00	73.67	78.00	79.33	0.972	2.241
Electrical conductivity (m Mho g^{-1} leaf fresh wt)	26	1.13	1.12	1.21	1.23	0.027	0.063
NRase activity (n mol NO_2^- h^{-1} g^{-1} leaf fresh wt.) in leaf	26	109.43	124.13	277.2	283.27	29.08	67.08
Nitrogen content (mg g^{-1}dry wt. of shoot)	26	6.040	6.010	7.612	7.809	0.292	0.811

leaves also found significantly higher in nitrate treated sets over control where as the nitrogen content found significantly higher in comparison to both *i.e.* DW treated and control sets at 26 DAS (Table 2).

These findings present a harmony with results of Bose *et al.* (1992) and Bose and Mishra (1999 and 2001). They found when maize seeds were soaked with $Ca(NO_3)_2$ and GA_3 and mustard seeds soaked with $Mg(NO_3)_2$ as a pre- sowing soaking treatment showed a better performance for root, shoot length, leaf area, chlorophyll etc. in both the crops over control. Further, studies showed that while rice seeds soaked with 45 ppm urea improved the seedling height, dry weight of above ground part of clum and dry matter (Guoxing and Wang, 1998). The data of EC reveled that when Mg $(NO_3)_2$ and KNO_3 were applied as soaking treatment to seeds the ionic concentration of growing leaves were found to be improved. This may be due to influx of the cations (K^+ or Mg^{++}) and anion (NO_3^-) during the soaking of seeds. These results may also support the views of Bose *et al.* (1982). They suggested that nitrogenous salts while introduced as a soaking treatment to maize seeds the leaching of metabolites from seeds inhibited. Data pertaining to nitrogen content and nitrate reductase activity were in accordance with the reports of Mativer and Dale (1979), Bose and Tandon (1991) and Verma and Srivastava (1998). All of them observed that while the seeds of various crops like barley, maize, mustard and pigeon pea were soaked with nitrate salts in form of Ca $(NO_3)_2$, Mg $(NO_3)_2$ and KNO_3 found to improve the nitrogen content, protein, amino acid content and nitrate reductase activity of all the growing crops.

On the basis of over all observations it is supposed that when the nitrate salts were used in form of pre-sowing soaking treatment then cation as well as anion part of salt influxed to seeds. These ions further may remain in the vacuolar part of the cells of the seeds in pool form. (Bose and Mishra, 1997 and 1999). The pooled nitrate, present in the seeds showed their carry over effect during seedling growth by inducing nitrate reductase activity as a result level of nitrogen increased in growing seedling (Cast *et al.,* 1995). The pooled nitrate may also act as a signaling agent which triggers changes in metabolism further, and developmental stages of crop growth (Forde, 2002). At physiological level, the NO_3^- ion can induce both the NO_3^- assimilatory pathway and the reprogramming of metabolism to provide reluctant and carbon skeleton for this pathway (Stitt, 1999), hence the over all performance of the crop may be improved. However, in India the state like Tamil Nadu has adopted the system of rice intensification (SRI) method for paddy cultivation which had been introduced by the farmers of Madagascar in 1980s;the method based on the principle of using less seed,less chemicals etc to get better root growth and improved input utilizing efficiency which lead towards increase in yield in rice. In the present, piece of work it has been realized that this type of pre sowing seed treatment with nitrate (15mM) not only improves germination /seedling vigor but also root growth and its number too which may also improve the nitrogen metabolism; therefore this kind of seed treatment if added to the SRI cultivation of paddy may be more beneficial in improving production potential of this crop.

REFERENCES

Bernfeld, P. (1955). In methods in Enzymology, 1, Eds. S.P.Colowick and M.O. Kaplan, *Academic Pr* Bose *ess,* London, 149.

Bose, B. and Mishra, T. (1999). Influence of pre sowing soaking treatment in *Brassica juncea* seeds with Mg salts on growth, nitrate reductase activity, total protein content and yield responses. *Physiol.Mol.Biol.Plants*, **5**: 3-88.

Bose, B. and Mishra, T. (2001). Effect of seed treatment with magnesium salts on growth and chemical attributes of mustard. *Indian J. Plant Physiol.*, **6** (4) (New Series) : 431-434

Bose, B., Srivastava, H.S. and Mathur, S.N.(1982). Effect of some nitrogenous salts on nitrogen transfer and protease activity in germinating *Zea mays* L. seeds. *Biol. Plantarum*, **24** : 89-95.

Bose, B. and Tandon, A. (1991). Effect of magnesium nitrate of nitrogen metabolism in germinating maize seeds. *Indian J. Plant Physiol.*, **34**:69-71.

Bose, B., Tyagi, D.N. and Medhavati, D. (1992). Influence of seeds soaking with Ca $(NO_3)_2$ and GA_3 on the growth of seedling of *Zea mays* L. (var. Pin Pakha) *Indian Biologist,* **24** (1):64-71.

Bose, B. and Mishra, T. (1997). Effect of Mg salts on seed germination of *Brassica* sp. *Cruciferae News Letter*, **19**:63-64.

Cast, D., Stalder, M., Ruegsegger, A., Galli, V. and Brumold, C.(1995). Effect of NO_2^- and nitrate reductase during water stress and recovery in maize. *Physiol.Plant.*, **49**: 495-500.

Cochran, W.G. and Cox, G.M. (1963). Experimental Designs, Asia Publishing House. New Delhi, 150-154.

Forde, B.G. (2002). Local and long range signaling pathways regulating plant responses to nitrate. *Annual Rev. Plant Biol.*, **53**: 203-224.

Guoxing, M. and Wang, Caiping (1998). Cold resistance of early rice seedling and the effect on cold resistance of different seed soaking treatments. *Kexue*, Z.N., **1**: 5-7.

Hilhorst, H.W.M. (1990). Dose response analysis of factor involvement in germination and second dormancy of *Sisymbrium officiale*, H. Nitrate. *Plant Physiol*., **94**: 1096-1102.

Kiss, A. S. (1979). Germination stimulation by magnesium and mechanism of action. *Acta Agron. Acad. Sci. Hung.,* **28**: 884-890.

Lang, C.A. (1958). Simple micro determination of Kjeldahl nitrogen in biological material. *Anal.Chem*., **30**: 1692-1694.

Metivier, J.R. and Dale, J.E. (1977). The effects of grain nitrogen and applied nitrite on growth, phothosynthesis and protein content of the first leaf of barley cultivars. *Ann. Bot*., **41**: 1287-1296.

Singh, A.I. and Chatterjee, B.N. (1980). Effect of seed pretreatment on rainfed dry land rice production and on water saturation deficit in leaves. *IRRN*, **5** (3): 19-20.

Srivastava, H.S. (1974). *In vivo* activity of nitrate reductase in maize seedling. *Indian J. Biochem. Biophys*., **2**: 230-232.

Stitt, M. (1999). Nitrate regulation of metabolism and growth. *Curr.Opin. Plant Biol*., **2**:178-486.

Taegsu, N. and Woo, L.B. (2000). Effect of seed soaked gibberlic acid and inorganic salts on mesocotyl and coleoptile elongation in rice. *Korean J. Crop Sci.*, **45** (1):50-54.

Verma, J. and Srivastava, A. K. (1998). Physiological Basis of salts stress resistance in pigeon pea (*Cajanus cajan* L.) –II. Pre-sowing seed treatment in regulatory early seedling metabolism during seed germination. *Plant Physiol. & Biochem*., **25**(2) : 89-94.

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