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Seed germination and seedling growth parameters of rice (*Oryza sativa*) varieties as affected by salt and water stress

VIBHUTI[1], CHARU SHAHI[2], KIRAN BARGALI[3] and S S BARGALI[4]

Kumaun University, Nainital, Uttarakhand 263 001

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ABSTRACT

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population. Globally, rice is grown on 161 million hectares, with an average annual production of 678.7 million tonnes. Drought and salinity are two major abiotic determinants due to high magnitude of their impact and wide occurrence. In the present study rice varieties were analyzed for water and salt stress tolerance at germination and early seedling growth stage. Seeds of three rice varieties (Narendra 1, Sabarmati and Hybrid 312) were collected and kept under four water stress and six salt stress levels. Seed germination, seedling length, dry weight, seed vigor and other parameters were recorded. The results showed that with increasing water stress, germination in all the varieties was delayed and decreased from 68.8% in control to 4.4 % in highest stress (-15 bar) level. Dry weight of shoot and root, shoot and root length, fresh weight of stem and root decreased in all rice varieties with the increase in water stress level. Narendra 1and Sabarmati showed better response while Hybrid 312 failed to germinate in all water stress levels. The increase in salt stress also reduced every measured trait significantly in all the varieties. Seed germination decreased from 100% in control to 65 % in highest (20 ds/m) salt stress level. Maximum germination percentage (100%) was observed in Hybrid 312 under all the salt stress levels. These results could be helpful in identification of the tolerant varieties which can be studied further and economically exploited.

Key words: Salt stress, *Oryza sativa*, Relative water content, Seed germination, Salt tolerance, Seed vigor, Water stress

Abiotic stresses can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth and development (Chutia and Borah 2012) and adversely affect agricultural productivity (Bartles and Sunkar 2005). In addition, abiotic stresses are the major cause of crop failure, decreasing average yield for major crops by more than 50% and threatening the sustainability of the agricultural industry (Mahajan and Tuteja 2005). Processes such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set are adversely affected by high salt and water stress, ultimately causing diminished economic yield and also quality of produce. Many plant species naturally accumulate protein and proline as major organic osmolytes when subjected to different abiotic stresses. These compounds are considered to play adaptive role in mediating osmotic adjustment and protecting sub- cellular structures in stressed plants.

Drought and salinity are two major abiotic determinants (Wang *et al.* 2009) due to high magnitude of their impact and wide occurrence (Bartels and Sunkar 2005). Salt stress is reported as a serious problem (Nedjimi and Daoud 2006), eventhough water deficit is the most limiting factor to cereal production (Chennafi *et al.* 2006). Rice is one of the most sensitive cultivated species to salt and drought stresses (Lefèvre *et al.* 2001), so both water/soil salinity and low water supply are growing obstacles to rice production worldwide (Flowers and Yeo 1995, Munns 2002). In recent years, salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increased rice production worldwide (Gregorio 1997).

Salinity stress negatively impacts agricultural yield throughout the world affecting production whether it is for subsistence or economic gain (Yokoi *et al.* 2002, Goumi *et al.* 2011). About 84% of rice production growth has been attributed to the use of modern technologies. Grain yield under stress environment is the primary trait for selection in breeding for drought tolerance. Drought effect on seed yield is due to the relation with duration of watering from flowering until physiological maturity. The plant response to salinity consists of numerous processes that must function in coordination to alleviate both cellular hyper osmolarity and ion disequilibrium. In addition, crop plant must be

[1] Research Scholar (e mail: vibhug20@gmail.com), [2]Research Scholar (e mail: charusha91@gmail.com), [3]Assistant Professor (e mail: kiranbargali@yahoo.co.in), [4]Professor (e mail: surendrakiran@rediffmail.com), Department of Botany, D S B Campus

capable of satisfactory biomass production in a saline environment (Yokoi *et al.* 2002).

Germination and seedling development is very important for early establishment of plants under stress condition. Selecting varieties for rapid and uniform germination under water and salt stress conditions can contribute towards early seedling establishment. In view of the above, in the present study effect of salt and water stress on the seed germination and seedling growth of rice varieties was carried out with the following objectives: (i) To identify the physiological and morphological response of rice varieties to mannitol- induced water deficit. (ii) To analyze the sensitivity and salt stress tolerance of rice varieties.

MATERIAL AND METHODS

Water stress experiment: In this study various levels of external water stress (-5,-10,-15 bars) were produced by mannitol solution, according to the formula given by Helmericls and Pfeifer (1954).

$$\text{Water stress} = \frac{1}{-25} \times \text{molality}/\psi_p$$

Where ψ_p is the osmotic potential of the mannitol solution (water stress), in addition one control (0 bar) water stress level was also maintained.

Salt stress experiment: In this study six salt stress levels (0, 4, 8, 12, 16, 20 ds/m) were prepared using NaCl.

Seeds of rice varieties grown in Bhabhar area of Nainital district (Narendra1, Sabarmati and Hybrid 312) were obtained from GB Pant University of Agriculture and Technology, Pantnagar. Healthy, uniform seeds of all varieties were surface sterilized and washed with distilled water. The seeds were placed in sterile petri dishes (9 cm diameter) lined with two sterile Whatman No. 1 filter paper with 5 ml of distilled water or the respective test solutions as described for water stress and salt stress experiment (Fig 1). There were 10 seeds per petri dish and three replicate in each treatment. Germination test were conducted under condition of 12 h light/dark cycle with 14^0C minimum and 24^oC maximum temperature. A seed was considered germinated when radicle was 2mm long. The germination percentage was determined counting the number of germinated seeds every day. The root and shoot length and early seedling fresh and dry weights were measured on the 15^{th} day. Shoot and root dry weight were recorded after oven drying at 60^oC for 48 hr. Statistical analysis was performed using SPSS version 16.

After final count, germination percentage (GP) and germination rate (GR) was calculated by the following formulae (Raun *et al.* 2002):

GP = Number of total germinated seeds/ Total number of seeds tested × 100

$$\text{GR} = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \text{———} + \frac{\text{Number of germinated seeds}}{\text{Day of final count}}$$

The shoots and roots were separated and the fresh weights were measured; after being oven dried at 60 °C for 24 hours, the dry weights were immediately taken. According to each salt treatment, the fresh and dry weights, referred to the controlled, were calculated in percent, by the following equations:

Fresh weight (FW) percentage reduction:
FWPR % = 100 × [1 - (fresh weight $_{\text{salt stress}}$/ fresh weight $_{\text{control}}$)]
Dry weight (DW) percentage reduction:
DWPR % = 100 × [1 - (dry weight $_{\text{salt stress}}$/ dry weight $_{\text{control}}$)]

Relative water content (RWC):
The water content respective to the fresh weight was calculated as described by Sumithra *et al.* (2006):
RWC % = 100 × [(FW – DW) / FW]

Seed vigor (SV):
This index was determined by following Abdul and Anderson (1970):
Strong seed index = {germination percentage × means of seedling length (Root + Shoot)/ 100

Fig 1 Seed Germination of Narendra 1 variety under the salt stress levels

Table 1 Variance analysis (ANOVA) for traits investigated for the three varieties of rice in response to drought stress

Parameter		Mean square					
	df	SL(cm)	RL(cm)	GP (%)	GR	SDW(g)	RDW(g)
Varieties (N-1, S, H-312)	2	9.217*	0.456 ns	11969.44*	14.423*	0.330ns	0.163ns
Water stress levels	3	61.85*	6.476*	8266.667*	9.257*	0.063ns	0.000ns

*: Significant at 5%, and ns: not significant. SL: shoot length, RL: root length, GP%: germination percentage, GR: germination rate, SDW: shoot dry weight, RDW: root dry weight

Salt Tolerance Index (STI):
It is quantified by the ratio, respectively to the controlled, of the total dry weight in salt stress, in percent, and calculated by the following equation:

$$STI = 100 \times (\text{Total DW}_{\text{salt stress}} / \text{Total DW}_{\text{control}})$$

RESULTS AND DISCUSSION

Effects of water stress: Germination and seedling growth of rice seeds was significantly influenced by varieties and drought stress levels (Table 1).

Effects on germination percentage: In this experiment, germination percentage decreased with increasing water stress (Fig 2). At -10 and -15 bar levels germination was completely inhibited in all the varieties. Among the three varieties, Hybrid 312 variety was very sensitive to drought stress as compared to other varieties (Narendra 1 and Sabarmati) as in this variety germination was completely inhibited at all the water stress (-5, -10, -15 bar) levels. Among the 3 rice varieties Sabarmati showed better performance against water stress.

In the present investigation drought stress greatly affects seed germination, but the response intensity and adverse effect of stress depend on the variety (Table 2). Drought stress was reported to adversely affect the seed germination, plant growth and development (Almaghrabi and Abdelomoneim 2012) and seedling growth (Ashraf *et al.* 2002). Among the varieties, Sabarmati showed the highest (70.7%) seed germination while Hybrid 312 showed the lowest (5%) germination percentage. Water stress reduced seed germination from 68.8% to 4.4% that was about 53.6% decrease (Table 2).

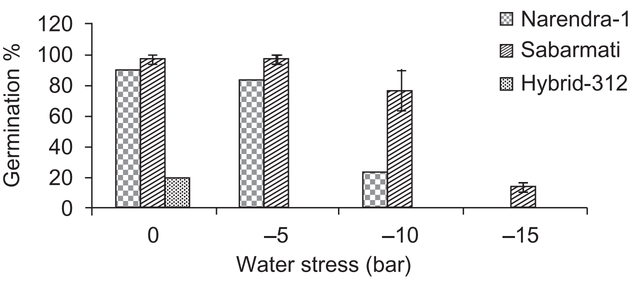

Fig 2 Effect of water stress on germination % of three rice varieties

Effects on germination rate: The rate of germination decreased with the increase in drought stress. In drought stress the highest germination rate was recorded at control and lowest at -15 bar level. Narendra 1 and Sabarmati (3.54) showed better rate of germination at -5 bar level and with further increase in drought stress towards-15 bar, where the rate of germination decreased. In case of Hybrid 312 variety, the germination rate was inhibited at all levels of drought stress.

Effects on initiation and completion of germination: Days taken to initiate and complete the germination processes varied in different rice varieties. At control level, the minimum time taken by all three varieties for initiation was 6 days and for completion was 12 days. However, with increasing water stress, emergence time also increased. Sabarmati show better initiation and completion at drought stress (-15 bar) while in Hybrid 312 initiation of seed germination was inhibited at -10 and -15 bar (Table 3).

Effects on root and shoot length: Plant height of different rice varieties were significantly affected by water stress. For both root and shoot, the maximum length was observed in the controlled condition and minimum in the highest drought stress level (Fig 3). Maximum shoot length was observed in Narendra 1 (4.22cm). At all the drought levels, Narendra 1(1.49cm) and Sabarmati (0.79cm) produced significantly higher root length compared to Hybrid 312 variety (0.70cm) which produced lowest root length (Fig 8).

Table 2 Means (± standard error) comparison of varieties, water stress levels and their interaction on the studied traits

Variety	SL (cm)	RL (cm)	TDW (g)	GP (%)
Narandra 1	1.20 ± 1.015	0.447 ± .354	0.020 ± .018	49.05 ± 22.17
Sabarmati	1.12 ± 0.963	0.337 ± 0.20	0.011 ± 0 .009	70.78 ± 19.72
Hybrid 312	0.20 ± 0	0.175 ± 0		5.00 ± 00
Treatment(bar)				
0	3.01 ± 1.09	0.99 ± 0.24	0.038 ±0 .021	68.86 ± 24.10
–5	0.36 ± 0.184	0.28 ± 0.163	0.003 ±0.001	59.96 ± 30.22
–10				33.3 ± 22.67
–15				4.44 ± 0

SL = shoot length, RL= root length, TDW = total dry weight, GP% = germination percentage.

Table 3 Effect of water stress on initiation and completion of seed germination of three rice varieties (I = initiation in days, C = completion days)

Water stress levels (bar)	Varieties					
	Narendra 1		Sabarmati		Hybrid 312	
	I	C	I	C	I	C
0	6	12	6	12	8	12
–5	6	14	6	12	10	16
–10	12	16	6	14		
–15			10	18		

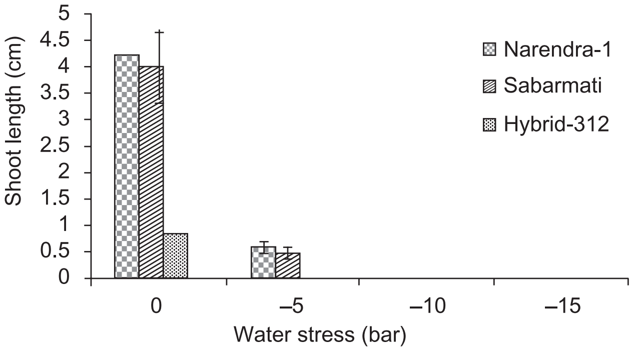

Fig 3 Effect of water stress on shoot length of three rice varieties

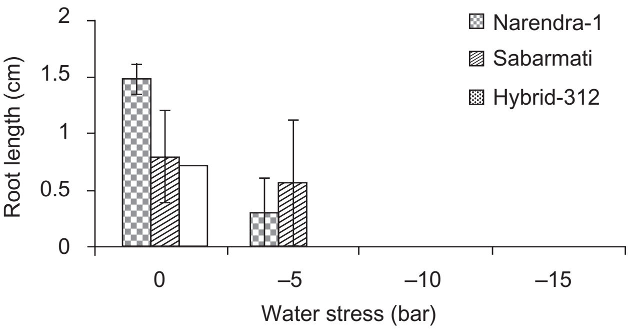

Fig 4 Effect of water stress on root length of three rice varieties

Effects on fresh and dry weight of seedlings: Shoot dry weight was inversely related to water stress. In Narendra 1 and Sabarmati variety shoot dry weight reduced with increasing water stress while in Hybrid 312 variety dry mass was completely inhibited at all the levels indicating that this variety was very sensitive to water stress. Dhanda *et al.* (2004) indicated that seed vigor index and seedling length are among the germination traits that are most sensitive to drought stress. In the present study, drought stress caused a significant reduction in seedling length and weight.

Effects of salt stress

Effect on germination percentage: Highest germination percentage was observed in Hybrid 312 (100%) as compared to other varieties (Narendar 1, Sabarmati) at all salt stress levels (Fig 5). The germination percentage significantly decreased in all the varieties due to the increase in salt stress levels. The sensitivity showed high significant difference between the varieties in response to salt stress. Comparison between means of different levels of salinity effects on germination rate and percentage (Table 4). The highest germination rate was observed at control (100%) while lowest at 20 ds/m (66.6%) salinity stress level. Hakim *et al.* (2010) suggested that in addition to toxic effects of certain ions, higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and result reduction in germination. At 20 ds/m salinity level germination was observed in the order Hybrid 312> Sabarmati> Narendra 1. The variability in salinity tolerance among rice varieties at germination have also been reported (Khan *et al.* 1997, Mondal *et al.* 1988, Hakim *et al.* 2010). According to Akbar and Ponnamperuma (1982) the osmotic effect due to salinity was the main inhibitory factor that reduces seed germination.

Effect on germination rate: The rate of germination decreased as the salinity stress increased. Hybrid 312 with 4.4 germination rate showed best performance as compared to other two varieties with 4.3 in Narendra 1 and 4.1 in Sabarmati. It means Hybrid 312 was more tolerant to salt stress.

Effect on initiation and completion of germination: The emergence time taken by Hybrid 312 was 4 to 10 days while Narendra 1and Sabarmati took 6 to 10 days to initiation and completion at all stress levels (Table 5).

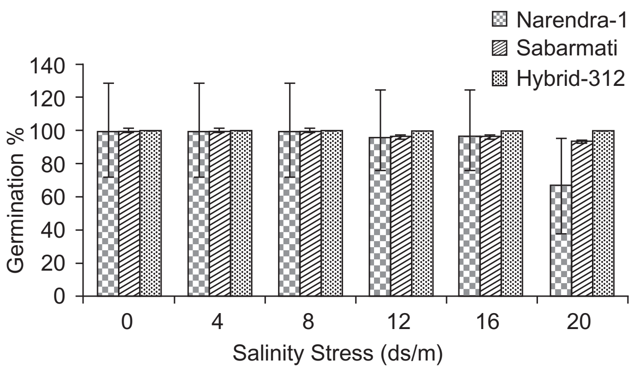

Fig 5 Effect of salinity stress on germination % of three rice varieties

Table 4 Variance analysis (ANOVA) for traits investigated for the three varieties of rice in response to salinity stress

Parameters	df	Mean square			
		SL(cm)	RL(cm)	GP (%)	TDW(g)
Varieties (N 1, S, H 312)	2	85.588*	5.170 ^{ns}	272.22 ^{ns}	0.016 ^{ns}
Salinity Stress levels (ds/m)	3	79.751*	33.656*	1352.222 *	0.001*

*: Significant at 5%, and ns: not significant. SL: shoot length, RL: root length, GP%: germination percentage, GR: germination rate, TDW: total dry weight.

Table 5 Effect of salinity stress on initiation and completion of seed germination of three rice varieties (I = initiation in days, C = completion days)

Salinity levels (ds/m)	Narendra 1		Sabarmati		Hybrid 312	
	I	C	I	C	I	C
0	4	10	4	6	4	6
4	4	10	4	6	4	6
8	4	12	4	8	4	6
12	6	10	4	8	4	6
16	6	12	6	10	4	8
20	6	12	6	14	4	10

Effect on root and shoot length: Plant height decreased with increase in salinity stress and Sabarmati showed maximum susceptibility to salinity stress. Hybrid 312 (3.3cm) variety showed better performance in comparison to other two varieties at all the salinity levels (Fig 6). Similarly, root length was also decreased with increase in salinity stress. Root length was more suppressed than shoot by salinity at each specific salt concentration level. Narendra 1 variety (5.83cm) showed higher root length (Fig 7). The effects of salinity stress on root and shoot length showed significant difference at 0.05% probability level (Table 4). Reduction in seedling height is common phenomenon of many crop plants grown under saline condition (Javed and Khan 1995). Shoot and root length provides an important clue to the response of plants to salt stress (Jamil and Rha 2004). The shoot and root length of seedlings grown in salt solutions also showed decreasing trend, indicating that the salt stress not only affected germination but also the growth of seedlings, which indicates that synthetic ability of seed

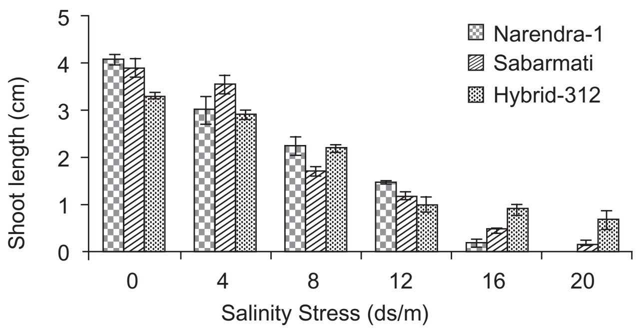

Fig 6 Effect of salinity stress on shoot length of three rice varieties

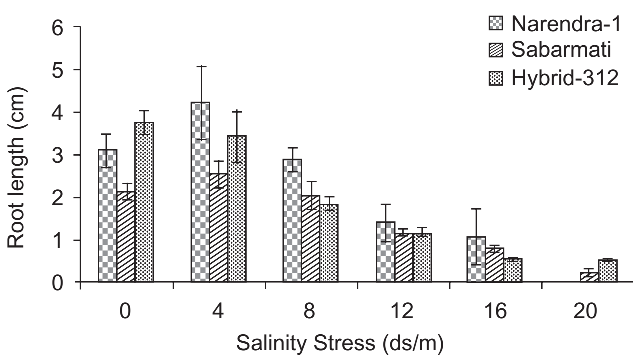

Fig 7 Effect of salinity stress on root length of three rice varieties

and thus dry matter production of the seedlings was affected. This is in conformity with the findings of Djanaguiraman *et al.* (2003) and Hakim *et al.* (2010) and they observed that shoot and root length was conspicuously affected by salt.

Effect on root and shoot dry weight: Shoot dry weight was inversely related to salt stress and was relatively less sensitive as compared to root dry weight especially at higher salt concentration. Hybrid 312 showed the highest reduction in shoot dry weight (0.31g) while Narendra 1 (0.006g) and Sabarmati (0.01g) showed lowest reduction. Shoot dry weight of Sabarmati was significantly less affected at all salinity levels. The fresh and dry weight percentage reduction was lower in the shoots than in the roots. Reduction in seedling growth as a result of salt stress has been reported in several others species (Achakzai *et al.* 2010, Akram *et al.* 2010). Salinity has both osmotic and specific ionic effects on seedlings (Dioniso and Tobita 2000).

Effect on relative water content: Relative water content was highly influenced by salinity levels. The maximum relative water content was observed at 12 dS/m. Sabarmati shows better performance under the salinity stress (Table 6).

Effect on salt tolerance index (STI)*:* Salt tolerance

Table 6 Effect of salinity stress on seed germination of different rice

Varieties / salt stress levels	FWPR%	DWPR%	RWC%	SV%	STI%
		0 dS/m			
Narendra 1	00	00	87	7.16	00
Sabarmati	00	00	87	6.01	00
Hybrid 312	00	00	85	21.26	00
		4 dS/m			
Narendra 1	20	31	88	7.2	69
Sabarmati	10	11	87	6.06	89
Hybrid 312	4	14	86	19	56
		8 dS/m			
Narendra 1	45	64	91	5.21	36
Sabarmati	67	58	86	3.73	38
Hybrid 312	30	57	90	12.1	43
		12 dS/m			
Narendra1	48	72	93	2.86	28
Sabarmati	68	72	89	2.14	28
Hybrid 312	59	98	99	6.5	12
		16 dS/m			
Narendra 1	50	91	97	1.24	9
Sabarmati	70	95	95	1.2	5
Hybrid 312	68		100	3.8	
		20 dS/m			
Narendra 1	99				
Sabarmati	96			3.4	
Hybrid 312	78	98		2.9	

FWPR%, Fresh weight percent reduction, DWPR%, Dry weight percent reduction, RWC%, Relative water content, SV, Seed vigor, STI%,Salt tolerance index.

Table 7 Means (± standard error) comparison of varieties, salinity stress levels and their interaction on the studied traits

Varieties	SL (cm)	RL (cm)	TDW (g)	GP (%)	RWC (%)	SV
Narendra 1	1.83 ±0.65	2.10 ±0.63	0.018 ±0.007	77.4 ±16.14	76 ±15.27	3.94 ± 1.24
Sabarmati	1.81 ±0.64	1.47 ±0.36	0.35 ± 0.329	97.77± 1.11	77± 11.54	3.24 ±0.99
Hybrid 312	1.83± 0.45	1.87 ±0.57	0.017 ±0.008	100	71 ±15.75	10.92±3.20
Treatment (dS/m)						
0	3.75 ±.23	2.99 ±.47	0.043 ±0.002	100	86.33± .66	11.47± 4.90
4	3.14 ±.19	3.39 ±.48	0.035 ±.002	100	77 ±10.50	10.75 ±4.12
8	2.07± .19	2.24 ±.32	0.017 ±.001	100	89 ±1.52	7.01±2.57
12	1.22± .12	1.22 ±.088	0.008 ±.003	97.75 ±1.12	93.66± 2.90	3.83± 1.34
16	0.51 ±.20	0.66 ±.66	0.007			
	86.66 ±10.18	97.33 ±1.45	2.08± .86			
20	0.27± .20	0.25 ±.15	0.002	65.96 ±32.37	6.66± 6.66	1.08 ±.91

SL, Shoot length, RL, root length;TDW, total dry weight; GP%, germination percentage; RWC%, relative water scontent.

index decreased with the increase in salt stress. Sabarmati (89%) showed highest STI as compared to Narendra 1(69%) and Hybrid 312 (56%). At 16 ds/m salinity stress all varieties showed the lowest salt tolerance (Table 6).

Effect on seed vigor: Seed vigor index declined with the increase in salt concentration. Hybrid 312 variety (21.6) showed strong seed vigor (Table 6). Salt stress reduced seedling vigor index from 11.5 to 1.1 (Table 7). Moisture content of seedlings plays an important role in various physiological processes including growth. It has also been reported that salinity suppresses the uptake of essential nutrients like P and K (Nasim *et al.* 2008), which could adversely affect seedlings growth and vigor. Prisco and Vieira (1976) reported that under stress conditions there is a decrease in water uptake both during imbibitions and seedling establishment and in the case of salt stress, this can be followed by uptake of ions. This results in physiological and biochemical changes in both anabolic and catabolic organs of the seeds and seedlings (Prisco *et al*. 1981, Gomes and Sodek 1988).

In conclusion, rice varieties were very sensitive to drought stress as compared to salinity stress. From the results of present investigation it can also be concluded that with increasing levels of water/ salt stress, seed germination and early seedling growth were adversely affected in all rice varieties. However, for all the physiological parameters like germination percentage, fresh and dry weight of shoot and root, length of shoot and root varietal difference were recorded and the difference was maximum towards higher stress levels. In response to water stress Sabarmati showed better performance in terms of germination while Narendra 1 showed better performance in terms of seedling length and weight. In salinity stress gradient, Hybrid 312 showed better response.

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