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Study of salinity tolerance in 3 varieties of finger millets (*Eleusine coracana* (L.) Gaertn.)

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Abstract
Abiotic salinity stress affects the overall growth of plants. It is a major issue in arid regions of the world and semi-arid regions of the world. Physiological changes like germination percentage, root-shoot length, turgidity, and Relative water content were determined in 3 ragi varieties (Indaf, Hasiru gambe, and Carr ragi) with salinity treatments at a concentration (of 0, 50, 75, and 100 mm). Biochemical changes like concentration of Proline, reducing sugar, protein, and chlorophyll content were also measured in comparison to the control. Salinity was given as a basal dose and sampling was done after 21 days. The salinity treatment was started after 5 days of germination. Out of 3 varieties, Carr is found to be salt tolerant and Hasiru gambe ragi is found to be susceptible to salinity stress.

Keywords: Salt stress, *Eleusine coracana*, germination percentage, Relative Water Content (RWC), turgidity, proline

1. Introduction
Finger millet (*Eleusine coracana* (L.) Gaertn.) is a allotetraploid cereal of Poaceae. Asians and Africans consume them in high amounts (Chivenge *et al.*, 2015) [13]. It is an exceptional nutrient source with compounds like essential amino acids and ions (Gupta *et al.*, 2017) [21]. Finger millet is beneficial because it is hypoglycemic, antiulceratic, and hypocholesterolemia (Chetan and Malleshi, 2007) [17]. Finger millet can be grown in many agronomical regions, and the crop also has a higher shelf life making it economically viable (Onyango, 2016) [32]. Finger millets are used as a raw material for producing ethanol (Tekaligne *et al.,* 2015) [45].
Soil salinity leads to hyperosmotic stress, and interferes with the various biological activities of plant roots and root microbes (Abbas *et al*., 2019) [1]. Salinity provided in the form of irrigation water near coastal areas is said to cause irreversible damage to plant systems (Zuazao *et al*., 2004) [49]. Affected plants show an increase in Caspase, ROS, Hydrogen and lipid peroxidase (Striker *et al.*, 2015) [44].
The decline in osmotic potential caused due to soil salinity can endorse the difficult translocation of nutrients. Salinity mainly shows two physiological effects on the affected plants i) Rate of emerging leaves decline and ii) Toxic symptoms (chlorosis and necrosis) on mature leaves (Rahaman *et al*., 2014) [35].
Plant development depends on seed germination (Tlig *et al*., 2008) [46]. Premature stages of finger millets are susceptible to stress like salinity and drought (Hema *et al*., 2014) [22]. In comparison to other cereal crops *viz*, barley, sorghum, oats, and wheat, finger millets are found to be highly susceptible to salinity stress (Bray *et al*., 2000) [9].
Reports suggest that short-time salinity stress can adversely affect the germination, root-shoot length, relative water contents, photosynthetic pigment concentration, proteins concentration, proline content, and amount of reducing sugars in finger millets (Dugasa *et al*., 2019; Kumar and Khare, 2016) [17, 26].
Finger millet is a staple crop grown in different agronomical regions of the world. With the changing geographical and climatic conditions there is limited information available on different varieties and their tolerance capacities with regard to salinity stress.
In the present study, three finger millet genotypes were evaluated for seedling characteristics like seed germination percentage, turgidity, relative water content, root length, shoot length, fresh weight, and dry weight. The biochemical characteristics like chlorophyll, Proline, Protein, and reducing sugar are also estimated. Five replicates of 100 seeds per genotype for each treatment were maintained.

Materials and Methods

Geographical location of the experiment

A multipot-based experiment was conducted May-August (2022) in the Department of Botany, Jnana Kaveri P.G. Centre, Kodagu, Karnataka, India.

Plant Material and experimental condition

3 finger millet genotypes *viz*, Hasiru gambe, Indaf, and Carr were collected from an agriculture shop in Hassan, Karnataka. Seeds were handpicked and washed and air dried. The seeds were sterilized with 1.5% $HgCl_2$ and were sowed in multi pots with a mixture of sterilized soil, sand and organic manure in a ratio of 2:1:1.

Finger millet seedlings were grown for 21 days, in laboratory conditions with temperature varying from 24 ºC - 18 ºC. The photoperiod provided was 16/8 hrs. The seedlings were treated with NaCl (50, 75, 100 mm) at a 3-day interval. The treatment was started after 5 days of seed sowing. The control set of seedlings was irrigated with distilled water (Mukami *et al.*, 2020) [30].

2.2 Morphological parameters

Finger millet (*Eleusine coracana*) of 3 different varieties *viz*, Indaf, Hasiru gambe, and Carr ragi were collected. Then Petri plates were prepared with the blotter technique. 3 replicates were kept for each variety. Salinity treatment was given in a concentration of 50 mm, 75 mm, and 100 mm treatment daily. The observation was noted after 7 days and results were calculated (Mukami *et al.*, 2020) [30]. Plants were cultivated in control and treated conditions, and parameters length of shoot (SL), length of root (RL), their ratios (S/R), as well as plant height (SLL), were measured (Cirillo *et al.*,2019; Soares *et al.*, 2018) [10, 14, 42]. Plants are carefully removed along with roots. Roots are rinsed with tap water and moisture is blotted. Plants were cultivated in control and treated conditions, and morphological parameters including shoot length (SL), root length (RL), and their ratios (S/R), as well as plant height (SLL), were measured.

Then the electronic weighing balance was used to measure the fresh weights (FW) of each plant. Plants were placed on aluminum foil and dried for 48 hours at 80 ºC to achieve dry weight (DW).

Physiological parameters

Leaf Relative water content

Leaflets are taken from 3 varieties of finger millets (n=3), fresh weight is recorded as W_1, Leafs are soaked in deionized water overnight, turgid weight W_2 is recorded. They are oven dried at 80 ^{0}C. The dry weight measured is W_3 (Sairam *et al.*, 2002) [37]. The relative water content (RWC) is estimated as follows (Polash *et al.*, 2018) [34].

RWC = W1- W3/ W2- W3 x 100.

Photosynthetic pigments

Using the Arnon (1949) [4] method, the different types chlorophyll contents were measured.

Biochemical changes

Proline content

In accordance with Bates *et al.*, (1973) [6] the fresh leaf from both the control and the treatment was utilized to determine the proline content. It was measured at 520 nm. The proline concentration is given as µmol/g FW.

Protein content

The acetone-trichloroacetic acid (TCA) preparation was used to extract the entire sample's protein (Damerval *et al*., 1986) [15]. Each treatment's 0.5 mg of leaf tissue was grinded in 10% TCA on ice and incubated at 40 ºC overnight. After centrifuging for 15 minutes, 100% acetone was used to wash the pellet. Based on a standard curve created using 1–10 mg of BSA (bovine serum albumin), the protein was calculated (Lowry *et al*., 1951) [27]. The protein concentration was given as mg/g FW.

Reducing sugar

Johnson *et al*., (1964) [24] procedure was used for estimation. Absorbance was measured at 520 nm. Reducing sugar was calculated in mg/FW and with glucose as a standard.

Statistical analysis

The results of five biological replicates (n = 5) were used to calculate the mean value and standard error of the morphological, physiological, and biochemical data. Tukey's multiple comparison tests were used in a two-way analysis of variance (ANOVA) to determine whether the salinity treatments significantly differed from the control treatments. Significant differences at $p<0.05$ were used to examine the variances between the means. Significant variations between three sets of control and treatment conditions are indicated by various alphabetical subgroups.

3. Results

3.1 Saltinity stress and seed germination

Table 1 shows the effect of salinity on finger millet germination. Results indicate a decrease in germination rate with increased salinity. A high germination rate of 90.6±2.3% to 94.6±4.6% was seen in the control. The lowest germination rate is found in the Carr variety with 44±4%, followed by 60±4% in Hasiru gambe and Indaf.

3.2 Shoot and root length in finger millet varieties under salt stress.

On observation the plants showed chlorosis (yellowish color), leaf scorching, delayed growth, as shown in table 2 the shoot length decreased with the increase in NaCl concentration. Particularly, the shoot height of Indaf has decreased by 44.6%, followed by Carr by 48% and Hasiru gambe by 50.1% with 100 mm treatment. The same result was found in case of roots also (Table 3).

3.3 Relative water content

Table 4 depicts the change in RWC with respect to salinity treatment. The RWC of all varieties under control conditions were ranging from 93.54±1.92 to 81.66±1.25. Increased salinity reduces water potential of leaves.. Indaf variety showed the least reduction.

3.4 Effects on Chlorophyll content

On analyzing the chlorophyll content there is a significant difference among varieties with salt treatments. The NaCl treatment has elicited a contrary relationship between the salinity and the chlorophyll. The higher chlorophyll concentration was found in Indaf 50 mm treatment- 6.8±0.40 mg/g FW, followed by Carr untreated with 6.4±0.4 mg/g FW. Under saline conditions, the chlorophyll values were reduced relatively in proportion to the treatment concentration. At 100 mm concentrations the chlorophyll concentration was 3.9±0.57 mg/g FW in Carr, 4.7±1.14 mg/g FW in Indaf and

4.4±0.48 mg/g FW in Hasiru gambe was found (Table 5).

3.5 Effects on Proline content
The effect on Proline production in different varieties is shown in Table 6. On increasing the salt concentration from 50 mm, 75 mm to 100 mm the concentration of Proline has increased. The maximum increase is seen in the Hasiru gambe variety (411±10.8 µg/g FW), followed by Indaf (401.36±µg/g FW) and Carr (340.26±27.72 µg/g FW). In the untreated varieties, the Proline content is in a similar concentration.

3.6 Effects on Reducing sugar content
In Table 7, the effects of salinity stress on reducing sugars are shown. The concentration of reducing sugar is proportional to concentration of salinity treatment in comparison to that of control. Plants under control conditions have the lowest reducing sugar content between 1.43 mg/g FW – 1.73 mg/g FW. The 100 mm treatment showed the highest concentration ranging from 4.36 mg/g FW to 4.98 mg/g FW.

3.7 Effects on Protein content
The impact of salinity triggered the increase in protein concentration in the stressed plants (Table 8). The 100 mm salinity treatments showed a protein concentration of 60±8.19mg/g FW in Hasiru gambe, 55±4.65 mg/g FW in Carr, and 57.66±2.29 mg/g FW in Indaf.

4. Discussion
According to genotype, varieties, and factors of environment, the response of plant varies and these are quantitatively inherited traits (Bertazzini *et al*., 2018; Shabala *et al*., 2013) [7, 41]. As per to Dugassa *et al*., (2019) [17], short-term salinity stress effects germination rate, root and shoot length, and relative water content. To comprehend their adaptations to saline conditions, it is crucial to screen for salt-tolerant varieties. Three different varieties of finger millets were tested in this study, and the results revealed variations within the tested parameters. The Carr variety is found to be salt sensitive and Indaf and Hasiru gambe is found to be salt tolerant. These findings were from previous work on finger millet (Mukami *et al*., 2020) [30], lettuce (Ahmed *et al*., 2019) [2], alfalfa (Sandhu *et al*., 2017) [38], and wheat (Tounsi *et al*., 2017) [47].

As per Soares *et al*., (2018) [42], shoots helps plants in adapting to stressful environments and survive by allocating a lot of resources to reduce stress. According to Carillo *et al*., (2019) [10, 14] and Hussain *et al*., (2015) [23].

Our findings suggest that Hasiru gambe is more salt tolerant and is followed by Carr and Indaf. Previous studies show that under saline conditions, salt-tolerant plant varieties grow more rapidly and exhibit less growth retardation than sensitive ones (Sarabi *et al*., 2017) [39].

As roots are responsible for nutrient uptake and translocation they play a significant role in the salt tolerance of plants. Root growth is also susceptible to salt stress because of their direct contact with saline environments (Munns and Tester, 2018) [31]. Relative water content is inversely proportional to salt stress (Polash *et al*. 2018) [34]. ABA-mediated stomal closure impacts transpiration resulting in little uptake by roots and an increase in cell water content (Blatt and Amstrong, 1993) [8].

With increasing salinity treatment concentration, the chlorophyll content dropped. Similar results with work on *Heritiera fomes* reported by Mitra and Banergee (2010) [29] provides additional support for the findings. In the study of salinity stress in rice plants, Rodreguez *et al*., (2006) [36] discovered a negative correlation between chlorophyll and salinity. Salinity stress and chlorophyll levels in bean plants are found to be negatively correlated, according to Stoeva and Kaymakanova's (2008) [43].

Proline accumulates in the plant under salinity condition, which helps the plant in adapting. Proline concentration is proportional to concentration of salinity treatment. The results are similar to the findings of Amini and Ehasanpour, (2016) [5] in *Lycopersicum esculentum*. The contents of proline increased with salinity concentration in gerbera as reported by Ganege don *et al*., (2010) [19]. Lokhande *et al*., 2011 [28] found an increase in free proline increasing of salinity treatment in *Sesuvium portulacastrum*.

In order for plant cells to adjust their osmotic balance under salinity stress, sugar must be reduced. Sugars are crucial in osmotic adjustment during salt stress, as evidenced by the high levels of reducing sugars that have been found in plants with high salinity tolerance. Numerous studies have documented the rise in reduced sugar concentration in response to salinity stress (Dubey and Singh, 1999; Flowers, 2004; Pattanagul and Thitisaksaksul, 2008) [16, 18, 33].

Plants exposed to NaCl stress have shown higher concentrations of protein production. The results obtained, agree with the work presented by Chao *et al.*, (1999) [11] in *Lycopersicon esculentum* (L.) with response to salinity treatment. Sibole *et al*., (2003) [40], have reported the same result in *Medicago sativa*. Tort and Turkyilmaz (2004) [48] found high protein content in *Hordeum vulgare* L. when subjected to salinity stress. Kapoor and Srivatsava (2010) [25] have obtained similar results with *Vigna mungo* (L.).

Table 1: Effects on the germination rate of 3-finger millets.

Variety	Germination rate (%)			
	0 mm	50 mm	75 mm	100 mm
Hasiru gambe	90.6±2.3^{a}	77.3±6.1^{ns}	68±4^{ns}	60±4^{a}
Carr	93.3± 2.3^{ns}	80±4^{ns}	64±4^{ns}	44±4^{ab}
Indaf	94.6±4.6^{a}	80±4^{ns}	72±4^{ns}	60±4^{b}

Readings within a column are marked with superscripts, superscripts represent if the means are significantly different between the varieties or not on the basis of Tukey's test at p<0.05 (Two way ANOVA). Every data is represented as mean± SD is the mean of 5 replicates.

Table 2: Effects on the shoot length of 3-finger millets.

Variety	Shoot length (cm)			
	0 mm	50 mm	75 mm	100 mm
Hasiru gambe	4.7 ± 0.4^{a}	3.9±0.4^{ns}	3.3±0.4^{ns}	2.4±0.29^{ns}
Carr	5.2±0.35^{ns}	4.8±1.8^{ns}	3.7±1.43^{ns}	2.5±0.15^{ns}
Indaf	6.56±0.78^{a}	2.9±0.8^{ns}	3.3±0.35^{ns}	2.9±0.61^{ns}

Readings within a column are marked with superscripts, superscripts represent if the means are significantly different between the varieties or not on the basis of Tukey's test at p<0.05 (Two way ANOVA). Every data is represented as mean± SD is the mean of 5 replicates.

Table 3: Effects of NaCl on the root length of 3-finger millets.

Variety	Root length (cm)			
	0 mm	50 mm	75 mm	100 mm
Hasiru gambe	8.4±1.4^{ns}	6.8 ± 1^{ns}	5.4±1.7^{ns}	4.3± 0.6^{ns}
Carr	6.9±0.8^{ns}	5.8±0.34^{ns}	4.9±0.73^{ns}	3.6±0.15^{ns}
Indaf	6.5±0.6^{ns}	5.6±0.6^{ns}	4.7± 1.7^{ns}	3.2± 0.5^{ns}

Readings within a column are marked with superscripts, superscripts represent if the means are significantly different between the varieties or not on the basis of Tukey's test at *p*< 0.05 (Two way ANOVA). Every data is represented as mean± SD is the mean of 5 replicates.

Table 4: Effects on Relative Water Content of 3-finger millets.

Variety	Relative water content (%)			
	0 mm	**50 mm**	**75 mm**	**100 mm**
Hasiru gambe	81.66±1.25^{ab}	68.18±2.5^{a}	51.52±2.6^{ac}	39.6±2.7^{c}
Carr	89.47±1.26^{a}	72.09±3.4	59.39±1.59^{ab}	41.6±4.3^{b}
Indaf	93.54±1.92^{b}	76.54±1.9^{a}	69.07 ± 2.3^{bc}	47.77±3.9^{bc}

Measurements within a column are marked with superscripts, superscripts represent if the means are significantly different between the varieties or not on the basis of Tukey's test at p< 0.05 (Two way ANOVA). Every data is represented as mean± SD is the mean of 5 replicates.

Table 5: Effects on Chlorophyll content of 3-finger millets.

Variety	Total chlorophyll content (mg/FW)			
	0 mm	**50 mm**	**75 mm**	**100 mm**
Hasiru gambe	5.5±0.86^{ns}	4±0.73^{ns}	5±0.32^{ns}	3.9±0.57^{ns}
Carr	6.2±0.40^{ns}	5±0.73^{ns}	4.3±0.32^{ns}	4.7±1.14^{ns}
Indaf	5.9±0.08^{ns}	6.8±0.08^{ns}	5.3±0.32^{ns}	4.4±0.48^{ns}

Readings within a column are marked with superscripts, superscripts represent if the means are significantly different between the varieties or not on the basis of Tukey's test at p< 0.05 (Two way ANOVA). Every data is represented as mean± SD is the mean of 5 replicates.

Table 6: Effects on Proline content of 3-finger millets.

Variety	Total Proline content (µg/FW)			
	0 mm	**50 mm**	**75 mm**	**100 mm**
Hasiru gambe	207.5±11.16^{ab}	322.9±17.89^{ac}	356.1±22.13^{ab}	411.9±10.8^{ac}
Carr	224.2±3.02^{bc}	262.2±4.74^{bc}	326.6±16.99^{bc}	340.26±27.72
Indaf	208.2±11.75^{ca}	309.63±6.21^{ca}	358.4±6.58^{ca}	401.36±21.2^{ca}

Readings within a column are marked with superscripts, superscripts represent if the means are significantly different between the varieties or not on the basis of Tukey's test at p< 0.05 (Two way ANOVA). Every data is represented as mean± SD is the mean of 5 replicates.

Table 7: Effects on Reducing sugar content of 3-finger millets.

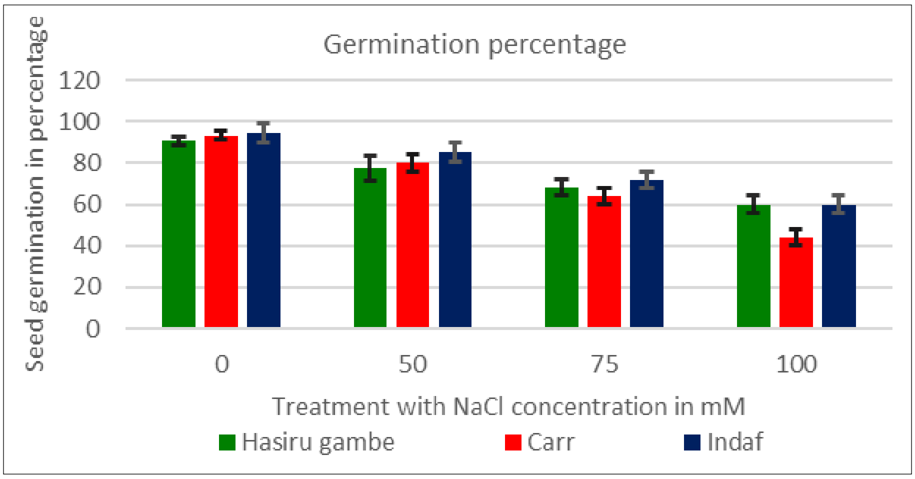
Variety	Total reducing sugar content (mg/FW)			
	0 mm	**50 mm**	**75 mm**	**100 mm**
Hasiru gambe	1.73±0.32^{a}	2.39±0.17^{ac}	2.83±0.08^{a}	4.36±0.36^{ns}
Carr	1.65±1.16^{b}	2.45±0.17^{bc}	3.3±0.12^{b}	4.47±0.23^{ns}
Indaf	1.43±0.17^{ns}	2.77±0.7^{ca}	3.7±0.21^{c}	4.98±0.09^{ns}

Readings within a column are marked with superscripts, superscripts represent if the means are significantly different between the varieties or not on the basis of Tukey's test at p< 0.05 (Two way ANOVA). Every data is represented as mean± SD is the mean of 5 replicates.

Table 8: Effects onProtein content of 3-finger millets.

Variety	Total Protein content (mg/FW)			
	0 mm	**50 mm**	**75 mm**	**100 mm**
Hasiru gambe	15.56±1.69^{ns}	20.3±0.64^{ns}	42.4±2.45^{a}	60±8.19^{ns}
Carr	13.83±1.11^{ns}	23.38±0.90^{ns}	43.83±1.12^{b}	55±4.65^{ns}
Indaf	20.2±1.55^{ns}	26.83±0.92^{ns}	45.3±2.48^{ns}	57.66±2.29^{ns}

Readings within a column are marked with superscripts, superscripts represent if the means are significantly different between the varieties or not on the basis of Tukey's test at p< 0.05 (Two way ANOVA). Every data is represented as mean± SD is the mean of 5 replicates.



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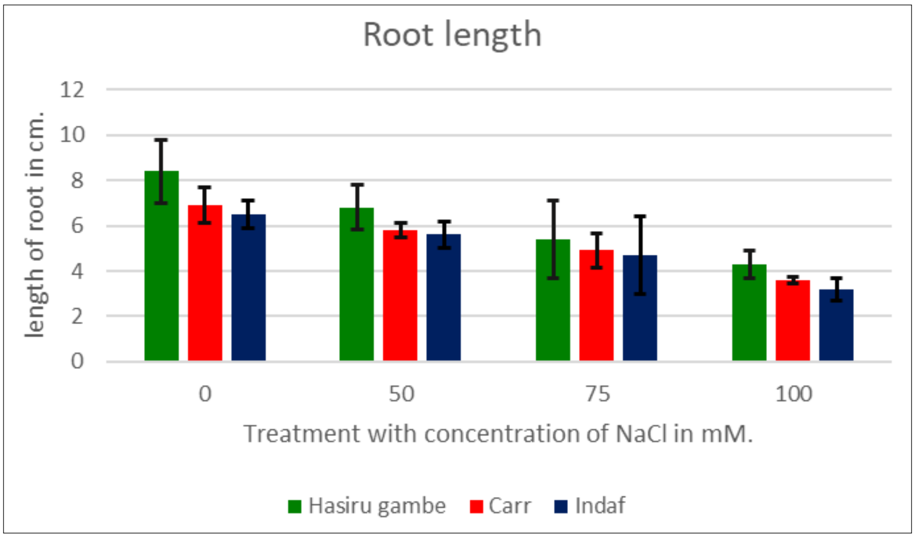

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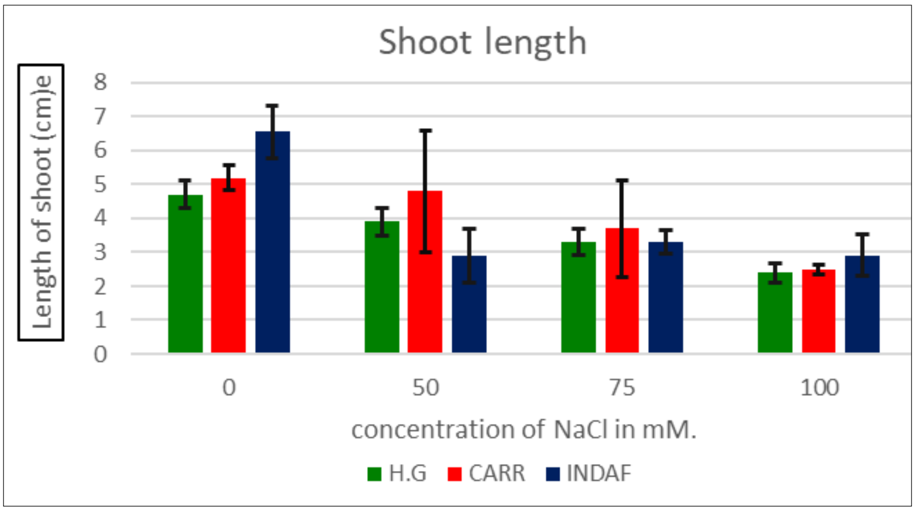

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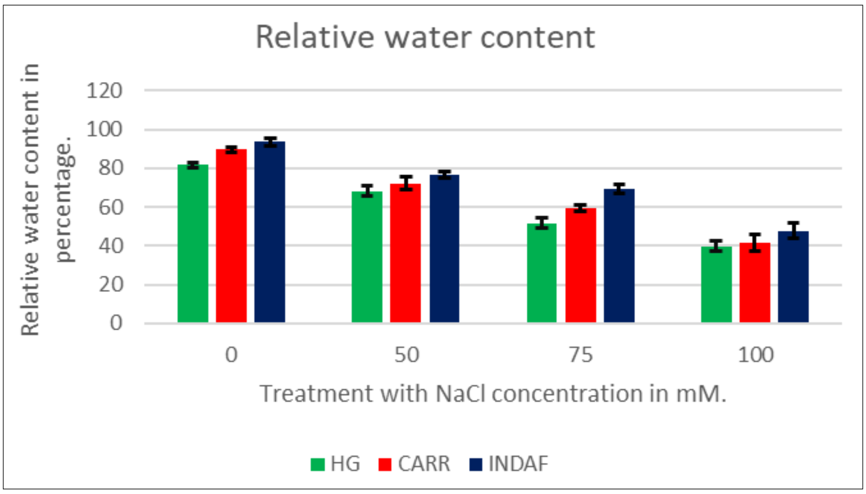

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Fig 1: A. Graphical representation of germination percentage, b. Graphical representation of shoot length, c. Graphical representation of root length, and d. Graphical representation of Relative Water Content.

5. Conclusion

In this study, the impact of NaCl stress treatments on the physiological characteristics of finger millet is analyzed. The results showed that finger millets exhibit a wide range of distinct salinity stress responses. It is concluded with an increase in salinity treatment concentration the seed germination, root length, shoot length, and relative water content decrease in the finger millets, whereas the biochemical parameters like, proline, protein, and reducing sugar concentration increase in salinity stressed plants. From the data of the Carr variety, we hypothesize that this variety has genetic resources with considerably high salinity tolerance. Understanding the genes involved in the regulation of salt tolerance in finger millets requires additional analysis.

Conflict of Interest

The authors declare no conflict of interest, as the paper has not been submitted anywhere else for publishing.

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