iJRASET
International Journal For Research in
Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 Issue: VII Month of publication: July 2019

DOI: http://doi.org/10.22214/ijraset.2019.7062

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue VII, July 2019- Available at www.ijraset.com

The Exogenous Effect of Gibberillic Acid on Enzyme Activities in Salt Stressed Rice Cultivars during Seed Germination

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Abstract: The amino acid content was increased under GA_3 treatment and also under combination of GA_3 and NaCl in both the cultivar. In salt sensitive Jyothi cultivar more protein was breakdown to amino acid hence the amino acid content increased, in salinity BPT Super cultivar the amino acid content decreased. It seems to be more tolerant when compared to the Jyothi cultivar. Therefore GA3 showed the salinity mitigation role.

α- and β-amylase activity was found to be decreased under salinity and the increase was observed under GA_3 application. Combination of GA_3 and NaCl also increased the α- and β-amylase activity, the least affected salinity in BPT Super. In Jyothi cultivar salinity was more affected. Catalase activity was increased under salinity in both the cultivar. Salinity was increased under GA_3 treatment and also under the combination of GA_3 and NaCl, so catalase activity was least affected due to the salinity in both the paddy cultivars. With respect to all the parameters Jyothi paddy cultivar is more salt sensitive when compared to the BPT Super paddy cultivar. BPT Super shows better salinity tolerance when compared to the Jyothi cultivar and salinity alleviating role of GA_3 was observed.

Keywords: Rice, NaCl, GA3, Protein, Amino acids, α and β amylase and catalase.

I. INTRODUCTION

Rice (*Oryza sativa* L.) is a salt sensitive monocot and widely grown crop in tropical and subtropical regions. It is one the main staple foods for nearly two third of the population of the World (Roy *et al.,* 2012), high rice consumption, degradation of soil and water quality around globe have focused 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* and Tavakkoli *et al., 2011*). 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, ion imbalance and can cause disorders in enzyme activities and metabolic activities in plants (Hasegawa *et al., 2000*; Basu *et al., 2002*; Murphy *et al., 2003*; Islam *et al., 2008*). These activities could affect morphological parameters and will reduce vegetative growth (Linghe and Shannon, 2000; Rogers *et al., 2004*) and chlorosis is common morphological and physiological characteristic in response to salt stress (Harinasut *et al.,* 2000). Chlorophyll pigment is a good indicator of plant nutrient stress during growing period and content of chlorophyll in the plant leaves indicates the growth of the 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; Roger *et al., 2009*) and dry matter production (Mansour and Salam, 2007) and ultimately crop yield (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, glycine betaine, treholose, auxin, gibberellins, (Kim *et al.,* 2006; Misratia *et al.,* 2013; Rahdari and Hoseini, 2015), 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 lead to improved growth and productivity (Roychoudhury *et al.,* 2011; Plaut *et al.,* 2013).

A few studies have however, demonstrated the ability of GA3 to overcome adverse effects of NaCl stress (Chakraborti and Mukherji, 2003; Misratia *et al.*, 2013) GA3 basically stimulated the inter node elongation, stem elongation and also control 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 GA3 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% mercuric chloride ($HgCl_2$) for two minutes. The seeds were washed thoroughly with distilled water for several 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 were used to soak the seeds as well as combination of NaCl and GA_3 seeds were also soaked. The germination studies were carried out as per International Seed Testing Association (2009). Five sets of each concentration of GA3, NaCl and combination of both were maintained along with control. The seeds allowed to germinate for 14days as per ISTA, 2009 and then analyse for morphological and biochemical parameters.

Estimation of total protein content by Lowry et. *al.*, (1951) had expressed in µg protein/ml and total amino acids contents by Moore and Stein (1948). α and β amylase activity was calculated as per Bernfeld (1955) and The enzyme activity is expressed as µmoles of glucose released/gm. Catalase activity was assayed according to Luck (1974).

III. RESULTS AND DISCUSSION

A. Protein

The test was conducted to study the effect of salinity on two rice cultivars and effect of GA_3 on Jyothi and BPT Super cultivar . Both the cultivar showed decrease in the protein content under salinity and the increase in protein content was observed under combination of NaCl and GA_3. The protein content was most affected in Jyothi than the BPT Super cultivar under salinity condition.

B. Effects Of Salinity On Protein Content

Protein content decreased in most of the plant species under Sodium chloride stress (Misra, *et al.*, 1997). A continuous decrease in protein content with increase in salt stress (Sunita Danai-Tambhale, 2011). The reason for decrease of protein content under salinity was breakdown of surface protein and production of free amino acid (Parvaneh rahdari., *et al.*, 2015).

C. Effects of GA_3 on Protein Content

Gibberellin treatment can lead to increased levels of protein, during the stress condition there will be increase in the nitrate reductase enzyme activity in the protein surface tension, and increase in the protein content (Masroor, *et al.*, 2006).

D. Amino Acid

In the present study the total amino acid content of two rice cultivar was estimated under salinity, total amino acid content of BPT Super was decreased but the total amino acid content of Jyothi cultivar was increased under salinity and under GA_3 treatment and combination of NaCl and GA_3 treatment in both the cultivars there was an increase in total amino acid content was observed.

E. Effects Of Salinity On Amino Acid Content

Total amino acid content was increased under salinity treatment and GA_3 treatment GA_3 treatment lead to the qualitative and quantitative changes in free amino acid, hence the increase in total amino acid content was observed in seeds treated with GA_3. Under salinity increase in amino acid was caused by proteolysis a great accumulation of protein amino acids, and disturbance of nitrogen metabolisms, and accumulation of non protein amino acids or ammonia was the reason for increase in total amino acid content under salinity (Yamamoto, *et al.*, 2011).

In general due to the salinity surface breakdown and production of free amino acids led to the increase in amino acid (Parvaneh rahdari., *et al.*, 2015). Total amino acid level in tolerant variety decreased under saline condition as reported by Saikat Paul., *et al.*, (2016). Total amino acids were also increased under salt stress as reported by Yamamoto, *et al.*, (2011).

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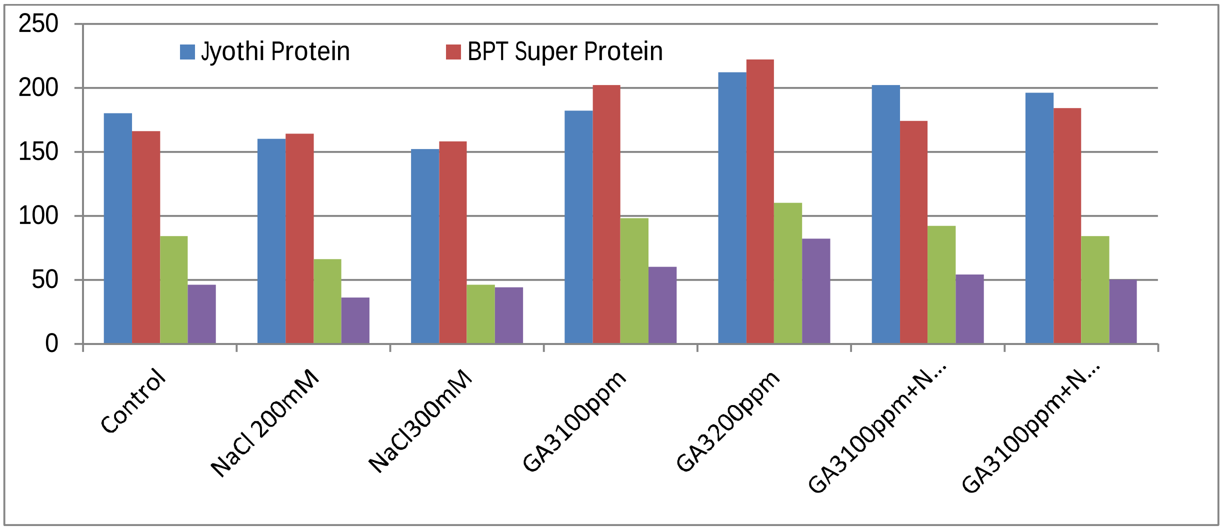
Table 1: Effect of different concentrations of NaCl, GA_3 and combination of NaCl and GA_3 on Protein and amino acids content (µg/ml) of Jyothi and BPT Super paddy cultivars on 14th day.

Cultivar	Parameters	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm + NaCl 200mM	GA_3 200ppm + NaCl 300mM
Jyothi	Protein	180^{c}	160^{d}	152^{e}	182^{e}	212^{a}	202^{b}	196^{b}
	Amino acids	84^{c}	66^{d}	46^{e}	98^{b}	110^{a}	92^{b}	84^{c}
BPT Super	Protein	166^{e}	164^{e}	158^{f}	202^{b}	222^{a}	174^{d}	184^{c}
	Amino acids	46^{d}	36^{e}	44^{d}	60^{b}	82^{a}	54^{bc}	50^{cd}

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



Graph 1: Effect of different concentrations of NaCl, GA_3 and combination of NaCl and GA_3 on protein and amino acids content (µg/ml) of Jyothi and BPT Super paddy cultivars on 14th day.

F. α-Amylase Activity

Starch is the most abundant reserve food material in rice seeds. α-amylase is a crucial enzyme that participates in the degradation of starch granules in to small organic molecules to provide energy and nutrients for seed germination. The study was done on the effect of salinity on α-amylase activity of rice seed where it was inhibited. Application of GA_3 on two rice cultivars there was an increase in α-amylase content. In the GA3 100ppm+NaCl 200mM quantitative data demonstrated that α-amylase activity was significantly increased compare to control.

G. Effect of Salinity on α-Amylase Activity

Seed germination is dependent on the degradation of storage reserves in mature seeds and the sugars from starch hydrolysis are the major source of energy for seedling emergence (Beck and Ziegler, 1989). α-amylase is the major enzyme involved in starch mobilization thus α-amylase activity is an important factor in seed germination. In this study quantitative data demonstrated that NaCl induced the inhibition of activity of α-amylase due to enhancing bioactive GA inactivation.

The reduction in α-amylase activity under salinity could account for the reduction in concentration and translocation of free sugars into the embryo axes during germination and early growth Saikat Paul., *et al.*, (2016) and due to the less imbibitions effect, because water as a solvent is necessary to stimulate the activity of this enzyme. Sodium chloride reduced α-amylase activity in germinating rice seeds to varying degrees even at low NaCl concentration (Shereen *et al.*, 2011). Salinity inhibits rice seed germination by decreasing the bioactive GA_3 content, as a result of an increase in bioactive GA_3 inactivation. Furthermore bioactive GA_3 deficiency inhibits seed germination by decreasing alpha amylase activity via down regulation of alpha amylase gene expression (Li Liu *et al.*, 2018).

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H. Effect of GA_3 on α-amylase Activity

Gibberllic acid is well known to induce the synthesis of α-amylase hydrolysis of starch in rice seeds (Palmiano and Juliano, (1972). Hence the α-amylase activity is more in GA_3 treated seeds. During seed germination, bioactive gas are synthesized in the embryo and transported to the aleurone layer to induce alpha amylase gene expression and alpha amylase synthesis. Then alpha amylase is secreted into the endosperm to hydrolyse the stored starch (Kaneko *et al.*, 2002). Alpha amylase is the major enzyme involved in the hydrolysis of starch to glucose, and accounts of 40-60% of de novo protein synthesis in grains (Sun and Henson, 1991).

I. β-Amylase Activity

A study was done on effect of salinity and GA_3 on the β amylase activity, decrease in the β amylase activity was found under saline treatment but increase in the β amylase under GA_3 treatment and also under combination of NaCl and GA_3 there was an increase in the β amylase activity.

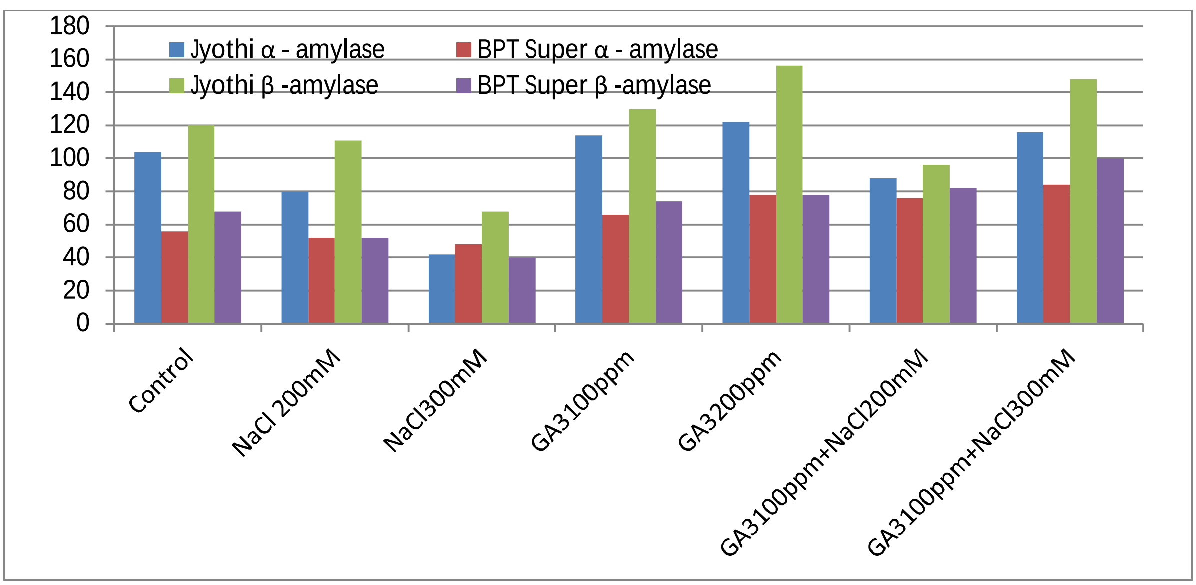
J. Effect of Salinity ON β-amylase Activity

Salinity stress led to a decrease in Beta amylase activity and there was by break down of starch into reducing and non-reducing sugars. Hence the amylase activity was reduced under salinity (Saikat Paul, *et al.*, 2016). Salt stress inhibited the express of β amylase isoenzyme activity (Liu Hua-long *et al.*, 2014).

Table 2: Effect of different concentrations of NaCl, GA_3 and combination of NaCl and GA_3 on α and β amylase (μm/gm) and catalase activity (unit min^{-1} mg^{-1}protein) 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	α - amylase	104^c	80^e	42^f	114^b	122^a	88^d	116^{ab}
	β -amylase	120^c	111^c	68^e	130^b	156^a	96^d	148^a
BPT Super	α - amylase	56^d	52^{de}	48^e	66^c	78^b	76^b	84^a
	β -amylase	68^d	52^e	40^f	74^{cd}	78^{bc}	82^b	100^a

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



Graph 2: Effect of different concentrations of NaCl, GA_3 and combination of NaCl and GA_3 on α - amylase and β -amylase content (μm/gm) of Jyothi and BPT Super paddy cultivars on 14th day.

K. Catalase Activity

A test was conducted to study the effect of salinity and GA_3 on the catalase activity. Increase in the catalase activity under the salinity under GA_3 treatment and in combination of NaCl and GA_3 was observed.

L. Effect of Salinity

The amount of H_2O_2 content in leaves gets increased with increasing salinity level than the increase in antioxidative enzyme catalase. Besides CAT detoxifying enzyme is reported to increase under various environmental stresses including salinity (Gueta-Dahan., *et al.*, 1997, Vaidyanathan *et al.*, 2003) and salinity stress increased the CAT activity, was stated by Paul and Roychoudhury. Catalase activity was increased in response to NaCl in rice cultivars with increasing salinity and the results were in concurrence with levels by Kanlaya *et al.*, (2012).

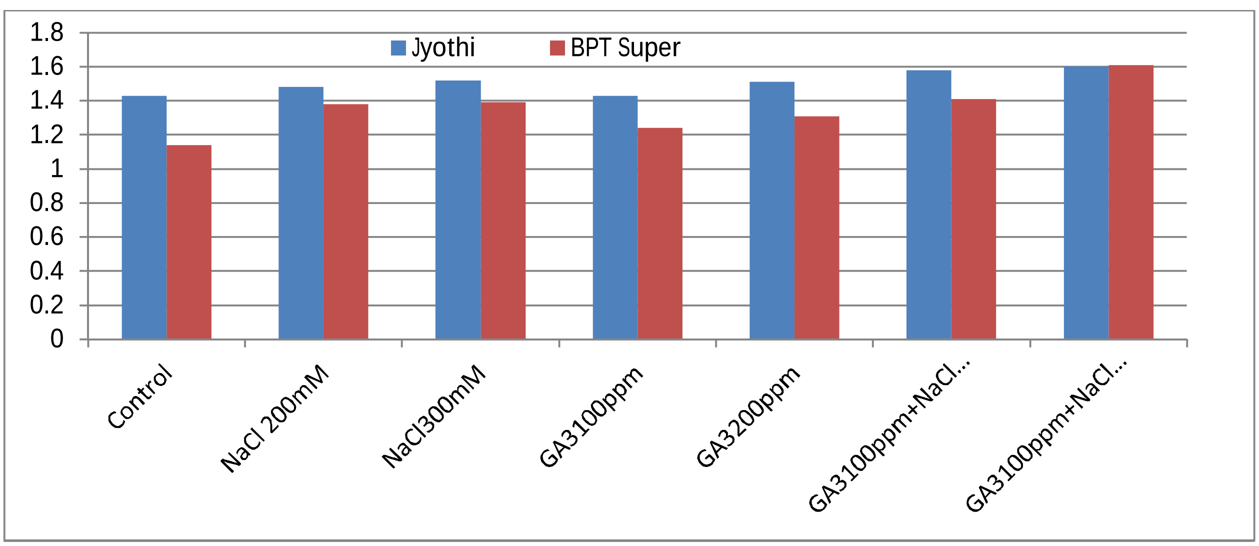
M. Effect of GA_3

The external use of GA_3 increases antioxidant level and will reduce the level of hydrogen peroxide hence the enzyme activity was increased under GA_3 treatment as was reported by Mohammed., et al., (2007)

Table 14: Effect of different concentrations of NaCl, GA_3 and combination of NaCl and GA_3 on Catalase activity (units' min^{-1} mg^{-1}protein) of Jyothi and BPT Super paddy cultivars on 14^{th} day.

Cultivar	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm + NaCl 200mM	GA_3 200ppm + NaCl 300mM
Jyothi	1.43[a]	1.48[a]	1.52[a]	1.43[a]	1.51[a]	1.58[a]	1.60[a]
BPT Super	1.14[c]	1.38[b]	1.39[b]	1.24[bc]	1.31[bc]	1.41[ab]	1.61[a]

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



Graph 2: Effect of different concentrations of NaCl, GA_3 and combination of NaCl and GA_3 on Catalase activity (units' min^{-1} mg^{-1}protein) of Jyothi and BPT Super paddy cultivars on 14^{th} day.

IV. CONCLUSION

α-amylase activity and β-amylase activity was found to be decreased under salinity and the increase was observed under GA_3 condition. Combination of GA_3 and NaCl also increased the α-amylase activity and β-amylase activity, the least affected from salinity was BPT Super cultivar and Jyothi cultivar was more affected. The salinity alleviating role of GA_3 was observed.

Catalase activity was increased under salinity in both the cultivars, and salinity was increased under GA_3 treatment and also under the combination of GA_3 and NaCl, so catalase activity was least affected due to the salinity in both the paddy cultivars.

With respect to all the above aspects Jyothi paddy cultivar is more salt sensitive compared to the BPT Super, and BPT Super shows better salinity tolerance when compared to the Jyothi cultivar, and salinity alleviating role of GA_3 was observed.

V. ACKNOWLEDGEMENT

The author is grateful to Principal Yuvaraja's College, Constituent autonomous college, University of Mysore. Mysore, Karnataka. For provide necessary facilities used for this work at the department of Botany and Biotechnology. Author also thank to Regional rice research station. V.C. Farm, Mandya.

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