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RAPID *IN VITRO* SELECTION OF SALT-TOLERANT GENOTYPES OF THE POTENTIALLY MEDICINAL PLANT *CENTAURIUM MARITIMUM* (L.) FRITSCH

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Abstract — We investigated differences of salinity tolerance between "salt-tolerant" (ST) and "salt-sensitive" (SS) genotypes of yellow centaury [*Centaurium maritimum* (L.) Fritsch] selected during the germination phase. The ability of *in vitro* cultured *C. maritimum* to complete the whole ontogenetic cycle in less than 6 months enabled us to deterine salinity tolerance during different growth phases. Based on the physiological attributes measured in this study (growth, morphogenesis, photosynthesis, flowering, seed germination), it can be concluded that *C. maritimum* genotypes differing in salinity tolerance showed a variable response to elevated salt concentrations during both the vegetative and the generative growth phase.

Key words: *Centaurium maritimum*, salt tolerance, growth, photosynthesis, flowering, seed germination, gentiopicrine

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INTRODUCTION

Yellow centaury, or sea centaury [*C. maritimum* (L.) Fritsch], an annual herbaceous plant from the family Gentianaceae, grows in forest glades and shrubby places of Western Europe and the Mediterranean region. It inhabits various saline soils. Like other *Centaurium* species, yellow centaury is a rich source of secoiridoid glucosides, among which the most important are swertiamarin, sweroside, and gentiopicrine (Van der Sluis, 1985; Jensen and Schripsema, 2002). Generally, secoiridoids show a number of biological activities, such as fungitoxic, antibacterial, choleretic, pancreatic, and hepatoprotective (Kumarasamy et al., 2003a, 2003b). Because of their bitterness, these compounds are also used in preparation of some commercial beverages (Kohlein, 1993). On the basis of the plant's ecological demand for saline soils, it would appear that *C. maritimum* could be successfully cultivated in coastal regions and different arid areas. Growing wild species that have economic value and perform well under saline soil conditions might be of global interest as a strategy to increase the agricultural utilization of saline soils. To attain optimum growth and productivity, various strategies can be employed, one of which is to produce "salt-tolerant" (ST) genotypes.

Yellow centaury is a facultative autogamous species, and it is therefore not clear whether variation in salinity tolerance exists in its natural populations. By using high NaCl concentrations as a selection pressure agent, we performed rapid *in vitro* selection of ST and SS genotypes during the germination phase. *In vitro* culture techniques – which represent an efficient tool for studying the salt tolerance of intact plants, plant organs, and tissues – have been widely used for the selection of salt-tolerant genotypes (Gangopadhya et al., 1997; Ochatt et al., 1999; Liu and van Staden, 2000; Zair et al., 2003; Gandonou et al., 2006; Queirós et al., 2007). Owing to brevity of the life cycle of *C. maritimum* under *in vitro* conditions, we were able to investigate the salt stress response during different growth phases.

It is well established that plant growth responses to salinity can vary with the degree and duration of stress encountered; the plant organ, variety, or species investigated; and the developmental stage (Neumann, 1997). One of the objectives of this study was to determine whether photosynthetic parameters can be used as indirect screening tools for evaluation of salinity tolerance and rapid selection of ST genotypes of *C. maritimum*. In the past, many attempts have been made to detect differences in salinity tolerance of crop species or cultivars by measuring photosynthetic parameters (Lutts et al., 1996; Tiwari et al., 1997; Ashraf et al., 2000; Loreto et al., 2003; Lee et al., 2004). The aim of this study was to obtain self-fertilization of *C. maritimum* under *in vitro* conditions and produce viable and disease-free ST seeds that can be further used for plantation establishment.

MATERIAL AND METHODS

Plant material

Centaurium maritimum seeds were collected in July of 2002 in the region of Podgorica (Montenegro). Seedlings obtained from them were potted in a greenhouse. Mature seeds of *C. maritimum* were collected in April of 2003 from plants grown in the greenhouse and stored at room temperature until use.

Selection of "salt-tolerant" and "salt-sensitive" genotypes

Seeds were surface-sterilized in a 20% solution of commercial bleach with two drops of liquid detergent for 10 min, then rinsed five times with sterile distilled water. They were aseptically transferred to half-strength MS medium (Murashige and Skoog, 1962) supplemented with 100 mg dm^{-3} *myo*-inositol, 30 g dm^{-3} sucrose, 7 g dm^{-3} agar (Torlak, Belgrade, Serbia), and 100 mM NaCl. The pH of the medium was adjusted to 5.8 before sterilization at 114°C for 25 min.

Two weeks after transfer to the selection media, some seeds germinated. Four-week-old seedlings were transferred to salt-free half-strength MS medium. These plants were treated as "salt-tolerant" (ST) genotypes. Seeds that did not germinate were separately transferred to half-strength MS medium, and the obtained seedlings were treated as "salt-sensitive" (SS) ones.

Experimental design and culture conditions

Eight-week-old seedlings of ST and SS genotypes were used in experiments to determine the effect of different salt concentrations on growth, morphogenesis, and photosynthetic activity of *C. maritimum*. The roots were cut off from seedlings and the explants were transferred to half-strength MS media supplemented with 0 to 200 mM NaCl. The length of shoots and roots, as well as their fresh weight and dry weight, were determined 8 weeks after the start of experiments. All treatments were repeated two times, with 30 explants each.

For all treatments, cultures were grown in 350-ml glass jars closed with transparent polycarbonate caps, each jar containing 60 ml of culture medium. All cultures were grown in a growth chamber under long-day conditions (16 h of light followed by 8 h of darkness) at a temperature of 25±2°C and relative humidity of 60-70%. Light was provided by 60-W white fluorescent tubes with photon flux density of 50 μmol $m^{-2}s^{-1}$ (Tesla, Pančevo, Serbia).

Photosynthetic efficiency

All photosynthetic mesurements were carried using a LI-6200 closed photosynthesis system (Li-Cor, Lincoln, NE, USA) under the following microclimate conditions: leaf chamber CO_2 concentration of 350 μl/l, temperature of 20°C, and relative humidity of 55%. The photosynthetic photon flux density of ambient radiation was measured with a selenium cell mounted on the leaf chamber, while gas exchange data at super-saturating PPFD (> 850 μmol m^{-2} s^{-1}) were taken in calculation of the mean photosynthetic rate (P_n). The average from five to 10 measurements, made evenly over a 5-min period, was calculated for each leaf CO_2 exchange (Pearcy et al., 1989). The value of P_n was expressed on a leaf area basis using Areameter software (Karadžić et al., 1999).

Chlorophyll fluorescence

Leaf photochemical efficiency was estimated by the

method of induced fluorometry (Oquist and Wass, 1988) using a Plant Stress Meter (Polartech, Umea, Sweden). The initial chlorophyll fluorescence yield (Fo), variable chlorophyll fluorescence yield (Fv), and maximum chlorophyll fluorescence yield (Fm) were recorded. Attached leaves were covered in a leaf chamber and dark-adapted for 20 min before measurements were conducted (by activating a pulse of 200 μmol m^{-2} s^{-1} of actinic light during 2 s). Maximum photochemical efficiency of photosystem II (Fv/Fm) in dark-adapted leaves was determined using the following equation: Fv/Fm = (Fm-Fo)/Fm. Measurements were carried out with 10 replicates each.

Chlorophyll content

Plants were harvested after 8 weeks of culturing on half-strength MS media with various NaCl concentrations, frozen in liquid nitrogen, and stored at -70°C until use.

For each extraction, 0.2 g of shoot tissue was homogenized in liquid nitrogen and total pigments were extracted in 6 ml of 80% acetone for 24 h. Extracts were centrifuged at 10000×g for 10 min and supernatant absorbance was measured with a UV-2501PC spectrophotometer (Shimadzu, Duisburg, Germany) at 470, 646.8, and 663.2 nm. Pigment concentrations were calculated according to Lichtenthaler (1987). All extractions and measurements were performed in triplicate or quadruplicate.

High-pressure liquid chromatography

Plant material was dried at 30°C and stored in paper bags at room temperature until use. Each sample (300 mg, dried and powdered) was extracted with 10 ml of methanol overnight. All samples were filtered through Spartan-3NY 0.45-μm nylon filters (S & S Biopath, USA) and stored at 4°C until use.

Analyses were performed on a Hewlett-Packard HPLC system, model 1100 with DAD. The column used for gentiopicrine analysis was Hypersil BDS-C18 (5 μ), 125 × 2 mm I.D. The mobile phase consisted of acetonitrile (CH_3CN; HPLC grade, Acros Organics, Geel, Belgium) and 0.2% phosphoric acid (H_3PO_4). Acetonitrile (A) and phosphoric acid (B) were applied in the following elution gradient: 100% B (0.00 min), 98% B (2.00 min), 90% B (5.00 min), 80% B (10.00 min), 0% B (20.00 min). The flow rate was set to 0.500 ml min^{-1} and the detection wavelength to 260 nm. All analyses were performed at 25°C. Additional peak confirmation was made by a peak spectral evaluation via HP Chemstation chromatographic software (Palo Alto, CA, USA), also used for data acquisition and method/run control. Standard solutions were prepared by dissolving 10 mg of gentiopicrine (Roth, Karlsruhe, Germany) in 10 ml of methanol. Further calibration levels were prepared by diluting the stock with methanol.

F_1 seed germination

Seeds were obtained from 24-week-old ST and SS plants grown on media supplemented with various NaCl concentrations. Lots of 30 seeds were placed in Petri dishes 6 cm in diameter containing 2 ml of distilled water or 200 mM NaCl solution. Seeds were germinated at a temperature of 24±2°C under conditions of a 16 h/8 h (light/dark) photoperiod. Germination was scored 6 weeks after the onset of imbibition, and radicle protrusion was the criterion of germination. Three replicates were prepared for each treatment.

Statistical analysis

Statistical analyses were performed using STATGRAPHICS software, version 4.2 (STSC Inc. and Statistical Graphics Corporation, 1985-1989, USA). The data were subjected to analysis of variance (ANOVA), and comparisons between the mean values of treatments were made by the least significant difference (LSD) test calculated at a confidence level of $p \leq 0.05$. Because of the binomial nature of data presented as percentages, the obtained data were normalized using the *arc sin sqrt* transformation before statistical analysis was performed.

Abbreviations: SS – salt-sensitive; ST – salt-tolerant; Pn – photosynthetic rate; Fv/Fm – photosystem II efficiency, PSII – photosystem II; FW – fresh weight; DW – dry weight; Chl – chlorophyll; Tchl – total chlorophyll; HPLC – high-pressure liquid chromatography.

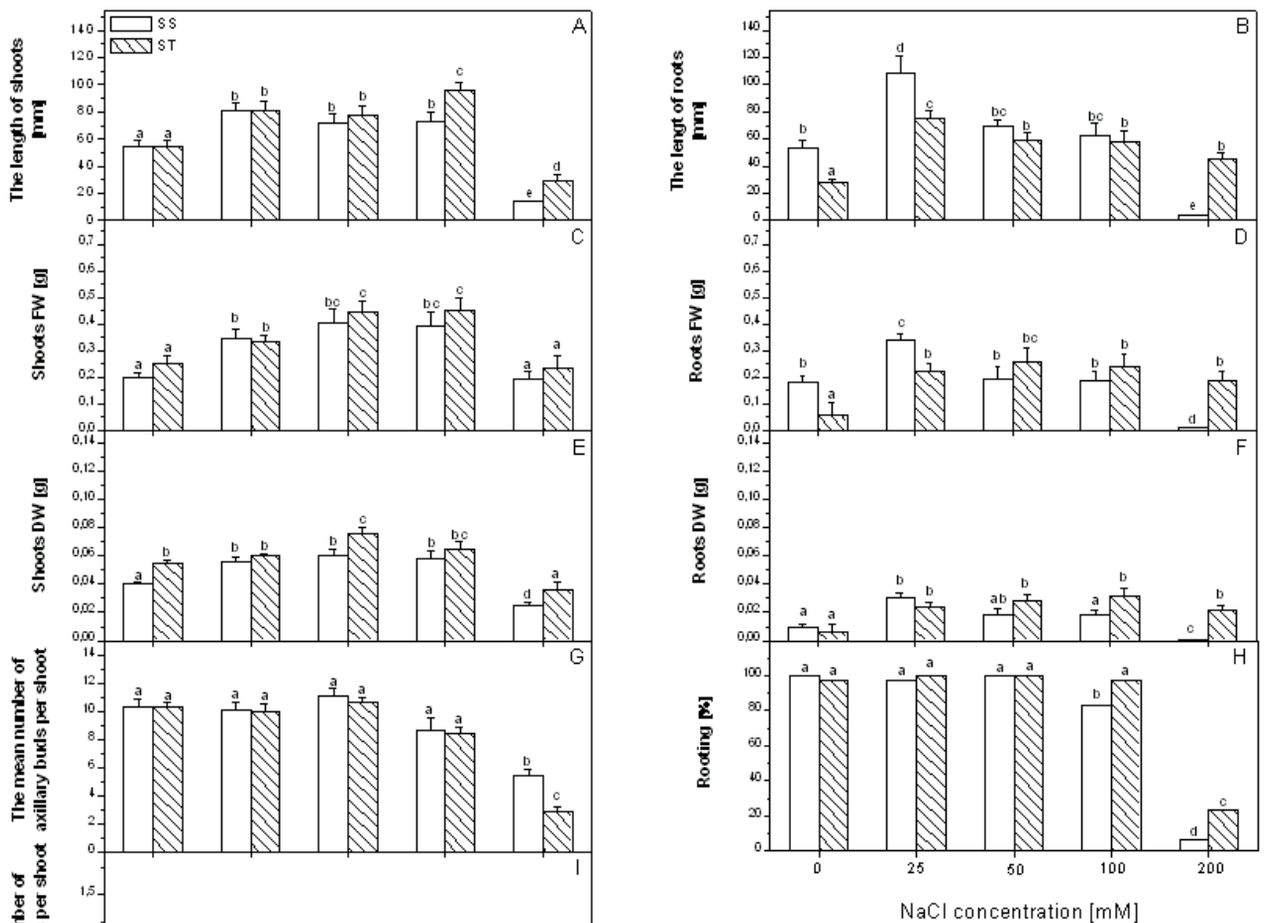

Fig.. Effect of different NaCl concentrations (0-200 mM) in culture media on growth and morphogenesis of *C. maritimum* SS genotypes (non-filled bars) and ST genotypes (filled bars): mean length of shoots (A) and roots (B); fresh weight of shoots (C) and roots (D); dry weight of shoots (E) and roots (F); formation of axillary buds (G); percentage of rooting (H); formation of adventitious buds (I); Within each parameter, values with the same letter are not significantly different at the $p \leq 0.05$ level according to the LSD test.

RESULTS

Selection pressure applied in the seed germination phase enabled us to separate *C. maritimum* ST genotypes. Approximately 30% of seeds germinated under these conditions. Seeds which were unable to germinate were transferred to NaCl-free medium, and seedlings obtained from those seeds were treated as SS ones. Eight-week-old ST and SS seedlings were used in the experiments.

Cultivation on media supplemented with 25 to 100 mM NaCl resulted in increased shoot length in both SS and ST genotypes (Fig. 1A). The length of ST roots increased under all salt treatments (Fig. 1B), while only 25 mM NaCl stimulated elongation of SS roots. The application of 200 mM NaCl significantly reduced the length of SS shoots and roots, as well as ST shoots. The FW and DW of both SS and ST shoots were increased under moderate salt stress (Figs. 1C and 1E). The rooting of shoots under moderate salt stress conditions was not impaired (Fig. 1H). Severe salt stress caused decrease in rooting of

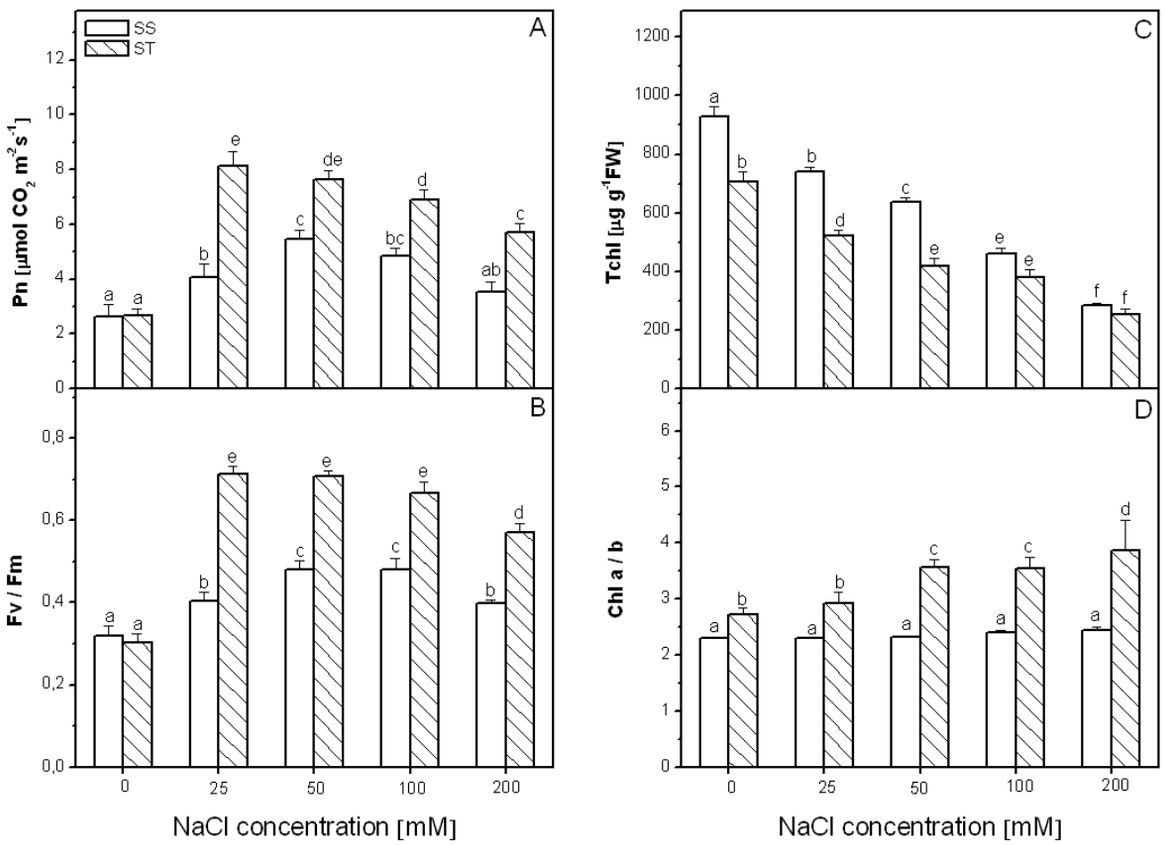

Fig. 2. Photosynthesis rate – Pn (A); photochemical efficiency – Fv/Fm (B); total chlorophyll content – Tchl (C); and chlorophyll a/b ratio (D) of SS (non-filled bars) and ST (filled bars) *C. maritimum* shoots grown at different NaCl levels. Within each parameter, values with the same letter are not significantly different at the p≤0.05 level according to the LSD test.

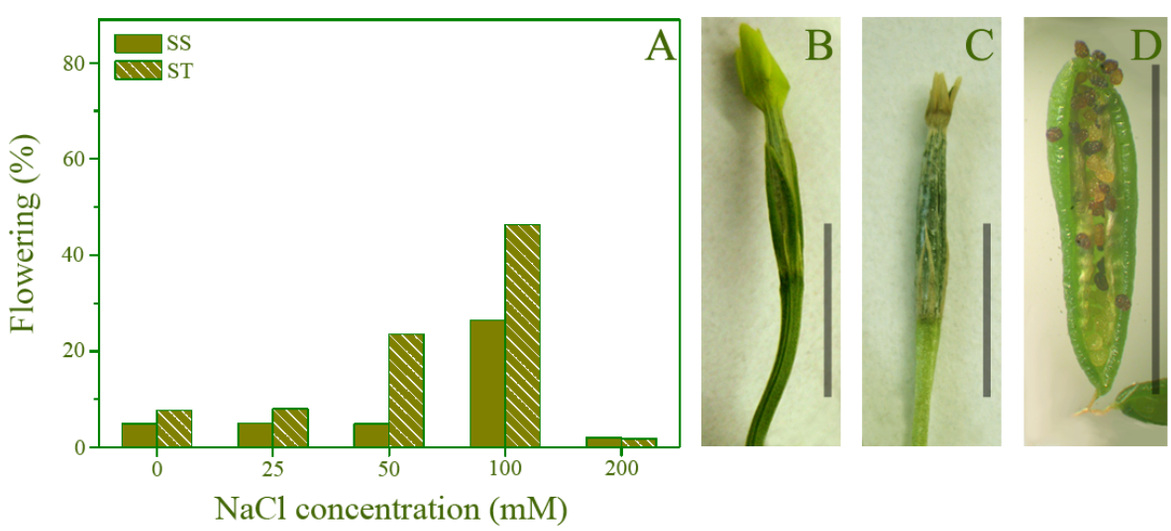

Fig. 3. *In vitro* reproduction of *Centaurium maritimum* during culturing on media containing 0-200 mM NaCl: A) flowering (%), recorded 8 weeks after the onset of experiments. Values with the same letter are not significantly different at the p≤0.05 level according to the LSD test; B) flower of yellow centaury formed on shoots grown on salt-free culture medium; C) fruiting of yellow centaury after self-fertilization; D) seeds produced in capsules after 16 weeks of culturing on salt-free medium. Scale lines = 1 cm.

Table 1. Gentiopicrine content in shoots and roots of *C. maritimum* grown on culture media supplemented with different NaCl concentrations (0-200 mM).

NaCl concentration [mM]	Gentiopicrine concentration [mg g^{-1} DW]	
	shoots	roots
0	3.19±0.30a	1.27±0.08a
50	3.15±0.11a	1.46±0.21a
100	2.68±0.14ab	1.74±0.38a
200	2.43±0.24b	1.82±0.00a

*Values are means of three repeated experiments ± standard error. In each column means followed by the same letter were not statistically different at the $p \leq 0.05$ level according to multiple range tests.

shoots, especially in SS genotypes. The FW and DW of ST roots increased under all salt treatments (Figs. 1D and 1F). Increased FW and DW of SS roots were observed when 25 mM NaCl was applied (Figs. 1D and 1F). Severe salt stress significantly reduced the FW and DW of SS roots. In terms of the measured growth parameters, a significant difference between SS and ST genotypes was observed when plants were grown on culture media containing NaCl at concentrations higher than 100 mM.

Formation of axillary and adventitious buds was observed in yellow centaury cultured *in vitro*. Salt concentrations higher than 100 mM significantly reduced the formation of axillary buds on shoots (Fig. 1G). The mean number of adventitious buds per shoot in ST genotypes could be increased by increasing NaCl concentration up to 50 mM (Fig. 1I). Further increase reduced the formation of adventitious buds to the control level. In SS genotypes, application of NaCl in concentrations higher than 100 mM completely inhibited the formation of adventitious buds.

In our experiments, with increasing NaCl concentration up to 100 mM, Pn increased in both SS and ST genotypes of *C. maritimum* (Fig. 2A). Salt concentrations higher than 100 mM (for SS genotypes) reduced Pn to the control levels. In ST genotypes, the application of 25 mM NaCl significantly increased Pn. Further increase of salt concentration reduced Pn values. However, under all salt treatments, values measured for ST genotypes were higher than the control values. The Pn values measured under all salt treatments were higher for ST than for SS genotypes.

Values of Fv/Fm showed a trend similar to that recorded for Pn (Fig. 2B). Under all NaCl treatments, ST genotypes of *C. maritimum* maintained significantly higher Fv/Fm values than in SS ones.

Chlorophyll a and b contents and total chlorophyll (Fig. 2C) had a decreasing trend with increasing supply of NaCl in the growth medium. The Chl a/b ratio was constant for SS genotypes, while ST genotypes showed a higher Chl a/b ratio under all NaCl treatments when compared to the control (Fig. 2D).

Quantification of the gentiopicrine content in shoots and roots of *C. maritimum* was performed by HPLC analysis. The HPLC system described made it possible to separate gentiopicrine from compounds of similar polarity within 10 min. Gentiopicrine was the main secoiridoid compound in roots and shoots, but considerable amounts of sweroside and swertiamarin as well were noticed in shoots. Shoots of yellow centaury showed greater accumulation of gentiopicrine than roots. Our results demonstrated no significant differences in gentiopicrine content between SS and ST genotypes of *C. maritimum*. The results were therefore summarized and presented in Table 1. By analyzing different samples, it was determined that NaCl had no effect on gentiopicrine content in roots of *C. maritimum* (Table 1). However, a slight decrease in the amount of this secoiridoid glucoside with increasing NaCl concentration was observed in shoots.

After 8 weeks of culturing *C. maritimum* at different salinity levels (16 weeks after placing the seeds on germination media), flowering was recorded in

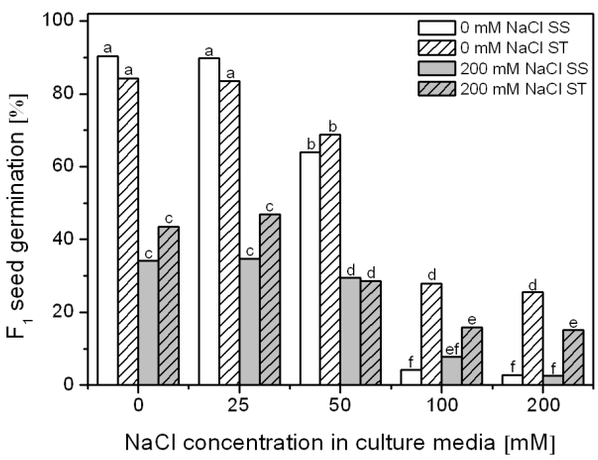

Fig. 4. Germination of C. maritimum seeds obtained by in vitro culturing of SS (empty bars) and ST (line-filled bars) genotypes on culture media containing 0-200 mM NaCl. The germination solution was distilled water (white bars) or 200 mM NaCl (gray bars). Values with the same letter are not significantly different at the p≤0.05 level according to the LSD test.

both SS and ST genotypes. An increasing flowering percentage, especially in ST genotypes, was observed with increasing salt concentration in the culture medium up to 100 mM NaCl (Fig. 3A). Significant differences between SS and ST genotypes were recorded when plants were grown under conditions of 50 and 100 mM NaCl, with ST genotypes showing a higher flowering percentage. Severe salt stress (200 mM NaCl) significantly reduced flowering, but also induced early flowering. After flowering (Fig. 3B), the plants under all treatments fruited (Fig. 3C) and subsequently produced seeds (Fig. 3D). In order to determine whether salt tolerance is preserved in the F_1 generation, seeds of SS and ST genotypes, collected after 16 weeks of culturing plants at different salinity levels, were placed in a germination solution (distilled water or 200 mM NaCl). It was observed that germination of seeds was affected by the previous culture history (Fig. 4). When seeds were germinated in distilled water, the highest germination percentage (80-85%) was observed for seeds derived on NaCl-free culture medium or on media supplemented with 25 mM NaCl. Generally, NaCl concentrations in the culture medium higher than 25 mM significantly reduced the viability of produced seeds. Furthermore, plants of both ST and SS genotypes grown under severe salt stress (100 and 200 mM NaCl), produced fewer seeds per capsule (data not shown). Significant differences between SS and ST seeds were observed for seeds obtained from plants grown at 100 mM and 200 mM NaCl. Seeds of ST genotypes showed a higher germination percentage. Generally, the addition of 200 mM NaCl to the germination solution resulted in a decreased germination percentage in both SS and ST seeds. However, only culturing under severe salt stress (100 mM and 200 mM NaCl) resulted in different salt tolerance of SS and ST genotypes during the germination phase. Seeds of ST genotypes showed a higher germination percentage than in seeds of SS genotypes cultured under the same experimental conditions.

DISCUSSION

Studies have shown that determination of the germination potential of seeds in saline conditions can be employed as a useful and efficient criterion in selection for salinity tolerance (Ashraf et al., 1987). However, in some plant species, salt tolerance during germination does not necessarily imply that mature plants will show similar resistance. We therefore performed selection of ST and SS genotypes during the germination phase of *C. maritimum* seeds, and subsequently investigated whether the differences in salt-tolerance observed during germination are representative of the salt-tolerance of selected genotypes during the whole growth cycle.

Numerous works comparing the general responses of some species to different salinity levels report growth reduction under conditions of salt stress (Brugnoli and Lauteri, 1991; Demir and Kocaçalişkan, 2002; Muscolo et al., 2003; Niknam et al., 2004; Karimi et al., 2005). Our results demonstrating stimulation of vegetative growth under moderate stress conditions might be surprising. It must be noted however, that the species investigated was identified as one that naturally grows in habitats with elevated salt concentrations. By measuring different growth parameters, it was determined that increase in salt concentration up to an optimum stimulated the growth of *C. maritimum*. Further increase reduced its growth. These findings are in agreement with some previous results (Ungar, 1996; Khan et al., 2000). Shoot growth reduction at

high salinity levels might be a consequence of the toxic effect of NaCl or increased osmotic pressure in whose presence plants are unable to uptake the required water. Growth of *C. maritimum* roots was found to be affected more adversely than that of shoots by an increasing supply of NaCl. Salt concentrations higher than 100 mM were inhibitory for the formation of roots and their growth. Some earlier studies also found roots to be among the first plant organs affected by salt stress and the most sensitive ones (Zidan et al., 1990; Muscolo et al., 2003). Our results showed no significant differences in shoot and root growth between ST and SS genotypes of yellow centaury subjected to treatments with NaCl concentrations up to 100 mM. Under severe salt stress (100 and 200 mM NaCl), the growth of roots and shoots of ST *C. maritimum* genotypes was significantly higher than in SS ones.

Reduced formation of axillary buds was observed when both ST and SS plants were grown on a culture medium supplemented with 200 mM NaCl. The mean number of adventitious buds per ST shoot was increased under moderate salt stress (25 and 50 mM NaCl), but reduced under severe salt stress. The formation of adventitious buds in SS shoots of *C. maritimum* was not observed under severe salt stress (200 mM NaCl). It was previously reported that NaCl stimulates the formation of axillary and adventitious buds in *Centaurium erythraea* (Šiler et al., 2007).

The decline in growth observed in many plants subjected to salinity stress is often associated with a decrease in their photosynthetic capacity (Qiu and Lu, 2003). The decrease in photosynthesis induced by salt stress is mainly associated with decrease in stomatal conductance or g_s (Jones, 1973; Sharkey, 1990; Centritto et al., 2003) and/or the non-stomatal limitation involved in the dark enzymatic process of CO_2 conductance and assimilation (Downton et al., 1985; Ziska et al., 1990; Brugnoli and Björkman, 1992; Delfine et al., 1999; Centritto et al., 2003). A decrease of Pn under saline conditions has been reported for some plant species (Delfine et al., 1999; Loreto et al., 2003; Qasim et al., 2003; Qiuet al., 2003). However, photosynthesis in some plant species is not reduced by the salinity and is even stimulated by low salt concentrations (Rajesh et al., 1998; Kurban et al., 1999). Many attempts have been made to detect differences in salinity tolerance of crop species or cultivars by measuring photosynthetic parameters (Lutts et al., 1996; Tiwari et al., 1997; Ashraf et al., 2002; Loreto et al., 2003; Lee et al., 2004). Our results show that the Pn of both SS and ST genotypes of *C. maritimum* was increased at moderate salinity. High salinity reduced Pn values to control levels. Similar results were recently reported for *C. erythraea* (Šiler et al., 2007). Salt-tolerant genotypes of *C. maritimum* were superior to SS genotypes under all salt treatments. Generally, the Pn values obtained in our experiments were low, regardless of the salt treatment. It is well known that photosynthetic activity of mixotrophic plant tissues *in vitro* is reduced, mainly due to the low light intensity, limited gas exchange, and low relative humidity in tightly closed vessels.

It has been reported that salinity stress can predispose plants to photoinhibition and photodamage of PSII (Mishra et al., 1991; Masojidek and Hall, 1992; Belkhodja et al., 1994; Jungklang et al., 2003). As a consequence of decreased photosynthetic rate (Pn), the plants are exposed to excess energy, which, if not safely dissipated, may be harmful to PSII. However, maximal efficiency of PSII photochemistry, i.e., the Fv/Fm ratio, has been shown to be highly resistant to salinity stress (Robinson et al., 1983; Morales et al., 1992). In some salt-tolerant species, the Fv/Fm ratio was unaffected by NaCl (Brugnoli and Bjorkman, 1992; Jungklang et al., 2003). Our results clearly indicate that PSII of *C. maritimum* plants is tolerant to salinity and has the capacity to adapt to salinity. Salt-tolerant genotypes maintained significantly higher Fv/Fm values than SS ones, suggesting that, in terms of photosynthetic efficiency, ST genotypes are more tolerant to salt stress compared to SS ones.

Reductions of chlorophyll content under conditions of elevated salinity were observed for some salt-susceptible plant species (Seemann and Critchley, 1985; Delfine et al., 1999; Ashraf et al., 2002; Jungklang et al., 2003; Lee et al., 2004; M'rah et al., 2006). Decrease of chlorophyll content was dependent on the salinity level, the time of expo-

sure to salts, and the plant species. In contrast, Chl content in salt-tolerant plants either does not decline or increases with rising salinity (Brugnoli and Björkman, 1992; M'rah et al., 2006; Qiu et al., 2003). In our experiments, chlorophyll a and b contents and total chlorophyll had a decreasing trend with increasing supply of NaCl in the growth medium. The Chl a/b ratio was constant for SS genotypes. An increase in the Chl a/b ratio under all NaCl treatments was observed in ST genotypes of *C. maritimum*. A high chlorophyll a/b ratio indicates that the PSII/PSI ratio changes in stressed leaves.

In vitro cultured yellow centaury retains the ability to produce gentiopicrine and other secoiridoid compounds. The obtained results showed greater accumulation of gentiopicrine in shoots than in roots. Some previous studies indicated that qualitative and quantitative content of secoiridoid glucosides in *in vitro* cultured *Centaurium* species depends on culture conditions, but also on the growth phase of the plants (Krstić et al., 2003; Piatzak et al., 2005). We here demonstrate that elevated salt concentrations have no significant effect on gentiopicrine content in roots of yellow centaury. However, shoots of *C. maritimum* showed a slight reduction in gentiopicrine content under severe salt stress (100 and 200 mM NaCl). No significant difference between SS and ST genotypes was observed.

Intact plants of yellow centaury were capable of *in vitro* flowering and seed production. They finished their ontogenetic cycle in less than 6 months. *In vitro* flower induction and production of viable seeds was previously reported for *Centaurium pulchellum* (Cvetić et al., 2004; Todorović et al., 2006). Under our experimental conditions, a decrease in flowering percentage was observed with increasing salt concentration in the culture medium. In our experiments, SS and ST genotypes showed different reproductive behavior under salt stress conditions, which is in agreement with some previous studies (Ruiz Carrasco et al., 2007). Early flowering was observed when plants were cultured under severe salt stress conditions. It has been previously reported that salinity can reduce the formation and viability of reproductive organs, but also alter the time of flowering and maturity (Khatun et al., 1995; Munns and Rawson, 1999; Munns, 2002; Achard et al., 2006). The "early flowering" phenomenon is a well-known adaptation strategy in saline environments, where plants use short-term favorable conditions in order to finish their growth cycle and produce seeds, which ensure further distribution and perpetuation of the species. In our experiments, due to the absence of pollinators, only self-fertilization was possible, and it resulted in the production of viable seeds. Genetic variability within the SS or ST genotypes therefore was not expected. Seeds of both ST and SS genotypes, from all salt treatments, were collected after 16 weeks of culturing (from 24-week-old plants) and tested for salt tolerance during the germination phase. Influence of the adaptation process during culturing under elevated salt conditions on the germination of seeds was confirmed: increase of NaCl concentration in the culture medium negatively affected the production of SS and ST seeds by reducing their number and viability. This may indicate that yellow centaury became salt-sensitive during pollination and fertilization or during seed maturation. The presence of 200 mM NaCl in the germination solution reduced the germination percentage of both SS and ST seeds. Regardless of the NaCl concentration in the germination solution, a significant difference between ST and SS genotypes was only apparent when seeds were germinated under severe salt stress. Salt-tolerant genotypes of yellow centaury showed a higher germination percentage of seeds under such conditions, which indicates that ST seeds can withstand more pronounced salt stress than SS ones. A delay of germination events was also observed under our experimental conditions (data not shown). It was previously reported that a high concentration of salt in germination media significantly delays the onset, reduces the rate, and extends the time of germination events in different species (Tobe et al., 1999; Mer et al., 2000; Yücel, 2000; Almansouri et al., 2001; Khan et al., 2001; Gul and Khan, 2003). It was suggested that decrease of seed germination under salt stress can be attributed to reduced water uptake followed by limited hydrolysis of food reserves from storage tissues, as well as to impaired translocation of food reserves from storage tissue to the developing embryo axis (Dubey, 1985; Ghoulam et al., 2002).

In conclusion, our results clearly demonstrate that salt tolerance of ST and SS genotypes of yellow centaury during the seed germination phase is related to salt tolerance in some later developmental stages. The measured growth parameters during the vegetative growth phase show significant differences between selected ST and SS genotypes. Photosynthetic parameters can be valuable and reliable tools in screening for salinity tolerance not only of *C. maritimum*, but also of other *Centaurium* species. Seeds of ST genotypes showed greater tolerance to elevated salt concentration in germination media compared to SS ones. Thus, *in vitro* selection of ST genotypes during germination and subsequent screening for salinity tolerance using photosynthetic parameters make it possible to achieve, in a short span of time, selection, large-scale propagation, and production of viable disease-free ST seeds, which can further be used for plantation establishment. Commercial cultivation of yellow centaury in saline soils is a good alternative for the large-scale production of gentiopicrine and other secoiridoid compounds having great application in the pharmaceutical and food industries. Our results clearly indicate that yellow centaury can be cultivated at moderate soil salt levels without any loss in growth or productivity. On the other hand, such production would reduce the exploitation of wild populations of *C. erythraea*, which is the officinal biological source of the drug *Centaurii herba* and is threatened on account of over-exploitation.

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ЕФИКАСНА *IN VITRO* СЕЛЕКЦИЈА "SALT-TOLERANT" ГЕНОТИПОВА ПОТЕНЦИЈАЛНО ЛЕКОВИТЕ ВРСТЕ *CENTAURIUM MARITIMUM* (L.) FRITSCH

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Вршена су истраживања у циљу утврђивања разлика у толерантности на повећани салинитет између *in vitro* селекционисаних "salt tolerant" (ST) и "salt sensitive" (SS) генотипова жуте кичице [*Centaurium maritimum* (L.) Fritsch]. Способност врсте *C. maritimum* да комплетира онтогенетски циклус у *in vitro* условима за мање од 6 месеци, омогућила је праћење толеранције на повећани салинитет током различитих фаза развића. Ако се узму у обзир сви физиолошки параметри који су праћени у овом истраживању (растење, морфогенеза, фотосинтеза, цветање, клијање семена), може се закључити да селекционисани ST и SS генотипови заиста показују различит одговор у условима у условима повећаног салинитета, како током вегетативне фазе, тако и током генеративне фазе развића.