



## **EFFECT OF SALINITY ON SODIUM & POTASSIUM UPTAKE AND PROLINE, CARBOHYDRATES CONTENTS OF TURMERIC PLANT PARTS**

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### **ABSTRACT**

Turmeric (*Curcuma longa* L.) is a rhizome-bearing perennial plant of the Zingiberacea family, which is native to Southeast Asia. Traditionally, turmeric has been used as a kind of spice, medicine, cosmetics, colorific matter and flavoring in food industries. Soil and water salinity is one of the main stresses that can drastically reduce the rate of plant production. Wide spread of saline soil in Iran and lack of information concerning this plant due to non-cultivation of Turmeric in Iran are reason for conducting this experiment. Therefore, the effect of saline water on the rate of sodium, potassium uptake by Turmeric and also proline and carbohydrates accumulation were evaluated at two times. So, rhizomes were grown in coco peat and perlite in controlled conditions. A month after germination, plants were irrigated with Hoagland's solution containing 0, 20, 60 and 100 mM, NaCl as treatment. One and two months after saline condition, the plants were harvested and sodium, potassium, proline and carbohydrate were measured in turmeric's plant parts. Experiment was conducted in a split plot design using completely randomized experiment with 6 replicates. According to the results, increase in salinity caused addition of sodium but less potassium in all plant parts at two different harvest times. Moreover, the amount of proline and the soluble and reduced carbohydrates were related to the interaction of salinity levels and exposed time to salinity. When the amount of Na<sup>+</sup> content was less than 1.74% DW, the amount of proline, carbohydrates and reduced sugars increase as salinity was increased. However, when salinity exceeded in plant, then the amount of carbohydrates and reduced sugar declined as salinity increases.

**Key words:** *Curcuma longa* L., Salinity stress, Proline, Carbohydrates, Sodium, Potassium.

### **INTRODUCTION**

Turmeric (*Curcuma longa* L.) is a rhizome-bearing perennial plant from the Zingiberacea family, which is native to Southern Asia and is cultivated in a wide range of tropical areas such as India, China, Pakistan, Japan, Thailand, Sri Lanka and Brazil. India comprises a monopoly in the production and export of turmeric and nearly 94% of turmeric in the world is produced in India<sup>2</sup>. The main active ingredients of turmeric are curcuminoids and volatile oils, which are synthesized in the leaves and transferred to rhizome wherein they are stored<sup>3</sup>. Traditionally, turmeric has been used as a kind of spice, medicine, cosmetics, colorific matter and flavoring in food industries<sup>1</sup>. Turmeric can grow in tropical conditions, at different altitudes from sea level to 1,500 meters above sea level. This plant needs 20 to 30°C and 1500 mm or more

rainfall or irrigation during growing season. It requires humid climate but can also grow in sunny conditions to partially shaded or in light and shade<sup>4,5</sup>.

Salinity of soil and irrigation water is one of the main stresses that can severely reduce the rate of production<sup>6</sup>. High salinity leads to hyperionic and hyperosmotic conditions, which causes the increase of osmotic pressure and consequently, reduction of water activity of cytosol. At the same time, penetration of sodium ions through  $K^+/Na^+$  channels and consequently, addition of their concentration in cytosol disturbs the enzymes activity<sup>7</sup>. In other words, the harmful effect of salinity for plant growth includes the direct effects of ion toxicity and the indirect effects of salt ions on water potential<sup>8</sup>.

The mechanisms of salinity tolerance fall into three categories: (i) Tolerance to osmotic stress. Some plants can adapt their osmotic potential to the low osmotic potentials of soils at low salinity<sup>9</sup>. However the osmotic stress immediately reduces cell expansion in root tips and young leaves, and causes stomatal closure, (ii)  $Na^+$  exclusion from leaf blades.  $Na^+$  exclusion by roots ensures that  $Na^+$  does not accumulate to toxic concentrations within leaves. A failure in  $Na^+$  exclusion manifests its toxic effect after days or weeks, depending on the species, and causes premature death of older leaves and (iii) Tissue tolerance, i.e., tolerance of tissue to accumulated  $Na^+$ , or in some species, to  $Cl^-$ . Tolerance requires compartmentalization of  $Na^+$  and  $Cl^-$  at the cellular and intracellular level to avoid toxic concentration within the cytoplasm, especially in mesophyll cells in the leaf. Usually, toxicity occurs with time, when  $Na^+$  concentration in older leaf increases than younger leaf exposed more to  $Na^+$  accumulation<sup>10</sup>.

In saline condition, a unique plant response to the external osmotic potential changes is accumulation of metabolites, which do not create any obstacle for normal metabolic reactions of cell and act as consistent solutions. If  $Na^+$  and  $Cl^-$  are not moved into the vacuole of the cell, organic solutes that are compatible with metabolic activity even at high concentrations must accumulate in the cytosol and organelles to balance the osmotic pressure of the cell<sup>11</sup>.

These metabolites so called “compatible solutes”, which have osmotic performance include: sucrose, fructose, sugar alcohols (e. g. glycerol, methylated inositol), complex sugars (e. g. trehalose and raffinose), ions like  $K^+$  and charged metabolites such as glycinebetaine and proline<sup>12</sup>. Osmolites protect cellular macromolecules against the damaging effects of water shortage and the increase in cell ion concentrations causing the plant’s salinity resistance<sup>13,14</sup>.

Mechanism of osmotic adjustment using different osmolites varies in different plant species. In many halophytes, proline or glycine betaine occur at sufficiently high concentrations in leaves (over 40 mM on a tissue water basis) to contribute to the osmotic pressure (over 0.1 MPa) in the cell<sup>11</sup>. In glycophytes, the concentrations of compatible solutes that accumulate are not so high, but if they partitioned exclusively to the cytoplasm. They could generate a significant osmotic pressure and function as an osmolyte. At low concentrations, these solutes presumably have another role, perhaps in stabilizing the tertiary structure of proteins, and function as osmoprotectants<sup>15</sup>. Among these organic compounds, which are accumulated in salinity-exposed plants. Proline and carbohydrates can play an important role in the salt tolerant plants.

Turmeric cultivation is not native to Iran. Lack of information related to its cultivation and existence of large areas of saline soils in Iran this experiment was conducted. In this study; (i) The rate of sodium, potassium uptake and accumulation in different plant parts was evaluated, (ii) The variation of proline and carbohydrates accumulation in turmeric plant parts was assay and (iii) Plant parts dry weight correlated with salinity.

## EXPERIMENTAL

### Planting, performing salinity and preparation of plant samples

The rhizomes of turmeric were prepared from India (Dept. of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, U.P.) and these were sterilized in Benomyle fungicide for 30 min. Rhizomes with uniform size (5 to 7 cm in length and 1 to 1.5 cm in diameter) were selected and then planted in small sterilized pots in a controlled condition (temperature  $30 \pm 5^\circ\text{C}$  and relative humidity  $60 \pm 10\%$  and light intensity 1200-1400 lux). The pots contained coco peat and perlite and these were watered regularly.

After two weeks, the rhizomes started to germinate. Two weeks after the emergence of rhizomes, identical and uniform germinated rhizomes were transferred to pots with 30 cm height and 15 cm diameter containing coco peat and perlite media. The pots were regularly watered daily using Hoagland's nutrient solution<sup>16</sup> to keep soil moisture at approximately field capacity. Salinity treatment (0, 20, 60 and 100 mM NaCl) applied to pots one month after germination with irrigation via Hoagland's solution. The experiment was conducted in a completely randomized design with 6 replicates.

Plant samples were obtained one and two months after application of salinity treatment and then separated into young and old leaves, pseudo-stems, root and the rhizomes. The young and old leaves, pseudo-stems, roots and rhizomes were placed in oven at  $70^\circ\text{C}$  for 48 hrs and the rhizomes were placed in the oven at  $40^\circ\text{C}$  for a week, respectively.

Sodium and potassium of plant samples were measured using flame photometer (Halstead, Essex-corning 410). The device was regulated by standard solutions of sodium and potassium. Then 10 mL sulfosalicylic acid (3%) was poured into glass containers containing 0.1 g dry plant samples. After 24 hours, samples were filtered and the amount of the sodium and potassium in the extract was read by flame photometer. Then concentration of plant samples was calculated by the standard curve based on dry tissue.

### Proline, soluble sugar and reduced sugar assay

Dried plant samples were grinded into powder and then 0.1 g of this powder was poured into glass dishes containing 10 mL of sulfosalicylic acid (3%) to measure proline<sup>17</sup>. After 24 hrs, samples were filtered, and then 2 mL of the extract were combined with 2 mL of ninhydrin and 2 mL of glacial acetic acid. The mixture was placed in  $100^\circ\text{C}$  for one hour using hot water bath. When tubes have been cooled to room temperature, 4 mL of toluene was added to each tube. After almost 20 seconds, tubes were intensity stirred up and the pink organic phase in the top was separated from colorless aqueous phase in the bottom. Then it was used for colorimetrically using spectrophotometer (Pharmacia LKB-Novaspec) at a wavelength of 520 nm. The amount of proline in plant samples was determined using a standard curve and relevant relationship.

Phenol-sulfuric acid method was used to measure soluble sugar<sup>18</sup>. For extraction of sugar from the plant tissue, 0.01 g grinded dry tissue was abraded in a mortar with 10 mL of hot distilled water. Finally, filtered 2 mL of extracted sample were added to 50 mL phenol (80% v/v) and then 5 mL of sulfuric acid was added and each tube was stirred. The mixture was placed in room temperature for ten minutes and then was laid in water bath at  $25^\circ\text{C}$  for 10 more minutes. 2 mL of distilled water was added to each tube and after homogenization, the optical absorbance was read using spectrophotometer (Pharmacia LKB-Novaspec) at wavelength of 490 nm and the amount of soluble carbohydrates was determined using a standard curve and relevant relationship.

Estimation of reducing sugar was done by colorimetric method, using 3, 5-dinitrosalicylate acid (DNS) as reagent.<sup>19</sup> For extraction of sugar from the plant tissue, 0.01 g of the dry tissue was grinded using

in a mortar with 10.0 mL of 80% ethyl alcohol and it was filtered. 2.0 mL of extract was mixed with 1.0 mL of DNS and placed in a boiling water bath for 10 min. Then 10.0 mL of distilled water were added to each tube and cooled under tap water. After homogenization of mixture, the absorbance was recorded using spectrophotometer (Pharmacia LKB-Novaspec) at 546 nm. The amount of reducing sugar was calculated using standard curve prepared from glucose. The quantity of reducing sugar was expressed as  $\text{mg g}^{-1}$  fresh weight of tissue.

### Statistical analysis

SPSS16 was used to determine ANOVA and Duncan's multiple test range was used to compare mean values. Excel was used to draw the graphs.

## RESULTS AND DISCUSSION

### Change in dry weight

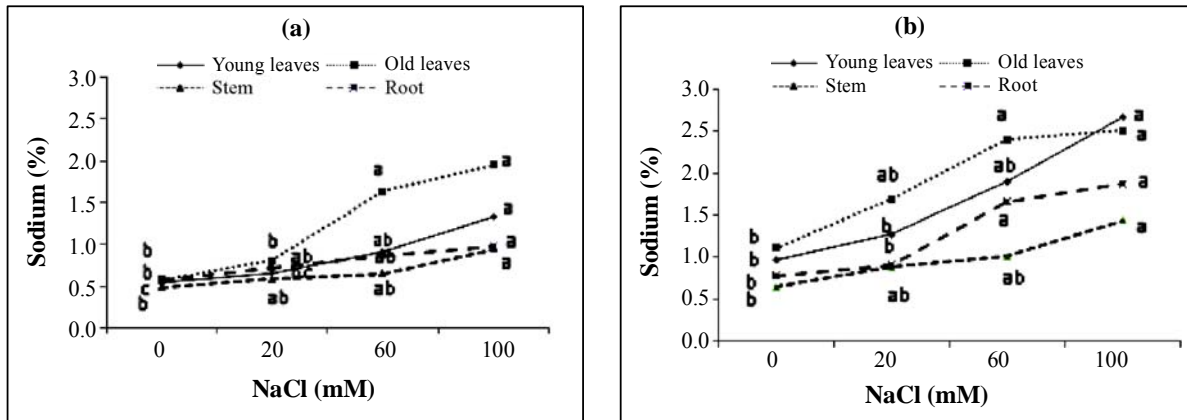
Dry weight reduction due to salinity was different in different plant parts as well as in different level of salinity (Table 1). According to the results of analysis of variance, the effect of salinity, plant parts, time of harvests and their interaction were statistically significant. Comparing control plants, dry weight reductions in 100 mM NaCl were 191%, 141%, 56%, 30% in leaf, pseudo-stem, root and rhizome, respectively, when plant was exposed to one month of salinity. In the second harvest, when plant was exposed to two months of salinity, the reductions were 198, 153, 39 and 40% in the same organs. This means that reduction was increased as the time of exposed to salinity was increased. The changes in dry weight of root and rhizome are not statistically significant, while the changes in pseudo-stem and leaf due to the change in salinity are significant ( $P < 0.01$ ).

**Table 1: Mean (n = 6) dry weights of curcuma plant parts after one and two months exposed to different level of salinity. Plants were two and three months old at the first and second harvest, respectively**

Time exposed to salinity	Salinity (mM NaCl)	Leaf ( $\text{mg plant}^{-1}$ )	Pseudo-stem ( $\text{mg plant}^{-1}$ )	Root ( $\text{mg plant}^{-1}$ )	Rhizome ( $\text{mg plant}^{-1}$ )
One month (First harvest)	0	1050	490	94	99
	20	680	380	66	96
	60	523	296	63	76
	100	360	203	60	76
Two months (Second harvest)	0	1600	743	266	210
	20	1300	446	253	156
	60	670	340	240	163
	100	536	293	191	150

### Change in sodium and potassium of plant parts

Increasing salinity from 0 to 100 mM NaCl in the media, sodium content of plant parts increased differently (Fig. 1). In the first harvest i.e. one month after application of salinity, the amount of sodium in different parts of control plants (0 mM NaCl) seems to be almost the same. By increasing salinity, the sodium was concentrated mostly in old leaves, while the young leaves and other organs accumulated less  $\text{Na}^+$ . When salinity was increased to 100 mM, the additional Na accumulation were 70, 59, 41 and 48% higher in old leaves, young leaves, roots, and pseudo-stems, respectively.



**Fig. 1: Mean (n = 6) values of sodium content based on dry weight of turmeric's plant parts (%) under different salinity (mM NaCl). Plants were exposed to one (a) and two months (b) of salinity. The mean values were compared using Duncan's multiple test range for each plant parts separately ( $P < 0.05$ )**

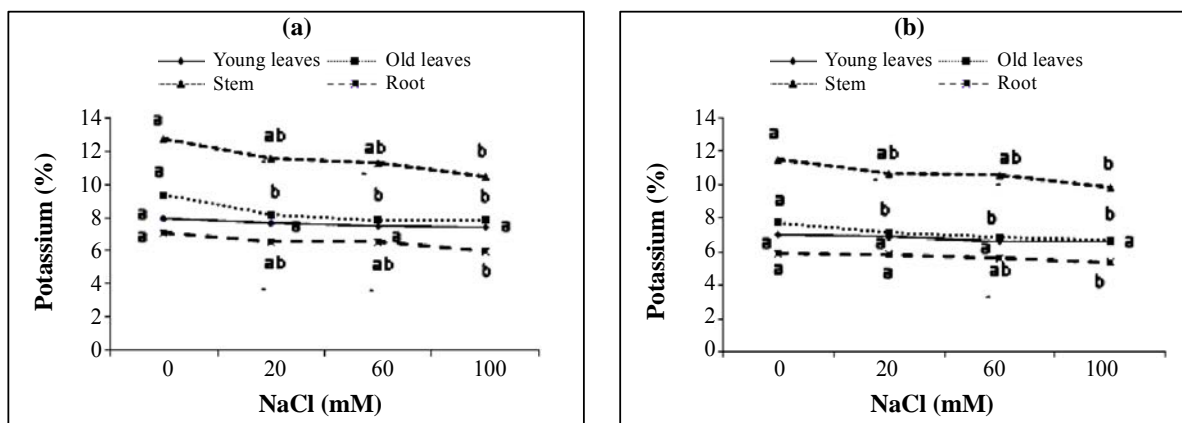
However, as salinity was extended to two months, the sodium in plant parts was increased as much as twice compared to one month salinity. This is an indication of more Na accumulation in plant parts as growing season extended in saline soil. The results show that the accumulation of Na mostly happens in the old leaves (Fig. 1a) at the beginning of salt application, and when the salinity was extended, the Na<sup>+</sup> accumulation was expanded to young leaves and roots.

Increase of salinity from 0 to 100 mM NaCl in the media, increased sodium content of plant parts. Plants avoid salinity damages by interdiction of ions uptake, excluding extra ions (Na<sup>+</sup> Cl<sup>-</sup>) from cell or store them in the vacuole. Under extra NaCl, sodium can enter cell faster than chloride, because of the low permeability of plasma membrane to chloride ions. Halophyte plants which absorb sodium chloride in high concentration have developed some strategies to survive under salinity condition. In such plants, ions are accumulated in vacuoles and also additional compatible metabolite synthesis to create osmotic potential without causing damage to chloroplast and cytosolic enzymes. The balance of water between cytoplasm and vacuole in such cases is maintained by accumulation of some metabolites such as proline and  $\beta$ -glycine in cytoplasm<sup>6</sup>. Resistant and sensitive species of glycophytes mostly categorized were based on the rate of ions uptake and the pattern of distribution in different parts of the plant. For example, in the resistant line to salinity of Hey, extra sodium accumulates in the leaflets, whereas in flax sodium accumulates in the leaves<sup>20</sup>.

In this experiment, when salinity was increased to 100 mM, the extra Na accumulated mostly in old leaves and then in young leaves, roots and pseudo-stems. Most of the Na<sup>+</sup> that is delivered to the shoot remains in the shoot, because for most plants, the movement of Na<sup>+</sup> from the shoot to the roots in the phloem can likely recirculate only a small proportion of the Na<sup>+</sup> that is delivered to the shoot<sup>10</sup>. Therefore, water potential adjustment needed to happen mostly in cytosol of old leaves via metabolite such as proline and hydrocarbons.

As salinity extended to two months, the pattern of sodium distribution changed within plant organs and more sodium was accumulated in young leaves as well as in old leaves. This might be due to the limited capacity of vacuoles to store sodium in the old leaves. This shows the severity of damages of sodium, which extend to whole plant parts due to more negative osmotic potential, which needs to be maintained to supply required water. However, the main site of Na<sup>+</sup> toxicity for most plants is the leaf blade, where Na<sup>+</sup> accumulates after being deposited in the transpiration stream, rather than in the roots<sup>21</sup>.

The amount of potassium was different in plant parts as well as in different stage of growth (Fig. 2a and B). At no salinity, potassium is more distributed in pseudo-stems and old leaves and less in roots and young leaves. However, when salinity was increased, the amount of potassium was reduced in all plant parts and the reduction was almost higher in plant parts containing higher potassium. This is the negative effect on the amount of potassium, which is more remarkable in parts with more potassium. The reductions were as much as 21, 19, 18 and 6% in pseudo-stems, old leaves, roots and young leaves, respectively under 100 mM NaCl in the first harvest, than the reductions 17, 15, 9, and 6% in the same plant parts in the same condition in the second harvest (Fig. 2a and b).



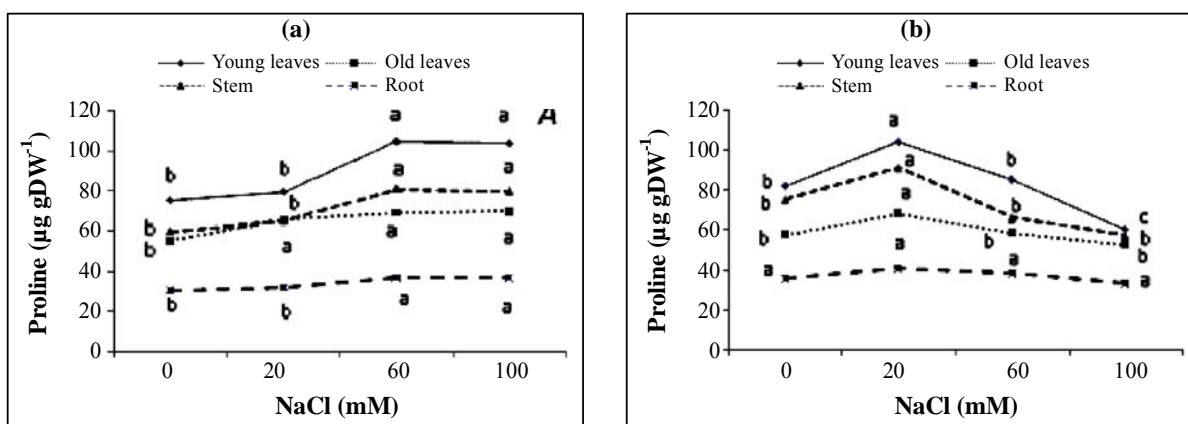
**Fig. 2: Mean (n = 6) values of potassium content based on dry weight of turmeric's plant parts (%) under different salinity (mM NaCl). Plants were exposed to one (a) and two months (b) of salinity. The mean values were compared using Duncan's multiple test range for each plant parts separately ( $P < 0.05$ )**

The addition of sodium chloride from 0 to 100 mM leads to a decrease in potassium content of different plant parts. This means that higher sodium in the media caused a reduction in potassium uptake and also affect distribution of potassium within plant parts. When salinity was extended to two months, the reduction in potassium content of plant parts increased significantly. Potassium can play an important role in osmotic adjustment and leads to increase the resistance of plant in response to high amounts of sodium chloride in the medium<sup>22</sup>. Wated et al.<sup>23</sup> observed that increasing sodium chloride in medium of tobacco cells caused an increase of sodium and decrease of potassium of intracellular in a non-linear fashion. In a review of mechanism of salinity, Munns and Tester<sup>10</sup> indicated that a specific relationship between leaf potassium and salinity has not been found. However, in some reports, the ratio of sodium to potassium has been used as an index in order to indicate resistance to salinity in a way that increase of sodium in plants resistant to salinity are usually accompanied by a lower reduction in potassium<sup>24</sup>. In this case, if the reduction of plant dry weight is assumed as the response of plant to salinity, then higher sodium and lower potassium uptake causes a higher sodium/potassium ratio, which is more correlated with plant dry weight reduction.

### Changes in proline

According to the results obtained from the plants exposed to one month of salinity (Fig. 3a), the amounts of proline increased differently in plant parts as salinity increased. The higher additions of proline were observed in young leaves (27%), pseudo-stem (24%), root (20%) and less in the old leaves (17%). In the older plants exposed to two months of salinity (Fig. 3b), the trend of proline accumulation was different and its amount increases first with addition of salinity (up to 20 mM) and then decreases as salinity increased to 100 mM NaCl. At 20 mM NaCl, the additions of proline at young leaf, pseudo-stem, old leaf

and root were 21, 17, 16, and 13, respectively compared to control plants. Then the amounts of proline were decreased as much as 36, 39, 9, and 5% in the same organs, respectively at 100 mM NaCl.



**Fig. 3: Mean ( $n = 6$ ) values of proline content ( $\mu\text{g gDW}^{-1}$ ) of turmeric's plant parts under different salinity (mM NaCl). Plants were exposed to one (a) and two months (b) of salinity. The mean values were compared using Duncan's multiple test range for each plant parts separately ( $P < 0.05$ )**

According to our result, when plant exposed to salinity (100 mM NaCl) for a month, sodium concentration increases in whole plant from 0.54 (in control plants) to 1.30% DW. This condition also increased the amount of proline from 55.34 to 72.66  $\mu\text{g g}^{-1}$  DW. Reports of different researches also show similar results for different plants. Paleg et al.<sup>25</sup> reported an increase of proline in different plants parts of *Licopersicumesculentum*, *Brassica juncea* and *Nicotianatabacum* under salinity. In *Aneurolepidiumchinense*, high salinity and pH decreased the plant survival, rhizome number, relative growth rate and potassium content while sodium and proline concentration were increased<sup>26</sup>. In another rhizome-bearing plant such as *Sporobolusvirginicus*, salinity causes loss of the stem weight, decreasing internode length and the size of leaves, while increasing proline content and ammonium compounds<sup>27</sup>. One of the reasons of proline accumulation under stress conditions may be related to its role as an osmotic regulator due to its bi-polar and hydrophilic properties<sup>28</sup> between the cytoplasm and vacuoles<sup>29</sup>. In addition, it is also responsible for protecting many cytoplasmic enzymes. These evidences demonstrate that proline accumulation could be a useful feature for adaption of plant to biotic stress<sup>30</sup>.

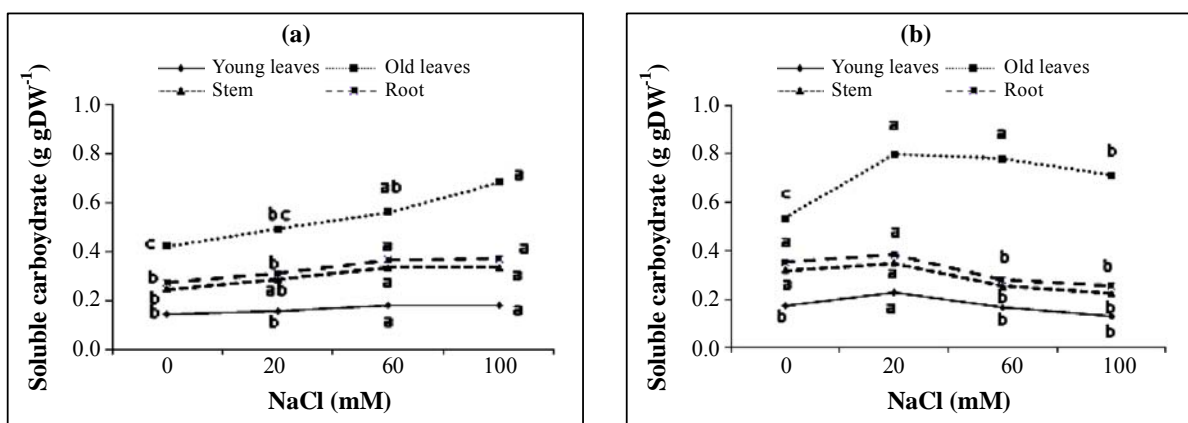
Although, under osmotic stress, proline accumulated in all vegetative parts of plant even its fruits, most of the accumulation occurs in the growing leaves. Our results indicated that when salinity increased to 100 mM NaCl, proline accumulated mostly in young leaves and then in pseudo-stem, old leaves and root. Generally exposing plant to osmotic stress increases free proline, especially in leaves compared to their roots<sup>31</sup>. Higher accumulation of proline in young leaves is accompanied with higher growth rate of young leaves.

When salinity was extended to two months, then the concentration of proline in plant parts varied according to the salinity levels. For example, when salinity increased to 20 mM NaCl (1.19% Na<sup>+</sup> content in plant based on dry weight), proline content increased to 17.86% compared to control plants. In contrast, extended salinity to 100 mM NaCl (2.12% Na<sup>+</sup> content in plant based on dry weight), proline concentration declines in whole plant up to 23.02%. The variation in proline content of plant parts, which regulates by Na<sup>+</sup> concentration in plant caused less and more damages to different organs as well as uptake of potassium. Reduction of proline was higher in organs with higher proline content, when salinity extended. This is accompanied with more accumulation of sodium.

It can be concluded that when accumulation of sodium in plant increased to more than 1.74% of dry weight (due to salinity or extension of salinity condition) proline content decreased due to less production and more consumption via physiological process. This might be the reason for less amount of proline in different plant parts.

### Changes in soluble and reduced carbohydrate

According to the results obtained for plants exposed to one month of salinity, the amount of soluble carbohydrate in different plant parts was increased with increasing salinity from zero to 100 mM NaCl (Fig. 4a). The accumulation of soluble carbohydrates in curcuma was mostly in the old leaves and less in young leaves. Increasing salinity to 100 mM in the first harvest (plant exposed to one month salinity) show addition of soluble carbohydrates much as 38.2, 22.5, 26.4 and 26.2% in old leaves, young leaves, pseudo-stems and roots, respectively. In the second harvest (plant exposed to two months of salinity), the soluble carbohydrate in the old leaves increased up to 20 mM and then decreased as salinity increased. However in the other organs, as salinity was increased, the accumulation of soluble carbohydrate was decreased (Fig. 4b).

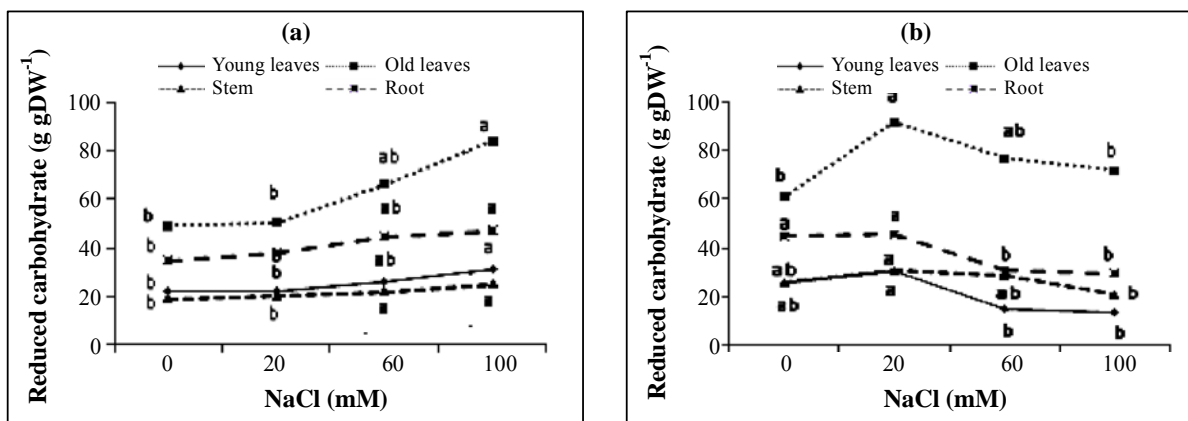


**Fig. 4: Mean (n = 6) values of carbohydrate content (g gDW<sup>-1</sup>) of turmeric's plant parts under different salinity (mM NaCl). Plants were exposed to one (a) and two months (b) of salinity. The mean values were compared using Duncan's multiple test range for each plant parts separately (P < 0.05)**

Changes in soluble sugars under salinity stress in some species of plants have been reported. Ashraf and Tufail<sup>32</sup> reported that soluble carbohydrates of 5 lines of sunflower increases as salinity increases. However, the lines resistant to salinity sustained more soluble carbohydrates in comparison to sensitive lines. Among soluble carbohydrates, saccharose and fructose play an important role in salinity and drought stresses. Saccharose can substitute for water to survive phospholipides of membrane during chrialization/liquidation phases and also to prevent structural changes in soluble proteins<sup>15</sup>. Sangam et al.<sup>34</sup> have concluded that any change in saccharose on mutant plants affect on photosynthesis of plants in salinity stress.

Reduced carbohydrate (Fig. 5) in different plant parts were increased with increasing salinity from zero to 100 mM NaCl, when curcuma was exposed to one month of salinity. The reduced carbohydrates are mostly accumulated in the old leaves and less in pseudo-stems (Fig. 5a). Increasing the salinity to 100 mM NaCl in the first harvest causes to have 41.6% more reduced carbohydrates in old leaves. The additions of reduced carbohydrate were 29.0, 24.0 and 25.5% in young leaves, pseudo-stems and roots, respectively. When plants were exposed to two months of salinity (100 mMNaCl), the reduced carbohydrate was decreased in all plant parts except in the old leaves (Fig. 5b).





**Fig. 5: Mean ( $n = 6$ ) values of reduced carbohydrate content ( $\text{g gDW}^{-1}$ ) of turmeric's plant parts under different salinity ( $\text{mM NaCl}$ ). Plants were exposed to one (A) and two months (B) of salinity. The mean values were compared using Duncan's multiple test range for each plant parts separately ( $P < 0.05$ )**

Variation in quality and quantity of carbohydrates is directly related to physiological activities of plants such as photosynthesis, translocation of materials and respiration<sup>32</sup> as well as adjustment of cell to environmental stress<sup>33</sup>. In this experiment, when plant was exposed to salinity ( $100 \text{ mM NaCl}$ ) for a month, sodium concentration increased in all plant parts especially in the old leaves. This condition increased the amount of soluble sugars from  $0.269$  to  $0.391 \text{ g g}^{-1} \text{ DW}$  and reduced sugars from  $31$  to  $46 \text{ mg g}^{-1} \text{ DW}$ . Generally, reduction of water potential due to drought<sup>34</sup> and salinity stresses leads to an increase in the amount of soluble and reduced sugars. This increase considered as metabolites adjustment for cell adaption via stabilization of osmotic balance<sup>35</sup>.

The role of reduced sugars (glucose and fructose) as adaptation mechanism in salinity stress is more arguable. There are some results regarding the effect of salinity and drought stress on reduced sugars accumulation in wheat and roses<sup>37</sup>. In contrast, others found that the sugar content was decreased<sup>38</sup> or was unchanged in wheat<sup>39</sup>. Glucose can participate in interaction with proteins by complicated glycolization reaction between amino and carbonyl groups and also as a respirational substrate causes an increase in respiration and mitochondrial transfer, which is able to change the metabolism pathways, energy production and formation of reactive oxygen species<sup>40</sup>.

Our results indicated that the amount of soluble and reduced carbohydrates accumulated mostly in the old leaves and then in roots, pseudo-stems and young leaves of curcuma. Moreover, as salinity increases, the accumulation occurs mostly in the old leaves. Whereas the old leaves are the first organs of  $\text{Na}^+$  accumulation in saline condition and therefore, it is expected to have more carbohydrates accumulated in this organ for water potential adjustment. However, the young leaves accumulate less carbohydrate because of high consumption of carbohydrate for growing aspect. Our results indicated that young leaves accumulate the highest amount of proline for water potential adjustment.

When salinity was extended to two months, then the concentration of soluble and reduced carbohydrates in plant parts varied according to the salinity levels. When salinity increased to  $20 \text{ mM NaCl}$  ( $1.19\% \text{ Na, DW}$ ) soluble and reduced carbohydrates increased to  $22.6$  and  $21\%$ , respectively compared to control plants. In contrast, extended salinity to  $100 \text{ mM NaCl}$  ( $2.12\% \text{ Na, DW}$ ) soluble and reduced carbohydrates concentration decline in whole plant as much as to  $3.57$  and  $15.3\%$ , respectively compared to

control plant. The reduction of soluble and reduced carbohydrates in higher salinity might be due to crucial point of Na<sup>+</sup> concentration in curcuma. For example after one month of salinity, the highest amount of Na<sup>+</sup> content was 1.30%, DW at 100 mM NaCl, up to this range, the amount of proline, carbohydrates and reduced sugars were increased as salinity increased. This means that the amount of Na<sup>+</sup> in curcuma is lower than crucial point. However, when salinity was extended to two months which Na<sup>+</sup> can accumulate more in plant, then at 20 mM NaCl, the Na<sup>+</sup> content reaches to crucial point, which is almost 1.74% dry weight. Beyond this point, the amount of carbohydrates and reduced sugar declined as salinity increases.

## CONCLUSION

According to the results, increase of salinity causes addition of Na<sup>+</sup> in all plant parts including root, pseudo-stem and leaf of turmeric. Simultaneously, the potassium content decreases. The addition of Na<sup>+</sup> in plant parts would be increased as the time of saline condition was increased. The amount of proline, soluble carbohydrates and reduced sugar increased as salinity increases. However, when salinity was extended to a longer time, then the amount of proline and carbohydrates decreased because of the accumulation and excess of Na in plant organs. In low salinity and limited accumulation of sodium in plant parts, plant can have required mechanisms properly in favor of plant growth by increasing the amount of proline and carbohydrates to reduce the harmful effect of stress on plant growth. However, by increasing salinity or extension of salinity, the accumulation of sodium in plant part causes toxic condition in the cell and consequently, the resistance mechanism would not work properly. This condition causes less growth or sometimes, even death of the plant. In this experiment, although different mechanisms for compatibility to salinities were used by turmeric plants, reduction of plant parts has happened as salinity increased. When plant was exposed to two months of salinity, the reductions were 198, 153, 39 and 40% in leaf, pseudo-stem, root and rhizome. Changes in dry weight of root and rhizome are not statistically significant, while the changes in pseudo-stem and leaf due to the change in salinity are significant ( $P < 0.01$ ). However, there are some reports that chemical component of turmeric rhizomes changes as salinity increases<sup>41</sup>.

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