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# Salinity Induced Changes in Amylases Activities and Phenol Content in Paddy Cultivars during Seed Germination

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**Abstract:** In the present study the two paddy cultivars were selected and the tests were conducted to examine the effect of salinity and different concentration of  $GA_3$  on the NaCl incubated seeds.  $GA_3$  and NaCl treated seeds showed increased content of phenol, reducing sugar, alpha and beta amylase activity during seed germination in both the cultivars. However, the one - way ANOVA performed for two paddy cultivars i.e., Jaya and Tanu value revealed a significant difference in control and different concentrations of  $GA_3$  on phenol, reducing sugar, alpha and beta amylase activity upon challenge inoculation with NaCl. Further the Scheffe (post hoc test) showed that the mean phenol, reducing sugar, alpha and beta amylase activity of Jaya and Tanu paddy cultivars in control was very less activity and different concentrations of  $GA_3$  and NaCl showed significantly different and high pronounced activity in  $GA_3$  concentration. Evidence that NaCl induced bioactive GA deficiency inhibits rice seed germination by decreasing alpha amylase activity via down regulation of alpha amylase gene expression and alpha amylase activity. Exogenous bioactive  $GA_3$  rescues NaCl inhibited seed germination by enhancing alpha and beta amylase activity, reducing sugar and also phenol content.

**Keywords:** Paddy,  $GA_3$ , NaCl, Phenol, Reducing sugar, alpha and beta amylase.

## I. INTRODUCTION

Abiotic stresses remain the greatest constraint to crop production worldwide. It has been projected that more than 50% of yield reduction is the direct result of abiotic stresses (Acquaah, 2007). The major abiotic stresses like drought, high salinity, cold and heat negatively influence the survival, biomass production and yield of staple food crops upto 70% (Ahmad and Prasad, 2012); hence, threaten the food production worldwide.

Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil. A considerable amount of land in the world is affected by salinity which is increasing day by day. On the other hand, increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% loss of cultivable lands by the middle of the twenty first century (Mahajan and Tuteja 2005).

High salt concentration in the soil or in the irrigation water can also have a devastating effect on plant metabolism, disrupting cellular homeostasis and uncoupling major physiological and biochemical processes. Biochemical and molecular studies of salt stress responses in plants have revealed significant increase of reactive oxygen species (ROS), including singlet oxygen ( $^1O_2$ ), superoxide ( $O_2^-$ ), hydroxyl radical ( $OH^\bullet$ ) and hydrogen peroxide ( $H_2O_2$ ) (Tanou *et al.*, 2009; Ahmad and Umar, 2011). However, the effect of salt stress on plants depends on the concentration and time of exposure of salt, plant genotypes and environmental factors.

Mechanisms of salt tolerance, not yet completely clear, can be explained to some extent by stress adaptation effectors that mediate ion homeostasis, osmolyte biosynthesis, toxic radical scavenging, water transport and long distance response co-ordination (Hasegawa *et al.*, 2000). However, attempts to improve yield under stress conditions by plant improvement have been largely unsuccessful, primarily due to the multigenic origin of the adaptive responses. Therefore, a well-focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to develop salt-tolerant crop varieties. Exploring suitable ameliorants or stress alleviant is one of the tasks of plant biologists.

Gibberellic acids are generally involved in growth and development; they control seed germination, leaf expansion, stem elongation and flowering (Magome *et al.*, 2004; Kim and Park, 2008). Additionally, GAs interacts with other hormones to regulate various metabolic processes in the plants.

However, many conflicting theories have been put forward concerning their interactions (Yang *et al.*, 1996; Van Huizen *et al.*, 1997). In order to alleviate deleterious effects of salinity, different types of phytohormones have been used. Among them, GA<sub>3</sub> have been the main focus of some plant scientists (Weiss and ori, 2007). Innumerable works have confirmed the potential of GA<sub>3</sub> to synergistically improve crop performance under normal conditions.

Hamayun *et al.* (2010) reported that exogenous GA<sub>3</sub> also mitigated the adverse effects of salt stress in *Glycine max* by regulating the level of phytohormones, thus aids the plant in resuming its normal growth and development. The application of GA<sub>3</sub> reduced the inhibitory effect of NaCl on growth attributes and photosynthetic pigments in *Hibiscus sabdariffa* by inducing the enzyme activity and enhancing RWC and thus GA<sub>3</sub> helped in the tolerance of plants to salt stress (Ali *et al.*, 2012). During the present investigation, an attempt has been made to study paddy cultivars primed with GA<sub>3</sub> and challenge with different saline conditions, seed germination and vigour index has been improved by application of GA<sub>3</sub> which is correlated with the studies of Kumar and Singh 1996.

Salinity has toxic effects on plants and causes of changes in metabolic activity such as reduced activity of chloroplasts, reduced photosynthetic pigments, reducing the rate of photosynthesis and increase of respiration rate, which ultimately leads to increased production of reactive oxygen species in plant. Content reduction of chlorophyll in plants such as *Poultownia imperialis* (Astorga *et al.*, 2010), Bean (Beinsan *et al.*, 2003) and *Carthamus tinctorius* (Siddiqi *et al.*, 2009) was reported.

The cause of this reduction was the increasing of destructive enzymes called chlorophyllase. Plant hormones such as gibberellin, has special effects on leaf anatomy and chloroplast structure. Salinity through the influence of on peroxidase enzymes and chlorophyll degradation can induce catabolism that gibberellin reduces the activity of these enzymes. The same result was reported by Aldesuquy (1992) in Wheat, El-Bastawisy (1999) in Wheat, El-Tayeb (2005) in Wheat and by Younis *et al.*, (1991) in *Pisium sativum*.

Gibberellins are a group of tetracyclic diterpenoid phytohormones and homeostasis plays important roles in regulating seed germination, plant growth and development (Mitsunaga and Yamaguchi, 1993; Richards *et al.*, 2001). GA<sub>3</sub> homeostasis is controlled by GA<sub>3</sub> metabolism including biosynthesis and inactivation (Hedden and Phillips, 2000, Frigerio *et al.*, 2006). However gibberellins including GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub>, and GA<sub>7</sub> are the bioactive forms in higher plants. Paddy cultivars primed with GA<sub>3</sub> and challenge inoculation with different concentration of NaCl showed increase photosynthetic contents in paddy cultivars. (Fahad *et al.*, 2015). In the present investigation, an attempt has been made to study the effect of salinity on paddy cultivars primed with GA<sub>3</sub>. Alpha amylase activities, phenol content, reducing sugar were studied at different concentration of saline conditions.

## II. MATERIALS AND METHODS

- 1) *Collection of seed Samples:* The seed samples of two cultivars of paddy viz., Jaya and Tanu were collected from V.C. Farm, Regional Agricultural Research Station, Mandya, Karnataka, India. The cultivars of paddy seeds were surface sterilized using 0.01% mercuric chloride for two minutes. The seeds were washed thoroughly with distilled water by 3-4 times and soaked in gibberellic acid for 24 hours at concentrations of 10ppm, 20ppm and 30ppm. The germination study was conducted by the between paper method recommended by ISTA, 2009. Hundred seeds of each cultivar were placed on Kraft paper saturated with known concentrations of salt (viz 0.01%, 0.02%). Seeds germinated in distilled water served as control. Each treatment including the control was replicated four times. The rolled papers were incubated in seed germinator at 28 ± 2°C. Total phenol was assayed as per the method of Mallick and Singh (1980). Reducing sugar was estimated following the method of Miller (DNS method 1959).  $\alpha$  and  $\beta$  - Amylase content was determined as per the procedure described by Peter Benfield (1955).

## III. RESULTS

The one - way ANOVA performed for two paddy cultivars *i.e.*, Jaya and Tanu value revealed a significant difference in control and different concentrations of GA<sub>3</sub> on phenol reducing sugar, alpha and beta amylase content upon challenge inoculation with NaCl. Further the Scheffe (post hoc test) showed that the mean phenol, reducing sugar, alpha and beta amylase content of Jaya and Tanu paddy cultivars in control and different concentrations of GA<sub>3</sub> and NaCl showed significantly different and phenol content, reducing sugar, alpha and beta amylase activity was more in GA<sub>3</sub> at 30ppm.

### A. Effect of 0.01% and 0.02% of NaCl on Phenol Content

Screening of paddy cultivars against 0.01% and 0.02% NaCl concentration revealed, that phenol content was more in both the cultivars from day 1 to 5<sup>th</sup> day in all the concentrations of GA<sub>3</sub> used *i.e.*, 10 ppm, 20ppm, 30ppm. Therefore compared to varieties and the concentration of phenol and salt tolerant was more pronounced in Jaya variety shown in Tables 1 and 2.

Table 1: Effect of 0.01% NaCl on phenol content of paddy cultivars pre soaked with GA<sub>3</sub> during germination (mg/g)

Paddy cultivars	Parameters	NaCl 0.01%	Control	Different concentration of GA <sub>3</sub>		
				10ppm	20ppm	30ppm
Jaya	Phenol	1 <sup>st</sup> day germination	0.241 <sup>c</sup>	0.249 <sup>c</sup>	0.401 <sup>b</sup>	0.693 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.243 <sup>d</sup>	0.342 <sup>c</sup>	0.495 <sup>b</sup>	0.751 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.255 <sup>d</sup>	0.471 <sup>c</sup>	0.522 <sup>b</sup>	0.899 <sup>a</sup>
		4 <sup>th</sup> day germination	0.257 <sup>c</sup>	0.554 <sup>b</sup>	0.721 <sup>b</sup>	1.121 <sup>a</sup>
		5 <sup>th</sup> day germination	0.260 <sup>d</sup>	0.704 <sup>c</sup>	1.102 <sup>b</sup>	1.247 <sup>a</sup>
Tanu		1 <sup>st</sup> day germination	0.211 <sup>c</sup>	0.202 <sup>c</sup>	0.321 <sup>b</sup>	0.456 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.215 <sup>c</sup>	0.311 <sup>b</sup>	0.344 <sup>b</sup>	0.572 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.222 <sup>c</sup>	0.354 <sup>b</sup>	0.412 <sup>b</sup>	0.695 <sup>a</sup>
		4 <sup>th</sup> day germination	0.232 <sup>c</sup>	0.426 <sup>b</sup>	0.553 <sup>ab</sup>	0.791 <sup>a</sup>
		5 <sup>th</sup> day germination	0.242 <sup>d</sup>	0.622 <sup>c</sup>	1.072 <sup>b</sup>	1.141 <sup>a</sup>

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

Table 2: Effect of 0.02% NaCl on phenol content of paddy cultivars pre soaked with GA<sub>3</sub> during germination of seeds (mg/g)

Paddy cultivars	Parameters	NaCl 0.02%	Control	Different concentration of GA <sub>3</sub>		
				10ppm	20ppm	30ppm
Jaya	Phenol	1 <sup>st</sup> day germination	0.242 <sup>c</sup>	0.491 <sup>bc</sup>	0.592 <sup>b</sup>	0.781 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.246 <sup>d</sup>	0.562 <sup>bc</sup>	0.761 <sup>b</sup>	1.245 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.259 <sup>c</sup>	0.696 <sup>b</sup>	0.844 <sup>b</sup>	1.369 <sup>a</sup>
		4 <sup>th</sup> day germination	0.261 <sup>d</sup>	0.714 <sup>c</sup>	1.224 <sup>ab</sup>	1.472 <sup>a</sup>
		5 <sup>th</sup> day germination	0.265 <sup>d</sup>	0.856 <sup>c</sup>	1.296 <sub>b</sub>	1.741 <sup>a</sup>
Tanu		1 <sup>st</sup> day germination	0.215 <sup>c</sup>	0.421 <sup>b</sup>	0.492 <sup>b</sup>	0.672 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.219 <sup>c</sup>	0.514 <sup>b</sup>	0.529 <sup>b</sup>	0.894 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.226 <sup>c</sup>	0.581 <sup>b</sup>	0.671 <sup>b</sup>	1.214 <sup>a</sup>
		4 <sup>th</sup> day germination	0.236 <sup>c</sup>	0.671 <sup>b</sup>	0.742 <sup>b</sup>	1.341 <sup>a</sup>
		5 <sup>th</sup> day germination	0.248 <sup>d</sup>	0.721 <sup>c</sup>	1.231 <sup>b</sup>	1.694 <sup>a</sup>

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

### B. Effect of 0.01% and 0.02% of NaCl on Reducing Sugar Content

Screening of paddy cultivars against 0.01% and 0.02% NaCl concentration revealed that, the reducing sugar concentration was more in both the cultivars from day 1 to 5<sup>th</sup> day in all the concentrations of GA<sub>3</sub> used *i.e.*, 10 ppm, 20ppm, 30ppm. Therefore compared to varieties the concentration of reducing sugar and salt tolerant was more pronounced in Jaya variety. The increase observed from 1<sup>st</sup> day to 5<sup>th</sup> day with 30ppm of concentration of GA<sub>3</sub> probably reflected an increase in carbohydrate metabolism, in response to the increased water uptake by germinating seeds as shown in Tables 3 and 4.

Table 3: Effect of 0.01% NaCl on reducing sugar content of paddy cultivars pre soaked with GA<sub>3</sub> during germination (mg g<sup>-1</sup> fresh wt)

Paddy cultivars	Parameters	NaCl 0.01%	Control	Different concentration of GA <sub>3</sub>		
				10ppm	20ppm	30ppm
Jaya	Reducing sugar	1 <sup>st</sup> day germination	0.133 <sup>d</sup>	0.178 <sup>c</sup>	0.348 <sup>b</sup>	0.571 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.142 <sup>d</sup>	0.567 <sup>c</sup>	0.606 <sup>a</sup>	0.991 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.145 <sup>d</sup>	0.893 <sup>c</sup>	1.051 <sup>b</sup>	1.107 <sup>a</sup>
		4 <sup>th</sup> day germination	0.148 <sup>d</sup>	1.014 <sup>c</sup>	1.101 <sup>b</sup>	1.253 <sup>a</sup>
		5 <sup>th</sup> day germination	0.150 <sup>d</sup>	1.119 <sup>c</sup>	1.163 <sup>b</sup>	1.273 <sup>a</sup>
Tanu	Reducing sugar	1 <sup>st</sup> day germination	0.113 <sup>d</sup>	0.123 <sup>c</sup>	0.162 <sup>b</sup>	0.241 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.117 <sup>d</sup>	0.189 <sup>c</sup>	0.215 <sup>b</sup>	0.382 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.119 <sup>d</sup>	0.294 <sup>c</sup>	0.471 <sup>b</sup>	0.559 <sup>a</sup>
		4 <sup>th</sup> day germination	0.120 <sup>d</sup>	0.355 <sup>c</sup>	0.561 <sup>a</sup>	0.672 <sup>a</sup>
		5 <sup>th</sup> day germination	0.125 <sup>d</sup>	0.521 <sup>b</sup>	0.618 <sup>b</sup>	0.819 <sup>a</sup>

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

Table 4: Effect of 0.02% NaCl on reducing sugar content of paddy cultivars pre soaked with GA<sub>3</sub> during germination (mg g<sup>-1</sup> fresh wt)

Paddy cultivars	Parameters	NaCl 0.02%	Control	Different concentration of GA <sub>3</sub>		
				10ppm	20ppm	30ppm
Jaya	Reducing sugar	1 <sup>st</sup> day germination	0.135 <sup>d</sup>	0.356 <sup>c</sup>	0.481 <sup>b</sup>	0.677 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.137 <sup>d</sup>	0.561 <sup>c</sup>	0.821 <sup>b</sup>	0.907 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.162 <sup>d</sup>	0.723 <sup>c</sup>	1.206 <sup>b</sup>	1.246 <sup>a</sup>
		4 <sup>th</sup> day germination	0.169 <sup>d</sup>	1.046 <sup>c</sup>	1.267 <sup>b</sup>	1.292 <sup>a</sup>
		5 <sup>th</sup> day germination	0.181 <sup>d</sup>	1.152 <sup>c</sup>	1.307 <sup>b</sup>	1.364 <sup>a</sup>
Tanu	Reducing sugar	1 <sup>st</sup> day germination	0.121 <sup>d</sup>	0.175 <sup>c</sup>	0.199 <sup>b</sup>	0.232 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.125 <sup>d</sup>	0.192 <sup>c</sup>	0.270 <sup>b</sup>	0.466 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.139 <sup>d</sup>	0.399 <sup>c</sup>	0.492 <sup>b</sup>	0.675 <sup>a</sup>
		4 <sup>th</sup> day germination	0.141 <sup>d</sup>	0.622 <sup>c</sup>	1.088 <sup>b</sup>	1.192 <sup>a</sup>
		5 <sup>th</sup> day germination	0.162 <sup>d</sup>	1.060 <sup>c</sup>	1.124 <sup>b</sup>	1.216 <sup>a</sup>

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

### C. Effect of 0.01% and 0.02% of NaCl on alpha Amylase Content

The results revealed that the mean alpha activity of Jaya and Tanu rice cultivars in control and different concentrations of GA<sub>3</sub> and 24 hrs incubated in NaCl at 0.01% and 0.02% of NaCl showed significantly different. Therefore compared to varieties the concentration of alpha - amylase and salt tolerant was more pronounced in Jaya variety as shown in Tables 5 and 6.

Table 5: Effect of 0.01% NaCl on alpha amylase content of paddy cultivars pre soaked with GA<sub>3</sub> during germination ( $\mu\text{mole min}^{-1}\text{g}^{-1}$ )

Paddy cultivars	Parameters	NaCl 0.01%	Control	Different concentration of GA <sub>3</sub>		
				10ppm	20ppm	30ppm
Jaya	alpha amylase	1 <sup>st</sup> day germination	0.013 <sup>d</sup>	0.034 <sup>c</sup>	0.047 <sup>b</sup>	0.053 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.018 <sup>d</sup>	0.038 <sup>c</sup>	0.072 <sup>b</sup>	0.124 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.023 <sup>d</sup>	0.114 <sup>c</sup>	0.155 <sup>b</sup>	0.164 <sup>a</sup>
		4 <sup>th</sup> day germination	0.027 <sup>d</sup>	0.161 <sup>c</sup>	0.171 <sup>b</sup>	0.189 <sup>a</sup>
		5 <sup>th</sup> day germination	0.030 <sup>d</sup>	0.215 <sup>c</sup>	0.222 <sup>b</sup>	0.237 <sup>a</sup>
Tanu	alpha amylase	1 <sup>st</sup> day germination	0.011 <sup>d</sup>	0.025 <sup>c</sup>	0.033 <sup>b</sup>	0.052 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.016 <sup>d</sup>	0.044 <sup>c</sup>	0.076 <sup>b</sup>	0.108 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.022 <sup>d</sup>	0.133 <sup>c</sup>	0.147 <sup>b</sup>	0.158 <sup>a</sup>
		4 <sup>th</sup> day germination	0.026 <sup>d</sup>	0.140 <sup>c</sup>	0.153 <sup>b</sup>	0.161 <sup>a</sup>
		5 <sup>th</sup> day germination	0.029 <sup>d</sup>	0.191 <sup>c</sup>	0.209 <sup>b</sup>	0.229 <sup>a</sup>

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

Table 6: Effect of 0.02% NaCl on alpha amylase content of paddy cultivars pre soaked with GA<sub>3</sub> during germination ( $\mu\text{mole min}^{-1}\text{g}^{-1}$ )

Paddy cultivars	Parameters	NaCl 0.02%	Control	Different concentration of GA <sub>3</sub>		
				10ppm	20ppm	30ppm
Jaya	alpha amylase	1 <sup>st</sup> day germination	0.018 <sup>d</sup>	0.022 <sup>c</sup>	0.040 <sup>b</sup>	0.070 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.024 <sup>d</sup>	0.041 <sup>c</sup>	0.057 <sup>b</sup>	0.065 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.029 <sup>d</sup>	0.115 <sup>c</sup>	0.143 <sup>b</sup>	0.151 <sup>a</sup>
		4 <sup>th</sup> day germination	0.047 <sup>d</sup>	0.128 <sup>c</sup>	0.185 <sup>b</sup>	0.199 <sup>a</sup>
		5 <sup>th</sup> day germination	0.066 <sup>d</sup>	0.191 <sup>c</sup>	0.209 <sup>b</sup>	0.231 <sup>a</sup>
Tanu	alpha amylase	1 <sup>st</sup> day germination	0.016 <sup>d</sup>	0.023 <sup>c</sup>	0.052 <sup>b</sup>	0.069 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.019 <sup>d</sup>	0.053 <sup>c</sup>	0.066 <sup>b</sup>	0.094 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.026 <sup>d</sup>	0.071 <sup>c</sup>	0.086 <sup>b</sup>	0.142 <sup>a</sup>
		4 <sup>th</sup> day germination	0.032 <sup>d</sup>	0.116 <sup>c</sup>	0.162 <sup>b</sup>	0.172 <sup>a</sup>
		5 <sup>th</sup> day germination	0.036 <sup>d</sup>	0.127 <sup>c</sup>	0.186 <sup>b</sup>	0.201 <sup>a</sup>

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

#### D. Effect of 0.01% and 0.02% of NaCl on beta Amylase Content

The results shown that the mean  $\beta$ - amylase activity of Jaya and Tanu rice cultivars in control and different concentrations of GA<sub>3</sub> and 24 hrs incubated in NaCl at 0.01% and 0.02% of NaCl were significantly different. Therefore compared to varieties the concentration of  $\beta$ - amylase and salt tolerant was more pronounced in Jaya variety as shown in Tables 7 and 8.

Table 7: Effect of 0.01% NaCl on beta amylase content of paddy cultivars pre soaked with GA<sub>3</sub> during germination ( $\mu\text{mole min}^{-1}\text{g}^{-1}$ )

Paddy cultivars	Parameters	NaCl 0.01%	Control	Different concentration of GA <sub>3</sub>		
				10ppm	20ppm	30ppm
Jaya	beta amylase	1 <sup>st</sup> day germination	0.012 <sup>c</sup>	0.014 <sup>c</sup>	0.025 <sup>b</sup>	0.045 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.015 <sup>d</sup>	0.032 <sup>c</sup>	0.041 <sup>b</sup>	0.052 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.020 <sup>c</sup>	0.039 <sup>c</sup>	0.043 <sup>b</sup>	0.059 <sup>a</sup>
		4 <sup>th</sup> day germination	0.024 <sup>d</sup>	0.041 <sup>c</sup>	0.052 <sup>b</sup>	0.064 <sup>a</sup>
		5 <sup>th</sup> day germination	0.030 <sup>d</sup>	0.052 <sup>c</sup>	0.069 <sup>b</sup>	0.078 <sup>a</sup>
Tanu		1 <sup>st</sup> day germination	0.009 <sup>d</sup>	0.012 <sup>c</sup>	0.021 <sup>b</sup>	0.035 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.012 <sup>d</sup>	0.015 <sup>c</sup>	0.029 <sup>b</sup>	0.036 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.015 <sup>c</sup>	0.017 <sup>c</sup>	0.032 <sup>b</sup>	0.040 <sup>a</sup>
		4 <sup>th</sup> day germination	0.021 <sup>c</sup>	0.030 <sup>b</sup>	0.039 <sup>b</sup>	0.040 <sup>a</sup>

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

Table 8: Effect of 0.02% NaCl on beta amylase content of paddy cultivars pre soaked with GA<sub>3</sub> during germination ( $\mu\text{mole min}^{-1}\text{g}^{-1}$ )

Paddy cultivars	Parameters	NaCl 0.02%	Control	Different concentration of GA <sub>3</sub>		
				10ppm	20ppm	30ppm
Jaya	beta amylase	1 <sup>st</sup> day germination	0.011 <sup>d</sup>	0.021 <sup>c</sup>	0.023 <sup>b</sup>	0.029 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.020 <sup>d</sup>	0.027 <sup>c</sup>	0.034 <sup>b</sup>	0.045 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.024 <sup>c</sup>	0.029 <sup>c</sup>	0.042 <sup>b</sup>	0.049 <sup>a</sup>
		4 <sup>th</sup> day germination	0.029 <sup>d</sup>	0.037 <sup>c</sup>	0.057 <sup>ba</sup>	0.062 <sup>a</sup>
		5 <sup>th</sup> day germination	0.039 <sup>d</sup>	0.049 <sup>c</sup>	0.130 <sup>b</sup>	0.141 <sup>a</sup>
Tanu		1 <sup>st</sup> day germination	0.010 <sup>d</sup>	0.015 <sup>c</sup>	0.030 <sup>b</sup>	0.040 <sup>a</sup>
		2 <sup>nd</sup> day germination	0.014 <sup>d</sup>	0.021 <sup>c</sup>	0.033 <sup>b</sup>	0.045 <sup>a</sup>
		3 <sup>rd</sup> day germination	0.024 <sup>c</sup>	0.028 <sup>bc</sup>	0.039 <sup>b</sup>	0.051 <sup>a</sup>
		4 <sup>th</sup> day germination	0.031 <sup>d</sup>	0.044 <sup>b</sup>	0.048 <sup>b</sup>	0.057 <sup>a</sup>
		5 <sup>th</sup> day germination	0.049 <sup>c</sup>	0.057 <sup>c</sup>	0.062 <sup>b</sup>	0.071 <sup>a</sup>

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. DISCUSSION

### A. Phenol Content

Secondary metabolites play an important role as antioxidants and antiradicals supporting plants to deal with oxidative stress (Gould *et al.*, 2002). Phenolic acids are secondary metabolites extensively spread throughout the plant kingdom (Tomas-Barberan and Espin, 2001). Phenolic compounds are crucial for plant growth and reproduction and are produced as response to unfavourable environmental factors and to defend injured plants (Valentine *et al.*, 2003). The increase of total phenols was observed during germination of rice seeds treated with GA<sub>3</sub> under salinity. Seeds would respond to the need of seedlings to free the non adverse condition due to salinity during germination. Long term physiological changes due to salinity to start accurate ions (Na<sup>+</sup> and Cl<sup>-</sup>) more quickly absorb during germination.

Reducing sugars increased with the age of seedling. But the increase was much more than control treated with different concentration of  $GA_3$ . Germination, growth, respiration and other related processes can be affected in seeds that are subjected to salt stress. Changes in any one of these processes can affect other metabolic activities, particularly the carbohydrate metabolism that plays an important role in germination. In this context, our results showing that occurred in soluble sugar content during exposure to salt stress of rice seeds. Seed carbohydrates metabolism under stress conditions can be considered a dynamic process involving often naturally accompanying occurring process of polysaccharide degradation and synthesis of new compounds. Reducing sugar content was more in treated with  $GA_3$  indicated that rapid utilization of sugar reserves leading to completion of germination. Successful utilization of germination process as it is an early source of energy and substrate (Mayer and Pojarkoff-Mayber 1975; Bewley 1997; Mei and Song 2008).

### B. Amylases

Exogenous supply of  $GA_3$  counteracted the adverse effect of salt on amylase activity. The  $\alpha$ -Amylase is a crucial enzyme that participates in the degradation of starch granules into small organic molecules to provide energy and nutrients for seed germination. 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 during seed germination.

Alpha amylase hydrolyses the reserve starch into sugars, which provide necessary energy and osmotic adjustment to the growing embryo (Ashraf *et al.*, 1991). Alpha amylase activity is an important factor in seed germination. In the present studies, NaCl induced bioactive GA deficiency inhibited during seed germination by reducing alpha amylase activity in control and  $GA_3$  treatments on alpha and beta amylase activity were increased with increasing the concentration of the  $GA_3$ . Whereas NaCl treated inhibits rice seed germination by decreasing the contents of bioactive gibberellins. That inhibition rescued by exogenous bioactive GA application. Liu *et al.*, (2018) suggested that NaCl inactivated genes, and the up regulated expression of GA biosynthetic genes might be a consequence of negative feedback regulation of the bioactive GA deficiency. Evidence that NaCl induced bioactive GA deficiency inhibits rice seed germination by decreasing alpha amylase activity via down regulation of alpha amylase gene expression. Exogenous bioactive GA rescues NaCl inhibited seed germination by enhancing alpha amylase activity. Thus NaCl treatment reduces bioactive GA content through promotion of bioactive GA inactivation, which in turn inhibits rice seed germination by decreasing alpha amylase activity of alpha gene expression.

In *Limonium bicolor* treated with melatonin significantly improved seed germination under salt stress. During seed germination, seeds pre-treated with melatonin contained high levels of melatonin and gibberlic acid ( $GA_3$ ) and high levels of amylase and alpha amylase activity. Melatonin treatment up regulated the expression of key genes involved in GA biosynthesis (*GA20ox* and *GA3ox*), and which mediate the changes in GA levels in seeds during germination. A high melatonin concentration in seeds promotes the utilization of nutrients and the synthesis of new proteins to enhance seed germination (Junpeng Li., *et al.*, 2019).

## V. CONCLUSION

Finally, it can be inferred that  $GA_3$  counteracts with salinity by improving membrane permeability and nutrient levels in leaves which ultimately leads to better seedling growth and also  $GA_3$  induced physicochemical changes responsible for induction of salt tolerance in paddy especially Jaya and Tanu. It is, therefore, possible that exogenous applications of  $GA_3$  could be a useful tool in promoting good seedlings growth and establishment under saline soil conditions.

It was concluded from the present investigation that with the increase in NaCl and  $GA_3$  concentration, applied to different paddy cultivars, the quality enhanced with regards to phenol content, reducing sugar content and amylase enzyme activity studies. However, the effect of salt stress on plants depends on the concentration and time of exposure of salt, plant genotypes and environmental factors.

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