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Salinity stress on rice (*Oryza sativa* L.) crop and its amelioration

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Abstract
Salinity is a major abiotic stress factor worldwide as more than 45 million hectares of irrigated land have been damaged, and 1.5 million hectares are taken out of production each year as a result of high salinity. High salinity may cause a physiological drought condition, and imparts toxicity, nutritional disorders, oxidative stress, and alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, and all together growth and development of crop plants. Therefore, laboratory and pot experiments were conducted to evaluate ten upland rice genotypes (*viz.*, Bahadur, Joymati, Ashoni Bara, Moleegacbhuru, Gitesh, Monoharsale and Moniram from Assam, and OM 5451, OM 6976, OM 4900 from Vietnam) for tolerance to salinity condition(@ 0-30 mM ≈ EC 0, 40dSm^{-1}). In the study, seed germination and shoot vigour index were reduced significantly commensuration with the increase in salt (NaCl) concentration from 10 to 40 mM as compared to control. The effects of pre-treatments of seeds overnight separately with chemicals viz., Gibberellic acid (100ppm), Kinetin (100ppm), Thiourea (100ppm), Glucose (100ppm), Calcium chloride (100ppm), MoP (100ppm), improved seed germination, and shoot vigour index many fold under saline condition as compared to control. Reductions in yield and yield attributes viz., panicle length, panicle weight, test weight, economic yield, biological yield, harvest index and HD grains were found in all the varieties under soil salinity condition with 30mM NaCl. In the experiments, Joymati and OM 6976 were found physiologically efficient among the varieties tested against salt stress condition.

Keywords: Rice, economic yield, salinity, vigour index, test weight, harvest index, HD grains

Introduction
Rice (*Oryza sativa* L.) is one of the most important cereal food crops in the world. Rice is a salt sensitive crop (Maas and Hoffman, 1977) [19] and salinity impedes rice production in many areas worldwide (Flowers and Yeo, 1995) [6]. Rice sensitivity to salt varies according to growth stages (Lutts *et al*. 1995 [18]. Shannon *et al*. 1998) [29]. During germination, rice is more tolerant to salt than during other stages (Khan *et al*. 1997) [12]. It becomes sensitive at seedling stage, recovers a relative tolerance at vegetative stage, and again is very susceptible at reproductive phase (Zeng and Shannon, 2000) [34].
In rice, salinity affects yield components such as panicle length, spikelet number per panicle and grain yield, and it also delays panicle emergence and flowering. Moreover, percentage seed set and hence rice yield, is clearly reduced by salinity mainly due to lower pollen viability (Khatun *et al.* 1995) [14]. When rice plants are exposed to high salinity (>50mM NaCl), they suffer a rapid and temporary drop in stomatal conductance and growth rate (Moradi and Ismail, 2007) [22]. In addition to growth drop, exposure to osmotic stress, induces an increase in abscissic acid (ABA) concentration usually correlated with increased leaf or soil water potential (Moons *et al.* 1995) [20] and closure of stomata (Zhang *et al*. 2006) [36].
Although rice is a salt sensitive species and lakhs of varieties had been screened for salt tolerance (Negrao, *et al.* 2011) [24] no land races from the non- saline conditions of Northeast India have been tested for salinity, so that some of these varieties could be cultivated in saline conditions elsewhere. For instance, the Mekong Delta in southern Vietnam, which produces nearly half of the country's rice, is particularly vulnerable to global sea level rise. Prolonged inundation of fields and increased salinity threaten the viability of three-season rice farming in this densely populated, low-lying region, and could directly affect one million people by 2050. Hence the present work has been undertaken to study the physiology of some differentially habituated rice varieties under salinity condition.

Methods and Material
The experiment was carried out during spring season 2016 at the Department of Crop physiology, Assam Agricultural University, Assam, India, with ten direct seeded rice genotypes from Assam (Bahudur, Joymat,, Ashoni Bara, Moleegacbhuru, Gitash, Movoharsale

And Moniram) and Vietnam (OM 5451, OM 6976, OM 4900) following Randomised block design, replicated three times. Preliminary study of rice varieties for salinity stress was based on (i) Germination per cent and (iii) Vigour index (mean shoot length x germination per cent) of seedlings at 7 days after treatment with NaCl (0, 10, 20, 30 and 40 mM). A concentration of NaCl (e.g. 30 mM), and the varieties were again studied in pot experiment. Subsequently, to evaluate the ameliorative effects of some chemicals on germination and vigour index of the rice varieties affected by salinity, seeds were pre-incubated (overnight) with Gibberellic acid (100ppm), Kinetin (100ppm), Thiourea (100ppm), Glucose (100ppm), Calcium chloride (100ppm), MoP (100ppm), and then treated the seeds with NaCl (0, 30mM) solution in petridis separately for one week. Care was taken to wipe out the excess pre-treated solution, and NaCl solutions were poured as and when necessary to get rid of seed drying during the germination process. In the pot culture (acid mineral soil and FYM @50:50), NaCl @ 0, 30 mM (≈ EC 0, $30 dSm^{-1}$), 1000 cm^3 was added to the soil at vegetative and flowering stages (retained for maximum 7 days and flush with water afterwards). Then, the physiological responses of the crop varieties to salt stress were studied. Electrical conductance was measured in dilute (<0.1 $mol^{-}kg^{-1}$) aqueous NaCl solutions using Conductivity meter, Model 304. The soil pH (7.97) was measured by pH meter, Model 510 Bench Meter. The total rainfall (1314.6 mm), monthly mean bright sunshine (7.7 to 161.1 hours), mean maximum (27.4-34°C) & minimum (16.1-24.4 °C) temperature during the period March-July, 2016 were recorded.

Results and Discussion

Data presented in Table 1 reveal that the germination per cent was affected significantly by the salinity stress. The highest germination percentage was observed in Gitesh (58.33%), followed by Bahadur, and the lowest germination percentage was observed in Difulce (23.87%). Among the NaCl doses, the highest germination was observed in 10 mM (43.13%), and the lowest was in 40 mM (17.93%). Below 70 per cent germination was recorded in Difulce (28.33%), which was showing the nature of susceptibility to salinity. Bahadur, Joymati, Mulagabharu, Moniram, OM5451, OM 6976, exhibited the higher germination per cent (> 90%) under control condition. Difulce, Kushal, Peoyle showed 23.83%, 25.93%, 27.53% germination per cent respectively. Germination per cent decreased with the increase in salt concentration. Germination of seeds involves the activation of enzyme systems as well as mobilization of reserve foods, and these processes are adversely affected by NaCl (Levitt, 1980) [15]. It is clear from the study that the varieties responded differently to the doses of NaCl in respect of germination per cent. Decrease in germination percentage with the increase in salt concentration was also reported by Singh and Rana (1989) [30]. Presumably, the osmotic effect due to salinity was the main inhibitory factor that reduced germination as reported by Akbar and Ponnamperuma (1982) [2].

There were significant differences of vigour index due to treatment (Table 2) Among the genotypes, Monoharsale recorded the maximum (210.63) and Difulce had the minimum (68.98) vigour index. NaCl at10mM caused the maximum (143.36) and NaCl at 40 mM decreased germination to the minimum (47.92). In the present study, there was decreasing trend in vigour index when salt concentration increased in the treatments. Among the genotypes, the lowest reduction in vigour index was observed in Difulce (28.98, 39.98, 25.85,) followed by Aghoni Bora (19.58, 42.9, 51.44,) at 10, 20 and 30mM NaCl respectively as compared to the control. At 40mM NaCl, only Difulcee (37.64) could maintain the lowest reduction in vigour index. Even, the higher reduction in vigour index was found in Joymati (328.14). There were 49.08%, 58.47%, 64.19%, and 82.97% reductions of it at 10, 20, 30 and 40mM NaCl concentrations respectively as compared to control. Dhanapackiam, *et al*. (2010) [5] also reported that salt tolerant genotypes showed lesser reduction of vigour index commensuration with the increase in salt concentration.

It has been clear from the that there were significant effects of pre treatment on germination of seeds. Among the genotypes the highest germination percentage was observed in Gitesh (88.96%), followed by Mulagabharu (85.83%), and the lowest germination percentage was observed in OM 5451 (79.58%). Among the chemicals, the highest germination was observed in Gibberellin acid (85.83%) treatment, and the lowest was in distilled water as well as glucose (78.33%). Rate of germination at distilled water (control) was higher than in 30 mM salt condition. All the events starting from imbibitions of water to the establishment of seedling are adversely affected by increased levels of salinity. It cripples the rate and percentage of germination partially through the osmotic effects on the imbibitions of water, and mainly is due to toxicity to metabolism of seed reserves (Hossin *et al*. 2012) [9]. In the present study, the effects of some chemicals viz., $CaCl_2$, MoP, Thiourea, Gibberellic acid, Kinetin, and Glucose were used to alleviate the effects of NaCl on germination of some rice varieties. Over all, all the chemicals including simple water improved germination per cent of all the varieties by more than 78% under the saline condition. Some of the promising varieties with least reduction of germination e.g., Gitesh (0.07%) and Monoharsale (4.67%) were also found in the pre incubation experiment. Gibberellin helps in stimulation, fresh synthesis or counteracting the inhibitor of α-amylase activity in seed and improves percentage of germination (Khan and Rizvi, 1994) [11]. Kinetin breaks dormancy; enhances protein synthesis; promotes radicle emergence; offsetting salt damage and increased water uptake (Khan and Huang, 1988) [10]. Thiourea is involved in reversal of salt-inhibited germination (Noor and Khan, 1995) [25]. Sugars promotes embryo growth (Poljakoff-Mayber *et al.* 1994) [28]. Among the ingredients, Calcium enhances membrane activity; stimulates plumule emergence (Morgan, 1989), Potassium is a counter inhibition of root growth (Lin and Kao, 1995) [16].

Data presented in Table 4 showed that there were significant effects of pre treatment on vigour index of rice varieties. Among the genotypes, the highest vigour index was observed in Bahadur (827.08) followed by OM 4900 (760.71). The lowest vigour index was observed in Moniram (525.55). Among the chemicals, the maximum vigour Index (1,030.0) was found in Giberillic acid followed by $CaCl_2$ (708.22), and the minimum vigour index (473.89) was found in distilled water. The pre treatments improved vigour index in almost all the rice varieties under saline condition in a wider range. Therefore, GA, $CaCl_2$, Thiourea, Kinetin, and MoP are suitable for mitigating the adverse effects of salinity (NaCl) on rice. Seed vigour index is related to the germination per cent, shoot length or root length as considered for calculating vigour index (Abbasian and Moemeni, 2013) [1]. Salt stress inhibits the seedling growth with root and shoots length (Asaadi, 2009) [4]. High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant, which may be another reason for this decrease in vigour index (Werner and Frankelstein, 1995) [32].

There were significant variations of economic yield among the varieties due to the treatments. The varieties Joymati (13.93 g/plant) > Aghoni Bora (13.36 g/plant) > Moniram (12.05 g/plant) decreased economic yield under salinity condition. The lowest economic yield was found in Gitesh (7.72 g/plant). On an average total treatment mean, varieties Joymati (14.96 g) had the highest economic yield, and the lowest was in Gitesh (7.74%). Overall, salinity reduced economic yield significantly in the varieties as compared to the control. Salinity reduced economic yield significantly (0.64-22.12%) in the varieties under the present study

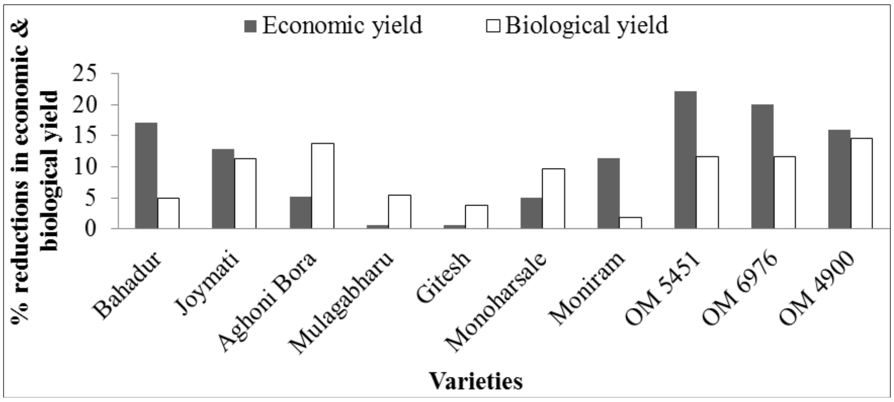

Fig 1: +ve values indicate decrease in economic and biological yields under salinity condition as compared to normal

Table 1: Effects of salinity on germination of rice varieties

Germination (%)						
Varieties	**Control**	**10 mM NaCl**	**20 mM NaCl**	**30 mM NaCl**	**40 mM NaCl**	**Mean**
Bahadur	98.66	53.33	50.66	48.33	17.72	53.74
Joymati	96.66	43.30	43.20	33.33	15.55	46.41
Aghoni Bora	85.00	45.60	45.00	41.60	25.87	48.61
Difulce	28.33	23.33	23.12	22.90	21.66	23.87
Mulagabharu	98.33	50.00	43.33	33.33	15.30	48.06
Gitesh	80.00	70.00	65.00	61.66	15.00	58.33
Kushal	83.33	15.00	11.66	11.66	8.00	25.93
Peoyle	75.00	20.00	16.00	15.00	11.66	27.53
Monoharsale	88.33	50.00	46.66	45.00	33.33	52.66
Moniram	96.00	63.33	51.66	46.66	15.79	54.69
OM 5451	95.00	28.33	21.66	19.84	16.66	36.30
OM 6976	98.33	55.00	51.66	41.66	18.75	53.08
OM 4900	84.46	43.50	38.56	34.72	17.84	43.82
Mean	85.19	43.13	39.09	35.05	17.93	
	S.Ed(±)	CD(0.05)				
Treatment (T)	4.69957	10.57558				
Variety (V)	2.349785	3.657044				
T × V teractions	3.323098	29.85685				

Among the varieties, OM 5451 (22.12%) had the highest reduction in economic yield followed by OM 6976 (20.11%) > Bahudur (17.04%) under salinity as compared to normal. The lowest reduction in economic yield was observed in Gitesh (0.64%) under salinity. Soil salinity is a major factor that limits the yield of agricultural crops, jeopardizing the capacity of agriculture to sustain the burgeoning human population increase (Flowers, 2004 [7]. Parida and Das, 2005 [26]. Munns and Tester, 2008) [23]. There were significant variations of biological yield among the varieties due to treatments. On an average, in natural condition, biological yield (24.94g/plant) was higher than in salinity (22.63g/plant) condition. Overall, salinity reduced biological yield significantly in the varieties as compared to the control.

There were significant reductions (3.77-14.53%) in biological yield among the varieties under salinity condition Among the varieties, OM 4900 had the highest reduction in biological (14.53%) followed by Ashoni Bara (13.81%) > OM 6976 (11.67%) under salinity as compared to normal. The lowest reduction was observed in Gitesh (3.77%) under salinity. The growing world-wide limitations on salinity stress and high-quality soils are becoming two major humanitarian and economic factors that limit crop yield (Toenniessen *et al*. 2003) [31].

There were significant variations in respect of panicle length among the varieties due to stress treatment. Overall, salinity reduced panicle length significantly in the varieties as compared to control. There were significant reductions (0.05-9.57%) in panicle length of the varieties under salinity condition Among the varieties, Monoharsale had the highest reduction in panicle length (9.57%) followed by OM 4900 (7.94%) under salinity as compared to normal. The lowest reduction was observed in OM 6976 (0.05%) under salinity. Increasing salinity reduces the average yield of major crops by more than 50%, and these losses are of great concern mainly for the agriculture based countries (Alam, 2014) [3].

There were significant variations of panicle weights among the varieties at harvest. Overall, salinity reduced panicle weight significantly in the varieties as compared to the control. There were significant reductions (0.62-13.24%) of panicle weight among the varieties under salinity condition

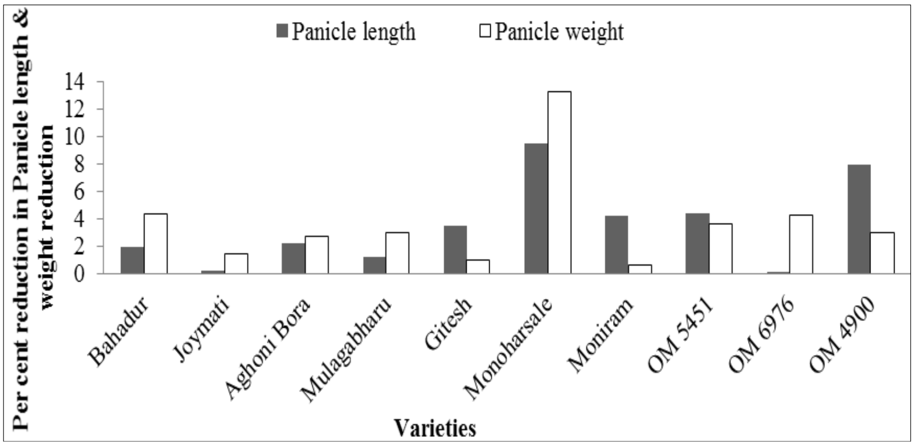

Fig. 2: +ve values indicate decrease in panicle length and weight under salinity condition as compared to normal

Table 2: Effects of salinity on Vigour index of rice varieties

Vigour index						
Varieties	Control	10 mM NaCl	20 mM NaCl	30 mM NaCl	40 mM NaCl	Mean
Bahadur	253.56	186.66	166.67	138.22	53.87	159.80
Joymati	376.97	194.42	171.07	130.65	48.83	184.39
Aghoni Bora	150.45	130.87	107.55	99.01	42.69	106.11
Difulce	95.47	66.49	55.49	69.62	57.83	68.98
Mulagabharu	223.50	120.83	73.77	65.82	34.15	103.61
Gitesh	327.49	52.50	32.65	31.72	28.56	94.58
Kushal	231.00	58.60	45.28	40.80	27.75	80.69
Peoyle	298.92	139.00	121.32	93.66	40.70	138.72
Monoharsale	362.40	276.50	200.85	176.96	36.45	210.63
Moniram	362.15	195.00	174.04	165.60	103.99	200.16
OM 5451	294.72	164.66	129.15	95.65	32.21	143.28
OM 6976	340.10	100.00	74.08	67.06	56.14	127.48
OM 4900	343.17	178.20	167.90	135.81	59.81	176.98
Mean	281.53	143.36	116.91	100.81	47.92	
	S.Ed(±)	CD(0.05)				
Treatment (T)	1.026645	22.25582				
Variety (V)	4.550275	81.76519				
T × V interactions	3.834123	68.89646				

Among the varieties, Monoharsale had the highest reduction in panicle weight (13.24%) followed by Bahudur (4.34%) > OM 6976 (3.59%) under salinity as compared to normal. The lowest reduction was observed in Moniram (0.62%) under salinity. These results indicate that the differential sensitivity at growth stages can be clearly shown when stages are well defined in the timing treatments and the stress is quantified at growth stages based on the same duration of salinization (Linghe, 2000) [17]. Salinity stress timing had a large influence on the overall sensitivity of rice to salinity (Zeng *et al*. 2001) [35].

In general, the 'Test weight' was higher under natural than the saline condition. Overall, salinity reduced 'Test weight' significantly in the varieties as compared to the control. There were significant variations of HI among the varieties due to the treatments

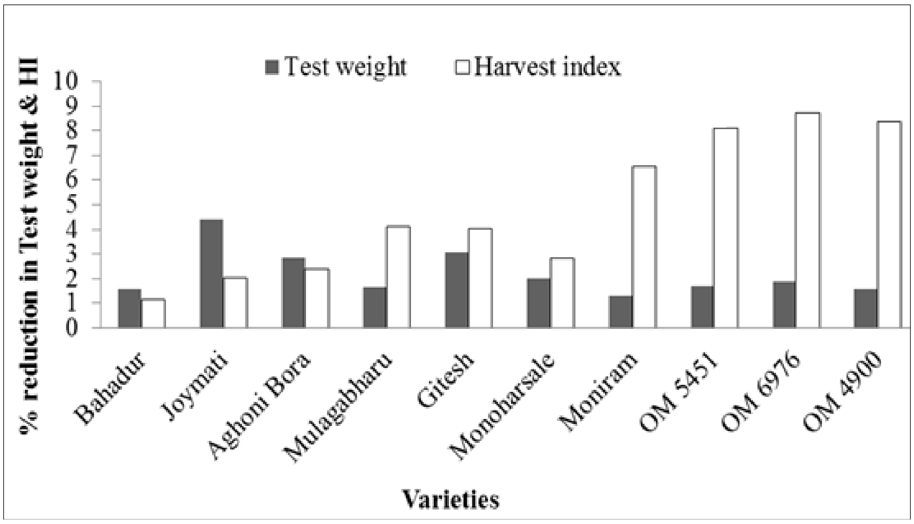

Fig. 3: +ve values indicate decrease in test weight and harvest index (HI) under salinity condition as compared to normal

Table 3: Effects of pretreatments of rice seeds with some chemicals on its germination following salinity

Germination (%)									
Varieties	Control (without chemical)	30 mM							
		Distilled water	Mop	$Cacl_2$	Kinetin	Thiorea	GA	Glucose	Mean
Bahadur	98.26	75.00	85.00	85.00	81.67	85.00	81.67	78.33	83.74
Joymati	98.33	83.33	80.00	98.33	76.67	71.67	88.33	86.67	85.42
Aghoni Bora	91.67	81.67	81.67	88.33	85.00	86.67	80.00	83.33	84.79
Mulagabharu	98.33	85.00	86.67	86.67	78.33	86.67	88.33	76.67	85.83
Gitesh	100.0	83.33	98.33	88.33	85.00	96.67	100.0	60.00	88.96
Monoharsale	80.33	73.33	81.67	78.33	75.00	85.00	85.00	85.00	80.46
Moniram	95.00	76.67	80.00	86.67	81.67	81.67	81.67	78.33	82.71
OM 5451	95.00	63.33	81.67	76.67	88.33	78.33	80.00	73.33	79.58
OM 6976	90.00	80.00	85.00	80.00	75.00	83.33	88.33	75.00	82.08
OM 4900	97.66	81.67	85.00	81.67	73.33	63.33	85.00	86.67	81.79
Mean	94.46	78.33	84.50	85.00	80.00	81.83	85.83	78.33	
	S.Ed(±)	CD(0.05)							
Treatment (T)	1.444472	25.95614							
Variety (V)	1.491811	6.80678							
T × V interaction	3.545843	11.56364							

Table 4: Effects of pretreatment of rice seeds with some chemicals on its vigour index following salinity

Vigour index									
Varieties	Control (without chemical)	30 mM							
		Distilled water	Mop	$Cacl_2$	Kinetin	Thiorea	GA	Glucose	Mean
Bahadur	935.44	735.00	1,116.90	1,065.9	917.97	630.70	1,156.45	418.28	872.08
Joymati	584.08	489.98	659.20	890.87	490.69	563.33	1,118.26	386.55	647.87
Aghoni Bora	462.02	432.85	573.32	473.45	737.80	809.50	1,003.20	581.64	634.22
Mulagabharu	448.38	397.80	402.15	861.50	747.27	747.10	1,015.80	415.55	629.44
Gitesh	730.00	631.64	827.94	669.54	810.90	827.50	1,380.00	528.00	800.69
Monoharsale	353.45	381.32	614.16	717.50	726.00	856.80	1,043.80	572.90	658.24
Moniram	389.50	305.15	622.40	483.62	529.22	666.43	770.96	437.08	525.55
OM 5451	543.40	372.38	362.61	726.83	565.31	452.75	1,028.80	293.32	543.18
OM 6976	410.40	408.00	661.30	438.40	342.00	738.30	660.71	709.50	546.08
OM 4900	523.46	584.76	778.60	754.63	775.83	747.29	1,122.00	799.10	760.71
Mean	538.01	473.89	661.86	708.22	664.30	703.97	1,030.0	514.19	
	S.Ed(±)	CD(0.05)							
Treatment (T)	3.18244	4.573859							
Variety (V)	2.20098	2.286929							
T × V interaction	12.7062	3.234207							

There was higher HI in rice crop under natural condition as compared to saline condition. Overall, salinity reduced HI significantly in the varieties as compared to the control. There were significant reductions (1.28 - 4.38%) in 1000grains weight (test weight) in the varieties under salinity condition Among the varieties, Joymati (4.38%) had the highest reduction in test weight followed by Gitesh (3.00%) > Ashoni Bara (2.85%) under salinity as compared to normal. The lowest reduction was observed in Moniram (1.28%) under salinity. Similary, there were significant reductions in HI in the varieties under salinity condition. Among the varieties, OM 6976 had the highest reduction in HI (8.69%) followed by OM 4900 (8.37%) > OM 5451 (8.09%) under salinity as compared to normal. The lowest reduction was observed in Bahudur (1.12%) under salinity. Salinity effects were highly significant on grain yield, plant stand, seed weight per panicle, and spikelet per panicle, but not significant on panicle density, kernel weight, and shoot weight per plant at seeding densities tested. (Peng, *et al*. 1999) [27].

Conclusion
The foregoing discussion convince that salinity chracterised by higher soil Ph, higher EC and lower range of N, P, K in soil, affects physiological processes of rice crop right from germination to growth and development, which reduces yield and vital yield attributing parameters of the crop ultimately. Besides, a few varieties viz., Joymati>Bahadur>Gitesh, Monoharsali from Assam, and OM5451> OM4900 have been sorted out as salt tolerant in the study. The up-regulation of germination, vigour index, panicle weight, HI, economic yield, biological yield of the varieties imparted salt tolerance to the rice varieties. The fact is that salinity can damage plants through its osmotic effect which is equivalent to a decreased in water activity, through specific toxic effects of ions, and by disturbing the uptake of essential nutrients.

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