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EFFECTS OF ELEVATED SALINITY OF THE GROWING MEDIUM ON RHIZOME ESSENTIAL OIL YIELD AND FATTY ACID COMPOSITION OF *Curcuma longa* L. (ZINGIBERACEAE) †

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Abstract. *This study was aimed at the examination of the effects of increased salinity of growing medium on the fatty acid composition and yield of rhizome essential oil of* Curcuma longa *L. The analyses (gas chromatography and gas chromatography/mass spectrometry) of the transesterified petroleum ether extract of rhizomes grown under non-saline conditions (0.0 mM NaCl; control) showed that the dominant fatty acids were oleic (48.6%), myristic (15.0%), linoleic (7.9%), palmitic (6.8%) and linolenic (2.4%). Increased growth medium salinity (25.0, 50.0 and 75.0 mM NaCl) provoked significant changes in the relative amounts of some acids. Increase of NaCl concentration caused an increase in the amount of linoleic and linolenic acids, but had the opposite or no effect on myristic and oleic acids quantity. The yield of the essential oil obtained from fresh control rhizome (non-saline conditions) was 1.7%. When* C. longa *was grown in a low-salinity medium (25.0 mM NaCl), the oil yield remained the same. However, under high salinity conditions the yield reached 2.9% (50.0 mM NaCl), i.e. 3.1% (75.0 mM NaCl). Our results clearly show that the studied plant species is susceptible to high salinity induced stress.*

Key words: Curcuma longa *L. (Zingiberaceae), salinity, rhizome, fatty acids, essential oil*

1. INTRODUCTION

Increased soil salinity–usually the outcome of the imbalance between evaporation and precipitation–is one of the most important environmental stresses which affects both yield and quality of the crops in arid and semi-arid regions of the world [1,2]. Salinity is known to affect both primary (e.g. lipids) and secondary metabolism (e.g. essential oil components) of different plant species: mustard (*Brassica junicea* L.), sunflower (*Helianthus annuus* L.), coriander (*Coriandrum sativum* L.) *etc.* [3-7].

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Turmeric, *Curcuma longa* L. (Zingiberaceae), is an important crop native to tropical South Asia, used as a spice and in the traditional medicine [8-10]. However, to the best of our knowledge, influence of the high salinity on the chemical composition of the fatty acid and volatile fractions (essential oil) of the rhizome of this plant species have not been studied previously. As qualitative/quantitative changes in fatty acid or volatile profiles of this species might influence its nutritive/medicinal properties, we have decided to study the effects of increased salinity (different concentrations of NaCl) of the growing medium on the fatty acid composition and yield of rhizome essential oil of *C. longa*.

2. Materials and Methods

2.1. Plant material

Curcuma longa rhizomes were collected from cultivated plant population in the southern region of India in April 2009. The rhizomes were planted in mid June 2009 under uniform agronomic management. After 10 days, rhizomes were transferred in quarter-strength Hoagland's solution to which 0.0 (control), 25.0, 50.0 or 75.0 mM of NaCl were added. The nutritive solutions were continuously aerated and were replaced every 4 days. The experiment was performed in greenhouse under control conditions (18-25 °C temperature; artificial light of 141 $\mu molm^{-2}s^{-1}$ and 6,000 lux). Plants were harvested in the end of January 2010.

2.2. Essential oil isolation

Fresh rhizomes (four different samples (each in triplicate) grown under different salinity conditions; 50 g each) were subjected to hydrodistillation during 90 min. Hydrodistillation conditions (duration) were chosen via preliminary kinetic survey which included measuring of the changes in the volume of the isolated essential oil after 30, 60, 90 and 120 min [11]. The distillates were extracted with diethyl-ether and dried over anhydrous sodium sulfate. The solvent was then removed on rotary evaporator. The pure oils were weighed on an analytical balance and multiple gravimetric measurements were taken during 24 h to ensure that all of the solvent had evaporated. After that, the yields of the essential oils were calculated (w/w); these are given in Table 1.

2.3. Extraction and transesterification of the non-volatile oil

Dry rhizomes (100g) were cut into small peaces and placed into the Soxhlet apparatus. Extraction was performed according to the AOCS method [12]. Petroleum ether (b.p. 40-60 °C) was used as a solvent, while extraction time was set to 6 h. For every individual sample, extraction was preformed in triplicate.

Every of the obtained non-volatile oil samples (0.2 ml) were subjected to methanolysis using 0.1M sodium methoxide solution in hexane. In this way obtained mixture of the fatty acid methyl esters (FAME) were further analyzed by gas chromatography (GC/FID) and gas chromatography/mass spectrometry (GC/MS).

2.4. Fatty acid composition

Analyses (GC/FID and GC/MS) of FAME-samples were carried out using a Thermo Fisher TRACE GC ULTRA, with a fused silica TR 50MS capillary column (60m × 0.25 mm, film thickness 0.25μm). The oven temperature was programmed as follows: it was kept on 70 °C for 5 min and then first increased to 120 °C at a heating rate of 2 °C/min and after that to 240 °C at a heating rate 3 °C/min; finally it was held isothermally at 240 °C for 5 min. Injector temperature was 220 °C while temperature of detector was 280 °C. Helium was used as a carrier gas, with the constant flow of 1.0 $mlmin^{-1}$. The samples were injected in a split mode. The identification of the constituents was based on: i) the comparison of their linear retention indices (RI), determined relative to the t_R of *n*-alkanes on the same column, with those reported in the literature [13]; ii) the comparison of their mass spectra with those of the commercial MS libraries (NIST/Wiley); iii) the co-injection with an commercial standard mixture of FAME.

2.5. Statistical analysis

Results of the chemical analyses (Table 2) were expressed as the mean ± SD. Statistically significant differences were determined by one-way analysis of variance (ANOVA) followed by Duncan test for multiple comparisons (STATISTICA [14]). Probability values (p) less than 0.005 were considered to be statistically significant.

Table 1 Effect of the salinity of the growing medium on the yield of *C. longa* essential oil

Salinity (mM of NaCl)	Yield[a] (%, w/w)
0.0	1.7 ± 0.1
25.0	1.6 ± 0.1
50.0	2.9 ± 0.1
75.0	3.1 ± 0.1

[a] mean (three repetitions) ± SD

3. Results and Discussion

3.1. Essential oil yield

Experimentally determined yields of the essential oils are listed in Table 1. Based on the obtained data, one might assume that the production of volatile metabolites is significantly affected by salinity stress: rhizomes grown under high-salinity conditions (50.0 or 75.0 mM of NaCl) yielded much higher relative amounts of the oil comparing to control sample (Table 1). It was previously reported that elevated salinity level of the growing medium can influence (at least quantitatively) production of the volatiles [15,16]. As an example, *Salvia officinallis* L. (sage) [17] and *Mentha pulegium* L. (squaw mint) [18] might be used. However, it was also shown that increasing levels of osmotic stress may provoke significant (exponential) decrease in the net biomass [19]. Thus, increase of the relative yield of *C. longa* could not be used as a direct proof of the elevated production of volatile metabolites. Instead, it clearly shows that plant species was highly affected by salinity induced stress (e.g. pronounced changes in the ratio of the net biomass and amount of volatile metabolites).

3.1. Fatty acid profile of the analyzed samples

Transesterified petroleum ether extract of the *C. longa* rhizomes grown under non-saline (0 mM NaCl; control) and saline (25, 50 or 75 mM of NaCl) conditions were analyzed using GC and GC/MS. The results of the chemical analyses are summarized in Table 2. The dominant fatty acids were as follows: oleic (48.6%), myristic (15.0%), linoleic (7.9%), palmitic (6.8%) and linolenic (2.4%). Increased growing medium salinity (25, 50 and 75 mM NaCl) provoked different changes in the relative amounts of these acids. Increase of the NaCl concentration caused increase in the amount of linoleic and linolenic acids, but had the opposite or no effect on myristic and oleic acids quantity.

Table 2 Fatty acid composition (relative amount, %) of *C. longa* rhizome grown under different saline conditions

Fatty Acid	Relative amount (%)[a]			
Salinity (mM of NaCl)	0.0	25.0	50.0	75.0
Myristic acid (14:0)	15.02 ± 0.10	14.65 ± 0.30	13.8 ± 0.08	13.15 ± 0.52
Palmitic acid (16:0)	6.85 ± 0.39	8.55 ± 0.50	5.93 ± 0.55	5.66 ± 0.31
Palmitoleic acid (16:1)	8.51 ± 0.58	11.65 ± 0.40	13.26 ± 0.09	13.15 ± 0.12
Stearic acid (18:0)	3.35 ± 0.28	5.73 ± 0.39	2.95 ± 0.18	2.77 ± 0.25
Oleic acid (18:1)	48.65 ± 0.26	38.71 ± 0.35	37.25 ± 0.27	38.32 ± 0.32
Linoleic acid (18:2)	7.95 ± 0.54	8.18 ± 0.72	9.15 ± 0.63	9.85 ± 0.29
Linolenic acid (18:3)	2.39 ± 0.28	4.82 ± 0.32	7.95 ± 0.09	7.73 ± 0.27
Eicosanoic acid (20:0)	7.18 ± 0.11	6.33 ± 0.10	6.62 ± 0.23	7.75 ± 0.24
C18:1/C18:2	6.11 ± 0.09	4.73 ± 0.08	3.78 ± 0.12	4.02 ± 0.15
US/S[b]	2.08 ± 0.18	1.79 ± 0.20	2.30 ± 0.16	2.35 ± 0.22

[a] mean (three repetitions) ± SD; [b] US-Unsaturated fatty acids, S-Saturated fatty acids

In respect to the control, the ratio of oleic and linoleic acid was reduced with raising NaCl concentration (Table 2). This decrease might be related to the higher activity of Δ12-oleate desaturase, which is responsible for the desaturation of oleate to linoleate [20, 27]. In fact, the ratio of net unsaturated to saturated fatty acid relative amounts (US/S; Table 2) increased with increasing NaCl concentrations. An increase in the level of unsaturation of rhizome lipids seems to be a common feature of salt-stressed plants, which might not be surprising knowing that the degree of fatty acids unsaturation is an important factor for the maintaining the membrane functions [22]. For example, membranes containing a high proportion of unsaturated fatty acids are more fluid [23].

4. CONCLUSIONS

This research showed that high salinity of the growing medium significantly affects chemical composition of *C. longa* rhizomes (e.g. fatty acid profile and relative amount of volatile secondary metabolites). Thus, we confirmed once again that increased soil could significantly influence both yield and quality of the crops, esspecially in arid and semi-arid regions of the world [1,2].

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UTICAJ POVEĆANOG SALINITETA PODLOGE ZA GAJENJE NA PRINOS ETARSKOG ULJA I SASTAV MASNIH KISELINA BILJNE VRSTE *Curcuma longa* L. (ZINGIBERACEAE)

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U ovom radu su prikazani rezultati ispitivanja uticaja povećanog saliniteta podloge za gajenje na prinos etarskog ulja i sastav masnih kiselina rizoma biljne vrste Curcuma longa *L. Analiza (gasna hromatografija i gasna hromatografija sa masenom detekcijom) transesterifikovanog petrol-etarskog ekstrakta rizoma gajenog pod kontrolnim uslovima (0,0 mM NaCl u podlozi) pokazala je da su najzastupljenije masne kiseline bile oleinska (48,6%), miristinska (15,0%), linolna (7,9%), palmitinska (6,8%) i linoleinska (2,4%). Povećani salinitet podloge za gajenje (25,0, 50,0 i 75,0 mM NaCl) izazvao je različite promene u relativnim zastupljenostima pomenutih kiselina. Povećanje koncentracije natrijum-hlorida prouzrokovalo je povećanje relativnih količina linolne i linoleinske kiseline. Istovremeno, relativni udeo oleinske kiseline je opao, dok je sadržaj miristinske kiseline ostao nepromenjen. Prinos etarskog ulja (svež kontrolni uzorak rizoma) bio je 1,7%. Nisu uočene promene u prinosu ulja onda kada je* C. longa *gajena u uslovima niskog saliniteta (25mM). Međutim, pri višim koncentracijama natrijum-hlorida u podlozi prinos ulja je dostigao 2,9% (50,0 mM NaCl), odnosno 3,1% (75,0 mM NaCl). Dobijeni rezultati jasno ukazuju na to da je ova biljna vrsta osetljiva prema fiziološkom stresu prouzrokovanom povećanim salinitetom.*

Ključne reči: *Curcuma longa L. (Zingiberaceae), salinitet, rizom, masne kiseline, etarsko ulje*