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Effect of salinity and alleviating role of gibberellic acid (GA_3) for improving the morphological, physiological and yield traits of rice varieties

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Abstract

An experiment was conducted in pots at the glasshouse of the Universiti Putra Malaysia during 2010 – 2011 to determine the salinity tolerance of two rice varieties exposed to GA_3. One internationally recognised salt tolerant rice variety (Pokkali) and one Malaysian well-cultivated rice variety were studied under five salinity levels with GA_3. The results revealed that the studied morphological traits such as plant height, tillers plant^{-1}, leaves plant^{-1}, leaf length and plant dry and the physiological attributes, chlorophyll *a*, *b*, total chlorophyll contents, photosynthetic rates, stomatal conductance and transpiration rate were reduced significantly with increasing saline condition in both of varieties. The transpiration rate was also reduced in both varieties, which showed less intercellular CO_2 at higher salinity. Identical findings were also noted for the vapour pressure deficit in leaves (VPDL). MR219 showed more slat affected than Pokkali in some parameters but the saline effects alleviated when GA_3applied. The present study concludes that GA_3, a safe plant growth regulator, could be effectively sprayed on rice variety MR219 in saline belts as it adequately proved its unique salinity alleviating role.

Keywords: Salinity, Gibberellic Acid, Rice, Malaysia

Abbreviations: GA_3_Gibberellic acid, VPDL_vapour pressure deficit in leaves, PGR_plant growth regulator, LSD_Least Significant Difference.

Introduction

Soil salinity is a global eco-threat to sustainable agriculture, and is also increasing over the time. It may be due to uses of fertilizers, soil erosion, rising in sea level from greenhouse emission leading to global warming and ice-belt melting, and natural disasters causing submersion of croplands under sea water (Brinkman, 1980). Salinity reduces growth and finally causes death through osmotic, ionic and nutritional imbalances (Afifi et al., 2010). Sensitive varieties loss vigour quickly by losing water from the stress shocks. But resistant genotypes can tolerate well and survive in severely saline soils (Blaylock, 1994). This eco-adjustment attracts global researchers to quest for the salt tolerant cultivar(s) from the surroundings. Gibberellic acid (GA_3), a safe plant growth regulator (PGR), alleviates salinity-induced inhibition of seed germination of glycolphytic plants (Basalah and Mohammad, 1999). Reversal of the harmful effects of NaCl on seed germination (Tipirdamaz et al., 1995) and seedling growth (Kaur et al., 1998) due to exogenous GA_3 are attributed to the stimulation of α-amylase activity. So, attention is now focused on uses of GA_3 in regulating plant responses to the external environment and controls a number of stress-induced genes (Naqvi, 1999). Innumerable works proved the potential of GA_3 to synergistically improve crop performance under normal conditions. But the focus on spraying during salt stress is scarce. A few studies; however, pinpointed the ability of its foliar pre-treatment to overcome adversities of NaCl (Chakraborti and Mukherji, 2003) as it alleviates the pessimistic effects on pigment contents and water use efficiency (Aldesuquy and Ibrahim, 2001).

More than half of the world population depends on rice as staple food. In Asia alone, there are near two billion consumers (Rao et al., 2007). In Malaysia, it is the third top raking crop covering 205,548ha (Ministry of Agriculture, 2007) meeting about 70% of the local demand (Bernama, 2008). To fulfill the increasing future need, Malaysia must expand rice area (Selamat and Ismail, 2009). It is an anxiety that the salinity may affect 100,000 ha of rice area by 2056 (Selamat and Ismail, 2008). Continuous intrusion of saline water may result in dwindling rice area leading to food shortages in domestic and global markets. So, researchers and policy makers must pave ways for the efficient exploitation of saline areas. The assortment of salt tolerant rice varieties might be its finest access to bring such areas under rice (Shereen et al., 2005). By now, much works are done to realize the role of salinity on seed germination, growth, reproduction and population dynamics of crops (Khan et al., 2002). Nonetheless, information on Malaysian rice varieties for saline zones is scanty. But the enhancement of salt tolerance in crops is challenging as it is linked with multiple physio-biochemical pathways. Therefore, the present work was undertaken to evaluate the rice varieties as well as fruitful rice production strategies on the Malaysian saline belt using spraying GA_3.

Results and Discussions

Effect of salinity and GA_3 on the morphological traits

Plant height (cm)

The plant height across all salinity levels increased as proceeded towards the 6^{th} week after GA_3 treatment. In Pokkali, the maximum heights (168 and 164 cm) were noted during the 4^{th} and 6^{th} weeks followed by MR219. Thus, GA_3 was much effective in having taller plants (Table 1). The overall observation also shows that Pokkali was taller than MR219. Irrespective of varieties, plant height was observed 179.9 and 174 cm in Pokkali and MR219, respectively under control treatment. But salinity decreased the plant height and that was proportional to the imposed salinity level. In the saline condition, Pokkali produced significantly taller plants than MR219. But under the higher salinity (200 mM), both varieties showed more decrease in height, 138.8 and 114.9 cm, in Pokkali and MR219, respectively (Table 2). The varieties responded well to GA_3 in recording taller plants as 178.4 and 176.1 cm in Pokkali and MR219, respectively, which was statistically similar. However, dwarf plants were observed in both varieties without GA_3 treatment (Table 3). GA_3 showed its salinity alleviating role significantly. In the non-saline conditions, height increased linearly with application of GA_3. But due to GA_3 application, the height was increased significantly under salinity. Plants at 50-200 mM GA_3 showed 124, 127.4, 115.6 and 109 cm height, respectively. By application of GA_3 the height increased 192.1, 181.6, 160.0 and 144.7cm, respectively. The decreasing trend in height was noted with the increased salinity. The effect of GA_3 was also consistent over the salinity levels (Fig. 1). The results showed a decreasing trend in height with increasing levels of salinity. But GA_3 alleviated the harmful effects of salinity and enhanced plant height. The height was higher in Pokkali than MR219 across salinity levels and GA_3. That could be predicted due to genetic potentials of varieties. Such variations for salt tolerance in rice and other species were already reported (Moud and Maghsoudi, 2008). It is clear that both varieties had high degree of pessimism for taller plants using GA_3 in saline condition. Reports are also available that salinity can reduce height in rice (Motamed et al., 2008). Gain et al. (2004) also noted that height was reduced significantly at 7.81dS m^{-1} salinity. In our study, both varieties were more responsive to GA_3 and to increase the height. Similar results were claimed by Watanabe and Saigusa (2004) that height increased significantly due to GA_3 over salinity. The results of this study are also in agreement with Suge (1985) who found that GA_3 enhanced growth through forming new cells in the intercalary meristem.

Tillers plant^{-1} (no.)

It was significantly different ($P<0.05$) in various sowing dates as well as in all forms of interactions. Maximum tillers (5.67-5.46) were produced during the 2^{nd} and 4^{th} weeks after GA_3 treatment in MR219 and Pokkali, respectively. But during the 6^{th} week, both the varieties produced the least tillers. That might be due to alleviating impact of GA_3 during the early growth (Table 1). Tiller number was decreased significantly as the salinity raised from 50 to 200 mM. The NaCl applied at 100-200 mM reduced the tillers (4.6-1.9) in MR219. Pokkali produced a little bit more tillers than MR219 under the salinity levels (Table 2). GA_3 significantly increased the tillers plant^{-1}, 5.4 and 5.2 in Pokkali and MR219, respectively. But the varieties differed notably among themselves. The less tillers were noted without GA_3 (Table 3). The linear regression amid salinity and GA_3 showed the declined trend for tillers plant^{-1} due to salinity. But GA_3 showed increasing trend over the salinity. Thus, it could be predicted that GA_3 was effective in overcoming the harmful effects of salinity (Fig. 1). The interaction of variety × salinity × GA_3 was significant. At 50-200 mM, MR219 recorded 6.2, 4.4, 1.9 and 1.6 tillers, respectively. But GA_3 raised the tillers notably, 6.8, 4.9, 2.4 and 2.3, respectively. Pokkali, at 50-200 mM had less, 6.1, 5.2, 2.3 and 1.9, respectively. In this situation, GA_3 enhanced tillers of Pokkali as 7.4, 6.2, 2.7 and 2.1. More tillers were produced in Pokkali than MR219 across GA_3 and salinity treatments (Table 4). Reports are available on fall in the growth rate due to salinity (Munns and James, 2003). The fall in tillers are occurred due to salinity over the control (Castillo et al., 2007). In the present study, GA_3 had optimistic effects on both the varieties. Similar results were argued by Emongor, (2007) that GA_3 was positive to enhance many growth and development processes. Kariali and Mohapatra, (2007) also noted that tillering in rice was dynamic, adjustable and phytohormones played a vital role in it. But the foliar spray of GA_3 changed the allocation pattern of carbohydrates in shoots, and thus inhibited tillers in rice (Yin et al., 1997).

Plant dry weight (g pot^{-1})

The highest dry weights were produced with GA_3: 14.4 and 14.2g pot^{-1} in MR219 and Pokkali, respectively, during the 6^{th} and 4^{th} weeks. Both varieties showed least affected at 50 mM salinity. However, further increase in the salinity reduced the dry weights: 5.6 and 4.8g pot^{-1} in Pokkali and MR219, respectively (Table 2). Both of the varieties (Pokkali and MR219) increased dry weights (13.59 and 11.60g pot^{-1}) when GA_3 applied but had less weight without GA_3 (Table 3). The linear regression showed the declining pattern in different salinity levels without GA_3. But it raised the dry weight over the salinity (Fig. 1). The dry weight decreased significantly with increasing the salinity levels. In MR219, under 50-200 mM salinity, the dry weights were decreased over the control: 10.8, 10.5, 5.5 and 4.2g pot^{-1}, respectively. However, under GA_3 this variety produced more dry weights: 13.4, 11.7, 8.3 and 5.4g pot^{-1} at salinity levels (Table 4). Pokkali produced the lower dry weights: 11.4, 9.8, 8.2 and 4.8g pot^{-1} under same saline condition but GA_3 had alleviating impact as it significantly increased the dry weights: 14.3, 14.3, 10.9 and 6.3g pot^{-1}, respectively (Table 4). The fall in the dry weight was echoed in the elevated metabolic energy cost and reduced carbon gain due to salinity (Karimi et al., 2005); thus, raised the harms of salinity for biomass production linearly in rice (Carmona et al., 2009). The reduced dry weight was also associated with photosynthetic rate (Ashraf, 2004). The stress suppressed plant height, leaf number and size, and tillers, which finally lowered the dry matter (Khan and Abdullah, 2003). Iqbal, (2008) noted reduction in dry weight of shoot due to salinity. Salinity lowered the growth rate and biomass production (Lin and Kao, 2001). Pattanagul and Thitisaksakul, (2008) also argued about reduce in dry biomass under higher salt stress, and that was possibly for the decrease in carbohydrate build

Table 1. Growth traits of two rice varieties at various weeks after GA_3 application across salt concentrations.

Vegetative growth traits	SE	LSD (5%)	Weeks after GA_3 application					
			MR219			Pokkali		
			2^{nd}	4^{th}	6^{th}	2^{nd}	4^{th}	6^{th}
Plant height (cm)	2.15	13.080	125 d	148 c	151 bc	146 c	164 ab	168 á
Tillers plant^{-1} (no.)	0.12	0.717	5.67 a	5.46 a	4.67 b	5.63 a	5.56 a	3.86 c
Plant dry wt. (g pot^{-1})	0.77	4.705	7.4 c	10.2 abc	14.4 a	9.2 bc	14.2 a	12.5 ab

In each row, the means with the common letter do not differ significantly at the 5% level of probability.

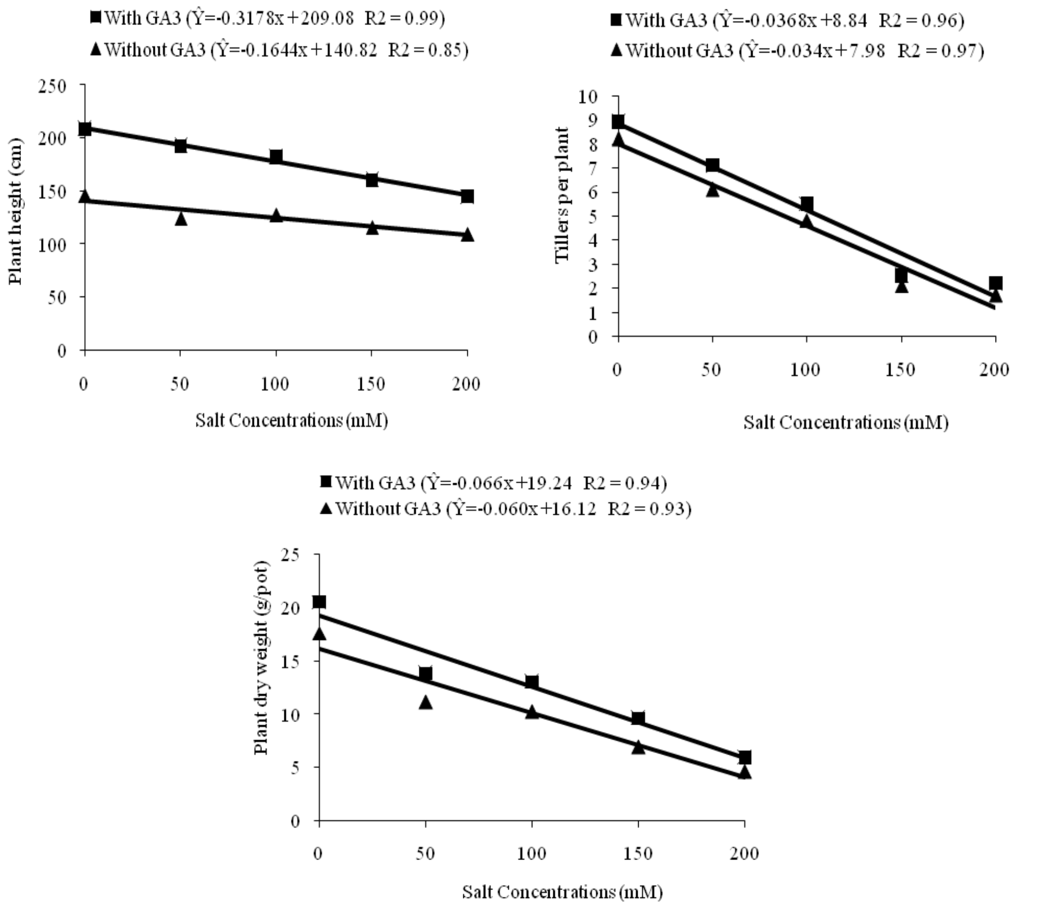

Fig 1. Linear regression among salinity levels and GA_3 on plant height, tillers plant^{-1} and plant dry matter of two rice varieties

up caused by reduced carbon assimilation. In our study, Pokkali and MR219 showed reduction in dry weight at different salinity levels with no GA_3. Conversely, plants under GA_3 treatments increased the dry weight. These results are in agreement with the findings of Ashraf et al. (2002) that GA_3 in salt stressed plants showed an increased photosynthetic capacity- a vital factor for higher dry matter synthesis.

Effect of salinity and GA_3 on physiological traits

Chlorophyll contents (mg g^{-1} of fresh weight)

Chlorophyll a content

It differed significantly (P<0.05) for the interaction of variety × salinity, variety × GA3, salinity × GA3, and variety × salinity × GA3. The higher chlorophyll *a* (2.36 mg g^{-1}) was found in Pokkali during the 2^{nd} week. Similar trend was also observed in MR219; being higher during the 2^{nd} week and reduced at the 4^{th} and 6^{th} weeks. Those could be due to salinity causing yellowing, abscission and wilting of foliage (Table 5). The chlorophyll *a* in both varieties was significantly higher under saline control condition. Both varieties showed insignificant variation of chlorophyll *a* in control condition and 50 mM salinity by having 2.04 and 2.0, and 1.78 and 1.70 mg g^{-1}, respectively. Thereafter, the raised salinity declined the chlorophyll *a* (Table 6). Pokkali under GA_3 spraying gave significantly higher chlorophyll *a* (1.86) than control. Similarly, MR219 had chlorophyll *a* (1.62) (Table 7).

The interaction of variety × salinity × GA3 had significant disparity for it. The raised stress notably lessened it in both varieties, in which MR219 had relatively less chlorophyll *a*: 1.47, 1.26, 1.12 and 1.01 at 50-200 mM, respectively. GA3 notably enhanced and produced 1.94, 1.49, 1.47 and 1.16 chl*a* at 50-200 mM, respectively. Pokkali at same salinity also showed least chl*a*: 1.82, 1.42, 1.17 and 1.08. But GA_3 raised it notably: 2.25, 1.64, 1.49 and 1.73 in Pokkali at 50-200 mM, respectively (Table 8). The response of a variety to salinity is predicted as its genetic background to tolerance (Moud and Maghsoudi, 2008). The ability of a tolerant variety to maintain higher level of chlorophyll *a* is probably one of the vital mechanisms aiding to salinity tolerance,

Table 2. Growth traits of two rice varieties as affected by different salt concentrations.

Varieties × Salt conc. (mM)		Plant height (cm)	Tillers plant^{-1} (no.)	Plant dry wt. (g pot^{-1})
MR219	0	174.0 ab	9.1 a	18.4 a
	50	145.4 de	6.5 c	12.1 bc
	100	148.4 d	4.6 e	11.1 bc
	150	126.7 f	2.2 fg	6.9 de
	200	114.9 g	1.9 g	4.8 e
Pokkali	0	179.9 a	8.1 b	19.7 a
	50	170.7 b	6.7 c	12.8 b
	100	160.5 c	5.7 d	12.1 bc
	150	148.9 d	2.5 f	9.6 cd
	200	138.8 e	2.0 g	5.6 e
SE		2.775	0.152	0.998
LSD 5%		7.771	0.426	2.795

In each column, the means with the common letter do not differ significantly at the 5% level of probability.

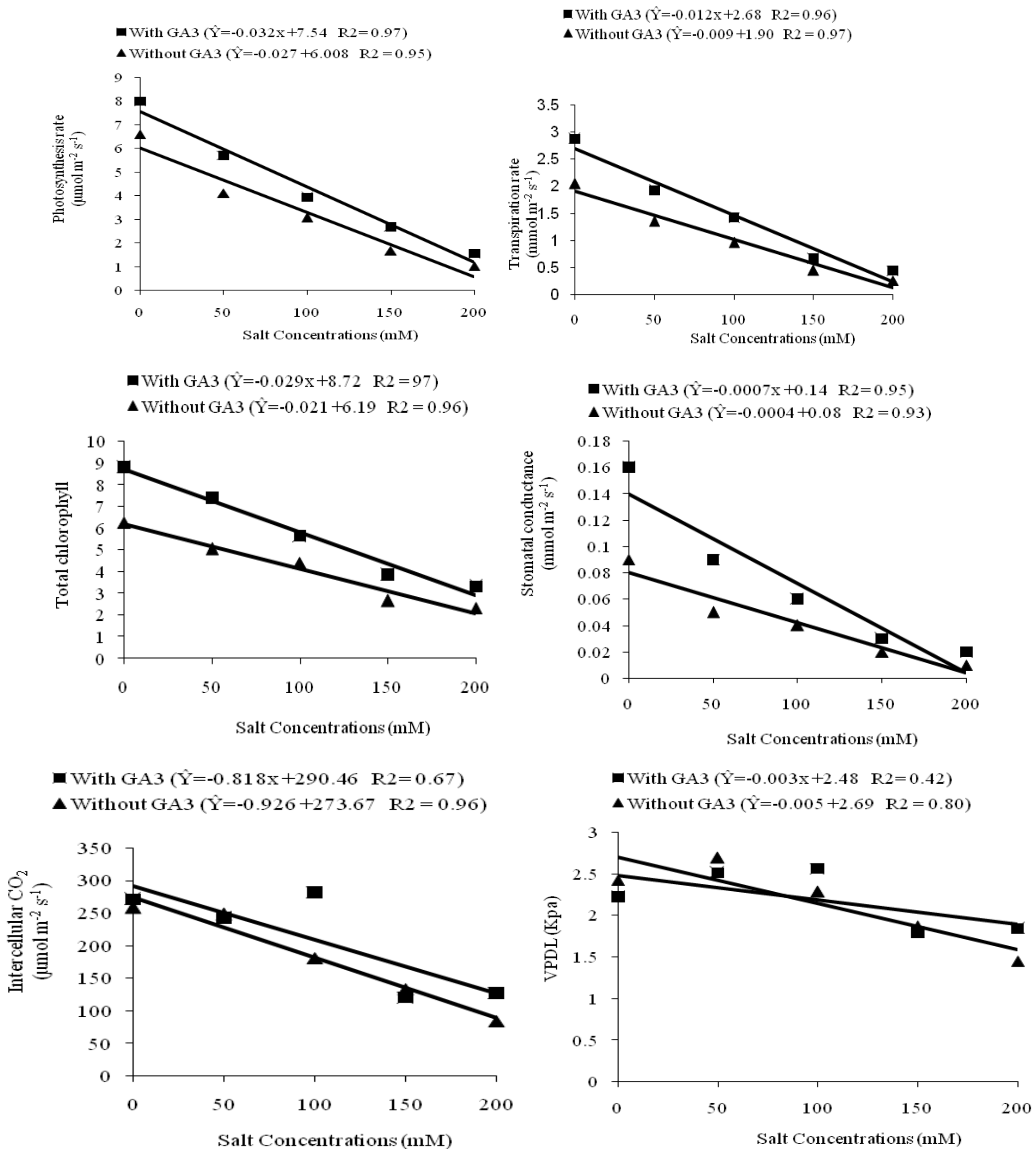

Fig 2. Linear regression amid salinity levels and GA_3 on the photosynthesis, transpiration, chlorophyll contents, stomatal conductance, intercellular CO_2 and VPDL of two rice varieties.

which results in higher photosynthesis vis-á-vis carbohydrate production (Datta et al., 2009).

Considering the chlorophyll a as the main pigment (Santo, 2004), reduce in the chlorophyll could probably be one of the vital cause of reduced photosynthesis under salt stress as noted in rice (Moradi and Ismail, 2007). Datta et al. (2009) also observed significant differences in chlorophyll *a* content, which could be predicted as the abilities of both varieties to maintain higher chlorophyll *a*, and the optimistic response to GA_3.

Chlorophyll b content

GA_3 showed significant positive role on chlorophyll *b*. Its level found to be higher (7.32) in MR219 during the 2nd week. Then, less chlorophyll *b* was found during the 6th week in both the varieties (Table 5). MR219 produced 5.13, 4.17, 1.94 and 1.44 chlorophyll *b* followed by 3.58, 2.97, 1.95 mg g^{-1} and 1.71 in Pokkali at 50-200 mM, respectively (Table 6). The relation of variety × GA_3 showed that chlorophyll *b* was notably low without application of GA_3, whereas it increased to 4.16 and 3.97, in MR219 and Pokkali, under GA_3 (Table 7). The interaction of variety × salinity × GA3 had notable disparities for it. The rising salinity significantly arrested in both varieties. In the saline conditions, MR219 produced 4.29, 4.0, 1.38, 1.25 chlorophyll *b* at 50-200 mM, respectively. However, MR219 produced greater chlorophyll *b* with GA_3, 5.96, 4.36, 2.50 and 1.64, respectively under the same saline conditions. It was noted that Pokkali produced: 2.51, 2.13, 1.65 and 1.30 at 50-200 mM, respectively, but with GA_3 application higher chlorophyll *b* were observed (4.65, 3.82, 2.26 and 2.12) (Table 8). The photosynthetic pigments were decreased at different salinity levels (Khan, 2003). The results are similar with those of Jaleel et al. (2008b). The decrease in chlorophyll content in the stressed plants could be due to increased activity of the chlorophyll-degrading enzyme chlorophyllase (Reddy et al., 1986).

Total chlorophyll content

Across the salinity levels and GA_3, it followed the same pattern as in the chlorophylls *a* and *b*, being higher (9.28) during the 2nd week in MR219 followed by Pokkali (6.63). The total chlorophyll is the combination of chlorophylls *a* and *b*. So, both (*a* and *b*) were increased linearly, which ultimately increased the total chlorophyll contents (Table 7). GA_3 had positive effect by raising the chlorophyll contents. The positive effect of GA_3 was constant by having more chlorophyll than stressed condition (Fig. 2). The interaction of variety × salinity × GA_3 appreciably reduced the total chlorophyll content. MR219 obtained less chlorophyll content: 5.76, 5.26, 2.51 and 2.26 mg g^{-1}, respectively under 50-200 mM. However, with GA_3 application the chlorophyll content was increased: 7.91, 5.84, 3.97 and 2.79 at 50-200 mM, respectively. Pokkali also produced: 4.34, 3.55, 2.82 and 2.38 mg g^{-1} under 50-200mM, while GA_3 raised the chlorophyll: 6.91, 5.45, 3.76 and 3.85, respectively (Table 8). The effects of salinity on chlorophyll synthesis varied with the levels of the stress (Santo, 2004). But significant differences amid genotypes were noted by Datta et al. (2009). Few conflicting reports pinpointed that the amounts of chlorophyll *a*, *b* and their total were higher under salinity conditions than the control one. Hence, the present findings disagree with Santo (2004). The depletion may be as the result of the hang-up of chlorophyll biosynthesis following an increase in ethylene production brought by the elevated NaCl content (Khan, 2003). It may be caused that the GA_3 generated sweetening of ultra-structural morphogenesis of plastids coupled with the retention of chlorophyll and delay of senescence caused by GA_3 (Arteca, 1997). Goswami (1995) claimed that the salinity caused the reduce in chlorophyll *a* and carotenoids in cotton but GA_3 raised those significantly. Radi et al. (1989) argued that in maize, chlorophylls (*a*+*b*) and carotenoids go up due to salinity stress but GA_3 caused a further rise in those pigments.

Photosynthetic rate (μmol m^{-2} s^{-1})

Salinity significantly inhibited photosynthesis in both varieties. But the rates were higher (8.09 and 6.49 μmol m^{-2} s^{-1}) under saline condition. However, GA_3 × variety increased the rates in both the varieties. The higher rate (4.86) was in MR219 followed by Pokkali (3.89) under GA_3 (Table 7). The linear regression visualizes that the rise in salinity declined photosynthesis (Fig. 2). The Photosynthetic rate significantly differed in the interaction of variety × salinity × GA_3. The photosynthesis notably decreased in both the varieties with increased salinity levels. But GA_3 showed the alleviating effect in both varieties. MR219 showed significantly higher photosynthesis under GA_3 treatments (6.69, 4.27, 3.10 and 1.34μmol m^{-2} s^{-1}) at 50-200 mM salinity, while in Pokkali the GA_3 raised the rate by noting higher values (4.71, 3.59, 2.28 and 1.78 μmol m^{-2} s^{-1}) at 50-200 mM, respectively, compared to no GA_3 condition (Table 8). The reasons of decreased photosynthetic rate might be attributed to the fall in chlorophyll contents, stomatal closure, transpiration, CO_2 assimilation by leaf tissues and finally, plant growth (Misra et al., 2002; Tardieu, 2005). The present findings opposed Yeo et al. (1985) who opined that photosynthesis was hardly retarded by salinity. Others claimed a notable fall under salinity (Kapulnik and Heuer, 1991). Such a decline was also pointed by Drew (1990). The photosynthesis fall could be crucial under salt stress (Moradi and Ismail, 2007).

Stomatal conductance (mmol m^{-2} s^{-1})

There was significant disparities ($P<0.05$) for the interaction of variety × weekly observation, variety × salinity, variety × GA_3, salinity × GA_3, and variety × salinity × GA_3. The weekly study across the salinity levels and GA_3 noted higher conductance (0.08 μmol m^{-2} s^{-1}) in MR219 during the 2nd and 4th weeks. But at the 6th week, the reduce was noted in both the varieties (Table 5). It could be due to drop in chlorophyll (*a*+*b)* contents, photosynthesis and unpleasant effects of salinity at the 6th week. The salinity levels notably inhibited the stomatal conductance in both varieties. But as the salinity was raised up to 200 mM, a sharp decline was noted in both the varieties (Table 6). GA_3 improved the stomatal conductance for both the varieties. But the higher value (0.09) was in MR219, while Pokkali showed reduced conductance (0.05 mmol m^{-2} s^{-1}) (Table 7). The linear regression shows decreasing trend in conductance with increasing salinity under no GA_3 conditions. But the salinity regression and GA_3 lines intersected at 200 mM indicating that GA_3 was not so effective at higher salinity conditions (Fig. 2). The interaction of variety × salinity × GA_3 showed significant effects in both varieties. In saline condition, MR219 had low conductance, 0.06, 0.03, 0.02 and 0.01 mmol m^{-2} s^{-1} at 50-200mM, respectively. But under GA_3 treatments we noted greater conductance, 0.14, 0.06, 0.03 and 0.02 mmol m^{-2} s^{-1} at saline condition. While Pokkali recorded 0.04 mmol m^{-2} s^{-1} at 50 and 100 mM but at the higher salinity (150 and 200mM) Pokkali noted 0.01mmol m^{-2} s^{-1}. In this study,

Table 3. Growth traits of two rice varieties under the influence of GA_3.

varieties x GA_3 (150 ppm)		Plant height (cm)	Tillers plant^{-1} (no.)	Plant dry wt. (g pot^{-1})
MR219	0	107.7 c	4.5 b	9.768 b
	GA_3	176.1 a	5.2 a	11.60 b
Pokkali	0	141.1 b	4.6 b	10.37 b
	GA_3	178.4 a	5.4 a	13.59 á
SE		1.755	0.096	0.631
LSD 5%		4.915	0.269	1.768

In each column, the means with the common letter do not differ significantly at the 5% level of probability.

Table 4. Growth traits of two rice varieties under the interaction of variety × salinity × GA_3.

Rice varieties	Salinity levels (mM)	Plant height (cm)		Tillers plant^{-1} (no.)		Plant dry weight (g pot^{-1})	
		GA_3 (ppm)		GA_3 (ppm)		GA_3 (ppm)	
		0	150	0	150	0	150
MR219	0	138.2	209.9	8.8	9.3	17.6	19.1
	50	102.3	188.4	6.2	6.8	10.8	13.4
	100	112.7	184.2	4.4	4.9	10.5	11.7
	150	96.78	156.7	1.9	2.4	5.5	8.3
	200	88.4	141.4	1.6	2.3	4.2	5.4
Pokkali	0	153.6	206.2	7.7	8.6	17.5	22
	50	145.7	195.7	6.1	7.4	11.4	14.3
	100	142.1	179	5.2	6.2	9.8	14.3
	150	134.4	163.3	2.3	2.7	8.2	10.9
	200	129.6	148	1.9	2.1	4.8	6.3
SE		3.925		0.215		1.412	
LSD (5%)		10.99		0.603		3.953	

Table 5. Physiological traits in various weeks after sowing across varieties salinity and GA_3.

Physiological traits	SE	LSD (5%)	Weeks after GA_3 application					
			MR219			Pokkali		
			2nd	4th	6th	2nd	4th	6th
Chlorophyll *a* (mg g^{-1})	0.520	0.320	1.96 b	1.87 b	0.49 c	2.36 á	2.18 ab	0.43 c
Chlorophyll *b* (mg g^{-1})	0.170	1.052	7.32 a	2.18 cd	1.71 d	4.27 b	2.85 c	2.18 cd
Total chlorophyll (mg g^{-1})	0.181	1.136	9.28 a	4.07 c	2.20 d	6.63 b	5.03 c	2.61 d
Photosynthetic rate (µmol m^{-2} s^{-1})	0.013	0.078	5.88 a	4.64 c	1.99 e	5.80 b	3.59 d	1.10 f
Stomatal conduct. (mmol m^{-2} s^{-1})	0.0002	0.111	0.08 a	0.08 á	0.04 c	0.05 b	0.04 c	0.02 d
Transpiration rate (mmol m^{-2} s^{-1})	0.0034	0.035	1.98 a	1.45 b	0.91 e	1.35 c	0.99 d	0.72 f
Intercellular CO_2 (µmol mol^{-1})	0.634	4.98	248.4 a	228.6 b	144.6 f	200.5 c	159.5 d	187.4 e
VPDL (KPa)	0.0012	0.011	2.44 c	2.11 d	1.38 f	2.52 b	2.69 a	1.81 e

In each row, the means with similar letters do not differ significantly at the 5% level of probability.

GA_3 slightly improved conductance and recorded 0.04, 0.05, 0.02 and 0.01mmol m^{-2} s^{-1} in Pokkali (Table 8). The reduction in growth is generally observed in plants under salinity. It may be partly due to lower water potential in cells, which caused stomatal closure and limited CO_2 assimilation. Salinity leads to dehydration and osmotic stress resulting in stomatal closure, reduced CO_2 supply and a high production of reactive oxygen species causing irreversible cellular damage and photo-inhibition (Darwish et al., 2009).

Transpiration rate (mmol m^{-2} s^{-1})

The transpiration rate across the salinity and GA_3 recorded higher rate (1.98) in MR219 during the 2nd followed by 4th weeks (1.45µmol m^{-2} s^{-1}). But Pokkali had low rates from the 2nd - 6th weeks. Both the varieties produced poor transpiration rates during the 6th week (Table 5). Both the varieties had better transpiration rate under control condition and it sharply declined with salinity (Table 6) but the rate increased significantly under GA_3. The highest rate (1.83) was noted in MR219 due to the positive impact of GA_3 but Pokkali was eventually 2nd (1.10 mmol m^{-2} s^{-1}). Both varieties showed reduced transpiration rate at no GA_3 condition (Table 7). The linear regression line shows decreasing trend in transpiration with the increase in salinity without GA_3. But higher rate was noted in GA_3 compared to the saline conditions. That increase could be predicted due to GA_3 and it was steady in response to salinity. However, at the highest salinity (200 mM) GA_3 failed to play its usual salinity relieving role (Fig. 2). The interaction of variety × salinity × GA_3 was remarkable. The increase in salinity significantly lowered the rates in both varieties MR219 at 50-200mM which had low rates: 1.60, 0.83, 0.57 and 0.22 mmol m^{-2} s$^{-1,}$ respectively, while under GA_3 had higher rates: 2.81, 1.46, and 0.87 and 0.49 mmol m^{-2} s^{-1} at 50-200 mM, respectively. In Pokkali, GA_3 acted positively and notably raised transpiration: 1.38, 0.44 and 0.39mmol m^{-2} s^{-1} at 100-200mM, respectively. In that case, Pokkali had non-significant differences for transpiration rate amid 50 mM and GA_3 (Table 8).

Table 6. Physiological traits of two rice varieties as affected by salt concentrations.

varieties × Salt conc. (mM)		Chloro-phyll *a* (mg g^{-1})	Chloro-phyll *b* (mg g^{-1})	Total chloro-phyll (mg g^{-1})	Photo-synthetic rate (μmol m^{-2} s^{-1})	Stomatal conduct. (mmol m^{-2} s^{-1})	Transpi-ration rate (mmol m^{-2} s^{-1})	Inter-cellular CO_2 (μmol mol^{-1})	VPDL (KPa)
MR219	0	1.78 b	5.99 a	7.77 a	8.09 a	0.17 a	2.82 a	273.9 a	2.25 e
	50	1.70 bc	5.13 b	6.83 b	5.55 c	0.10 b	2.21 b	274.0 a	2.42 c
	100	1.37 de	4.17 c	5.55 c	3.70 e	0.05 d	1.15 e	204.9 e	2.17 f
	150	1.29 e	1.94 e	3.24 e	2.44 g	0.03 f	0.72 g	155.8 f	1.73 i
	200	1.08 f	1.44 e	2.52 f	1.06 j	0.01 g	0.36 i	127.6 g	1.34 j
Pokkali	0	2.04 a	5.28 b	7.28 ab	6.49 b	0.09 c	2.09 c	255.6 c	2.39 d
	50	2.00 a	3.58 cd	5.62 c	4.25 d	0.04 e	1.06 f	216.5 d	2.79 a
	100	1.53 cd	2.97 d	4.50 d	3.29 f	0.05 d	1.24 d	258.0 b	2.66 b
	150	1.33 de	1.95 e	3.29 e	1.93 h	0.01 g	0.39 h	98.06 h	1.92 h
	200	1.40 de	1.71 e	3.11 ef	1.52 i	0.01 g	0.34 j	84.13 i	1.95 g
SE		0.067	0.223	0.240	0.013	0.002	0.005	2.281	0.002
LSD 5%		0.187	0.624	0.674	0.036	0.005	0.016	0.819	0.005

In each column, the means with similar letters do not differ significantly at the 5% level of probability.

The reduction in transpiration might be ionic toxicity which is caused by an undue quantity of salt entering the transpiration stream and eventually injured cells in the transpiring leaves and may further reduce growth (Munns et al., 2006). Due to salinity, stressed plants reduce transpiration and uptake of Na^+ (Flowers and Yeo, 1989). This reduce with the increased salinity can be imputed to a reduced capacity to absorb H_2O by the decrease in the osmotic component of soil water potential (Tester and Davenport, 2003). In this study, the alleviating role of GA_3 showed enhanced transpiration rate. Khan, and Azuma, (2003) also viewed that GA_3 relieved the adverse effects of salinity and maintained transpiration rate in rice.

Intercellular CO_2 (μmol mol^{-1})

The interactions of variety × weekly observation, variety × salinily, variety × GA_3, salinity × GA_3, and variety × salinity × GA_3 showed significant differences ($P<0.05$). The CO_2 concentration across the salinity levels and GA_3 was higher (248.4 μmol m^{-2}) in MR219 during the 2nd week followed by 4th week (228.6 μmol $m^{-2)}$. In Pokkali, lower CO_2 was found during the 2nd - 6th weeks. The results clarified that both the varieties had lower CO_2 contents during the 6th week (Table 5). The intercellular CO_2 was notably higher in MR219 under saline control condition and 50mM: 273.9 and 274 mol mol^{-1}, respectively followed by 255.6 and 258μmol mol^{-1} s^{-1} in Pokily with H_2O and 100mM, respectively (Table 6). GA_3 remarkably enhanced CO_2 by noting 234.7 μmol mol^{-1} in MR219. In Pokkali, GA_3 also raised CO_2 (182.6 μmol mol^{-1} s^{-1}) but was insignificant amid GA_3 and the control treatment (Table 7). The linear regression shows declining trend of CO_2 due to increased salinity, and so, its line came below the GA_3 pointing that GA_3 had positive role (Fig. 2). The relation of variety × salinity × GA_3 accounted significant differences in the CO_2 levels. MR219 produced higher CO_2: 267.1, 132.5 and 76.5μmol mol^{-1} s^{-1} at 50, 100 and 200mM, respectively. In this variety, GA_3 showed positive effect and elevated CO_2 (280.9, 277.3 and 178.6 μmol mol^{-1} s^{-1}). At 150mM, MR219 had uncertain results as showed higher CO_2 (162.8 mol mol^{-1} s^{-1}) but less value (148.9 mol mol^{-1} s^{-1}) with GA_3. In Pokkali, CO_2 was higher: 229.4, 104.1 and 91.9 μmol mol^{-1} s^{-1} at 50, 150 and 200mM, respectively. At 50, 150 and 200 mM salinity, GA_3 showed no optimistic effect as had low CO_2 levels of 203.6, 148.9 and 76.2μmol mol^{-1} s^{-1}, respectively. The cv. Pokkali had higher tolerance at 100 mM having higher value of 286.4 against 229.6 μmol mol^{-1} (Table 8). Reduced photosynthesis under increasing salinity is attributed to stomatal closure leading to the drop in the intercellular CO_2 (Bethke and Drew, 1992). Leaf internal CO_2 affected photosynthesis, and it is now obvious that stomatal closure and leaf internal CO_2 are the two vital reasons to lessen photosynthetic rates under mild or moderate salinity (Flexas et al., 2004). The present study also proved that the intercellular CO_2 went-up in both the varieties with GA_3.

Vapour pressure difference in leaf (VPDL)

There were significant differences ($P<0.05$) for Vapour pressure difference in leaf and salinity declined the VPDL. The VPDL across salinity and GA_3 levels was higher in Pokkali (2.69) at the 4th week followed by 2.52 kpa in the same variety during the 2nd week. Oppositely, the lower VPDL was noted in MR219 in all weekly observations. The VPDL in both varieties showed notably lower with increasing the salinity levels (Table 6). The VPDL had higher values (2.39 Kpa) with no GA_3 but GA_3 slightly decreased it (2.29 Kpa) in Pokkali. In MR219, different results were found as higher (2.07) with GA_3 and lower (1.89Kpa) in the absence of GA_3 (Table 7). The linear regression involving salinity and GA_3 did not show any specific trend in decrease or increase in the VPDL (Fig. 2). The relation of variety × salinity × GA_3 was significant. The increasing salinity notably reduced the VPDL. MR219 produced 2.62, 1.82, 1.72 and 0.87Kpa under 50-200mM, respectively but GA_3 showed little disparity. Pokkali at 50-200mM had no specific trend in increase or decrease for GA_3. At 50mM, Pokkali showed higher VPDL due to GA_3. But it gave negative response due to GA_3 at 100-200 mM as had the less VPDL (Table 8). The VDPL decreased probably due to cohesive forces amid the NaCl and water. Stronger inter/intra-molecular forces in solution possibly caused the VDPL to decrease. The higher VPDL lowered the stomatal conductance due to deficit of turgor pressure. The gaseous exchange became more arrested as evaporative demand and potential transpiration rates were high. Even in well-irrigated plants, stomata tend to close at the VPDL above 1.5kPa (Machado et al., 2005). But here, GA_3 was not able to prove the VPDL recovery.

Table 7. Physiological traits of rice varieties under the influence of GA_3

varieties x GA_3 (ppm)		Chloro-phyll *a* (mg g^{-1})	Chloro-phyll *b* (mg g^{-1})	Total chloro-phyll (mg g^{-1})	Photo-synthetic rate (µmol m^{-2} s^{-1})	Stomatal conduct. (mmol m^{-2} s^{-1})	Transpi-ration rate (mmol m^{-2} s^{-1})	Inter-cellular CO_2 (µmol mol^{-1})	VPDL (KPa)
MR219	0	1.27 d	3.31 b	4.58 b	3.49 c	0.05 b	1.07 c	179.8 c	1.89 d
	150	1.62 b	4.16 a	5.78 a	4.86 a	0.09 a	1.83 a	234.7 a	2.07 c
Pokkali	0	1.46 c	2.22 c	3.69 c	3.11 d	0.04 c	0.95 d	182.3 b	2.39 a
	150	1.86 a	3.97 a	5.83 a	3.89 b	0.05 b	1.10 b	182.6 b	2.29 b
SE		0.042	0.141	0.152	0.008	0.001	0.004	0.518	0.001
LSD 5%		0.118	0.395	0.426	0.023	0.003	0.010	1.442	0.003

In each column, the means with similar letters do not differ significantly at the 5% level of probability.

Table 8. Physiological traits of rice varieties under the influence of salt concentrations and GA_3.

Rice varieties	Salinity levels (mM)	Chlorophyll a (mg g^{-1})		Chlorophyll b (mg g^{-1})		Total chlorophyll (mg g^{-1})		Photosynthetic rate (µmol m^{-2} s^{-1})		Transpiration rate (mmol m^{-2} s^{-1})		Stomatal conductance (mmol m^{-2} s^{-1})		Inter-cellular CO_2 (µmol mol^{-1})		VPDL (KPa)	
		GA_3 (ppm)		GA_3 (ppm)		GA_3 (ppm)		GA_3 (ppm)		GA_3 (ppm)		GA_3 (ppm)		GA_3 (ppm)		GA_3 (ppm)	
		0	150	0	150	0	150	0	150	0	150	0	150	0	150	0	150
MR219	0	1.5	2.06	5.63	6.35	7.13	8.42	7.31	8.88	2.14	3.49	0.11	0.23	260	287.7	2.42	2.07
	50	1.47	1.94	4.29	5.96	5.76	7.91	4.42	6.69	1.6	2.81	0.06	0.14	267	280.9	2.62	2.22
	100	1.26	1.49	4	4.36	5.26	5.84	3.14	4.27	0.83	1.46	0.03	0.06	132.5	277.3	1.82	2.52
	150	1.12	1.47	1.38	2.5	2.51	3.97	1.78	3.1	0.57	0.87	0.02	0.03	162.8	148.9	1.72	1.73
	200	1.01	1.16	1.25	1.64	2.26	2.79	0.79	1.34	0.22	0.49	0.01	0.02	76.5	178.6	0.87	1.81
Pokkali	0	1.82	2.19	3.55	7.01	5.37	9.19	5.89	7.09	1.93	2.26	0.09	0.09	256.6	254.6	2.42	2.37
	50	1.82	2.25	2.51	4.65	4.34	6.91	3.79	4.71	1.09	1.04	0.04	0.04	229.4	203.6	2.77	2.81
	100	1.42	1.64	2.13	3.82	3.55	5.45	2.99	3.59	1.09	1.38	0.04	0.05	229.6	286.4	2.74	2.59
	150	1.17	1.49	1.65	2.26	2.82	3.76 f	1.59	2.28	0.33	0.44	0.01	0.02	104.1	92.1	2.01	1.84
	200	1.08	1.73	1.3	2.12	2.38	3.85 f	1.27	1.78	0.29	0.39	0.01	0.01	91.9	76.2	2.03	1.86
SE		0.095		0.315		0.341		0.018		0.008		0.003		1.158		0.003	
LSD (5%)		0.265		0.883		0.954		0.051		0.023		0.007		3.225		0.007	

Materials and Methods

Experimental site and soil characteristics

The experiment was conducted in pots (24.5 cm diameter and 28 cm depth) at the glasshouse of the Universiti Putra Malaysia ((3◦00′21.34″N, 101◦4′ 15.06″ E, 37m elevation) during 2010-2011. The day and night temperature were 34±2 and 27±2^{0}C with the relative humidity of 50-75% during the experimental period. The pots were filled up with 10 kg prepared clay soil of rice field of Perak. The experimental soil was clay in texture (18.3% sand, 43.7% silt, 38% clay) and acidic in reaction (pH 6.1) with 1.02% organic carbon, EC-1.56 dSm^{-1}, soil nutrient status was 0.19% total N, 11.12 ppm available P, 122 ppm available K, 620 ppm Ca, 290 ppm, 7.63 ppm S and 0.96 ppm Zn.

Plant materials

Two rice varieties Pokkali and MR219, collected from Malaysian Agriculture Research and Development Institute (MARDI), was used as the plant material in this study, of which Pokkali used as salt tolerant check (Pokkali is the international salt tolerant rice variety) and MR219 is the well-known cultivated Malaysian variety and it was performed better under saline conditions in laboratory studies.

Experimental design and treatments

The experiment was laid out as the factorial with the treatments arranged in the randomized complete block design with four replications. Five salinity levels viz. 0, 50, 100, 150 and 200 mM and 150 ppm GA_3 as foliar spray were used in this study.

Methodology

The seeds of rice varieties were sterilized with 0.1% $HgCl_2$ solution (Hewitt, 1961) and dipped for 5 min in "Zap Padi Angin" solution to enhance sprouting. Those were sprouted by placing on a moist filter paper for 24 hours were sown in a well prepared wet seedbed. The soil was prior mixed well with urea, triple supper phosphate (TSP) and muriate of potash (MOP) at the rate of 120, 70, and 80kg ha^{-1}, respectively. Whole TSP and MOP, and ¼th urea were added during the pot preparation and mixed thoroughly with the soil. The remaining urea was equally split at 3-leaf, active tillering, booting and heading stages. Three-week-old rice seedlings were transplanted, allocating six hills per pot giving one seedling per hill. Water was applied into the pot to maintain saturated condition at transplanting. After two weeks, the salt solutions according to the treatments were applied and renewed after every two days. The control treatment was only irrigated with water (0 mM salinity). Two weeks after the inception of the salinity treatments, plants were sprayed GA_3 at the rate of 150ppm with a hand sprayer at 5p.m. to avoid evaporation. Conductivity of soil was compared with the conductivity meter (model: ECTestr, Spectrum Technologies, Inc.).

Data measurements

The plant height of five hills was measured from the ground level to tip of the top most leaf at 30, 60 and 90 DAT. The number of tillers from five hills was counted and the average was worked out as tiller number hill^{-1}. Shoot and root samples were oven dried at 70 °C for 3 days and total dry matter hill^{-1} was recorded. Relative water content (RWC) was determined as described by Gonzales and Gonzales (2003). Chlorophyll content such as chlorophyll-a, chlorophyll-b and total chlorophyll were determined from the leaf samples using the method of Witham et al. (1986). Stomatal conductance, photosynthetic rate and transpiration rate were measured from flag leaf of rice by the LI-COR 6400, Nebraska, USA. Intercellular CO_2 concentration (Ci) and the vapour pressure deficit in leaves (VPDL) were noted using the LI-COR 6400, Nebraska, USA.

Statistical analysis

Data on the growth and the yield parameters were analysed using the Analysis of Variance (ANOVA) technique and the mean separation was done with the Least Significant Difference (LSD) test at 5% probability level using the computerized Statistical Analysis System Software (SAS version 9.0).

Conclusions

The present work focused that the high salinity levels notably inhibited morpho-physiological traits of both studied rice varieties. MR219 showed more affected in some parameters than control variety Pokkali under saline condition. However, less reduction was observed when GA_3applied. The study pinpointed that the spray of 150 ppm GA_3 could also be effectively utilized for rice production in saline belts due to its unique salinity alleviating role to improve the growth parameters of the cultivated rice.

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