Botanica SERBICA 39 (2): (2015) 187-195

Original Scientific Paper

Chenopodium murale hairy root exudates inhibit growth and induce oxidative stress in collard greens *(Brassica oleracea* L. var. *acephala)*

Nevena Mitić, Mariana Stanišić, Jelena Savić, Snežana Zdravković-Korać, Slavica Ninković*

Institute for Biological Research "Siniša Stanković", University of Belgrade, Despot Stefan Blvd. 142, 11060 Belgrade, Serbia

ABSTRACT: Goosefoot (*Chenopodium murale* L.) is a cosmopolitan weed species whose root produces substances with allelopathic effects to various plant species, including agricultural ones. To investigate the allelopathic effect of *C. murale* on vegetable plant collard greens (*Brassica oleracea* L. var. *acephala*), the sterilized seeds of collard greens were treated with liquid nutrient medium in which *C. murale* hairy root clone R5 was grown for 4 weeks and which contained its exudates. Results indicated that final germination rate was not affected by R5 while the growth and development of collard greens seedlings were significantly inhibited. The roots were more affected than shoots. Comparing to the control, seedlings treated with R5 had reduced number of roots per seedling (27%), root length (33%) and root fresh weight (59%). Further, R5 led to the seedlings bending, loss of chlorophyll in the leaves, root necrosis and finally seedlings decay, indicating the ultimate lethal effect of *C. murale* allelochemicals. The seedling growth inhibition was accompanied with alteration in antioxidant enzymes activities illustrated by increased peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activity in shoots and emphasized CAT and decreased SOD activity in roots. The results could be contributed to the dissemination of knowledge about allelopathic influence of *C. murale* on cultivated plants and can be helpful in determining the mode of action against this weed species to protect collard greens plants.

Key words: Allelopathy, *Chenopodium murale*, transformed roots, collard greens, inhibition, germination/ seedling growth

Received: 30 March 2015 Revision accepted 07 July 2015

UDK: 581.144.2

INTRODUCTION

Allelopathy is a phenomenon involving either direct or indirect and either beneficial or adverse effects of a plant (and some microorganisms) on another plant through the release of chemicals in the environment (Blum 2011). Interest in allelopathic research has been growing up in the last decades allowing allelochemistry to attain leading role in an explanation of plant invasion and plant-plant communication in the rhizosphere (Weir *et al*. 2004). On the other side, allelochemicals, such as the secreted or volatile compounds, root exudates and chemicals released into the soil during tissue decay, have applicative potential for agroecosystems (Soltys *et al*. 2013). In agroecosystems allelophaty released chemicals could have detrimental effects on the growth of associated and next-season crops or beneficial, as in the case of natural weed control (Fay & Duke 1977; Soltys *et al*. 2013). In addition, weeds can exhibit allelopathy against crop plants (Qasem 1993, 1995; Nishida *et al*. 2005; Batish *et al*. 2007a, b).

Nettleleaf goosefoot (*Chenopodium murale* L.) is a fast-growing annual weed plant native to Europe, Asia and northern Africa (Holm *et al*. 1997), with strong

*correspondence: slavica@ibiss.bg.ac.rs

allelopathic properties. It provides a good example of plant exerting allelopathic effects on different, particularly cultivated, plant species (El-Khatib *et al.* 2004; Batish *et al.* 2007a, b) by releasing phenolics into the soil as putative allelochemicals that are produced just by the roots (Batish *et al.* 2007a, b).

Determining the mode of action of allelochemicals is one of the challenging aspects in allelopathic studies. However, the study of plant-plant interactions mediated by roots is aggravated by the complexity of soil (El-Khatib *et al.* 2004), thus interfering with allelopathic interactions. The use of *in vitro* systems can overcome limitations in studying the root exudates-mediated allelopathy by eliminating soil as a complex factor. *C. murale* hairy roots culture established by *Agrobacterium rhizogenes*-mediated transformation *in vitro* (Mitić *et al.* 2012), provides a suitable tool for further investigation of the potential and nature of root-mediated allelopathic interference of *C. murale* in the absence of interference by soil microflora. The inhibitory activity of *C. murale* hairy roots, released by exuded allelochemicals affecting the tested plant species such as wheat and lettuce (Mitić *et al.* 2012), *Arabidopsis thaliana* (Dmitrović *et al.* 2015a) or *Capsela bursa pastoris* (Dmitrović *et al.* 2015b), confirmed allelopathic feature of *in vitro* grown *C. murale* roots. Affected plant species demonstrated species-specific mode of sensitivity (Mitić *et al.* 2012, Dmitrović *et al.* 2015b) that further stimulated investigation of *C. murale* phytotoxicity to the new targets.

Plant survival under allelopathy stress conditions depends on plant defense leading to allelochemical detoxication, the process which may go on in parallel to cell defense reaction to oxidative stress (Gniazdowska & Bogatek 2005). As a consequence, an increased activity of the antioxidant enzymes upon treatment with allelochemicals has been reported in many cases (Yu *et al.* 2003; Weir *et al.* 2004; Ye *et al.* 2006; Ghareib *et al.* 2010). Phytotoxicity mode of *C. murale* root exudates has been also linked with alterations in antioxidant enzyme activities in affected target species (Dmitrović *et al.* 2015a,b)

Collard green (*Brassica oleracea* L. var. *acephala*), one of the most common green vegetables in Europe is endangered by different weed species including *C. murale*. The economic consequences of weed infestations in these plants are decreases in crop quality and yield. An integrated weed management program includes proper identification of weeds mechanisms. To elucidate some of the *C. murale* allelopathic mechanisms on collard greens, *in vitro*-grown seedlings were treated with *C. murale* hairy root phytotoxic media (R5) and the growth parameters, phenotype alterations and the changes in the activities of antioxidant enzymes-catalases (CAT), peroxidases (POD) and superoxide dismutases (SOD) were investigated.

MATERIALS AND METHODS

Plant material. Seeds of the *Brassica oleracea* L. var. *acephala* used in this experiment were collected during 2011/2012 from an open-pollinated plant population grown in Trebinje, Bosnia and Herzegovina, located 19 km away from the Adriatic Sea (275 m altitude, latitude 42° 42' 18''N, longitude 18° 19' 18''E).

The seeds were surface sterilized using 70% ethanol for 1 min followed by 20% commercial NaOCl bleach (4% active chlorine) with addition of a few drops of detergent (Fairy, Procter and Gamble), as well as fungicide Previcur® (Bayer CropScience, Monheim, Germany) and 500 mg l^{-1} antibiotic Ampicilin (Panfarma d.o.o, Belgrade, Serbia) for 2 h, rinsed 4 times with sterile distilled water and blotted dry on a piece of sterile filter paper.

***C. murale* root cultures.** The genetic transformation, regeneration of hairy roots and their growth potential were previously described (Mitić *et al.* 2012). Briefly, transgenic hairy root clones were induced by *A. rhizogenes* A4M70GUS from roots, cotyledons, leaves, and internodes of *C. murale* seedlings. Transformed roots were detached from the explants and grown further in 100 mL Erlenmeyer flasks containing 50 mL of liquid MS (Murashige & Skoog 1962) medium and cultured on a rotary shaker (70 rpm) for four weeks. Cultures were subcultured on fresh MS medium in 4-week intervals. Root-derived hairy root clone R5 was chosen for bioassay because growth medium of R5 displayed significantly higher total phenolics content - TPC (about 0.250 mg gallic acid equivalents (GAE) mL^{-1}) than growth medium from all other hairy root clones (< 0.180 mg GAE mL^{-1}, Dmitrović *et al.* 2015b).

Bioassay with *Brasica oleracea* L. var. *acephala*. The potential phytotoxicity of the hairy root liquid MS medium wherein 25 mg initial weight of hairy root clone R5 of *C. murale* was grown for four weeks (designated as R5 medium) was evaluated against seeds of *Brassica oleracea* L. var. *acephala*.

Twenty seeds were placed in a Petri dish (Ø 90 mm), on the sterile filter paper wetted with 5 mL of either R5 or fresh liquid MS medium without root exudates used as a control. Five Petri dishes were used for each treatment. The bioassay was repeated three times. Petri dishes were sealed with parafilm (Bemis Flexible Packaging, Neenah, WI, USA) and incubated in a growth chamber under standard cool-white fluorescent tubes (16 h light/ 8 h dark cycle) with a photon flux density of 70 $\mu mol^{-2}s^{-1}$, at 25 ± 2°C.

The allelophatic activity of R5 was evaluated by germination percentage after 3 and 14 days, by measuring the shoot and the longest root length, shoots and roots fresh weight, by determination of number of roots per seedling after 14 days and additionally by measuring

seedling fresh weight after 18 days. The percentage of inhibition compared to the control was calculated using the following formula:

% inhibition = (1 − R5/MS) × 100,

where R5 is the parameter of R5 treated variants and MS is the parameter of control variants. In the case the positive value was calculated it indicates stimulation by R5 treatment.

Protein extraction and determination of antioxidant enzyme activities

Total proteins isolation. Total proteins were extracted from the shoots and roots of collard green seedlings grown for two weeks on R5 or MS media. For each treatment 200 mg of plant tissue were ground to fine powder in liquid nitrogen with addition of ice-cold extraction buffer in 2:1 (v:w) proportion. The extraction buffer consisted of 50 mM Tris-HCl pH 7.6, 10 mM ethylenediaminetetraacetic acid (EDTA) pH 8.0, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10% (w/v) glycerol and 5% (w/v) insoluble polyvinylpolypyrrolidone (PVP). The homogenates were cleared by centrifugation at 12 000 x *g* for 10 min at 4°C. Total soluble protein contents in crude extracts were determined by the method of Bradford (1976).

Spectrophotometric determination of POD, CAT and SOD activity. The activities of POD, CAT and SOD were determinate spectrophotometrically. Specific activity of each enzyme was determinated as the rates of respective product formed or substrate disappeared per mg of total soluble proteins per min (U mg^{-1}).

The reaction mixture for total POD activity measurements was consisted of 50 mM K-phosphate buffer pH 6.5, 10 mM pyrogallol and aliquots of 30 µL of crude protein extracts. The reaction was started by addition of 3.3 mM H_2O_2, and an increase in absorbance at 430 nm caused by production of purpurogallin was followed using Agilent 8453 spectrophotometer (Life Sciences, USA). Peroxidase activity was calculated using the extinction coefficient for formed purpurogallin (ε_{430} = 2.47 $mM^{-1}cm^{-1}$).

The activity of CAT was measured by reading absorbance decrease at 240 nm, as a consequence of H_2O_2 consumption (ε_{240} = 0.0436 $mM^{-1}cm^{-1}$), according to the method of Aebi (1984). The reaction mixture (1.5 mL) contained 50 mM Na-K-phosphate buffer pH 7.0 and 10 µL of enzyme extract. The reaction was initiated by the addition of 30 mM H_2O_2.

Total SOD activity determination was based on capacity of the enzyme to inhibit the reduction of light yellow nitro blue tetrazolium (NBT) and conversion to blue formazan (Beyer & Fridovich 1987). The reaction mixture contained 50 mM K-phosphate buffer pH 7.8, 0.1 mM EDTA, 12 mM L-methionine, 75 µM NBT, 2 µM riboflavin and 0-10 µL of crude protein extract. Reaction mixtures were exposed to illumination for 30 min at 25°C. Absorbance was recorded at 540 nm using an ELISA microplate reader (LKB Vertriebs GmbH, Austria).

Statistical analyses. The experiment was carried out three times using a completely randomized design with five replications per treatment. The mean values are presented in Figures with standard error (SE). The percentage data were subjected to angular transformation and the mean root number data to square root transformation before statistical analysis and inverse-transformed for presentation. The data were subjected to one-way analysis of variance (ANOVA). Differences between control and R5 treatments means were evaluated by the *t*-test for dependent samples at the confidence level of $P < 0.05$. All data were analyzed by SAS software (SAS Institute, 2002; SAS/STAT, ver. 9.00. SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Since growth medium of *C. murale* L. hairy root clone R5 obtained by *A. rhizogenes*-mediated transformation contained significantly higher total phenolic content (TPC) than medium of other hairy root clones (Dmitrović *et al.* 2015b), and exhibited prominent phytotoxic effect on previously tested species (Mitić *et al.* 2012; Dmitrović *et al.* 2015a,b), it was selected for use in allelopathic study with collard greens (*Brassica oleracea* L. var. *acephala).*

The allelopathic potential of R5 was tested on seed germination and seedlings growth of *B. oleracea* L. var. *acephala*. Results revealed differential allelopathic effects on this species depending on the tested parameter. Thus, the hairy root exudates exerted the strong inhibitory effect on seedlings growth, but at the same time final germination was not significantly affected (Fig. 1A). Moreover, germination was initially stimulated and after 3 days of the treatment number of germinated seeds on R5 medium was higher for 50% than on the control medium (Fig. 1A). This effect was annulled in time and no significant difference in final germination rate on both R5 and MS was observed after 14 days (Fig. 1A).

Beside collard greens, insensitivity of final germination rate to *C. murale* allelochemicals was also reported for *Arabidopsis thaliana* seeds (Dmitrović *et al.* 2015a), that is opposite to the findings of strong inhibitory effect of *C. murale* hairy root growth medium on the germination of wheat, lettuce, *Capsela bursa-pastoris* and *Vicia angustifolia* seeds (Mitić *et al.* 2012; Dmitrović *et al.* 2015b). Differential response of the various plant species to the same allelopathic source indicated selectivity in its allelopathic activity confirming necessity for testing each species in

particular (Amoo *et al.* 2008). Differential response in case of the germination could be also connected with seed size since the smaller seeds are generally more sensitive (Gonçalves *et al.* 2009), but that was not the case in collards seeds.

Stimulative effects allelochemicals displayed only in the first three days of collard greens seeds germination is not unusual phenomena and has also been reported for allelopathic effects of weed species *Euphorbia helioscopia*s on lentil seeds germination (Tanaveer *et al.* 2010). This type of seed response could be considered as an aspect of programmed output from the stress conditions or adaptive advantage that reduced the time of the seeds exposure to the allelochemicals and accelerated the life cycle to the reproductive stage.

Contrary to germination, which is more dependent on the seed reserves and less susceptible to exogenous factors (Tigre *et al.* 2012), seedlings growth was more sensitive to R5 exudates exerting significant decrease in almost all tested parameters (Fig.1B-F). Gained shoot length on R5 medium (0.74 cm) indicated suppression of 20% compared to control ones grown on MS medium (0.93 cm) (Fig. 1B). In spite of the significant shoot length decrement in the presence of R5 exudates, fresh weights of collards shoots were almost identical to that of the control (Fig. 1C) indicated additional thickening of R5 treated shoots by accumulation of liquid medium or possibly by tissue callusing.

Apart from shoot length decreasing, comparing to seedlings grown on control MS medium that were erected and healthy (Fig. 2A), R5 exudates also caused appearing of "U" shape bending of shoots with roots elevated from the medium (Fig. 2B).

An inhibitory effect of R5 medium was observed in collard greens roots, too. Mean number of roots per seedling was significantly decreased from 1.93 in MS control to 1.40 in R5 treated seedlings. Beside suppression of root formation (-27%) (Fig. 1D) R5 also inhibited root elongation (-33%) (Fig. 1E). The most sensitive parameter was root fresh weight with a decrement of 59% comparing to MS control (Fig. 1F). Most of the roots appeared on R5 medium were thin, fibrous, with large necrosis and as was mentioned before, they were elevated from the medium (Fig. 2B).

Prolonged cultivation on R5 medium (more than 2 weeks) induced further collard seedling bending, necrosis, fresh weight decrement (Fig. 3A), and finally total seedling drying (Fig. 3B)

This study clearly revealed the inhibitory allelophatic effect of R5 exudates of *C. murale* roots on collard greens seedling growth, especially demonstrated on the root parameters, confirming once again existence of root-root allelopathic interactions. Most allelopathic studies indicated root growth to be more sensitive compared to shoots (Haouala *et al.* 2008; Omezzine *et al.* 2011; Ladhari *et al.* 2013) since the roots are in the direct

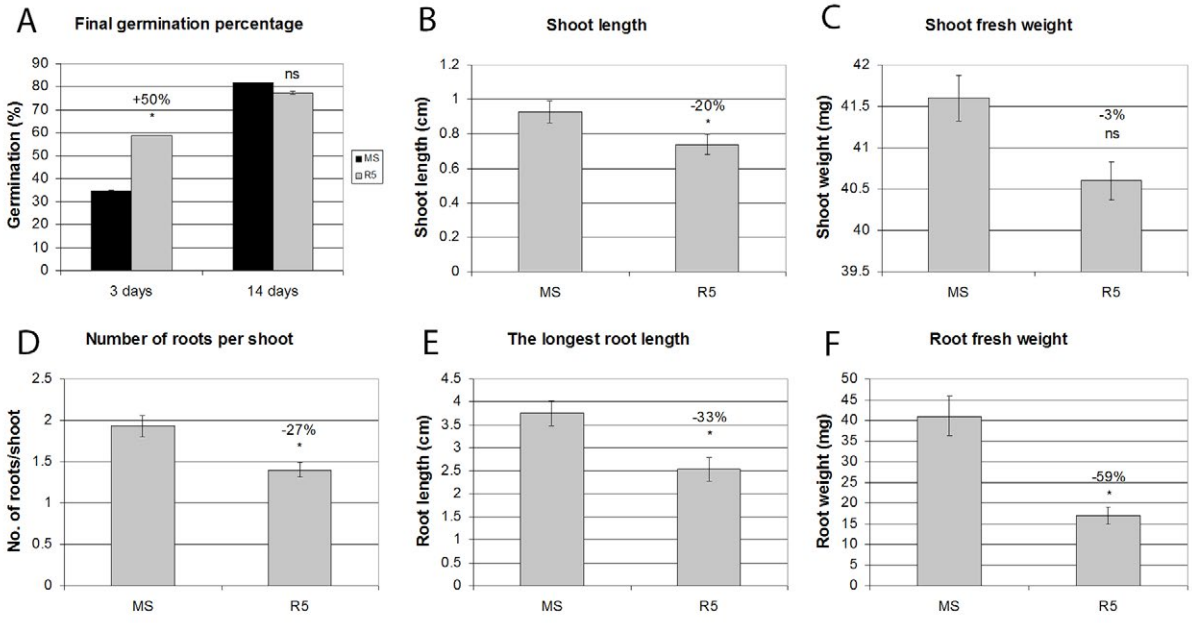

Fig. 1. The effect of 4-week-old growth liquid medium of *C. murale* hairy root clone R5 on collard greens seeds germination (A) and seedling growth (B-F). Final percentage of germinated seeds and seedling growth characteristics were determined 14 days after the treatment. Control treatment contained fresh MS instead of R5. The results were presented as mean values of three independent experiments with bars showing standard errors. Asterisks indicate statistically significant difference at $P < 0.05$ in comparison to the control based on *t*-test, while numbers above the bars indicate the percentage of inhibition (−)/stimulation (+) over control that was calculated as indicated in Materials and Methods. ns- statistically nonsignificant

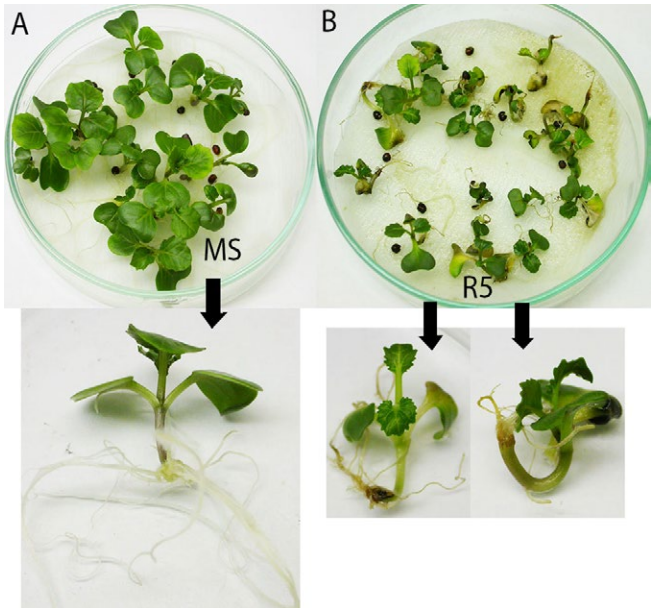

Fig. 2. Allelopathic effect of *C. murale* hairy root clone R5 growth medium on collard greens seedlings. Collard seeds were germinated on filter papers wetted with 5 mL of either MS (A) or R5 (B) for 14 days. R5 retarded development, induced necrosis and "U" shape bending of collard seedlings.

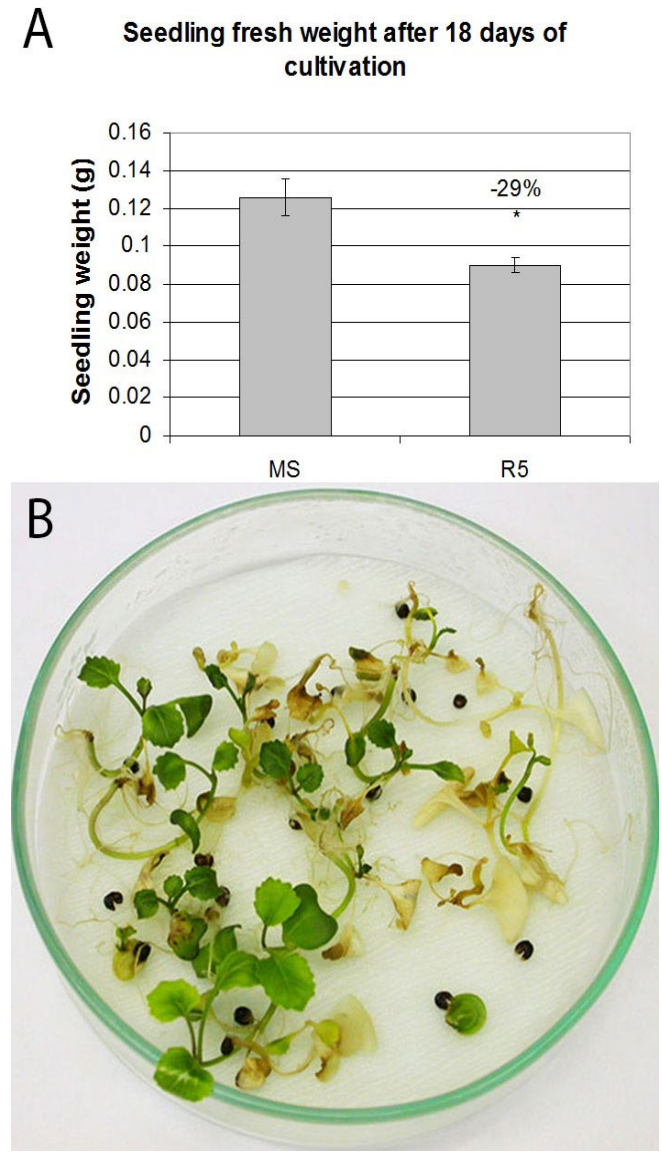

Fig. 3. The effect of *C. murale* hairy root clone R5 growth medium during prolonged cultivation (18 days) on collard greens seedling fresh weight (A), and phenotypical appearance (B). The results were presented as mean values of three independent experiments with bars showing standard errors. Asterisks indicate statistically significant difference at $P < 0.05$ in comparison to the control based on *t*-test, while numbers above the bars indicate the percentage of inhibition (–) of R5 treatment over MS control that was calculated as indicated in Materials and Methods.

contact with allelochemicals that causes the inhibition in the meristematic and elongation zone of root (Romero-Romero *et al.* 2005). These visible inhibitory effects can be accounted for by certain alterations at the cellular and molecular levels including hormonal homeostasis (Jones *et al.* 2010; Aquea *et al.* 2012). Several hormonal pathways have been shown to be involved in the regulation of the stress response, although the specific mechanisms of action are still largely unknown. The recent study of Dmitrović *et al.* (2015a) indicated that the mode of allelopathic action of *C. murale* hairy root exudates on wheat and *Arabidopsis* was based on down-regulation of the core cell cycle genes, accompanied by generation of oxidative stress in both shoots and roots of affected plants.

To determine whether *C. murale* hairy root medium provokes oxidative stress in *B. oleracea* var. *acephala* plants the activity of antioxidant enzymes POD, CAT and SOD was investigated in shoots and roots of *in vitro* grown collard seedlings exposed to R5 medium (Fig. 4A-C).

SOD is known to play a central role as the major scavenger of ROS in the process accompanied by formation of H_2O_2 that can be eliminated by CAT or POD. Catalases are tetrameric hem-containing enzymes that dismute H_2O_2 by reducing it to water and oxidizing it to molecular oxygen. With an exception of maize mitochondrial CAT3, plant catalases are primarily targeted to peroxisomes (Prasad 1997). Peroxidases (POD) represent large superfamily of hemcontaining enzymes, divided into three classes based on their primary structure, function, substrate specificities and reaction mechanisms (Cosio & Dunand 2009).

Spectrophotometric measurements revealed that only for CAT, total activities in shoot and root of control, untreated plants were at the similar levels (7.60 and 7.30 U mg^{-1} of total proteins in shoots and roots, respectively; Fig. 4B). Activities of POD and SOD were elevated in roots compared to shoots. Measured SOD activities were 122 U mg^{-1} of total proteins in shoots and 33 U mg^{-1} of

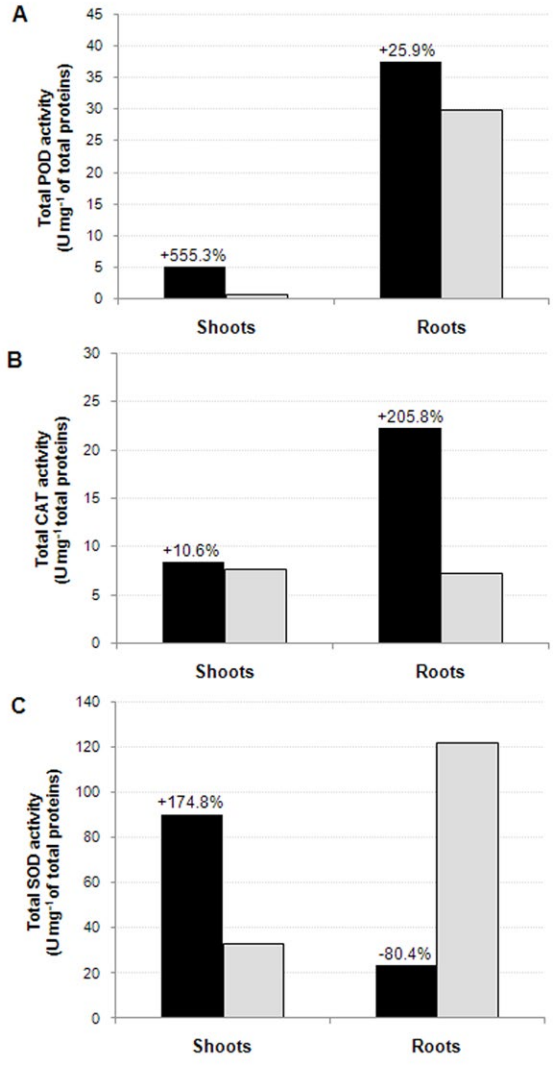

Fig. 4. Total activities of peroxidases - POD (A), catalases -CAT (B) and superoxide dismutases - SOD (C) in collard green seedlings treated with *C. murale* R5 hairy root growth medium (black bars). Control treatment (gray bars) contained MS instead of R5. Percentage of activity decrease (–)/increase (+) over control was calculated as indicated in Materials and Methods.

total proteins in roots (Fig. 4C). The greatest difference in antioxidant enzyme activity level between separated parts of plantlets was recorded for POD, with total activity of 0.8 and 30 U mg^{-1} of total proteins in shoots and roots, respectively (Fig. 4A).

However, the activities of all analyzed antioxidant enzymes were significantly affected by allelopathic compounds present in R5 liquid medium used for collards seedlings treatment *in vitro*. Total activity of POD, CAT and SOD increased in collards shoots exposed to allelotoxic media. The most intensive response was recorded for POD with more than 555% increment compared to the activity in untreated shoots (Fig. 4A), while SOD and CAT activity increased less, for 175% and 11%, respectively (Fig. 4B and C).

Enhancements of enzymes activity comparing to control is in accordance with the fact that antioxidant enzymes are involved in responses to biotic and abiotic stress (Cosio & Dunand 2009), including allelopathic interactions (Bais *et al.* 2003). Similar pattern of enzymes response was also detected in shoots of wheat seedlings treated with *C. murale* hairy root exudates (Dmitrović *et al.* 2015a) indicating that these two plants reacted to specific alleloinfluence by activating a potent defense response, with the main role of POD in tolerance and defense activity. It is well known that the plant response to unfavorable or damaging conditions is mainly related to SOD activity, while POD could hamper peroxidation and thus reduce the injury of cell membranes (Sunmonu & Van Staden 2014). Preserving the membrane integrity is one of the key aspects in the plant cell stress response (Gniazdowska & Bogatek 2005).

The most sensitive allelophaty-induced responses of collard roots were illustrated through a significantly elevated increase of CAT (206%) and POD (26%) activity (Fig. 4A and B), and a decrease of SOD activity (-80%) (Fig. 4C). Reduction of SOD activity under stressful conditions has been attributed to the inactivation of enzyme protein due to uncontrolled generation of reactive oxygen species. Decrease of SOD activity could be connected to the conspicuous growth retardation in the collards root tissue by interfering with absence of the potent defense response that overcame oxidative damage. Over threefold increment in the activity of CAT enzymes was obviously not sufficient to overcome induced alteration in the antioxidant machinery as a response to R5.

It is obvious that collards roots are more markedly affected by R5 than shoots. Higher sensitivity of roots could be explained by longer and most direct exposure to the stress factors leading to the increased ROS production. Organ-specific activity of antioxidative enzymes observed in collard greens seedlings was also reported in young maize organs (Chorianopoulou *et al.* 2012) as well as in cucumber seedlings (Du *et al.* 2010) under salt stress. Similar phenomenon has been observed in *V. angustifolia* shoots and roots where CAT and SOD activity was inconsistently and mutually inversely affected by allelochemicals of *C. murale* (Dmitrović *et al.* 2015b). However, fluctuations in the SOD but not in the CAT activities, with an organ specific manner, was observed in wheat seedlings treated with *C. murale* hairy root (R5) medium indicated once again existence of species-specific response to the same type of the allelochemicals.

CONCLUSION

Exhibited stunted growth and phenotype pattern, as well as an increment of antioxidant enzyme activities, in collard greens seedlings exposed to *C. murale* hairy

root growth medium indicated root-root allelopathic interference between *C. murale* and *B. oleracea* var. *acephala* plants. Results obtained in this study represent significant contribution to the pool of knowledge of *C. murale* phytotoxicity mechanisms in different target plant species, especially in cultivated ones. From the practical standpoint, these results may provide an insight for herbicide using studies in collards production for successful protection of these plants.

Acknowledgments - This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia-Project ON173015.

REFERENCES

Aebi H. 1984. Catalase in vitro. *Methods Enzymol.* **105**: 121 – 126.

Amoo SO, Ojo AU & Van Staden J. 2008. Allelopatic potential of *Tetrapleura tetraptera* leaf extracts on early seedling growth of five agricultural crops. *S Afr J Bot.* **74**: 149 – 152.

Aquea F, Federici F, Moscoso C, Vega A, Jullian P, Haseloff J & Arce-Johnson P. 2012. A molecular framework for the inhibition of *Arabidopsis* root growth in response to boron toxicity. *Plant Cell Environ.* **35**: 719 - 734.

Bais HP, Vepachedu R, Gilroy S, Callaway RM & Vivanco JM. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science.* **301**: 1377 – 1380.

Batish DR, Lavanya K, Pal Singh H & Kohli RK. 2007a. Root–mediated allelopathic interference of nettleleaved goosefoot (*Chenopodium murale*) on wheat (*Triticum aestivum*). *J Agr Crop Sci.* **193**: 37 – 44.

Batish DR, Lavanya K, Pal Singh H & Kohli RK. 2007b. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. *Plant Growth Regul.* **51**: 119 – 28.

Beyer WF Jr & Fridovich I. 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal Biochem.* **161**: 559 – 566.

Blum U. 2011. Plant–Plant Allelopathic Interactions: Phenolic Acids, Crop Cover and Weed Emergence. Springer, London (Dordrecht, Heidelberg).

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* **72**: 248 – 254.

Chorianopoulou SN, Giamouroglou M & Bouranis DL. 2012. Differential early fluctuations in superoxide dismutase and catalase activities are included in the responses of young maize organs to S-deprivation. *Am J Plant Sci.* **3**: 338 – 345.

Cosio C & Dunand C. 2009. Specific functions of individual class III peroxidase genes. *J Exp Bot* **60**: 391–408.

Dmitrović S, Simonović A, Mitić N, Savić J, Cingel A, Filipović B & Ninković S. 2015a. Hairy root exudates of allelopathic weed *Chenopodium murale* L. induce oxidative stress and down-regulate core cell cycle genes in *Arabidopsis* and wheat seedlings. *Plant Growth Regul.* **75**: 365 – 382.

Dmitrović S, Mitić N, Budimir S, Janošević D, Živković S, Skorić M & Ninković S. 2015b. Morpho-histological and bioherbicidal evaluation of wild-type and transformed hairy roots of goosefoot. *S Afr J Bot.* **96**: 53 – 61.

Du CX, Fan HF, Guo SR, Tezuka T & Li J. 2010. Proteomic analysis of cucumber seedling roots subjected to salt stress. *Phytochemistry* **71**: 1450 – 1459.

El-Khatib AA, Hegazy AK & Galal HK. 2004. Allelopathy in the rhizosphere and amended soil of *Chenopodium murale* L. *Weed Biol. Manag.* **4**: 35 – 42.

Fay PK & Duke WB. 1977. An assessment of allelopathic potential in Avena germplasm. *Weed Sci.* **25**: 224 – 228.

Ghareib HRA, Abdelhamed MS & Ibrahim OH. 2010. Antioxidative effects of the acetone fraction and vanillic acid from *Chenopodium murale* on tomato plants. *Weed Biol. Manag.* **10**: 64 – 72.

Gniazdowska R & Bogatek R. 2005. Allelopathic interactions between plants. Multi site action of allelochemicals. *Acta Phys. Plant.* **27**: 395 - 407.

Gonçalves S, Ferraz M & Romano A. 2009. Phytotoxic properties of *Drosophyllum lusitanicum* leaf extracts and its main compound plumbagin. *Sci Hortic.* **122**: 96 – 101.

Haouala R, Khanfir R, Tarchoune A, Hawala S & Beji M. 2008. Allelopathic potential of *Trigonella foenum-graecum* L. *Allelopathy J.* **21**: 307 – 316.

Holm L, Doll J, Holm E, Pancho J & Herberger J. 1997. World Weeds: Natural Histories and Distribution. Wiley, New York.

Jones B, Andresson-Gunnerås S, Petersson SV, Tarkowski P, Graham N, May S, Dolezal K, Sandberg G & Ljung K. 2010. Cytokinin regulation of auxin synthesis in *Arabidopsis* involves a homeostatic feedback loop regulated via auxin and cytokinin signal transduction. *Plant Cell* **22**: 2956 - 2969.

Ladhari A, Omezzine F, DellaGreca M, Zarrelli A, Zuppolini S & Haouala R. 2013. Phytotoxic activity of *Cleome arabica* L. and its principal discovered active compounds. *S Afr J Bot.* **88**: 341 – 351.

Mitić N, Dmitrović S, Đorđević M, Zdravković-Korać S, Nikolić R, Raspor M, Đorđević T, Maksimović V, Živković S, Krstić-Milošević D, Stanišić M & Ninković S. 2012. Use of *Chenopodium murale* L. transgenic hairy root in vitro culture system as a new tool for allelopathic assays. *J Plant Physiol.* **169**: 1203 – 1211.

Murashige T & Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant.* **15**: 473 – 497.

Nishida N, Tamotsu S, Nagata N, Saito C & Sakai A. 2005. Allelopathic effects of volatile monoterpenoids produced by Salvia leucophylla: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *J Chem Ecol.* **31**: 1187 – 1203.

Omezzine F, Ladhari A, Rinez A & Haouala R. 2011. Potent herbicidal activity of Inula crithmoides L. *Sci Hort.* **130**: 853 – 861.

Prasad TK. 1997. Role of catalase in inducing chilling tolerance in pre-emergent maize seedlings. *Plant Physiol.* **114**:1369–1376.

Qasem JR. 1993. Allelopathic effect of nettle-leaved goosefoot (*Chenopodium murale*) on wheat and barley. *Dirasat Pure Appl Sci.* **20B**: 80 – 94.

Qasem JR. 1995. Allelopathic effects of *Amaranthus retroflexus* and *Chenopodium murale* on vegetable crops. *Allelopathy J.* **2**: 49 – 66.

Romero-Romero T, Sanchez-Nieto S, Sanjuan-Badillo A, Amaua AL & Cruz-Ortega R. 2005. Comparative effects of allelochemical and water stress in roots of *Lycopersicon esculentum* Mill. (Solanaceae). *Plant Sci.* **168**: 1059 – 1066.

Soltys D, Krasuska U, Bogatek R & Gniazdowska A. 2013. Allelochemicals as bioherbicides present and perspectives. In: Price, A.J., Kelton, J.A. (Eds.), Herbicides—Current Research and Case Studies in Use, pp. 517–542, In tech.

Sunmonu TO & Van Staden J. 2014. Phytotoxicity evaluation of six fast-growing tree species in South Africa. *S Afr J Bot.* **90**: 101 – 106.

Tanveer A, Rehman A, Javaid MM, Abbas RN, Sibtain M, Ahmad AUH, Ibin-I-Zamir MS, Chaudhary KM & Aziz A. 2010. Allelopathic potential of *Euphorbia helioscopia* L. against wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris*Medic.). *Turk J Agric For.* **34**: 75 – 81.

Tigre RC, Silva NH, Santos MG, Honda NK, Falcao EPS & Pereira EC. 2012. Allelopathic and bioherbicidal potential of *Cladonia verticillarison* on the germination and growth of *Lactuca sativa*. *Ecotox Environmen Safe.* **84**: 125 – 132.

Weir TL, Park SW & Vivanco JM. 2004. Biochemical and physiological mechanisms mediated by allelochemicals. *Curr Opin Plant Biol.* **7**: 472 – 479.

Ye SF, Zhou YH, Sun Y, Zou LY & Yu JQ. 2006. Cinnamic acid causes oxidative stress in cucumber roots, and promotes incidence of *Fusarium* wilt. *Environ Exp Bot.* **56**: 255 – 262.

Yu JQ, Ye SF, Zhang MF & Hu WH. 2003. Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. *Biochem Syst Ecol.* **31**: 129 – 139.

Botanica SERBICA

REZIME

Eksudati transformisanih korenova *Chenopodium murale* L. inhibiraju rast i izazivaju oksidativni stres kod klijanaca raštana *(Brassica oleracea* L. var. *acephala)*

Nevena Mitić, Mariana Stanišić, Jelena Savić, Snežana Zdravković-Korać, Slavica Ninković

Pepeljuga (*Chenopodium murale* L.) je kosmopolitski korov čiji koren produkuje supstance sa alelopatskim efektom prema drugim biljnim vrstama, uključujući povrtarske i ratarske kulture. U cilju izučavanja alelopatskog efekta korenova *C. murale* na povrtarsku biljku kupus raštan *(Brasica oleracea* L. var. *acephala*), semena raštana su tretirana *in vitro* hranljivim medijumom u kome su 4 nedelje gajeni transformisani korenovi *C. murale* (R5) i koji je sadržao njihove eksudate. Utvrdjeno je da R5 nije uticao na smanjenje procenta klijanja semena, ali je delovao inhibitorno na rast i razvoj klijanaca, kako izdanaka tako i korenova. Najviše su bili inhibirani korenovi klijanaca kupusa i to broj korenova po klijancu (27%), dužina (33%) i sveža težina (59%) u odnosu na kontrolni tretman. Tretman sa R5 doveo je i do savijanja izdanaka, gubitka hlorofila u listovima, nekroze korena i najzad uvenuća celih klijanaca ukazujući na krajnji letalni uticaj alelohemikalija poreklom iz korena *C. murale*. Inhibicija rasta klijanaca raštana je bila praćena promenom aktivnosti antioksidativnih enzima koja se ogledala u povećanju aktivnosti peroksidaza, SOD i katalaze u izdancima, dok je u korenovima došlo do smanjenja aktivnosti SOD i povećanja aktivnosti katalaze. Dobijeni rezultati doprinose širenju saznanja o alelopatskom uticaju *C. murale* na gajene biljke i mogu biti od pomoći u određivanju mera borbe protiv ovog korova radi zaštite biljaka kupusa.

Ključne reči: Alelopatija, *Chenopodium murale*, transformisani korenovi, kupus raštan, inhibicija, klijanje/rast klijanaca