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Original Article

Salinity alters curcumin, essential oil and chlorophyll of turmeric (*Curcuma longa* L.)

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Abstract

Turmeric (*Curcuma longa* L.) is a perennial rhizomatous plant from the family of Zingibraceae, native in South Asia. The main components of turmeric are curcuminoids and essential oil which are responsible for turmeric characteristic such as odor and taste. Due to the large areas of saline land in Iran and less information related to cultivation of turmeric, in this research, the effect of salinity on growth, curcumin and essential oil of turmeric was evaluated. Rhizomes were planted in coco peat and perlite for germination. Then uniform germinated rhizomes transferred to hydroponic condition containing Hoagland's solution. Two months old plants were exposed to salinity (0, 20, 60 and 100 mM NaCl) for two months via hydroponic media using Hoagland's solution. Then dry weight of different plant parts, chlorophyll, curcumin and essential oil components of turmeric were determined. The result indicated that, dry weight reductions in 100 mM NaCl were 191%, 141%, 56%, 30% in leaf, pseudo-stem, root and rhizome, respectively (This is almost equal to 6.9, 2.87, 0.34 and 0.23 mg plant^{-1} mM^{-1}NaCl reduction of dry weight, respectively). The reductions in chlorophyll a and b are almost 3.32 and 0.79 µg/gFW respectively due to one unit addition of NaCl ($P <$ 0.05). The addition of curcumin of rhizome for four months old plant versus three months were almost 5 fold for 0 mM NaCl and 2 fold for 100 mM NaCl due to one month of delay in harvest. Low salinity has positive effect in curcumin production but higher salinity (higher than 60 mM) had adverse effect and causes 24% reduction of curcumin compared to control plants. There were more para-cymene and terpineol in volatile oils of turmeric rhizome than the other components, most of the volatile oil compounds were unchanged or varied slightly as salinity changed.

Keywords: *Curcuma longa* L.; Dry weight; salinity stress; Curcumin; Essential oil

INTRODUCTION

Turmeric (*Curcuma longa* L.) is a rhizome-bearing perennial plant from the Zingiberaceae family which is native to Southern Asia and is cultivated in a wide range of tropical areas such as India (1). Nearly 94% of turmeric in the world is produced in India (2).The main active ingredients of turmeric are curcuminoids and essential oil which are synthesized in the leaves and stored in the rhizome (3). Curcumionoids, which form the yellow color of turmeric, consist of curcumin, methoxy curcumin and bismethoxy curcumin. The essential oil of turmeric consists of several sesquiterpenoids such as *ar*-turmerone, curlone, α-turmerone, β-turmerone and bisacumol. Other sesquiterpenoids are zingibrene, curcumenone, curcumenol, procurcumenol and dehydrocurdione and germacrone-13-al. The analysis of curcuminoids and volatile oil in turmeric are considered as a significant character in order to evaluate the quality of plant materials (4,5). However, it was reported that curcuminoid and essential oil components of turmeric are varied at different stages of growth (6).

Traditionally, turmeric has been used as a kind of spice, medicine, cosmetics, colorific material and flavoring in food industries (1). Curcuminoids and essential oil in turmeric are used as anti-inflammation, anti-poison, anti-tumor, anti-fungi, HIV1 virus preventive agents and are useful in Alzheimer, urinal diseases abatement and diabetes treatment (7).

Stress is an environmental factor which limits plant growth and consequently decreases plant

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biomass. The biomass reduction is mainly due to deviation of metabolic pathway toward synthesis of defense metabolites which mostly called secondary metabolites. Salinity in soil or irrigation water is the main cause of salinity stress in rhizosphere especially in arid and semi-arid region which severely decreases the plant production. The production of different active oxygen species (ROS) in the form of free radical could be considered as a result of osmosis stress (8). Consequently secondary metabolites such as phenolic compounds, terpenoids, tocopherol, ascorbate and glutathione are the main components of the cellular antioxidants network that can be activated as a consequence of osmosis stress for reduction of damages to the cell via scavenging action (9). Curcuminoids and essential oil in turmeric as secondary metabolites may be affected under saline condition due to the salinity stress or the process of ROS removal.

Due to the fact that turmeric is not planted in Iran and therefore there is not enough information related to cultivation of turmeric as well as the possibility of planting this plant in wide spread saline soil of Iran, this study was conducted to evaluate the possibility of planting turmeric in Iran, the effect of salt stress on the growth and chlorophyll synthesis of plant leaves and on the amount of secondary metabolism such as essential oil components.

MATERIALS AND METHODS

Plant growth and application of salinity

The rhizomes of turmeric were provided from India (Uttar Pradesh, Banaras Hindu University, Department of Pharmaceutics). Then uniform rhizomes (5 to 7 cm in length and 1 to 1.5 cm in diameter) were sterilized using Benomyle fungicide and planted in sterilized pots containing coco peat and perlite. The pots kept under control condition (temperature 30 ± 5°C, relative humidity 60 ± 5% and light intensity 1200-1400 lux) and irrigated every three days. Two weeks after germination, identical and uniform germinated rhizomes were transferred to bigger pots containing same media. The pots were watered daily by Hoagland's nutrient solution (10). One month after germination, when plants had two leaves, sodium chloride (0, 20, 60 and 100 mM NaCl) was applied through irrigation along with Hoagland's solution. A completely randomized design with three replications were used as experimental lay out. Plants were harvested after one month of sodium chloride supplement in nutrient solution except for the rhizome samples which were harvested twice (one and two months after sodium chloride applied). Plant samples were splitted into young and old leaf, pseudo-stem, root and rhizome and their fresh weights (FW) were measured. Then each plant part was divided into two subsamples for further analysis. One subsample was oven dried for dry weight (DW) calcutation and the other was frozen for further analysis.

Chlorophyll assay

The rate of chlorophyll a, b and the total chlorophyll (mg/gFW) was calculated according to Arnon method (11). 0.2 g fresh tissue of young leaves of curcuma were rubbed in 10 mL of 80% acetone, mixed well and kept at 4°C overnight in dark. Supernatant was then removed after centrifugation (1957 g) and filtered before transfering into a new tube. Finally the absorbance was recorded at 663 and 645 nm using spectrophotometer (Pharmacia LKB-Nova spec). The amount of chlorophyll was calculated according to Arnon's equations (12).

$$\text{Chlorophyll a} = 12.7\ (D_{663}) - 2.69\ (D_{645})\ (V/1000\ FW)$$

$$\text{Chlorophyll b} = 22.9\ (D_{645}) - 4.93\ (D_{663})\ (V/1000\ FW)$$

$$\text{Total chlorophyll} = 20.2\ (D_{645}) + 8.12\ (D_{663})\ (V/1000\ FW)$$

where D, V and FW are absorbance, final volume (ml) and fresh weight (g) of leaf samples, respectively

Preparation and GC analysis of essential oil

Essential oil was collected from 50 g of rhizome's powder which was prepared from each unit experiment using hydro-distillation method (13). The essential oil was analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectroscopy (GC-

MS). GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with FID detector and a BP-1 capillary column (30 m × 0.25 mm; film thickness 0.25 µm). Nitrogen was used as the carrier gas with a flow rate of 2 ml /min and oven temperature was initially set at 60°C and then increased at a rate of 4°C/min until reached to the temperature of 150°C, then increased to 280°C at a rate of 15°C/min. Injector and detector temperatures were set at 280°C. The quantity of constituent was obtained base on GC analysis and component identified was based on GC mass analysis. The quantities assessment of essential oil composition was carried out based on relative surface area under the peaks.

GC-MS analysis of essential oil

The mass spectra were recorded using a Hewlett Packard (HP) 6890 MS detector with EI source, coupled with Hewlett Packard 6890 gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The gas chromatographic conditions were set as described above for GC analysis. The ionization potential was70 eV, and source temperature was 200°C in mass spectrometer.. Identification was based on retention data and computer matching with the WILEY275.L library as well as by comparison of electron-impact-mass spectra with those of relevant reference materials and literature (14).

Spectrophotometric analysis of curcumin

A known amount of rhizome's powder (0.1 g) prepared from collected samples, was quantitatively extracted with sufficient amount of redistilled methanol. The intensity of their absorbance was measured using a spectro-photometer (Secomam 1000, France). The methanolic extract of sample was quantitatively diluted in order to use linear part of the calibration curve and the absorbance of each sample was then measured at 530 nm against the acetic acid as blank. Curcuminoids content of each sample was then calculated using the published pharmacopoeia equation (% Curcumin = 0.426 (E/b); when E and b are absorbance and sample weight respectively) and their total contents were reported as curcumin (15).

Statistical analysis

SPSS16 software was used to prepare analysis of variances data and Duncan's multiple test range was used to compare the mean values.

RESULTS

Change in dry weight

Dry weight of different plant parts decreased as salinity was increased from zero to 100 mM NaCl (Fig. 1). The reductions were different in different plant parts as well as in different levels of salinity. Comparing to control plants, the dry weight reductions in 100 mM NaCl were 191%, 141%, 56%, 30% in leaf, pseudo-stem, root and rhizome, respectively. This is almost equal to 6.9, 2.87, 0.34 and 0.23 mg/plant dry weight reduction per one mM of NaCl for leaf, pseudo-stem, root and rhizome, respectively.

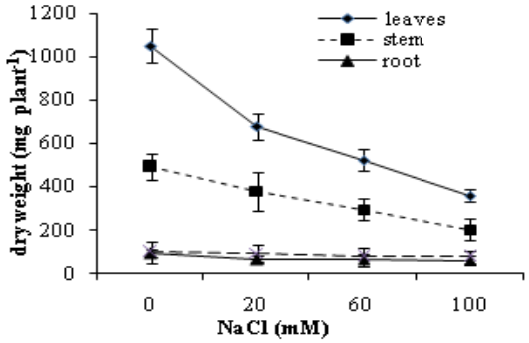

Fig. 1. Effect of sodium chloride (mM) on plant parts dry weight (mg/plant) of turmeric. Error bars on mean values are ± SD.

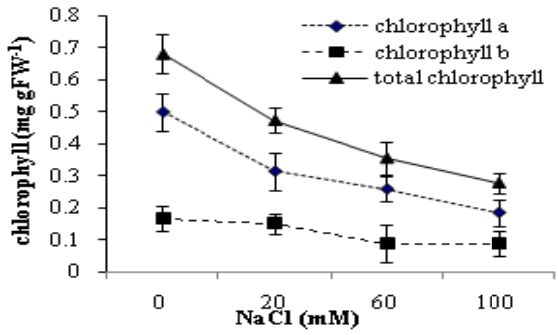

Fig. 2. Effect of sodium chloride (mM) on chlorophyll (mg/gFW) of turmeric leaves. Error bars on mean values are ± SD.

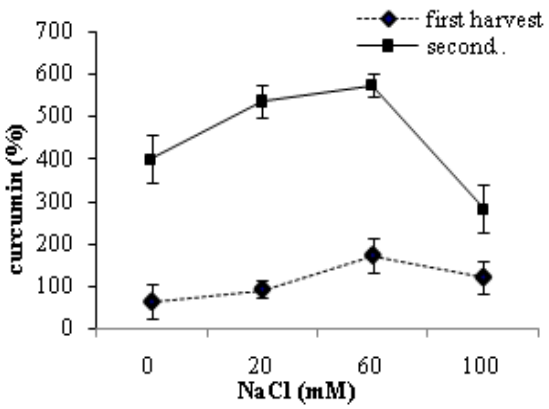

Fig. 3. The effect of sodium chloride (mM) on curcumin (%, mg per gDW) of rhizome. Error bars on mean values are ± SD.

The reduction of dry weight of leaf and pseudo-stem in saline condition were higher than that of root and rhizome. 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).

Change in chlorophyll

Changing salinity from zero to 100 mM NaCl caused reduction of chlorophyll a, b and total chlorophyll. However the reduction was higher for chlorophyll a than b (Fig. 2). In comparison to control plant, the reduction of chlorophyll a, b and total were 171%, 90% and 145%, respectively. The reductions are almost 3.32 and 0.79 µg/gFW for chlorophyll a and b respectively due to one mM addition of NaCl (P<0.05). This shows higher sensitivity of chlorophyll a to salinity in comparison to chlorophyll b.

Change in curcumin and essential oil

The amount of curcumin of rhizome (%, mg/gDW) at different harvests (three and four months old plants) showed that plant age (time of harvest) significantly affects the amount of curcumin. As plant gets older the accumulation of curcumin is more in rhizome (Fig. 3). For this case the addition of curcumin in unit dry weight of rhizome were almost 5 fold for control plants due to one month delay in harvest.

The addition of curcumin was almost 2 fold for 100 mM NaCl due to one month delay in harvest. The effect of salinity on curcumin content was linear in the first harvest but non liner in the second harvest. In the second harvest, its amount increased with increasing salinity up to 60 mM, and then started to decrease.

The addition of curcumin at 60 mM NaCl was almost 43% more compared to control plants and then its amount was decreased to 24% lower than control plant when 100 mM NaCl applied. This means that low salinity has positive effect on curcumin production but higher salinity (more than 60 mM) causes 24% reduction in its amount.

Although the amount of curcumin was lower than that of control in 100 mM NaCl by 24% in the second harvest, this value is still higher by 133% compared to the first harvest. This means that the time of harvest in this case is more crucial than salinity in curcumin production.

Composition of volatile oil of turmeric

Due to the significant effect of harvesting time on curcumin content in rhizome, the GC-MS analysis of the volatile oil of the second harvest (four months old turmeric plant rhizome) are presented in Fig. 4 and will be discussed. The GC-MS analysis of the volatile oil showed the presence of 7 peaks which were identified as α- phellanderene, α- terpinen, para-cymene, 1,8- cineole, terpineol, zingeberene and α-turmerone.

The composition of volatile oil of turmeric rhizome of four-months old plants after two months of salinity imposed is illustrated in Table 1. Although there were more para-cymene and terpineol in volatile oil of turmeric rhizome than the other components, most of the volatile oil compounds were unchanged or varied slightly as salinity changed.

As salinity increased to 60 mM NaCl, α-phellanderene, zingeberine and α- terpinen were relatively increased slightly however other components were decreased. The prediction of volatile oil components at higher salinity than 100 mM is not clear; however at 100 mM sodium chloride, para-cymene, terpineol and 1, 8-cineole contributed more than 80% of volatile oil components.

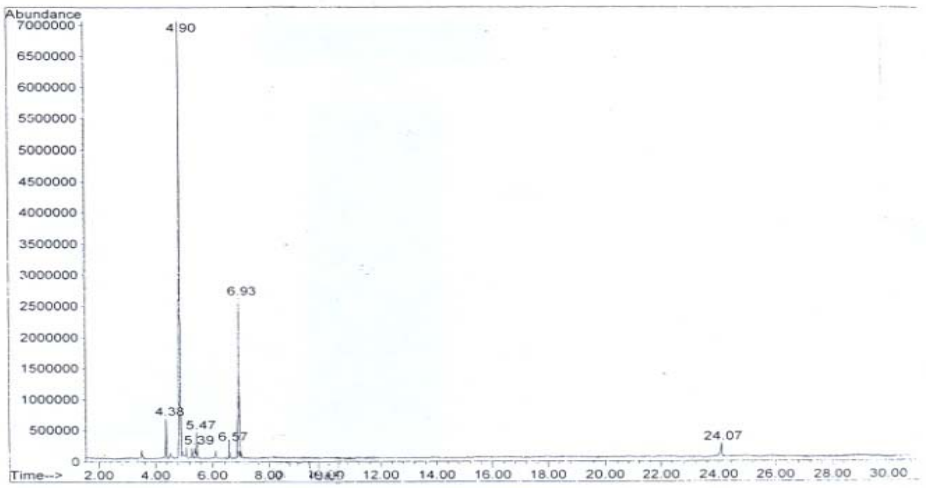

Fig. 4. The Gas chromatogram of volatile oil of turmeric leaves.

Table 1. The variation of volatile oil composition (%; mg/gDW) in turmeric rhizome due to salinity (mM).

Volatile oils	KI	Salinity (mM)				Average (%)
		0	20	60	100	
α- phellanderene	1005	2.8	2.3	6.0	2.0	3.27 ± 1.84*
zingeberine	1495	0.9	4.7	4.1	5.0	3.67 ± 1.88
α- terpinen	1018	2.1	2.9	8.0	7.6	5.15 ± 3.08
para-cymene	1026	44.1	40.3	38.0	40.3	40.67 ± 2.52
1,8- cineole	1062	13.1	12.2	12.8	10.9	12.25 ± 0.97
terpineol	1088	31.8	31.8	29.0	32.4	31.25 ± 1.52
α-turmerone	1664	5.6	6.5	2.5	2.4	4.25 ± 2011

*Average values ± SD

DISCUSSION

Change in dry weight

Usually plants have less access to water under salinity stress and consequently resulted in reduction of growth rate. Due to excessive ion transfer into plants, in high-salinity condition, osmotic equilibrium fails and plant growth is limited. Ions accumulation in vacuoles and cytosol of cell causes reduction of water potential which can lead to reduction of plant growth (16). In addition, when plant exposed to higher salinity, metabolic energy is consumed in the process of intracellular osmotic regulation for plant survival. Energy consumption for the regulation of osmotic stress can reduce availability of energy needed for growth (17). The synthesis of organic molecules that play an osmotic role can also be an additional metabolic duty for plant. Hence, when carbohydrates are used in the osmotic regulation, they cannot contribute in biomass production (18,19). Therefore reduction of 174% in total plant dry weight of turmeric in this experiment due to 100 mM NaCl is a mean for achieving survival.

The sensitivity of different plant parts to salinity condition could be another face of plant behavior. Cramer and Bowman (20) found that maize roots in comparison with its branches are less sensitive to salinity. In some salinity levels which hindered the growth of the branches, no effect on root growth was seen. Salinity may be the cause of morphological and anatomical changes in the root of some species (21). For instance, roots of plants such as *Atriplex* and *Suaeda monica* can even reach the soil up to 5 meters deep under salinity condition (22). In this study, turmeric plant showed that the leaves are more sensitive to salinity compared to the root and the sensitivity can be arranged as leaf, pseudo-stem, root and rhizome, respectively. However difference in dry weight of rhizome and root is

not statistically significant however leaf and pseudo-stem affected significantly by salinity stress. The reduction of dry weight of leaves more than that of the roots indicated the higher sensitivity of this organ compared to others which it may be has happened due to more accumulation of NaCl transferring into leaves in saline condition.

Change in chlorophyll

Our result indicated that total chlorophyll reduced by 145%. The reduction appeared more in chlorophyll a than in b. According to several studies, change (increasing or decreasing) in leaf chlorophyll due to the salinity stress have been reported in different plant species (23). Based on many researches, salinity decreases chlorophyll content of plants. Koocheki and coworkers (24) reported that increasing salinity reduced chlorophyll content of thyme (*Zataria multiflora*), kakooti (*Ziziphora clinopodioides*), garden thyme (*Thymus vulgaris*), and cat thyme (*Teucrium polium*) plants. While other studies indicated that salinity increases chlorophyll content (25). Nimbalkar and Bhivare (26) observed that in the leaves of *Phaseolus* beans under salinity stress, the chlorophyll content of the leaves decreased. This reduction occurred as the result of decline in the amount of magnesium of leaves which appeared under salinity stress. Chlorophyll reduction can also occur as a result of increase in degradation as well as decrease in the synthesis of chlorophyll (27). It has been reported that salinity stress can reduces number of chloroplast and also affect thylacoids and plastids membrane via their decomposition. Chloroplasts decomposition may also increase the chlorophylase enzyme activity and decrease the amount of chlorophyll (28). According to Soad and coworkers (29) the reduction in chlorophyll a and b associated with low photosynthetic activity and transpiration rate in *Catharanthus roseus* plants subjected to salinity were considered as a defense mechanism against damaging reactive oxygen species by diminishing light absorbing capacity that reduces the flow of electrons through the photosystems. The reduction of total chlorophyll (145%), which is contributed to almost 3.32 and 0.79 µg/gFW reduction of chlorophyll a and b respectively due to addition of one mM NaCl causes disruption of photosynthesis and alters metabolic pathways. These processes cause more production of different metabolites and less biomass in plant (30).

Changes of the curcumin content and essential oil composition

Production of different type of active oxygen species in the form of free radicals has a special importance among the adverse effects of osmotic stress to plant activities.(31). Some parts of sub-cellular organelles such as mitochondria and peroxisomes are regarded as potential generators of ROS, since enzyme activities and the reactions concerning the electron transport chain occurs or related to these organelles (32). Thus, osmotic stress causes a significant increase in the oxidative potential which significantly resulted degradation of proteins, especially proteins of the mitochondria and peroxisomes (33). Negative effects of stresses, may be partly due to oxidative damages resulted from lack of balance and coordination between the amount of active oxygen species and the antioxidant defense mechanisms (31). In these cases, synthesis processes divert to secondary metabolites as protection mechanisms for plant survival.

Secondary metabolites such as phenolic compounds (e.g. curcumin), terpenoids (such as volatile oil found in turmeric), tocopherol, ascorbate and glutathione are considered as the key components of cellular antioxidant network, whose changes occure as a result of environmental stresses. The antioxidant effects of these metabolites are in a way that disables free radical physically through resonance energy transfer and removes them chemically from the cell environment (34).

The changes in these metabolites in response to environmental stresses are revealed in two phases: the first phase mostly happens in moderate stress is the increase in the synthesis of secondary metabolites which leads to plant protection through a decrease in reactive oxygen species and the prevention of fat peroxidation. The second phase occurs in

the severe stress and in the plants sensitive to stress, in which degradation of secondary metabolites occurs more than its synthesis (35). Therefore, it can be concluded that the curcumin of rhizomes and the volatile oil composition such as para-cymene and α-terpinen of turmeric's leaves relatively increased as salinity increased up to 60 mM and then decreased as salinity increased to 100 mM. Findings of other reports appear to be similar with our results concerning secondary metabolites in plants. For instance it was indicated that the amount of trigonelline increased significantly in *Glycine max* due to salinity stress (36). Another example is *Hordeum vulgare* where salinity stress increased flavonoid content significantly (37). Likewise in *Grevillea spp*, the anthocyanins content increased significantly due to salt stress (38).

It is also shown that essential oil of coriander leaves were stimulated only under low and moderate stress, while it decreased at high salinity levels. At low stress, (E)-2-decenal, (E)-2-dodecenal and dodecanal contents increased (39). In *Salvia officinalis* the main essential oil compound was viridiflorol in control plant and at 25 mM NaCl, 1,8-cineole became the predominant compound at 50 and 75 mM and manool prevailed at 100 mM (40). However, the major components of the essential oil of *Ocimum basilicum* var. Purpurascens were eugenol and linalool. In another experiment, soil salinity of 1500 and 4500 mg/kg of soil increased the content of linalool and decreased eugenol content (41).

There are reports of an increase in essential oil due to lowering water potential in vegetative growth of *Satureja hortensis* (42). The highest amount of carvacrol and the lowest amount of γ-terpinene were obtained by increasing the salinity levels. The stimulation of essential oil production under a moderate degree of salinity could be due to a higher oil gland density and an increase in the absolute number of glands produced prior to leaf emergence (43). Salt stress may also affect the essential oil accumulation indirectly through its effects on either net assimilation or the partitioning of assimilates among growth and differentiation processes (44). Also, the formation and accumulation of essential oil in plants was also attributable to the action of environmental factors. It might be claimed that the formation and accumulation of essential oil was directly dependent on perfect growth and development of the plant's producing oil (45). The decrease in oil production might be due to the decrease in plant anabolism.

The increase in oil content in some of the salt stressed plants might be attributed to the decline ofthe primary metabolites due to the effects of salinity, causing intermediary products to become available for secondary metabolites synthesis (46). Therefore, nonlinear addition of curcumin and essential oil in the rhizomes and leaves of turmuric in response to salinity stress show two phases of reaction. In the first phase as well as in balanced salinity of 20 and 60 mM, it seems that turmeric tries to reduce the effects of free radicals and in the case of high salinity (100 mM) enters the second phase and degradation of these metabolites might somewhat surpasses its synthesis.

CONCLUSION

Salinity stress has more adverse effect on leaf and pseudo-stem dry weight of turmeric plant as compared to root and rhizomes as well as chlorophyll of leaf specially chlorophyll a. Meanwhile even in 100 mM salinity, the root and rhizome failed to show a significant reduction on their dry weight. This might be attributed to the high amount of curcumin metabolites in rhizomes, or even due to an increase in their amounts under moderate salinity stress, which reduces the stress effect by its antioxidant properties. If total curcumin of plant rhizome considered as the total curcumin production then the salinity would show its effect differently. In our case the total curcumin obtained from one plant would be 0.06, 0.09, 0.13 and 0.09 mg/plant which are equal curcumin concentrations of 400, 546, 573, 283 (%, mg/gDW) for 0, 20, 60 and 100 mM Sodium chloride, respectively. This means, although low salinity condition is not a favor to growth of plant, it is an advantage to curcumin production.

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