Govindaraj C *et al*, International Journal of Advances in Agricultural Science and Technology,
Vol.7 Issue.7, July-2020, pg. 22-39 ISSN: 2348-1358
Impact Factor: 6.057
NAAS Rating: 3.77

EFFECT OF PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSE OF *MUSA ACUMINATA* UNDER *IN VITRO* STRESS CONDITION WITH DIFFERENT SALINITY LEVELS

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Abstract: Salinity represents one among the foremost abiotic stress factors influence plant growth and production round the world. In this experiment, in vitro multiplication of Musa acuminata has been standardized. The physiological and morphological responses of the crop against salt stress has been identified. Seven different NaCl levels [0(control), 50, 100, 150, 200, 250and 300mM] were maintained at shoot multiplication stage for 6-weeks.To study the effects of Salicylic acid (SA) and Abscisic acid (ABA) on salt stress in banana (Musa acuminata), the explants were treated with varying SA and ABA concentrations (0.5, 1, 1.5, 2, 2.5 and 3mM) and incubated on MS media containing different levels (0, 50, 100, 150, 200, 250, 300mM) of NaCl in vitro. After two months, proliferation rate, fresh weight increase, relative water content, chlorophyll level and proline accumulations were measured and analyzed. The results indicated that with increasing levels of NaCl, proliferation rate, fresh weight, relative water content and chlorophyll concentrations were significantly decreased. The SA and ABA concentrations improved plant performance by increasing proliferation rate, fresh weight increase and relative water content. Although, non SA and ABA treated plants were not significantly responsive to increasing levels of NaCl in terms of elevated proline content, they responded positively to supply of SA and ABA by showing significant increase in proline, fresh weight and chlorophyll contents under water stressed conditions. The results revealed that exogenous application of SA and ABA helped to reduce the harmful effects of salt deficient on banana regenerates in vitro and in vivo condition.
Keywords: in vitro, Musa acuminata, proline, phytohormones, stress tolerance

1. Introduction

The Banana belongs to the family of Musaceae. Banana is grown in wide scale in tropical and subtropical regions. Banana is that the "queen of tropical fruits" and is one among the oldest fruits known to mankind from prehistoric times [4]. Salinity is one among the main growth limiting abiotic factors, decreasing propagation rate of plant under natural also as artificial conditions. Salt stress are induce slow growth, stomatal closure and thus reduces photosynthesis. Plants have evolved to measure in environments where they're often exposed to different stress factors together [1]. Plants activate a selected and unique stress response when subjected to a mixture of multiple stresses [1, 4]. Utilization of tissue culture techniques for quantifying stress tolerance of varied crops has been increasing rapidly. Also, In vitro culture techniques minimize environmental variations thanks to defined nutrient media, controlled conditions and homogeneity

of stress application [6]. A standard strategy is that the synthesis and accumulation of compatible solutes like proline, glycine, betaine, polyamines. Today, agricultural acreage is losing its fertility due to soil salinity increasing with the passage of your time in arid to semi-arid regions of the plant. It's resulting either by natural processes or crop irrigation with saline water [27]. Normal plant growth is assumed, when cultured under normal and balance nutritional conditions. While, if any of the nutritional components (may be inorganic or organic) isn't properly supplied (deficient or exceeded) than it results in abnormal expression in them [15]. Most of the salinity problems are caused by excess NaCl. It imposes different three sorts of problems, like on exceed external pressure than internal. Disruptions of balanced nutritional ions in cells could develop toxic effects on membranes and enzymes directly [11]. Moreover, plant growth depends on proper metabolic processes. Metabolic dysfunction is caused by ion toxicity, osmotic stress and nutritional deficiency [14]. Under stressed conditions, plants need to synthesis and accumulate various compatible solutes like free amino acids (proline, betaine etc), sugars and polyols [20]. These are playing a pivotal role to compensate developed osmotic stress thanks to salinity in several organisms including plants [16]. The fluctuations in metabolites are observable in growing plantlets through micro-propagation [3]. Among physical conditions, salinity may be a critical factor, limiting plant propagation efficiency in ex-vitro also as Invitro. So plant part culture system has been acting as a useful gizmo to assess effectiveness of varied salts on plant growth related characters [13]. However, salt tolerance mechanisms involved at the entire plant level growing under natural conditions might be quite different from *In vitro* cultures [1]. Therefore, the objectives of this study were to work out the effect of salts on the some morphological and physiological exchanges in banana whether application of NaCl to banana could be a technique for increasing the salt tolerance.

2. Materials and Methods

Collection of Culture Plant

The mother plant stocks were collected from the Banana plantation fields of Perandapalli, Hosur Krishnagiri District of Tamil Nadu. Suckers of mother stocks were maintained in the garden of KSRCT, Tiruchengode and simulation established in plant tissue culture laboratory.

Culture Condition

Standardize the temperature and light intensity condition for propagation was managed. It can be observed for *Musa acuminata* micro propagation kept in under dark condition for 8 hours and culture were placed under a fluorescent lights for 16 hours [22]. The cultures were maintained at 25±2°C at the 80% of humidity.

Preparation of MS Medium

MS medium along with different concentration and various combination auxin and cytokinin was used in the media. The pH of the developed medium was altered at 5.7 prior to inclusion of agar at 5 g/l. The medium was give out into the culture flask (325 ml.), where each flask carried 30 ml of the medium [34]. The culture jars were autoclaved at 121ºC at 15 lb/inch for 20 min. Cultures were allowed to grow for 8 weeks at 27 °C of day and night temperature. Illumination was as long as by white fluorescent tubes allow intensity of about 1500 Lux at the culture level [22].

Sterilization of Shooting Media

Shoot proliferation medium consist of the MS medium IAA 1mg/l and BAP 3mg/l and solidifying agent of 2.8 g/l clerigel fortified with pH of the medium was maintained with 5.7 [13]. To add salt stress inducing agent various concentration (50, 100, 150, 200, 250 and 300mM) of NaCl and another salt tolerance inducing agent ABA and SA concentration level (0, 0.5, 1, 1.5, 2, 2.5 and 3mM) was added to the MS media [21]. The shooting media sterilized at 121°C for 20 minutes at 15 psi.

Proline Assay

Proline content of the leaf sample was determined by the tactic of [7]. It is necessary to study the accumulation of proline in the plant for water deficit stress activity. Fresh plant leaf samples of 0.3 g were macerated with 10ml of 3% aqueous sulphosalicyclic acid by using mortar and pestle. The leaf extract were spun at 4000 rpm for 10 minutes. Discard the pellet and then collect the supernatant solution for each samples was taken in a 2 ml of test tube and to this 2 ml of acid ninhydrin (Preparation for acid ninhydrin: 2.5 g of ninhydrin was taken and mixing to the 6 M 40 ml of orthophosphoric acid and 60 ml of glacial acetic acid with agitation until dissolved for water bath. Kept in 4°C for reagent stored at 24 hours) were added for each samples and then 2 ml of glacial acetic acid were added for all test tubes.

The test solution were heated to kept in water bath for one hour incubation at 100°C and it was cooled under tap water for few minutes. After cooling, the test solution was transferred into a separating funnel. By adding 4ml of toluene were added in the separating funnel. The tube was similarly shacked during 15-30 seconds. Two different layers were separated. The upper color layer was collected and another bottom colorless layer was discarded. The optical density was measured at 520 nm against using toluene for blank solution. The proline concentrations were determined during from standard curve by using D-proline [19]. Proline reaction mixture of 1:1:1 solution of D-proline, acid ninhydrin and glacial acetic acid was incubated for one hour at 100°C. The reaction was arrested in an ice bath and the standard solution was collected into a separating funnel and 4ml of toluene were added. The absorbance were analyzed during 520 nm using spectrophotometer.

ISSN: 2348-1358
Impact Factor: 6.057
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Photosynthetic Pigments

A fresh plant leaves were washed and rinsed with running tap water and then with in the distilled water [14]. A fresh leaf sample of 0.2 g of was macerated with 20 ml of 80% acetone by using mortar and pestle. Leaf extract were collected in the centrifuge tube by adding 80% acetone were spun at 4000 rpm for 10 minutes [9, 10]. The pellet was discarded and all the samples of supernatant were collected. The optical density of absorbance was measured at 663 and 645 nm for chlorophyll 'A' and chlorophyll 'B'. The amount of total chlorophylls were analyzed by the following equations [23].

$$(1)\ \text{For chlorophyll a/g tissue} = \frac{12.7(A663) - 2.69(A645) \times \text{Volume of Extract}}{1000 \times \text{Weight of tissue extract}}$$

$$(2)\ \text{For chlorophyll b/g tissue} = \frac{22.9(A665) - 4.68(A645) \times \text{Volume of Extract}}{1000 \times \text{Weight of tissue extract}}$$

$$(3)\ \text{For total chlorophyll /g tissue} = \frac{8.02(A645) + 20.2(A663) \times \text{Volume of extract}}{1000 \times \text{Weight of tissue extract}}$$

Relative Water Content

Discs (1 cm in diameter) from middle portion of fully developed leaf were randomly taken from chosen plants of each replicate [32]. Discs were weighed (FW) and then immediately floated on distilled water for 5 hours in the dark. Turgid weights (TW) of plant leaf discs were obtained after parched excess outside water with tissue paper. Dry weights (DW) of discs were measured after drying at 75ºC for 48 h. Relative water content (RWC) was determined using the succeeding formula [25].

$$\text{Relative water content} = \frac{\text{(Fresh weight-Dry weight)}}{\text{(Turgid weight-Dry weight)}}$$

3. Result and Discussion

Mass Multiplication of *Musa acuminata*

The plants which are inoculated on the MS medium supplement with BAP (1- 9mg/l) was initiated after three week of inoculation in the culture room. After three weeks duration the initiated culture were sub cultured in the same medium for multiplication. The importance of the appliance of more BAP aggregation to begin bud developed from explants. A discount within the number also as length of root that appear with exposure to increased levels of BAP

alone (9 mg/l) in banana plant (Table 1). Harmonious effects of explant growth regulators have affected the cultural reaction in banana shoot multiplication and elongation. The media was optimized with BAP 3 mg/l is produced multiplication of shoot and mass culture.

Table 1: Effect of BAP for the Multiplication of *Musa acuminata* on MS Medium

S.NO	Concentration of BAP (mg/l)	Mean no of plants multiplication (m±sd)	Length of culture roots after 5th cycle (m±sd) cm	Effect of BAP for the multiplication of *Musa acuminata*
1	1	5.37±2.296	4.2±0.89	
2	3	10.52 ±6.77	4.6±0.47	
3	5	4.67±1.032	3.8±0.37	
4	7	1.85±0.872	3.2±0.72	
5	9	0.55±0.332	3.01±0.15	

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Plant Cultivate Under Different NaCl Stress Condition

Salinity can eventually cause to decreased crop productivity. In numerous areas secondary salinization, as a solutions of irrigation practices, water quality or drainage, are primary component contributing to the reduction of productive farming land. Increasing salinity reduces plant development by affecting the plant's osmotic and ionic homeostasis. *In vitro* studies have analyzed physiological and phenotypic reaction of banana to salinity under *in vitro* conditions. Identification of salt-tolerant genotypes from the banana culture is required. *In vitro* cultivation of *Musa acuminata* species in the medium augmented with different concentration of NaCl (50, 100, 150, 200, 250 and 300 mM) and hormone concentration IAA (1mg/l) and BAP (3mg/l). After one month sub culture will be carried out for every plant sample. Plant growth and shoot development will be measured for every plant sample.

Table 2: Plant growth in different NaCl stress

S.NO	Hormone concentration	Salt concentration NaCl (mM)	Mean no of plants multiplication (m±sd)	Multiplication of *Musa acuminata* on MS media with different NaCl levels
1	BAP-3mg/l IAA-1mg/l	50	0.4±0.405	
2		100	0.3±0.171	
3		150	0.27±0.176	
4		200	0.15±0.042	

5		250	0.05±0.007	
6		300	0.01±0.005	

Banana micro propagation under NaCl with Salicylic acid and Abscisic acid

Salicylic acid (SA) is a phenolic phytohormone and is found in plants with roles in plant growth and development, photosynthesis, transpiration and ion uptake. Salicylic acid induce innate plant immunity, the role of SA is the function of guarding cells. SA also makes specific changes in leaf chloroplast and exploration structure. SA is intricate in endogenic signaling, mediating in plant protection against microbes. The plants which are inoculated on the MS medium supplement with NaCl with SA was initiated after three week of inoculation in the culture room. A reduction in the number as well as length of root that occurred with exposure to high levels of NaCl alone (250mM) in banana plant. The result showed that Plant height, leaf and root length was augmented by adding salicylic acid instead of Abscisic acid.

Table 3: Plant growth in different levels of NaCl with SA

S.no	Hormone concentration	Salt concentration NaCl (mM)	Salicylic acid concentration (mM)	Mean no of plants multiplication (m±sd)	Plant growth in different concentration of NaCl treated with SA
1	BAP-3mg/l IAA-1mg/l	50	0.5	6.23±0.233	
2		100	1	4.53±0.22	

ISSN: 2348-1358
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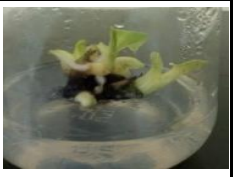
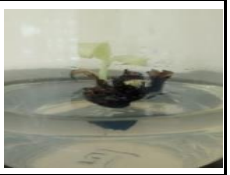
NAAS Rating: 3.77

3		150	1.5	3.23±0.87	
4		200	2	3.00±0.55	
5		250	2.5	1.87±0.54	
6		300	3	0.98±0.22	

Abscisic acid mediated signaling also plays an important part in plant responses to environmental stress and plant pathogens. ABA plays an important role in plant adaptation to salinity stresses. ABA act as differential signal transduction pathways on cells which are the least and most affected by the imposed stress. ABA may regulate through some genes/gene products, which control the expression of stress or adaptive-specific genes. The plants which are inoculated on the MS medium supplement with NaCl with ABA was initiated after three week of inoculation in the culture room. A reduction in the number as well as length of root that occurred with exposure to high levels of NaCl alone (250mM) in banana plant. The result showed that Plant height, leaf and root length was augmented by adding salicylic acid instead of Abscisic acid. Abscisic acid (1.5 and 2mM) plays an important role in produced salt tolerance plant as compared with salicylic acid treated plants.

Table 4: Plant growth in different concentration of NaCl with ABA

S.NO	Hormone concentration	Salt concentration NaCl (mM)	Abscisic acid concentration (mM)	Mean no of plants multiplication (m±sd)	Plant growth in different concentration of NaCl treated with ABA
1	BAP-3mg/l IAA-1mg/l	50	0.5	5.33±0.47	
2		100	1	5.00±0.577	
3		150	1.5	8.33±0.333	
4		200	2	6.43±0.33	
5		250	2.5	2.67±0.33	
6		300	3	1.98±0.75	

Proline Content analysis in *Musa acuminata*

Proline is liable for protecting plant tissues from salinity stress injury. It's liable for imparting salt stress tolerance to plant part, but proline deposition seems to be a symbol of cells injury instead of to a measure for salinity tolerance. In our work it had been determined that salinity (NaCl) stress are dramatics as critical

factor for banana micro propagation productivity. Exogenous application of SA and ABA did not significantly affect Proline content under normal conditions (treatments without the addition of NaCl to the media (control)). The results showed that the Proline contents increases significantly under NaCl stress (fig 1, 2). The highest amounts of proline (0.082 mg g^{-1}) were achieved in severe stress condition and lowest amount (0.061 mg g^{-1}) achieved in stress condition in NaCl and SA media (test). There are many reports about proline increasing under salt stress conditions. In the NaCl and ABA culture media plant shows that the higher amount of proline (0.093 mg g^{-1}) were achieved in severe stress condition and lowest amount (0.041 mg g^{-1}) achieved in stress condition.

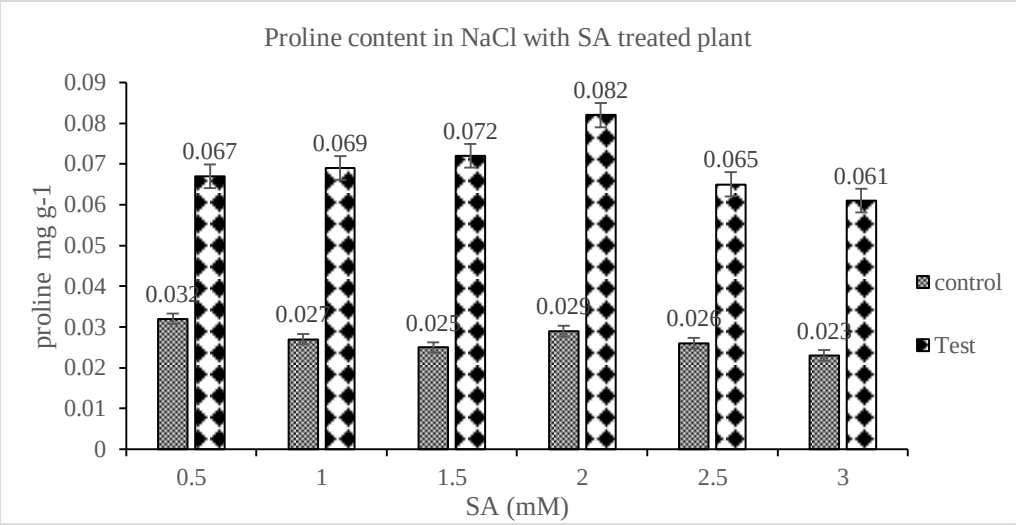


Figure 1: Proline content present in the SA with NaCl treated plant mg g^{-1}

The data showed that proline content in leaves highly increased as ABA concentrations increased and slightly increase as SA concentration increase. In the present study, SA and ABA induced an accumulation of proline in the leaves under salt stress, when SA and ABA was applied an induction occurred in the leaves under stress. Thus, proline can be considered to be one of the important factors involved in SA and ABA induced protective mechanism in Banana leaves in response to salt stress.

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Impact Factor: 6.057
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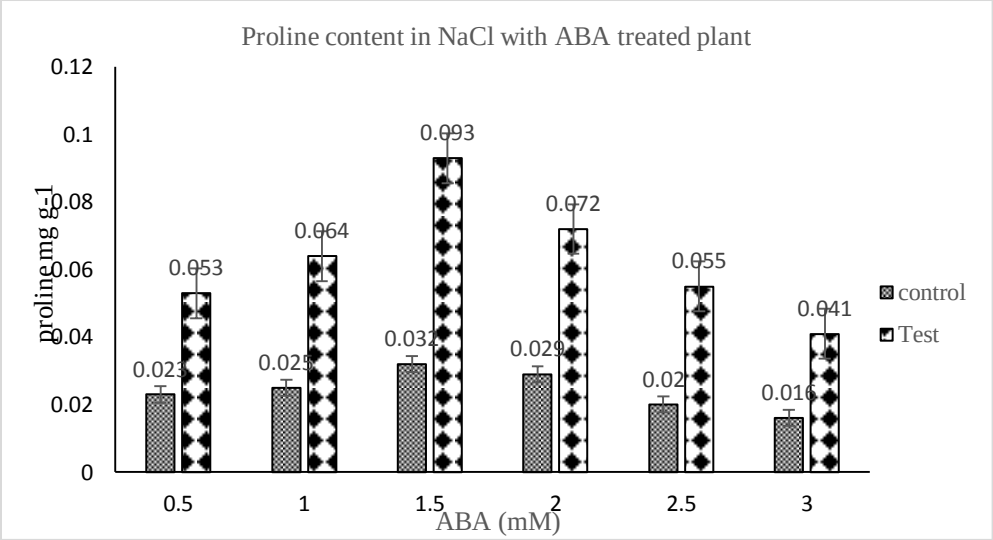

Figure 2: Proline content present in the ABA with NaCl treated plant

Relative Water Content (RWC)

Result showed that RWC was decreased by increasing salt intensity (fig 4.14). Highest relative water content (89.2%) was observed under stress condition with (0.5 mM SA) and lowest relative water content (77%) achieved in stress condition (3mM). This depletion in RWC strength be resulted in depletion of plant extension attributes. RWC in plant had a positive correlation with soil relative water content. Highest relative water content (95%) was observed under stress condition with 1.5mM ABA and lowest relative water content (85%) achieved in severe stress condition 300mM ABA (fig 4). RWC was decreased by increasing evapotranspiration in plants and reducing root growth and activity. In this experiment, the enhancement rate of RWC in SA-treated plants was low and RWC in ABA-treated plants was high.

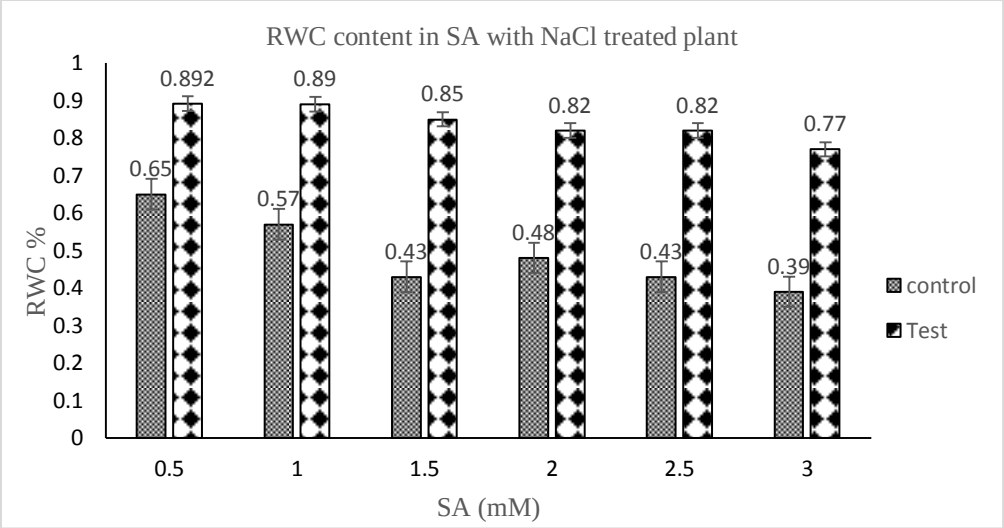

Figure 3: RWC present in SA with NaCl treated plant

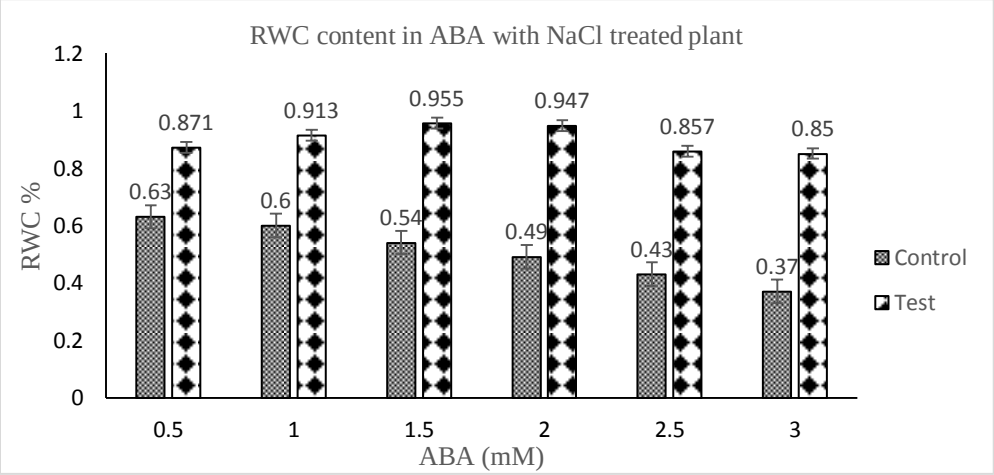

Figure 4: RWC present in ABA with NaCl treated plant

Photosynthetic Pigments (Chlorophyll)

Photosynthetic pigments (chlorophyll) are affected by salt stress condition. Under stress condition, the photosynthetic pigments greatly decreased. The reduction of photosynthetic compounds were due to unreliability of protein composite and demolition of chlorophyll by high activity of chlorophyll humiliating enzymes and chlorophylls under *in vitro* stress condition. SA and ABA treatments increased carotenoids content of the plants. The

application of 2mM SA, 2.5mM SA (fig 5) and 1.5mM, 2mM (fig 6) 0f 0.04958mg g-1, 0.0383mg g-1 and 0.09911mg g-1, 0.0672 mg g-1 of chlorophyll content increases in respectively. Comparison of untreated plants. ABA regulates physiological and biological processes in plants and can be used as a potential growth regulator to improve plant growth under environmental conditions, but the efficiency of exogenous ABA depends on multiple causes such as the species, developmental stage, application method and ABA concentration. These results shows that the SA and ABA increases the chlorophyll content in the plant and ABA give maximum activity compare to SA treated plants.

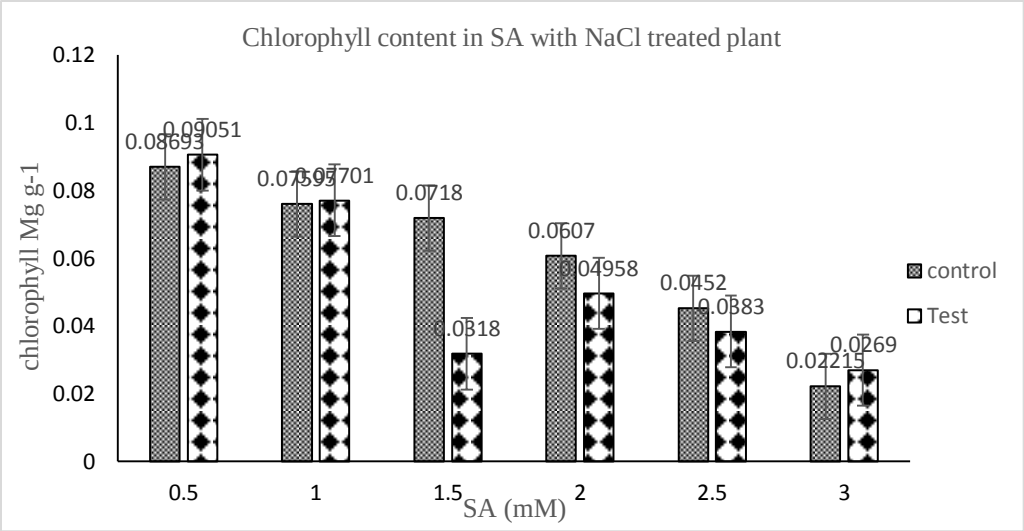

Figure 5: chlorophyll content present in SA with NaCl treated plant

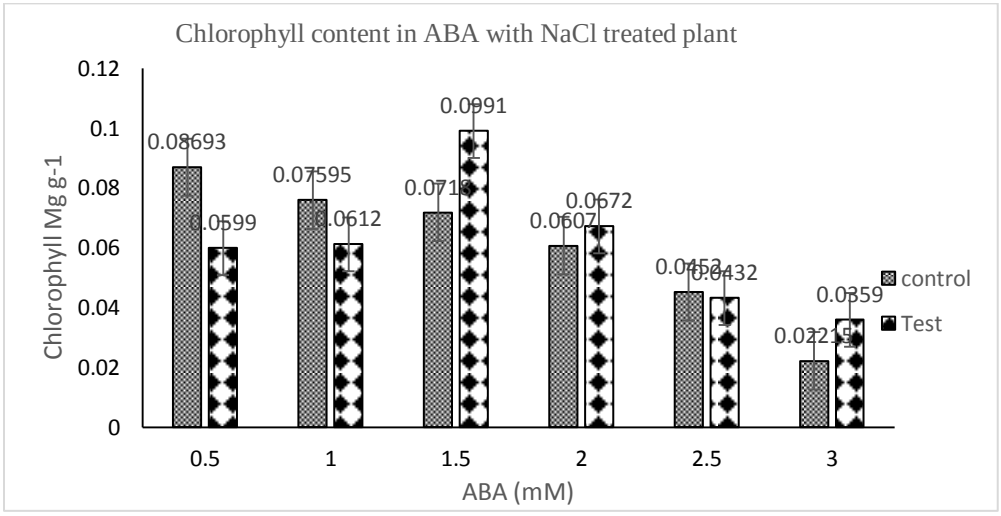

Figure 6: chlorophyll content present in ABA with NaCl treated plant

Plant Height, Root and Leaf Length of *Musa acuminata*

The results represented in illustrated that explants treated with higher concentrations of SA and ABA (1.5mM) and sub cultured on free NaCl media produced the highest plant height, leaf and root length. On the other hand, increasing concentrations of NaCl in the culture media led to decreasing gradually. Plantlets sub cultured on medium containing the highest NaCl concentration (300mM) revealed insignificant differences between various SA and ABA treatments. These results shows that the SA (Fig 7) and ABA (Fig 8) increases the root length in the plant and ABA give maximum activity compare to SA treated plant. NaCl concentrations in the culture media decreased number of roots gradually. Explants received the higher ABA concentration (1.5mM) produced largest number of roots and leafs compared to SA in the presence of the highest NaCl concentration (150mM) in the culture medium.

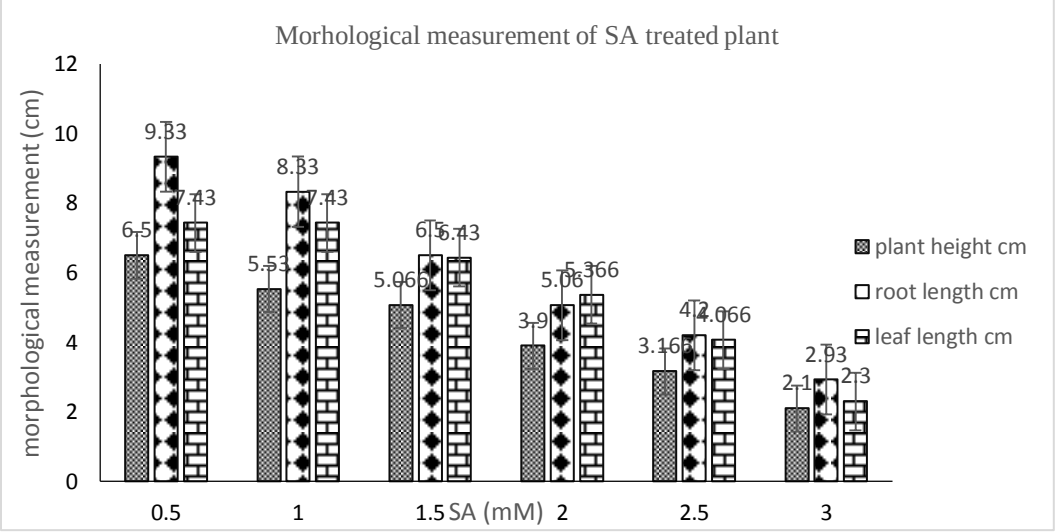

Figure 7: plant height, root and leaf length of SA with NaCl treated plant

ISSN: 2348-1358
Impact Factor: 6.057
NAAS Rating: 3.77

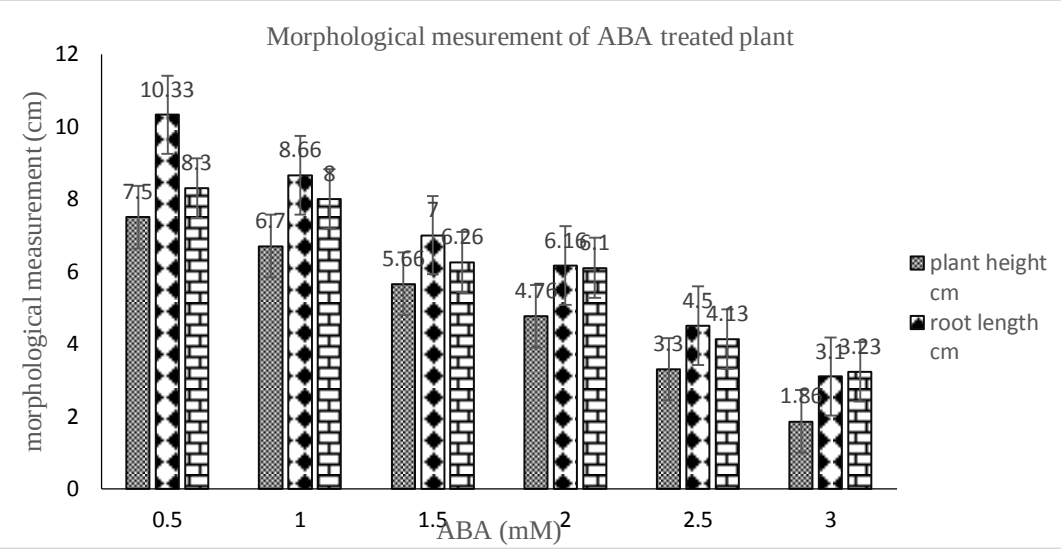

Figure 8: plant height, root and leaf length of ABA with NaCl treated plant

4. Conclusion

Abiotic stress signaling pathway is a crucial area with reference to increase in plant production. Salinity is one of the abiotic stresses caused by excess NaCl levels, extreme temperatures and low atmospheric humidity and it ultimately affects the plant productivity. Development of new varieties is one of the ultimate methods to overcome the problem associated with the salinity stress. *Musa acuminata* plant was developed under salt stress condition by giving different concentration of NaCl. The media was optimized with BAP 3mg/l is produced multiplication of shoot and mass culture. The subculture was done up to fifth cycles to maintain genetic purity. The plants which are supplemented with NaCl (50, 100, 150, 200, 250 and 300mM). The Chlorophyll pigment present in the plant was decreased due to stress and significantly increase by adding Salicylic acid and Abscisic acid (1.5 and 2mM). Salicylic acid and Abscisic acid induced an accumulation of proline in the leaves under salinity stress. Abscisic acid (1.5 and 2mM) plays an important role in accumulation of proline content in banana plant as compared with salicylic acid treated plants. Plant height, leaf and root length was augmented by adding salicylic acid instead of Abscisic acid. In salinity condition number of leaves and number of roots were developed by adding Salicylic acid and Abscisic acid. In salicylic acid treated plants quite significantly increase the number of leaves and roots of the banana plants. NaCl (150, 200mM) and ABA (1.5, 2mM) was found to be effective against salinity stress with the help of Morphological and Physiological test.

 ISSN: 2348-1358
Impact Factor: 6.057
NAAS Rating: 3.77

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