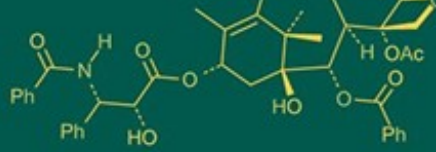
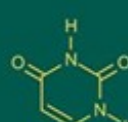
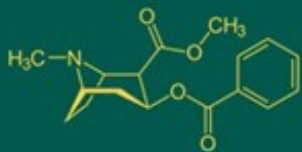


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Biochemical responses of banana cultivars G9, B5 and B3 to in vitro NaCl induced salt stress

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ABSTRACT

Banana (*Musa* spp.) is a sparingly important fruit crop that is sensitive to salinity, which unpleasantly disturbs plant growth, photosynthetic activity and cellular metabolism. The existing training was showed to appraise the biochemical replies of three banana varieties, Grand Naine (G9), Nendran (B5) and Poovan Chandan (B3) to different levels of NaCl under in vitro conditions. Healthy shoots were cultured on Murashige and Skoog basal medium accompanied with NaCl at 0, 2, 4 and 8 gL⁻¹. After 21 days of treatment, changes in chlorophyll a, chlorophyll b, total chlorophyll, proline, total soluble sugars, total phenols and malondialdehyde (MDA) were assessed. Increasing NaCl concentration occasioned in a advanced decline in chlorophyll a, chlorophyll b and total chlorophyll contents representing a reduction in photosynthetic pigment stability under salt stress. In contrast, proline and MDA contents increased with increasing NaCl concentration signifying improved osmotic adjustment and oxidative stress, respectively. Amid the three varieties, G9 and B5 preserved moderately higher chlorophyll contents than B3, meanwhile B3 displayed the highest proline accretion at the highest NaCl concentration. G9 unveiled the lowest MDA accumulation, representing comparatively lower membrane lipid peroxidation under salt stress. The disparity biochemical responses among the varieties demonstrated variation in their responses to salinity. The education specifies that biochemical parameters, mainly chlorophyll, proline and MDA can serve as useful gauges for in vitro screening of banana varieties for salinity tolerance. The conclusions may afford a basis for identifying promising banana genotypes for further physiological, tissue culture and salinity-tolerance improvement studies.

Keywords: Banana, NaCl, Salinity Stress, In Vitro Culture, Biochemical Responses, Membrane Stability, Abiotic Stress Tolerance

1. Introduction

Banana (*Musa* spp.) is one of the world's most important tropical fruit crops and goes to the family *Musaceae*. It is a triploid crop (2n = 3x = 33) that instigated in Asia and is widely refined for its nutritional and economic value (Rahman *et al.*, 2013) [1]. In India, banana dwell in a prominent position amongst fruit crops because of its high productivity and year-round demand. In spite of its commercial importance, banana cultivation is progressively inhibited by abiotic stresses between which salinity has appeared as one of the most serious factors restraining plant growth, productivity and fruit quality.

Salinity distresses agricultural production by plummeting water availability, disturbing nutrient uptake and instigating excessive accretion of sodium (Na⁺) and chloride (Cl⁻) ions in plant tissues. Roughly 70% of crop yield losses worldwide are qualified to abiotic stresses with salinity being a major contributor. In banana, salt stress originally persuades osmotic stress that confines water absorption and cell expansion followed by ionic toxicity resulting from the accretion of toxic ions within plant cells. These changes ultimately partiality photosynthesis, membrane stability and metabolic actions chief to poor plant growth and reduced yield (Munns *et al.*, 2008) [2].

Banana is generally measured sensitive to salinity particularly going to the *Musa acuminata* group (Israeli *et al.*, 1986) [3]. Salt injury is first expressed as leaf margin necrosis, reduced pseudostem thickness, delayed flowering, and poor fruit development. Since conventional breeding for salinity tolerance is limited by polyploidy, parthenocarp, low female fertility, and narrow genetic variability, alternative approaches such as tissue culture-based screening have gained considerable importance. In vitro culture provides a controlled environment for revealing plant tissues to distinct levels of NaCl permitting rapid and reliable evaluation of varietal replies during the early stages of plant development.

Biochemical replies serve as sensitive indicators of plant adaptation to salt stress. Salinity induced oxidative stress improves the production of reactive oxygen species, which damage cellular membranes, proteins and pigments except effectively counteracted by protective mechanisms (Rai *et al.*, 2011) [4]. Plants respond by accruing osmo-protectants such as proline and soluble sugars which help maintain osmotic balance and stabilize cellular structures. Likewise, phenolic compounds donate to antioxidant defense by searching free rad

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icals, whereas changes in chlorophyll content imitate the extent of photosynthetic impairment. Malondialdehyde, lipid peroxidation is widely used as a biochemical marker of membrane damage under stress conditions. Jointly, these biochemical qualities deliver valuable insights into the tolerance mechanisms functioning under saline environments.

Consequently, the present investigation was assumed to assess the effect of different NaCl levels on key biochemical parameters, namely chlorophyll, proline, total phenol, soluble sugar and malondialdehyde under in vitro conditions in three banana varieties, G9, B5 and B3. The study purposes to relate their biochemical responses and recognize varietal differences accompanying with salinity tolerance throughout the early stages of growth.

2. Material and Methods

2.1 Plant material and in vitro culture

Suckers of Grand Naine (AAA), Nendran, (AAB), Povillachandum (AAB) variety of banana were collected from Agriculture Research Station AAU, Jabugam and sterilized with the help of sterilizing

agents. Healthy shoots were cultured on Murashige and Skoog basal medium accompanied with NaCl at 0, 2, 4, and 8 gL⁻¹ to assess salinity tolerance.

2.2 Biochemical analysis

Biochemical analyses were achieved after 21 days of NaCl treatment. Total chlorophyll content was appraised by the DMSO extraction method using absorbance at 663 and 645 nm. Proline content was resolved by the acid ninhydrin method (520 nm), total phenol by the folin-ciocalteu method (620 nm), total soluble sugar by the phenol sulphuric acid method and malondialdehyde by the thiobarbituric acid calculate using absorbance at 535 and 600 nm. Calculations were performed using standard equations for each assay.

(1) Chlorophyll content

$$\text{Chlorophyll a (mg/g fresh wt.)} = 12.7 \times \text{O.D. at 663} - 2.63 \times \text{O.D. at 645}$$

$$\text{Chlorophyll b (mg/g fresh wt.)} = 22.7 \times \text{O.D. at 645} - 4.68 \times \text{O.D. at 663}$$

$$\text{Total chlorophyll (mg/g fresh wt.)} = 22.2 \times \text{O.D. at 663} + 8.02 \times \text{O.D. at 645}$$

(2) Total phenol (μg/g)

$$\text{Phenol } \mu\text{g/g} = \text{sample O.D} \times \mu\text{g phenol per unit O.D} \\ 0.0002 \text{ g}$$

(3) Total sugar (mg/g)

$$\text{Percentage soluble sugar sample} = \frac{X \times 250 \times 100}{2 \times 100000}$$

(4) Malondialdehyde (μg/g)

3. Results and Discussion

3.1 Biochemical response of three banana varieties to NaCl under in vitro conditions

1. Chlorophyll a content (mg/g)

The data made from the two factorial interaction clearly indicated that the chlorophyll a has reduced upon increase in the concentration of NaCl in media from 0 to 8 gL⁻¹. The lowest and highest chlorophyll a recorded were 0.71 (8 gL⁻¹) and 6.68 (0 gL⁻¹). It was obvious from the

data that between all three varieties B3 variety has less chlorophyll a competence as compared to G9 and B5 varieties. The order of varieties in relations of chlorophyll a was B5>G9>B3. The thorough results are accessible in Table 1 and Graph 1. El-Meleigy *et al.* (2004) [5] assessed six tomato genotypes under 0–6000 ppm NaCl and labelled substantial reductions in chlorophyll a with decreases of 77%, 66% and 61% in Castrock, Oriet and Super Marmande, respectively. Rahnesan *et al.* (2018) [6] accurate a similar trend in pistachio (*Pistacia vera* L.) cultivars uncovered to 0–150 mM NaCl. Chlorophyll a improved slightly at 50 mM but weakened markedly at 100 and 150 mM, representing that higher salinity shortened photosynthetic pigments.

MDA (ppm) (μg/g)	Corrected O.D.	X	72	X	10	X	1000000
	155.0 x L		1000		200		

2. Chlorophyll b content (mg/g)

The data produced from the two factorial interaction visibly specified that the chlorophyll b has condensed upon increase in the concentration of NaCl in media from 0 to 8 gL⁻¹. The lowest and highest chlorophyll b noted were 0.13 (8 gL⁻¹) and 0.96 (0 gL⁻¹). It was obvious from the data that between all three varieties B3 variety has less chlorophyll b efficiency as associated to G9 and B5 varieties. The direction of varieties in terms of chlorophyll b was G9 > B5 > B3. The comprehensive results are existing in Table 2 and Graph 2. El-Meleigy *et al.* (2004) [5] described that chlorophyll b amplified slightly at restrained salinity in tomato. While BL-1079 displayed less pigment decrease at higher NaCl levels. Rahnesan *et al.* (2018) [6] detected a alike trend in pistachio with chlorophyll b growing at 50

mM but lessening at higher salinity. Similarly, Mazmudar *et al.* (2019) [7] originate that chlorophyll b in banana cv. Berangan reduced from 15 to 5 mg g⁻¹ with increasing salinity. This deterioration was accredited to ROS induced pigment deprivation under salt stress.

3. Total chlorophyll content (mg/g)

The data generated from the two factorial interaction clearly indicated that the total chlorophyll has reduced upon increase in the concentration of NaCl in media from 0 to 8 gL⁻¹. The lowest and highest total chlorophyll recorded were 1.49 (8 gL⁻¹) and 13.59 (0 gL⁻¹). It was evident from the data that among all three varieties B3 variety has less total chlorophyll b efficiency as compared to G9 and B5 varieties. The order of varieties in terms of total chlorophyll was G9 > B5 > B3. The detailed results are presented in Table 3 and Graph 3. Rahnesan

et al. (2018) [6] conveyed that total chlorophyll in *Pistacia vera* augmented to some extent at 50 mM NaCl but deteriorated at 100 and 150 mM with the BZ cultivar showing greater salt tolerance than BS. Correspondingly, Mazmudar *et al.* (2019) [7] detected a decline in total chlorophyll from 50 to 10 mg g⁻¹ in banana cv. Berangan below increasing salinity (0–52.7 dSm⁻¹ which was accredited to ROS encouraged pigment degradation (Ashraf, 2003) [8]. Siddiqui *et al.* (2009) [9] also described a advanced reduction in total chlorophyll with increasing NaCl levels in 10 genotypes of *Brassica juncea*.

4. Malondialdehyde (μg/g)

The data generated from the two factorial interaction clearly indicated that the malondialdehyde has increased upon increase in the concentration of NaCl in media from 0 to 8 g l⁻¹. the lowest and highest malondialdehyde recorded were 184.19 μg/g (0 g l⁻¹) and 319.25 μg/g (8 g l⁻¹). It was evident from the data that among all three varieties G9 variety has less malondialdehyde efficiency as compared to B5 and B3 varieties. The order of varieties in terms of malondialdehyde was B5 > B3 > G9. The detailed results are presented in Table 4 and Graph 4. Mazmudar *et al.* (2019) [7] described that MDA content in banana increased from 0.15 to 0.50 mg g⁻¹ with increasing salinity (0–52.7 dSm⁻¹) representing heightened lipid peroxidation under salt stress. Correspondingly, Queiros *et al.* (2007) [10] detected a progressive increase in MDA content in *Solanum tuberosum* callus with increasing NaCl concentrations (0–200 mM), reflecting greater oxidative damage at higher salinity levels.

5. Proline (mg/g)

The data generated from the two factorial interaction clearly indicated that the proline has increased upon increase in the concentration of NaCl in media from 0 to 8 g l⁻¹. The lowest and highest proline recorded were 0.12 (0 g l⁻¹) and 0.66 (8 g l⁻¹). It was evident from the data that among all three varieties B5 variety has less proline efficiency as compared to B3 and G9 varieties. The order of varieties in terms of proline was B3 > G9 > B5. The detailed results are presented in Table 5 and Graph 5. Rahnesan *et al.* (2018) [6] described similar trend for proline in *Pistacia vera* L. cv., whereas proline amplified at moderate and high salinity with the BZ cultivar viewing higher accumulation. Correspondingly, Mazmudar *et al.* (2019) [7] described that proline in banana (*Musa acuminata* cv. Berangan) amplified from

5.00 to 14.00 mg g⁻¹ with growing salinity (0–52.7 dSm⁻¹). Sotiropoulos (2007) [11] also detected a substantial increase in leaf proline content in *Malus domestica* rootstocks refined in vitro under 100 and 200 mM NaCl associated to the control.

4. Conclusions

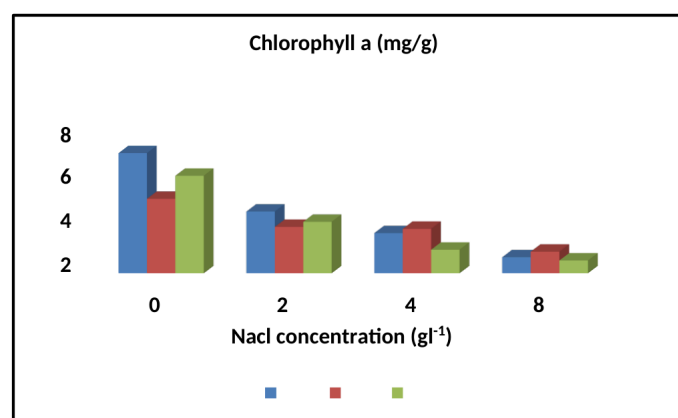
The present investigation established that increasing NaCl concentration under in vitro conditions significantly inclined the biochemical replies of the three banana varieties, specifically G9, B5 and B3. Salinity gradually reduced chlorophyll a, chlorophyll b and total chlorophyll contents representing weakening of the photosynthetic system with increasing salt stress. Between the tested varieties, G9 and B5 preserved higher chlorophyll levels than B3 signifying better protection of photosynthetic pigments under saline conditions. In disparity, proline and malondialdehyde contents amplified with increasing NaCl concentration, shimmering improved osmotic adjustment and oxidative stress under salinity. The highest proline accretion was recorded at 8 g l⁻¹ NaCl with B3 unveiling the greatest accumulation, however G9 exhibited the lowest malondialdehyde content representing comparatively lower membrane damage. These conclusions recommend that the three varieties assumed different biochemical strategies to cope with salt stress with G9 displaying greater membrane stability in spite of relatively lower proline accumulation. Complete study confirmed that biochemical markers such as chlorophyll, proline and malondialdehyde are consistent indicators for gauging salinity tolerance during the early stages of banana growth below in vitro conditions. The difference responses between the tested varieties offer valuable information for picking talented genotypes for tissue culture based screening and upcoming salinity tolerance improvement programmes. These conclusions contribute to the expansion of efficient approaches for attractive banana efficiency in salt-affected environments and deliver a useful substance for additional physiological and molecular investigations.

5. Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text to image generators have been used during the writing or editing of this manuscript.

Table 1. Effect of various concentrations of NaCl on chlorophyll a (mg/g) in three different genotypes of banana.

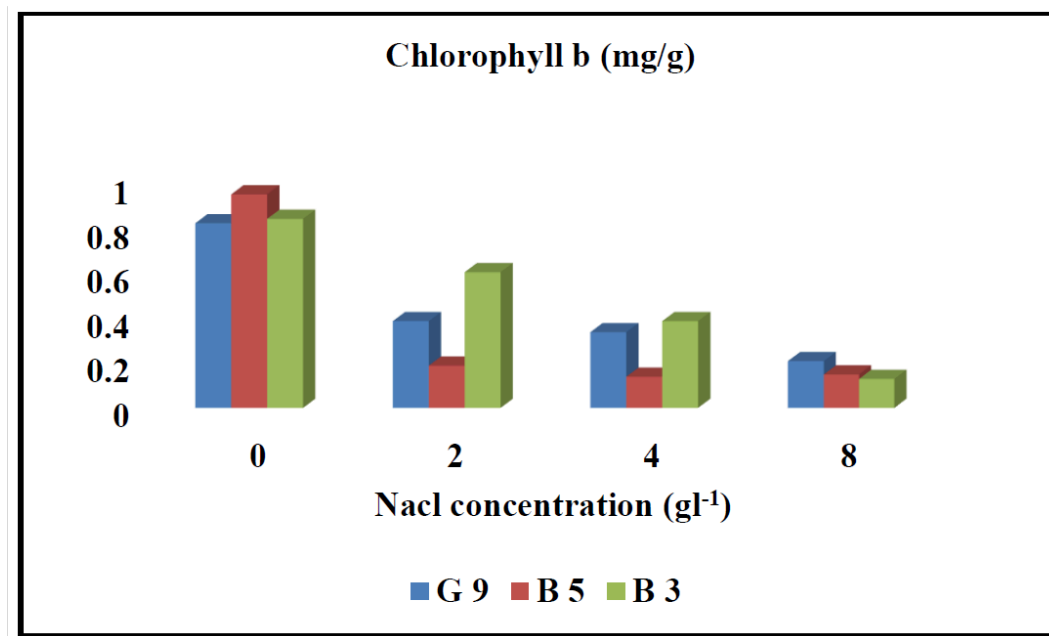
Variety X Concentration	C1 (0 g l ⁻¹)	C2 (2 g l ⁻¹)	C3 (4 g l ⁻¹)	C4 (8 g l ⁻¹)	Var. Mean
V1 (G9)	6.68	3.43	2.23	0.89	3.30
V2 (B5)	4.14	2.58	2.47	1.20	2.59
V3 (B3)	5.42	2.86	1.31	0.71	2.57
Conc. Mean	5.41	2.96	2.00	0.93	



Graph 1. Effect of different NaCl concentrations and incubation periods on chlorophyll a in three different varieties of banana (A) G9 - Grand Naine (B) B5 - Povilachandam and (C) B3 - Nendran.

Table 2. Effect of various concentrations of NaCl on chlorophyll b (mg/g)

Variety X Concentration	C ₁ (0 gl ⁻¹)	C ₂ (2 gl ⁻¹)	C ₃ (4 gl ⁻¹)	C ₄ (8 gl ⁻¹)	Var. mean
V1 (G9)	0.83	0.39	0.34	0.21	0.44
V2 (B5)	0.96	0.19	0.14	0.15	0.36
V3 (B3)	0.85	0.61	0.39	0.13	0.49
Conc. Mean	0.88	0.40	0.29	0.16	

**Graph 2.** Effect of different NaCl concentrations and incubation periods on chlorophyll b**Table 3.** Effect of various concentrations of NaCl on total chlorophyll (mg/g) in three different genotypes of banana.

Variety X Concentration	C ₁ (0 gl ⁻¹)	C ₂ (2 gl ⁻¹)	C ₃ (4 gl ⁻¹)	C ₄ (8 gl ⁻¹)	Variety mean
V1 (G9)	13.59	6.95	4.58	1.87	6.75
V2 (B5)	8.68	5.18	4.93	2.44	5.31
V3 (B3)	11.13	5.98	2.81	1.49	5.35
Concentration mean	11.13	6.03	4.10	1.93	

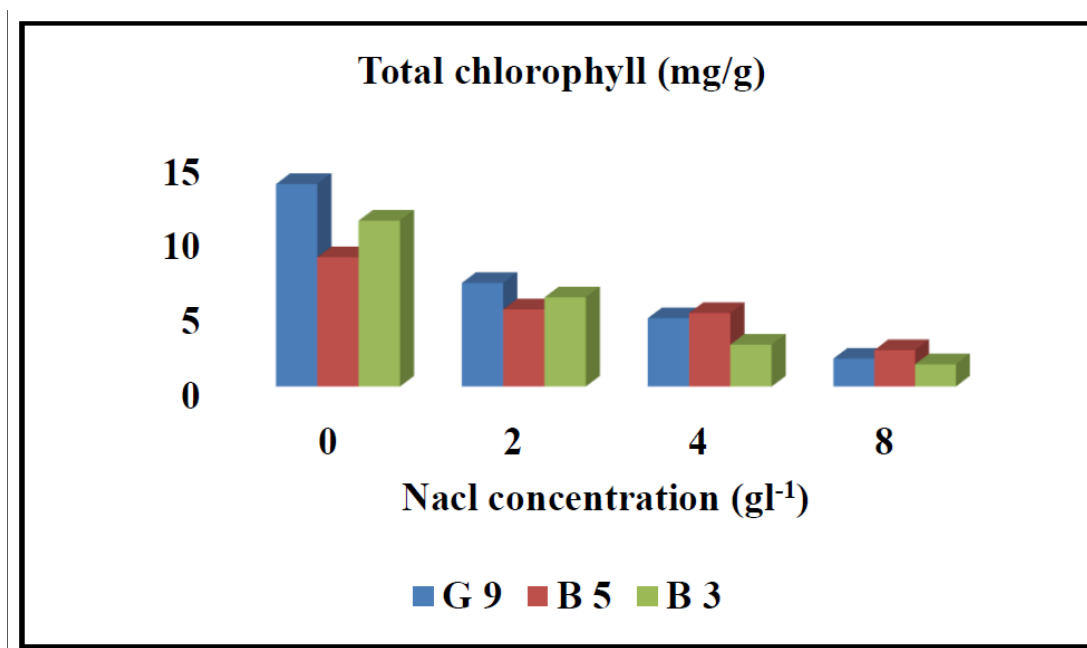
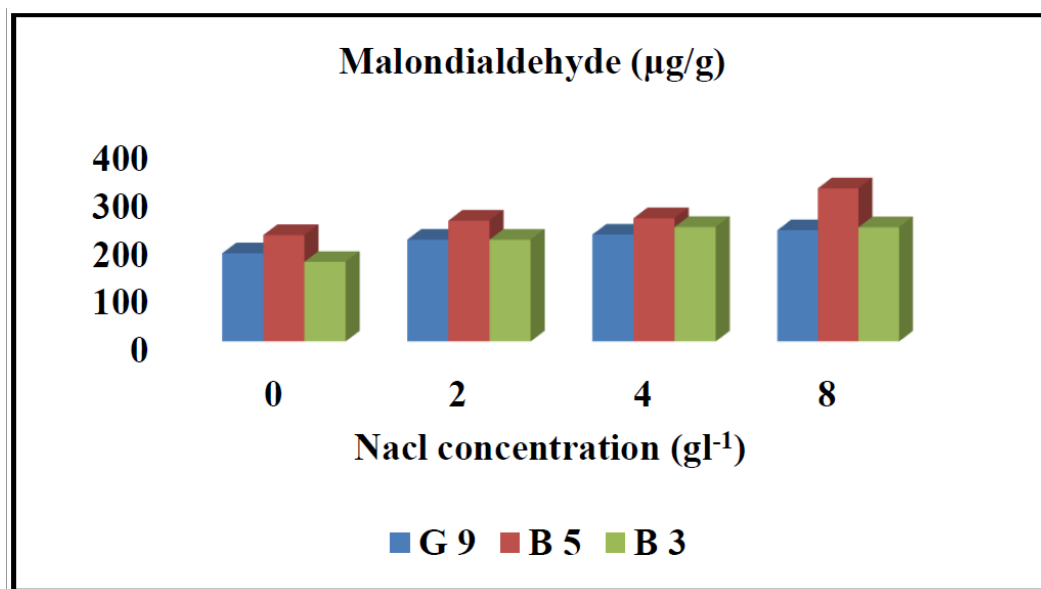
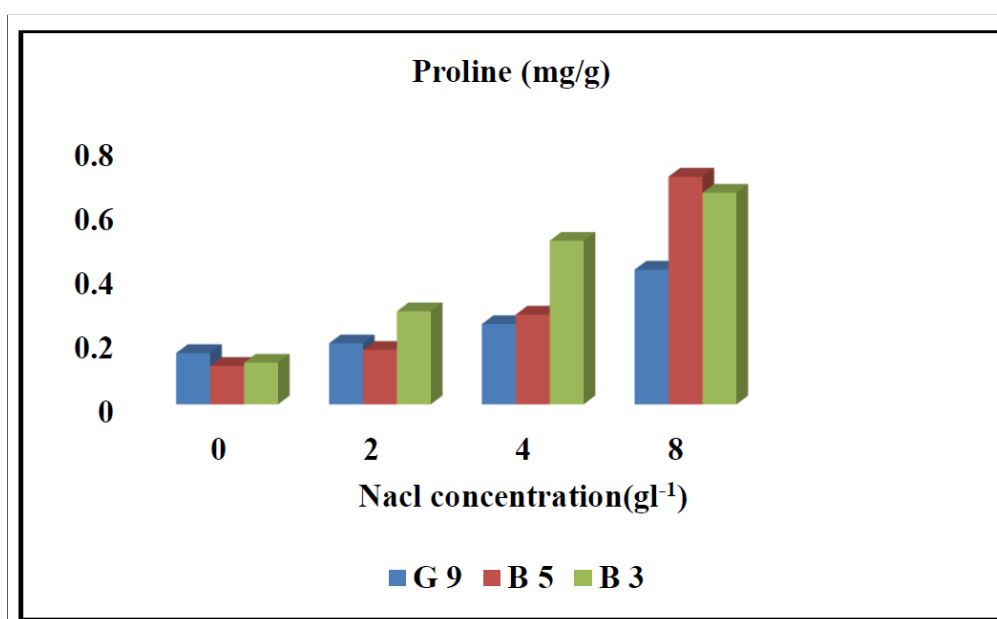
**Graph 3.** Effect of different NaCl concentrations and incubation periods on total chlorophyll

Table 4. Effect of various concentrations of NaCl on Malondialdehyde ($\mu\text{g/g}$)

Variety X Concentration	C ₁ (0 gl-1)	C ₂ (2 gl-1)	C ₃ (4 gl-1)	C ₄ (8 gl-1)	Var. mean
V1 (G9)	184.19	212.65	223.25	232.18	213.07
V2 (B5)	222.14	252.28	257.30	319.25	262.74
V3 (B3)	166.32	212.65	238.88	238.32	214.04
Conc. mean	190.88	225.86	239.81	263.25	

**Graph 4.** Effect of different NaCl concentrations and incubation periods on Malondialdehyde in**Table 5.** Effect of various concentrations of NaCl on proline (mg/g)

Variety X Concentration	C ₁ (0 gl-1)	C ₂ (2 gl-1)	C ₃ (4 gl-1)	C ₄ (8 gl-1)	Var. mean
V1 (G9)	0.16	0.19	0.25	0.42	0.26
V2 (B5)	0.12	0.17	0.28	0.71	0.32
V3 (B3)	0.13	0.29	0.51	0.66	0.39
Conc. mean	0.14	0.21	0.35	0.59	

**Graph 5.** Effect of different NaCl concentrations and incubation periods on proline

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