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The Effect of Exogenous Application of GA_3 to Mitigate the Salt Induced Damages in Rice Cultivars during Germination

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***Abstract:** In the present study the two paddy cultivars were selected to examine the effect of salinity, GA_3 and the effect of GA_3 on the salinity stressed seeds, the tests were conducted at different concentrations. The salinity decreased the germination percentage, vigour index and seedling length and chlorophyll content. GA_3, combination of GA_3 and NaCl treated seeds showed increased germination percentage in both the cultivars, but the decrease in germination percentage due to salinity was more in Jyothi cultivar compared to the BPT Super cultivar. GA_3 played the salinity attenuating role, Jyothi cultivars seems to be more salt sensitive when compared to the BPT Super cultivar. The Chlorophyll a, Chlorophyll b and total chlorophyll content was decreased in salinity in both the varieties, but a gradual increase was found in GA_3 treatment and also combination of NaCl and GA_3 treatments. The decrease in chlorophyll content under salinity was more in Jyothi cultivar when compared to the BPT Super cultivar.*
***Keywords:** Rice, NaCl, GA_3, seed germination, seedling length, chlorophyll.*

I. INTRODUCTION

Rice (*Oryza sativa* L.) is a salt sensitive monocot and widely grown crop in tropical and subtropical regions. It is one of the main staple foods for nearly two thirds of the population of the world (Roy *et al*., 2012). High rice consumption, degradation of soil and water quality around the globe has focussed urgent attention to understand the response of this important crop towards abiotic stresses. Salinity is estimated that over 800 million hectares of land in the world are affected (Munns, 2005; Kumar *et al*., 2010). Salt stress in soil is one of the major stresses especially in arid and semi arid regions and can severely limit plant growth and productivity by reducing osmotic potential, ion toxicity creation, uptake disarrangement and ion imbalance and can cause disorders in enzyme activities of membrane and metabolic activities in plants (Gorham, 1993; Hasegawa *et al*., 2000; Basu *et al*., 2002; Murphy *et al*., 2003; Islam *et al*., *2008*). These activities could affect morphological parameters and plant growth and will reduce vegetative growth (Linghe and Shannon, 2000; Sairam and Tyagi, 2004). Chlorosis is common morphological and physiological characteristic of mulberry in response to salt stress (Harinasut *et al*., 2000). Chlorophyll is a good indicator of plant nutrient stress during growing period and content of chlorophyll in the leaves indicates the growth of crops. Synthesis and integrity of chlorophyll level may vary due to salt stress (Santo, 2004; Rout *et al*, 1997). Chlorophyll content of salt stressed rice can be described as functions of the sodium content (Yeo and Flowers, 1983). Excess of NaCl to plants involves changes in their morphology, physiology and metabolism (Hilal *et al*., 1998; Rahman *et al*., 2008) and consequently reducing plant dry weight (Zeng and Shannon, 2000; Razzaque *et al*., 2009) and dry matter production (Mansour and Salam, 2007) and ultimately its effect on crop yield (Shannon *et al*., *1998*; Jamil *et al*., 2010; Osakabe *et al*., 2011). Several strategies have been proposed to alleviate the degree of cellular damage caused by abiotic stress and to improve crop salt tolerance. Among them exogenous application of compatible osmolytes such as proline, glycinebetaine, treholose, auxin, gibberellins, (Kim *et al*., 2006; Rahdari *et al*., 2012), Methyl Jasmonate, Sucrose (Siringam *et al*., 2012), Spermidine (Saleethong *et al*., 2016) had gained considerable attention in mitigating the effect of salt stress. Plant growth regulators and other groups of chemicals have been used to treat rice plants exogenously at various growth stages to increase salt tolerance by alleviating salt induced damages and led to improved growth and productivity (Roychoudhury *et al*., 2011; Plaut *et al*., 2013). A few studies have however, demonstrated the ability of GA_3 to overcome adverse effects of NaCl stress (Chakraborti and Mukherji, 2003). GA_3 basically stimulated the inter node elongation, and also controls the various aspects of seed germination, mobilization of reserves, floral initiation, sex determination and fruit set. The present work was conducted to examine whether NaCl inhibited seed germination, growth and synthesis of chlorophyll content and to determine the influence of GA_3 when exposed to NaCl stress.

II. MATERIALS AND METHODS

Paddy seed sample of cultivars Jyothi and BPT Super were procured from VC Farm, Regional Agricultural Research Station, University of Agriculture Science, Mandya, Karnataka. Seeds of uniform size were selected and surface sterilized using 0.01% of mercuric chloride for two minutes. The seeds were washed thoroughly with distilled water for 4-5 times and soaked for 24 hours in distilled water (control) and different concentrations of GA_3 i.e 100ppm and 200ppm and in NaCl i.e. 200mM and 300mM and in combination of NaCl and GA_3. The germination studies were carried as per International Seed Testing Association (2009). Five sets of each concentration of GA_3, NaCl and combination of both were maintained along with control. The seeds were allowed to germinate for 14days as per ISTA, 2009 and then analysed for morphological and biochemical parameters.

The test was conducted by the between paper towel method recommended (ISTA, 2009). Hundred seeds of each cultivar were placed on craft paper saturated with known concentration of sodium chloride and GA_3. The number of seeds germinated in each treatment was counted on 5th day of germination and total germination percentage was calculated. On the 14thday root length, shoot length and seedling length was measured and The seedling vigour index was calculated by using the formula proposed by Abdul Baki and Anderson (1973) and expressed as seedling vigour index (SVI) = % of Germination X Mean seedling length. The chlorophyll content viz., Chlorophyll-a and Chlorophyll-b and the total chlorophyll were estimated as per the method of Arnon (1949).

III. RESULTS AND DISCUSSION

A. Seed Germination

Rice is one of the most important cereal crops all over the world with exceptional agricultural and economic importance as a staple food for more than 50% population worldwide. Asian farmers produce more than 90% of the total rice. The test was conducted to study the germination percentage of two rice cultivars subjected to salinity stress. As per investigation there was decrease in germination percentage under saline treatment and there was increase in the germination percentage under GA_3 treatment GA_3 alleviated the negative effect of NaCl.

B. Effect of Salinity on Germination

Salinity is a common environmental stress seriously affecting on crop growth and crop yield in many regions, particularly crops in arid and semi-arid regions (Jamil *et al.*, 2010; Osakabe *et al.*, 2011). It is estimated that over 800 million hectares of land in the world are affected by salinity (Munns, 2005; Kumar *et al.*, 2010).

The salinity decreased germination percentage, vigour index and seedling length (table 1). Salinity induces numerous disorders in seeds during germination, it reduces the imbibitions of water because of lower osmotic potential of the medium, (Akbar and Ponnamperuma, 1982, Almansouri, *et al.*, 2001). Salinity causes mineral imbalance and toxicity in the environment, so that the mineral imbalance and toxicity of the saline environment often affects the structure and chemical composition of bilayer lipid membrane of the seed. It also affects the membrane selective ability to transport of the solutes and ions inward and also becomes leaky to solutes they contain and hence the germination percentage is affected (Cushman, *et al.*, 2001).

Salinity decreased the germination percentage, magnitude of reduction increased with increasing salinity stress (Hakim, *et al.*, 2009 and Mirza Hasamuzzaman *et al.*, 2009 and Anbumalarmathi, *et al.*, 2013). Salinity of soil and water is a major environmental stress that drastically affects on crop productivity in term of growth and yield, all over the world (Zhu, 2001).

C. Effect of GA3 on Germination

Gibberellin increased the cell division and elongated the cells and it effected positively on the germination (Parvanehrahdari *et al., 2015*). The interaction of GA_3 and NaCl concentrations was no significant, but optimal concentration of GA_3 treatment was slightly increased germination percentage. The exogenous application of plant growth regulator like gibberellins (Afzal *et al., 2006*), reduce the adverse effects of salt stress and also improves germination, growth, development and seed yields and yield quality (table 1).

D. Effect of salinity and GA3 on germination

The test was conducted to study the germination percentage of two rice cultivars subjected to salinity stress (table 1). As per our investigation there was decrease in germination percentage under saline treatment due to osmotic potential of salinity on imbibitions of seeds was the main cause for reduced germination (Akbar and Ponnamperuma 1982; Almansouri., *et al., 2001*) and increase in the germination percentage under GA_3 treatment. The NaCl treated seeds were treated with GA_3 the germination percentage was increased, hence GA_3 alleviated the negative effect of NaCl.

E. Effect of Salinity and GA3 on Vigour Index

The vigour index was decreased under saline treatment in both the varieties. There was increase in vigour index under GA_3 treatment. The combination of GA_3 and NaCl also showed increase in the vigour index. Vigour index of Jyothi cultivar was most affected under salinity when compared to the BPT Super cultivar. Vigour index is directly dependent on germination percentage and seedling length. Germination percentage and the seedling length of saline treated seeds were decreased. Therefore the vigour index was also decreased. Under GA_3treatment and combination of GA_3 and NaCl seeds showed increase in germination percentage and seedling length and hence there was increase in the vigour index too.

Table 1: Effect of different concentration of NaCl, GA_3 and combination of NaCl and GA_3 on germination percentage (%) and vigour index of Jyothi and BPT Super paddy cultivars on 5th day.

Cultivar	Parameters	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm + NaCl 200mM	GA_3 200ppm + NaCl 300mM
Jyothi	Germination %	96.66^{ab}	92.00^{c}	87.33^{d}	96.00^{abc}	98.66^{a}	94.00^{bc}	96.66^{ab}
	Vigour index	1985.2^{bcd}	1545.4^{de}	1313.1^{e}	2218.5^{abc}	2669.2^{a}	1808.6^{cde}	2457.5^{ab}
BPT Super	Germination %	92.66^{bc}	90.00^{cd}	86.66^{d}	96.00^{ab}	98.66^{a}	92.00^{bc}	95.33^{ab}
	Vigour index	1395.9^{bc}	1162.4^{cd}	1040.3^{d}	1650.5^{ab}	1941.7^{a}	1440.6^{bc}	1715.4^{ab}

Means followed by the same letter within a row are not significantly different as indicated by Scheffe ($P \leq 0.05$) significant at $P \leq 0.001$.

F. Effect Of Salinity On Seedling Length

The seedling length was decreased under salinity and increase in the seedling length was found in GA_3 treatment. Combination of NaCl and GA_3 also showed increase in seedling length in both Jyothi and BPT Super cultivars. Seedling length was less decreased in BPT Super cultivar under saline treatment compared to the Jyothi cultivar (table 2). The gradual decrease in root length with the increase in salinity was observed might be due to more inhibitory effect of NaCl salt on the root growth (Rahman, *et al., 2001*).

The concentration of NaCl modifies the metabolic activities of the cell wall causing the deposition of various materials which limit the cell wall elasticity, secondary cell walls become rigid and consequently the turgor pressure efficiency in cell enlargement is decreased, it affects the growth of seedling (Ali *et al., 2004*). Due to salinity seed fertility was damaged hence the seedling length was decreased (Thanaphol *et al.*, 2012). Salinity led to the reduction in shoot and root length of rice varieties and the magnitude of reduction increased with increasing salinity stress (Hakim, *et al., 2009*; Anbumalarmathi, *et al., 2013* and Mirza Hasamuzzaman *et al.*, 2009).

G. Effect of GA_3 on Seedling Length

GA_3 treatment and combination of GA_3 and NaCl increased the germination percentage of both the cultivars (table 2) due to increases the cell division, elongation of cells and helps in the increase of seedling length (Parvanehrahdari *et al.,* 2015). GA_3 enhanced the growth by forming new cells in the intercalary meristem (Khadija *et al*., 2013).

Table 2: Effect of different concentrations of NaCl, GA_3 and combination of NaCl and GA_3 on root, shoot and seedlings length (cm) of Jyothi and BPT Super Paddy cultivars on 14th day.

Cultivar	Parameters	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm + NaCl 200mM	GA_3 200ppm + NaCl 300mM
Jyothi	Root length	8.87^{b}	7.59^{bc}	6.92^{c}	11.32^{a}	12.36^{a}	8.39^{bc}	11.06^{a}
	Shoot length	11.65^{abc}	9.21^{c}	8.13^{c}	11.80^{abc}	14.69^{a}	10.84^{bc}	14.36^{ab}
	Seedling length	20.62^{bcd}	16.94^{de}	15.12^{e}	23.16^{abc}	27.14^{a}	19.30^{cde}	25.46^{ab}
BPT Super	Root length	6.71^{cd}	5.77^{de}	5.01^{e}	7.66^{bc}	8.92^{a}	6.88^{bcd}	8.38^{ab}
	Shoot length	8.33^{a}	7.14^{a}	7.00^{a}	12.06^{a}	10.75^{a}	8.76^{a}	9.61^{a}
	Seedling length	15.15^{bcd}	13.01^{cd}	12.07^{d}	17.29^{ab}	19.74^{a}	15.75^{bc}	18.08^{ab}

Means followed by the same letter within a row are not significantly different as indicated by Scheffe ($P \leq 0.05$) significant at $P \leq 0.001$.

H. Chlorophyll

1) *Effect of salinity on chlorophyll:* The chlorophyll content was decreased under saline condition as the salinity increased (table 3) due to increased activity of the chlorophyll degrading enzyme chlorophyllase (Reddy *et al., 1986*), ion accumulation and functional disorders observed during opening and closing of stoma under salinity stress (Seemann and Critchley, 1985; Khalehi *et al*, 2012; Nawaz *et al*, 2010). The reason for the decrease of chlorophyll content under salt conditions is the rapid maturity of leaves (Yeo, *et al.,* 1991). The depletion of chlorophyll under salinity may be the result of the hang-up of chlorophyll biosynthesis following an increase in ethylene production brought by the elevated NaCl content (Khan, 2003). Chlorophyll a, chlorophyll b, total chlorophyll content was decreased under salinity (Mohammad Reza Amirjani., *et al., 2011*, Khadija., *et al.*, 2013, and Abhilash Joseph., *et al.*, 2014).
2) *Effect Of Ga_3 On Chlorophyll:* GA_3 played significant role in salinity alleviating and increased the chlorophyll content in the saline treated seeds (table 3). The positive effect of GA_3 was constant by having more chlorophyll than stressed conditions (Khadija, *et al., 2013*). 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). The salinity reduces in chlorophyll but GA_3 raised significantly (table 3).

Table 3: Effect of different concentrations of NaCl, GA_3 and combination of NaCl and GA_3 on chlorophyll a, chlorophyll b and total chlorophyll (mg/g) of Jyothi and BPT Super paddy cultivars on 14th day.

Cultivar	Parameters	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm + NaCl 200mM	GA_3 200ppm + NaCl 300mM
Jyothi	Chl-a	0.028^{d}	0.010^{e}	0.008^{e}	0.032^{cd}	0.075^{b}	0.036^{c}	0.089^{a}
	Chl-b	0.009^{d}	0.007^{d}	0.006^{d}	0.027^{b}	0.044^{a}	0.008^{d}	0.019^{c}
	Total chl	0.070^{d}	0.026^{e}	0.023^{e}	0.084^{c}	0.192^{b}	0.089^{c}	0.219^{a}
BPT Super	Chl-a	0.030^{c}	0.027^{c}	0.027^{c}	0.036^{bc}	0.041^{bc}	0.038^{bc}	0.060^{b}
	Chl-b	0.007^{bc}	0.005^{cd}	0.003^{d}	0.01^{ab}	0.013^{a}	0.008^{bc}	0.009^{b}
	Total chl	0.073^{de}	0.060^{e}	0.060^{e}	0.086^{cd}	0.103^{b}	0.093^{bc}	0.151^{a}

Means followed by the same letter within a row are not significantly different as indicated by Scheffe ($P \leq 0.05$) significant at $P \leq 0.001$.

IV. CONCLUSION

In our present study the two paddy cultivar were selected to examine the effect of salinity, GA_3 and the effect of GA_3 on the salinity stressed seeds and the tests were conducted at different conditions with different concentrations. The various aspects were investigated. The salinity decreased germination percentage, vigour index and seedling length, GA_3 treatment and combination of GA_3 and NaCl increased the germination percentage of both the cultivars, but the decrease in germination percentage due to salinity was more in Jyothi cultivar compared to the BPT Super cultivar. GA_3 played the salinity attenuating role and Jyothi cultivars seems to be more salt sensitive when compared to the BPT Super cultivar. The chlorophyll content was decreased under saline condition as the salinity increased. GA_3 played significant role in salinity alleviating and increased the chlorophyll content in the saline treated seeds. The salinity alleviating role of GA_3 was observed and BPT Super cultivars chlorophyll content was less affected when compared to the Jyothi cultivar hence the BPT super cultivar is more tolerant compared to the Jyothi cultivar.

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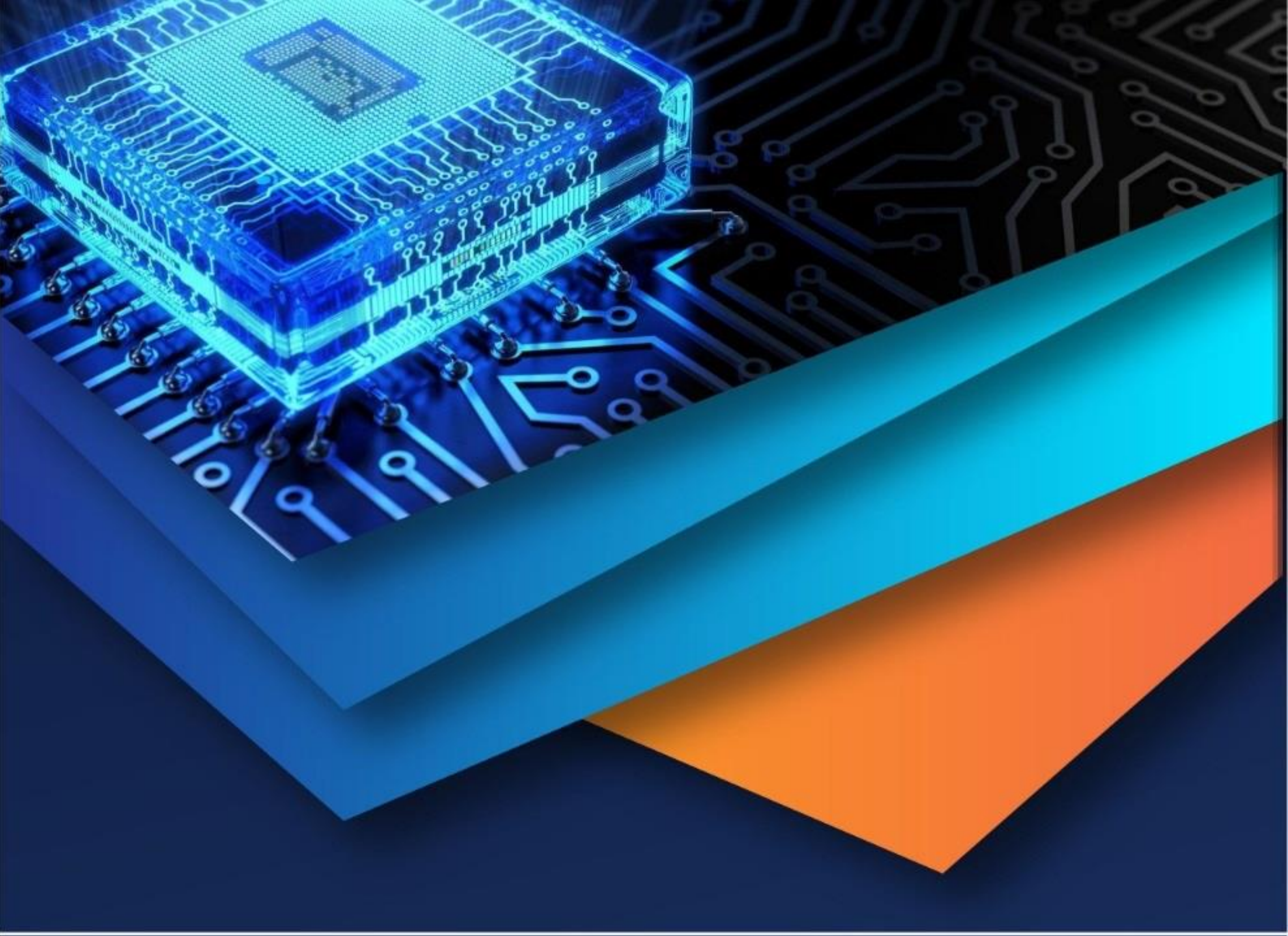

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