Original Research Article

On Improving Seed Germination and Seedling Growth in Rice under Minimal Soil Salinity.

ABSTRACT

Aims: It was assumed that two- way approach i.e. nutrient-priming with potassium salt of the seeds and later on ammonium sulphate application may be binary beneficial for growth of rice besides evidencing genetic variability under salt stress

Study Design: The experiment was laid out in Complete Randomized Design with three replications.

Place and Duration of Study: The study was conducted in laboratory and glass house of Soil Salinity Research Programme of Land Resources Research Institute at National Agricultural research Centre, Islamabad, Pakistan during the period from May to August, 2016.

Methodology: Seeds of *Oryza sativa* (cv. KS-282 and BAS 385) were primed with potassium nitrate. In the second phase of the study, the primed seeds were raised in a minimal saline soil with ammonium sulphate nutrition gradually up to 150 mg Kg^{-1}.

Results: Bas-385 was more responsive for mean germination time than KS-282. In Bas-385 and KS-282 germination was 100 and 90 percent. Germination rate index of Bas-385 was 16 percent higher than that of KS-282. Biomass of Bas-385 seedlings was higher than that of KS-282 with the treatments. In both the cultivars of rice, Na^+/K^+ ratio was in antagonistic relation R = (- 0.99) with the gradual increase in ammonium sulphate application. Potassium ion was accordant with sulphate ion and N concentration. Bas-385 was more tolerant to KS-282 based on Na^+/K^+ ratio and bio mass.

Conclusion: BAS 385 (salt sensitive) superseded to KS-282 (salt tolerant) under minimal salt stress due to nutrient priming and then enhanced nutrition.

Key words: Ammonium sulphate, priming, rice, salinity, tolerance

1. Introduction

The embryo in the seed seems to be prone to surplus ionic effects from the micro-external environment. These consequences may change its cellular responses either proceeding to seed germination or its senescence. Excess of salts in the form of 'salinity monster' is restricting the crop production, whereas research findings report that seeds when treated with some salts in minute quantity, a process scientifically known as priming, is beneficial for its germination. After the priming process, once the seeds are germinated, provided the other processes/microenvironments are favorable and concomitant, seedling growth may take place in a normal direction under salt stress. Salinity is a major abiotic stress [1]. Saline soils contain multiple types of soluble salt components, each of which has a different effect on the initial growth of plants [2]. Soil salinity may affect the germination of seeds either by creating an osmotic potential external to the seed, preventing water uptake or through the damaging effects of Na^+ and Cl^- ions on the germinating seed [3]. Salt and osmotic stresses are responsible for either inhibition or delayed seed germination and for seedling establishment [4]. NaCl is the predominant salt causing salinization [5]. Germination parameters and biomass of rice decreased with increasing salt stress [6]. To have a healthy mature plant, it is vital that its early stages of nourishment such as germination and seedling must be prone to any set back in a metabolic process as [7] reported that germination and seedling growth are critical stages regarding salt stress. The entry of dissolved ions except the toxic one, trigger metabolic processes especially the enzymatic activities in water-imbibed seeds. For such activities, the role of potassium ion is significant. Potassium salts are the widely used source of seed priming [8]. Potassium ions with different combinations of anions are in use. Nutrient priming of seeds also improved nutrient supply [9]. Use of potassium source as seed priming mediator improved germination process and decreased Na^+/K^+ ratio in the follow-on seedlings [10]. After seed germination, seedlings need the optimum environment and balanced nutrition for growth. Since nutrition priming support germination process, post germination processes also need to overcome salinity effects. Salt diminution process necessitates decreasing sodium ion impact in the growth medium. To decrease its shock, it becomes significant to covenant it with a suitable nutrition ion. Potassium ion is the most suitable ion due to its chemical or physical structure. Its suitability is measured in the form of Na^+/K^+ ratio in plant material under salt stress. The lower this ratio, the lower is the impact of sodium ion on a glycophytes growth. The sulphur application can be beneficial for rice growth [11]. The sulphur application is well-known for amino acids formation. It has been observed that sulphur application also decreases Na^+/K^+ ratio in plant organ and sulphur is in synergistic relation with potassium ion [12]. Since sulphur and nitrogen are in synergistic relations and nitrogen has its own importance in the metabolism of plant tissue build up, therefore application of sulphur in the form of ammonium sulphate could play a better role for the growth of seedlings under salt stress. In Pakistan, rice is the second most consumed cereal after wheat. Pakistan is blessed with multitude of agro-climatic condition for growing several types of rice i.e. aromatic (Basmati) and non aromatic (non–Basmati) [13]. Basmati-385 and KS-282 are rice varieties released by Rice Research Institute, Kala Shah Kaku, Lahore, Pakistan [14]. Basmati-385 is a short stature, fine and short duration variety. It attains height of 133 cm and matures in 95–100 days. Yield is about 4,000 kg ha^{-1}. KS-282 is a high yielding coarse variety for southern Punjab of the country. It is 118 cm tall. It matures in 100 days with about 6,000 ha^{-1}yield. To what extent sulphur nutrition could be helpful for plant growth especially when the seeds of rice are nutrient-primed. It was assumed that two- way approach i.e. nutrient-priming with potassium salt of the seeds and later on ammonium sulphate application may be binary beneficial for growth of rice besides evidencing genetic variability under salt stress.

2.MATERIALS AND METHODS

Treated seeds of *Oryza sativa* (cv. KS-282 and BAS 385) with 1 % sodium hypochlorite for 15 minutes [15] and then washed these seeds three times with distilled water. For nutrient-priming, placed the seeds in a 100 ml beaker and applied one percent (w/v) of potassium nitrate salt solution. Kept these seeds in the solution for five hours. Then dehydrated these seeds with tissue papers, and air dried. Placed ten dried seeds on filter paper in Petri dish (11cm dia.) in quadruplicates for germination. Counted germinated seeds on 2^{nd} to 7^{th} day. Calculated mean germination time (MGT) according to the equation of Ellis and [16]. Converted observed germination percentage into ASIN form for statistical analysis purpose as worked by [17]. Computed rate of germination index (RGI) as given by [18]. In the next phase of the study, analyzed the soil for physicochemical characteristics (Table 1) and applied ammonium sulphate

@ 0, 30, 60, 90, 120, 150 mg kg^{-1} in this saline soil (pH = 7.84, EC = 4.55 dSm^{-1}). Maintained adequate moisture in the soil of the pots. The pots were placed in a glass house under sunlight, controlling temperature at 25±2 ºC. The primed seeds of the varieties were sown in this soil. The pots were arranged in complete randomized design with three replications and were placed in glass house under controlled conditions. After 30 days of seedling establishment, excised the aerial portion of the plants. Rinsed the shoots with demonized water, surface dehydrated with tissue paper and recorded fresh mass (FM). Dried shoots at 65 °C and recorded dry mass (DM). Cut each sample into small pieces and digested separately in a perchloric-nitric (1:2) di-acid mixture [19]. Determined sodium and potassium ions in the digested material by flame photometry. Analyzed sulphur in the digested material as given by [20]. Determined nitrogen in the plants according to [21]. Computed the data statistically according to factorial CRD and compared treatment means using LSD test [22].

Table1 . Physico-chemical characteristics of the saline soil.

Characteristics	Values
pH (1:1)	7.84
ECe (1:1) (dS m^{-1})	4.55
Cl^{-1} (meq L^{-1})	5.13
CO_3^{-2} (meq L^{-1})	0.35
HCO_3^{-1} (meq L^{-1}))	1.71
SO_4^{-2} (mg kg^{-1})	6.22
NO_3^--N (mg kg^{-1})	14.3
P (ABDTPA Extractable) (mg kg^{-1})	5.21
SAR (mol $L^{-1})^{1/2}$	5.24
Saturation (%)	32
Clay (%)	36
Silt (%)	50
Sand (%)	14
Text. Class	Silty Clay Loam

3. RESULTS AND DISCUSSIONS

Nutrient-priming of rice seeds with potassium nitrate and post-germination application of ammonium sulphate significantly (*P* = 0.01) affected seed germination parameters and seedling growth respectively.

3.1 Germination Parameters

In KS-282 germination percentage of unprimed seeds was 80 percent, but the seeds of the same variety, when primed with potassium nitrate salt, the germination was higher (90 %). In Bas-385 germination of un-primed seeds was 90 percent; however, it was 100 percent when the seeds of the same variety were primed with the same salt (Table 2). Rice varieties physio-genetically responded to seed priming treatment. Genetic traits of Bas-385 supported to seed-surface treatment with potassium nitrate than the hereditary attributes of KS-282 for germination at initial salinity. [23] found that germination is inversed to the increasing salt stress. During the treatment time, useful metabolic processes in the form of enzyme activation passed successfully through the lag phase. Usage of potassium nitrate as the seed priming agent was useful from nutritional aspect also. Priming treatment usability was revealed in the later stages of growth. Germination rate index (GRI) improved 22 percent in the primed seeds than the un-primed one in KS-282 (Table 3). In Bas-385, GRI improved 33 percent in the primed seeds than the un-primed one. The performance of Bas-385 was better than KS-282 by 16 percent. Mean Germination Time (MGT), remained low in the primed seeds than the un-primed one in KS-282. Overall performance difference for MGT of both the varieties was non-significant. Germination rate index expresses the speed of the germination. With increasing level of salinity, GRI decreased [24]. Therefore seed-priming improved the speed of germination. Higher GRI values indicated higher and faster germination. Early germination is reciprocal to MGT [25] as MGT is related to the time during which radicle appeared. Priming softens seed coat adherence due to imbibition, which may permit to emerge out radicle without resistance as reported by [26].

Table 2 Germination of rice seeds with nutrient -priming using potassium nitrate.

Priming Treatment	Germination (%)		Means	ASIN		Means
	Cv. KS-282	Cv. Bas-385		Cv. KS-282	Cv. Bas-385	
Primed	90 b	100 a	95 A	71.6 b	90.0 a	80.8 A
Un-primed	80 c	90 b	85 B	63.4 c	71.6 b	67.5 B
Means	85 B	95 A		67.5 B	80.8 A	

Means sharing similar letter(s) for a parameter do not differ significantly at $p < 0.01$
Cv. 0.01 %

Table 3 Effect of potassium nitrate as nutrient halo-priming on GRI and MGT of two varieties of rice.

Priming Treatment	Germination Rate Index (GRI)		Means	Mean Germination Time (MGT), (days)		Means
	Cv. KS-282	Cv. Bas-385		Cv. KS-282	Cv. Bas-385	
Primed	10.9 b	13.2 a	12.05 A	4.1 c	4.1 c	4.1 B
Un-primed	8.9 d	9.9 c	9.35 B	4.3 a	4.2 b	4.3 A
Means	9.9 B	11.5 A		4.2 A	4.2 A	

Means sharing similar letter(s) for a parameter do not differ significantly at $p < 0.01$
Cv. 0.86 %

3.2 Seedlings Growth

With the increasing of ammonium sulphate (AS) application, fresh mass (FM) and dry mass (DM) of both the cultivars were affected (Table 4). In KS-282 and Bas-385, FM was increased 4, 6, 8 and 10 percent; and 6, 9, 12, 15 and 18 percent than the respective control from 30 to 150 mg kg^{-1} of AS application. In KS-282 and Bas-385, DM was increased 12, 15, 17, 19 and 21 percent; and 14, 18, 21, 24 and 27 percent than the respective control from 30 to 150 mg kg^{-1} of AS application. Biomass of Bas-385 was higher than that of KS-282 with AS treatments. Fresh mass of plant material is a product of water and other chemicals in the form of tissue. Water potential is affected by included salts in a plant. Water retention of a plant tissue indicates its health and turgidity [27]. Antagonistic relations between plant biomass to increasing salinity stress has been reported [28]. Dry mass of plant material is the net outcome of the resultant metabolic activities [27].

Table 4 Effect of ammonium sulphate application on fresh and dry mass of shoot of rice grown from nutrient –primed seeds in saline soil

Ammonium sulphate ($mgKg^{-1}$) applied	Fresh mass (mg $plant^{-1}$)		Dry mass (mg $plant^{-1}$)	
	Cv. KS-282	Cv. Bas-385	Cv. KS-282	Cv. Bas-385
Control	140.2 l	146.1 k	9.81 k	10.1 3j
30	146.3 j	155.2 f	11.07 i	11.53 fg
60	149.2 i	159.6 d	11.23 h	12.03 d
90	152.1 h	164.2 c	11.43 g	12.33 c
120	154.6 g	168.3 b	11.63 f	12.63 b
150	157.3 e	172.5 a	11.8e	13.03 a

Means sharing similar letter(s) of a parameter do not differ significantly at $p < 0.01$
Cv. 2.05 %

3.3 Sodium Potassium Ratio

In both the cultivars of rice, Na^+/K^+ ratio was in antagonistic relation (r = - 0.99) with the applied AS (Fig.1) . In KS-282, Na^+/K^+ ratio decreased 8, 13, 23, 30 and 38 percent than the control at 30, 60, 90, 120 and 150 mg kg^{-1} of AS application respectively. In BAS-385, Na^+/K^+ ratio decreased 10, 19, 30, 40 and 50 percent than the control in same sequence of AS application as above. Potassium ions are synergistic to sulphate ion and nitrogen application. These ions are beneficial to crop plants. Potassium and sulphate ions are inversed to Na^+ in glycophyes. In both the varieties, the linear equation shows that every 100 units increase in sulphur application decreased 9 units of Na^+/K^+ ratio. In addition, KS-282 needed 1.31 times higher the application of AS application than that of Bas-385 to maintain low Na^+/K^+ ratio. Sodium ion impedes positive biochemical activities resulting in decreased dry mass. Therefore sodium ion maneuvers senescence in reduced growth of glycophytes. It has been evident by [29] that reduction in seedling growth under saline conditions is due to increase in sodium chloride toxicity. In many species, salt sensitivity is associated with the accumulation of sodium ion in photosynthetic tissues [30]. Externally decreased in water potential created by NaCl might have affected fresh mass by preventing water uptake. The capacity of plants to maintain a high K^+/Na^+ ratio is one of the key determinants of plant salt tolerance [31]. KS-282 sequester and accumulate K^+ according to the external application of sulphate ion. According to [32] change in ratios of ions in plants may be a resultant due to same pathways of Na^+ and K^+. Na^+ competes with K^+ uptake through Na^+- K^+ co-transporters and may also block the K^+ specific transporters of root cells under salinity [33].

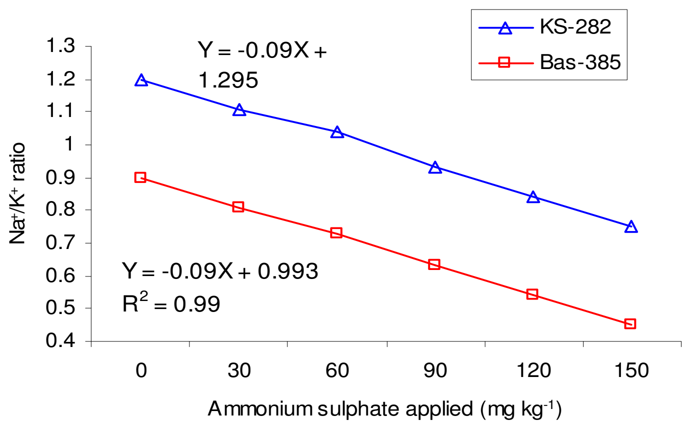

Figure 1 Relation between ammonium sulphate applied and Na^+/K^+ ratio in rice.

3.4 Relation between sulphur and nitrogen

Under minimal soil salinity conditions, with the application of ammonium sulphate @ 30, 60, 90, 120 and 150 mg kg-1 soil, N concentration in shoot of Cv. Bas-385 was higher 15, 18, 21, 24 and 27 percent than that in KS-282 (Fig. 2). Availability of sulphur along with S-containing compounds such as ATP-sulfurylase is considered as the first rate-limiting enzyme of the sulphur assimilation pathway and is up-regulated under salinity stress [34]. In salt-treated plants, sufficient sulphur supply allows glutathione synthesis necessary to prevent the adverse effects of Reactive oxygen species on photosynthesis. Plants with higher levels of thiol were more salinity tolerant [35]. It seems K ion accordant with sulphate ion increases tolerance against Na^+. For rice growth, application of nitrogen is beneficial under salt stress [36]. In addition it was observed that cultivar response may be variable as Bas- 385 was more responsive than KS-282 for N- concentration in such conditions.

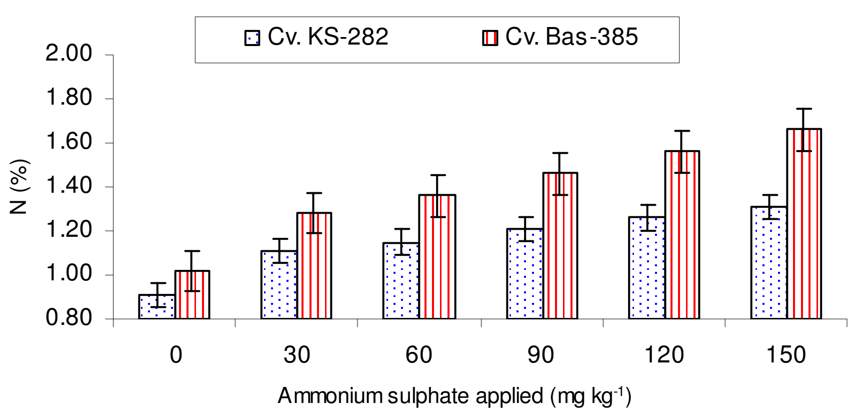

Figure 2 Level of total -N in shoot of two varieties of rice under salt stress.

4. CONCLUSIONS

Under salt stress, nutrient-primed with potassium nitrate eased germination 100 and 90 percent in rice varieties i.e. Bas-385 and KS-282. For germination rate index, the performance of Bas-385 was better than that of KS-282 by 16 percent. Biomass of Bas-385 was higher than that of KS-282 with sulphur treatments. In both the cultivars of rice, Na^+/K^+ ratio was in antagonistic relation (R = - 0.99) with the applied sulphur, however, Bas-385 accumulated more potassium ions than that of KS-282.

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