VARIATION OF FLAVONOIDS AND TOTAL POLYPHENOLS CONTENTS IN TWO PARSLEY (*PETROSELINUM CRISPUM*) VARIETIES UNDER SALIN CONDITIONS

VARIAȚIA CONȚINUTULUI DE FLAVONOIDE ŞI POLIFENOLI LA DOUĂ VARIETĂȚI DE PĂTRUNJEL (*PETROSELINUM CRISPUM*) SUB INFLUENȚA STRESULUI SALIN

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Abstract. *The aim of this work was to investigate some of the biochemical responses in two parsley cultivars (cultivated for roots and leaves production) under salt stress. Thus, the total polyphenols and flavonoids contents (well known for acting as antioxidants), were determined in two parsley varieties, in order to study their possible role as biochemical markers for salt stress responses. Three saline treatments were applied to parsley plants, in parallel with a control, free of salt exposure plant series. The level of total phenols and flavonoids varies slightly among parsley varieties cultivated for roots production, under NaCl stress. While NaCl treatments stimulate the total phenols content in parsley variety cultivated for leaves, the flavonoids level was found to be lower, in the same variety.*
Keyords: *parsley, flavonoids, polyphenols, NaCl stress*

Rezumat. *Scopul acestei lucrări a fost investigarea unor răspunsuri biochimice la două soiuri de pătrunjel (cultivat pentru rădăcină şi frunze), sub influența stresului salin. Astfel, conținutul de polifenoli totali şi flavonoide (cunoscuți ca şi antioxidanți) s-a determinat în soiuri de pătrunjel pentru a studia posibilul rol ca markeri biochimici pentru răspunsul la stres. Au fost aplicate trei tratamente saline, plantelor pătrunjel iar în paralel, s-a realizat şi un martor, fără tratament. Nivelul de polifenoli şi flavonoide variază uşor între soiurile de pătrunjel cultivate pentru rădăcină, în condiții de stres salin. În timp ce tratamentul cu NaCl stimulează conținutul total de fenoli la varietatea de pătrunjel pentru frunze, nivelul de flavonoizi a fost mai mic, în cazul aceluiaşi soi.*
Cuvinte cheie: *pătrunjel, flavonoide, polifenoli totali, NaCl stres*

INTRODUCTION

Petroselinum crispum (Mill.) A. W. Hill is a member of *Apiaceae* family that is employed in the food, pharmaceutical and cosmetic industries (Lopez et al., 1999). Parsley has a very high content of vitamins (β-carotene, thiamin, riboflavin, ascorbic acid and tocopherol) and is a rich source of calcium,

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iron and folate; it contains also fatty acids and an essential or volatile oil (Parthasaranthy et al., 2008). Phytochemical screening of parsley *P. crispum* has revealed the presence of several classes of flavonoids (Fejes et al., 2000). The major flavonoids found in parsley and other apiaceous vegetables are flavonols (kaempferol and quercetin) and flavones (apigenin and luteolin), which occur as glycosidic form in nature (Peterson et al., 2006). The predominant mechanism of their biological actions is thought to result from antioxidant activity and the capacity to scavenge free radicals (Lin et al., 2002, Potapovich and Kostyuk, 2003). The antioxidant activity of plants mainly contribute by the active compounds present in them. It is believed that increase in secondary metabolites synthesis in response to stressful conditions protect the cellular structures against oxidation (Chanwitheesuk et al., 2005). In a study by Hinneburg et al., 2006, hydrodistilled extracts from basil, laurel, parsley, juniper, aniseed, fennel, cumin, cardamom and ginger were assessed for their total antioxidant activities by several *in vitro* methods. Although parsley showed the best performance in the iron chelation assay, it was less effective at retarding the oxidation of linoleic acid in the linolenic acid peroxidation assay.

Many researches have reported that phenolic constituents of plant provide protection against oxidation (Amarowicz et al., 2003; Pokorny, 2001). Flavonoids are reported as antioxidant agents by scavenging ROS, which are functioned by virtue of the number and arrangement of their hydroxyl groups attaches to ring structures. Their ability to act as antioxidants depends on the reduction potentials of their radicals and accessibility of the radicals (Rice-Evans, 2006, Heim et al., 2002). Polyphenol synthesis and accumulation is generally stimulated in response to biotic or abiotic stresses (Dixon and Paiva, 1995, Muthukumarasamy et al., 2000). Increase in polyphenol content in different tissues under increasing salinity has also been reported in a number of plants (Parida and Das, 2005, Navarro et al., 2006).

The main aim of the present study was investigation the effect of different concentration of NaCl salt on total polyphenols and flavonoids contents at two parsley varieties (cultivated for roots and leaves).

MATERIAL AND METHOD

Seedling growth conditions

The researches were conducted with two parsley seeds varieties (for roots and leaves). The seeds surface was sterilized with 3% H_2O_2 for three minutes then thoroughly rinsed with distilled water. During the course of experiment, the seeds were placed in plastic pots containing soil and watered every three days with NaCl solution. Thus, for each variety it was used three different concentrations of NaCl (50 mM, 100 mM and 150 mM NaCl). Distilled water, without NaCl addition, was used for control. The watering was carried out for 14 days, after which the plantlets of two parsley varieties were collected for biochemical determinations. At 150 mM NaCl concentration parsley seedling for leaves production did not survives.

Extraction of flavonoid and total polyphenolic content

For determination of total flavonoid and total polyphenolic contents it was used the same extract. Thus, the fresh seedling samples of two parsley varieties were extracted with the 80% methanol, at room temperature using an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000 g for 15 min.

Total flavonoid content

The total flavonoid content was measured following a spectrophotometric method (Dewanto et al., 2002). Briefly, methanol extract of each parsley cultivar were appropriately diluted with distilled water. Initially, 5% $NaN0_2$ solution was added to each test tube; at five minutes, 10% $AlCl_3$ solution was added and then at six minutes 1.0 M NaOH was added. Finally water was then added to the test tube and mixed well. Absorbance of resulting pink-coloured solution was read at 510 nm against the blank (distilled water). Flavonoid content was expressed as mg catechin equivalents (CE) per g of fresh weight parsley (mg CE/g FW) (R^2 =0,98). Three readings were taken for each sample and the result averaged.

Total polyphenolic content

The total polyphenolic content was determined by using a modified Folin-Ciocalteau method (Singelton *et al*., 1999). The appropriately diluted sample was added Follin-Ciocalteau reagent and mixed thoroughly. After four minutes, 15% Na_2CO_3 was added. The absorbance of resulting bleu-coloured solution was read at 760 nm after two hours, against the blank (distilled water). The amount of the total phenolic content was expressed as mg galic acid equivalents (GAE) per g of fresh weight parsley (mg GAE/g FW) (R^2 =0,99). Three readings were taken for each sample and the result averaged.

Statistical analysis

Statistical analysis of the results was carried out according to student test.

RESULTS AND DISCUSSIONS

Plant phenolics constitute one of the major groups of compounds that may function as antioxidants. Therefore, it was beneficial to determine the amount of phenolics and flavonoids in parsley extracts. From the results present, in figure 1 it was observed that NaCl salinity different influenced the total polyphenol content in plantlets of two parsley varieties. It is evident that total polyphenol content of parsley seedling variety for roots decreased comparatively with control at 50 mM and 150 mM levels of salinity, from 0,671 mg GAE/g FW to 0,577 and 0,774 mg GAE/g FW, respectively. At 100 mM NaCl salinity it was remarked the highest polyphenol content.

Seedling of parsley variety for leaves under the same salinity conditions indicated an increase of total polyphenol level at 50 mM and 100 mM NaCl. This increase of polyphenols content under increasing salinity level possibly shows that the induction of secondary metabolism is one of the defence mechanisms adopted by the plants to face saline environment. The maximum increase in total polyphenols was observed at 50 mM for parsley variety for leaves whereas at the second variety, for roots at 100 mM concentration.

Increase in polyphenol content in different plants tissues under increasing salinity has also been reported in a number of plants like red matured fruits of pepper (Navarro et al., 2006), some mulberry genotypes (Agastian et al., 2000) in mangrove *Aegiceras corniculatum* (Parida et al., 2004).

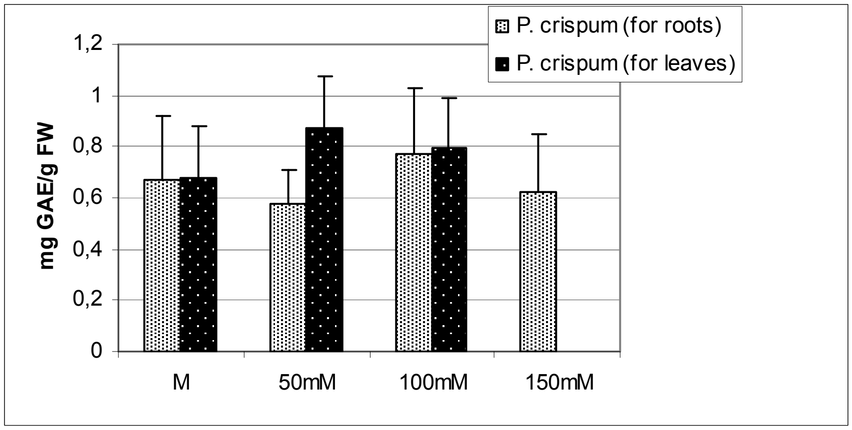

Fig. 1 - Salt stress effects on total polyphenol content in seedling of two *parsley varieties* (for roots and leaves) at 14 day of experiment

The ancient and widespread flavonol metabolism has been widely reported to be mostly involved in the response mechanisms of plants to a wide range of stressful conditions (Winkel-Shirley, 2002). In parsley variety for roots, the salinity concentrations had the similar trend on total flavonoid accumulation like phenolic compunds (Fig. 2).

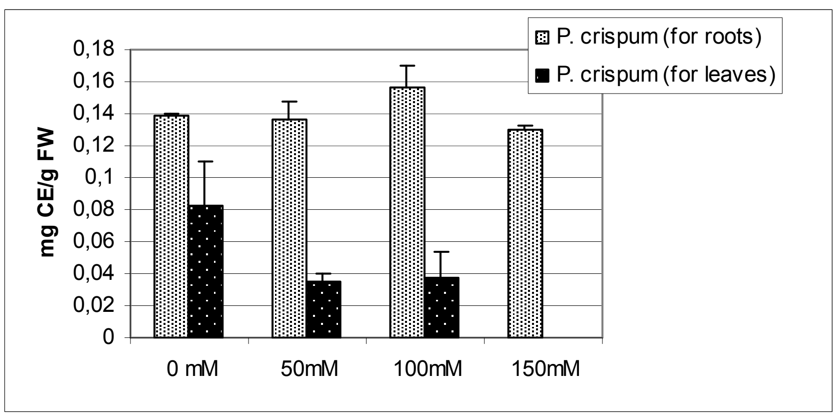

Fig. 2 - Salt stress effects on total flavonoids level in seedling of two *parsley varieties* (for roots and leaves) at 14 day of experiment

Total flavonoid contents in salt-stressed seedling of parsley variety for roots ranged between 0,130 mg - 0,156 mg CE/g FW at concentrations 100 mM and, 50 mM,, respectively. At both low NaCl concentrations, in seedling of parsley variety for leaves, the flavonoid level was actually similar (0,034 mg CE/g FW at 50mM and 0,036 mg CE/g FW at 100mM), being significant reduced comparatively with control (0,082 mg CE/g FW).

CONCLUSIONS

The results regarding nonenzymatic antioxidants responses, at 14 day after the experiment start, in both parsley varieties for roots and leaves, induced by salinity are complex. Thus, at seedling parsley variety for roots, polyphenols and flavonoids contents have had the same variation tendency as regards the salinity effect; only 100 mM concentration has had stimulating effect comparatively with control. The salin effect stimulated the polyphenols contents at seedling parsley variety for leaves but inhibited the flavonoids contents.

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