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EFFECTS OF SALINITY ON IN VITRO GROWTH AND PHOTOSYNTHESIS OF COMMON CENTAURY (*CENTAURIUM ERYTHRAEA* RAFN.)

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Abstract – The effects of salinity on *in vitro* growth, morphogenesis, photosynthetic rate (Pn), PSII efficiency (Fv/Fm), and chlorophyll content were investigated in *Centaurium erythraea* Rafn. Root growth was more adversely affected by increasing NaCl concentration than was shoot growth. High salt concentrations were effective in induction of axillary and adventitious buds on shoots, with 400 mM NaCl being the most efficient one. Values of Pn and Fv/Fm increased at moderate salt levels, but decreased when 400 mM NaCl was applied. Chlorophyll a and b contents and total chlorophyll had a decreasing trend with increasing supply of NaCl in the growth medium.

Key words: *Centaurium erythraea*, salt stress, growth, buds, photosynthetic rate, PSII efficiency, chlorophyll content

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INTRODUCTION

Soil or water salinity is considered to be the major environmental factor limiting plant growth and productivity, especially in arid and semi-arid regions. Salinity has a two-fold effect on plants: the salt in the soil solution decreases the availability of water to the roots (osmotic stress), and the salt taken up by the plant can accumulate to toxic levels in certain tissues (ionic stress) (M u n n s et al., 1995).

Reduction in growth under saline conditions is a consequence of several physiological responses, including modification of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, and carbon allocation and utilization (F l o w e r s et al., 1977; M u n n s and T e r m a a t, 1986). The decrease in photosynthesis caused by salt stress is mainly associated with decrease in stomatal conductance- g_s (C e n t r i t t o et al., 2003) and/or the non-stomatal limitation related to carbon fixation (B o n g i and L o r e t o, 1989; B r u g n o l i and B j ö r k m a n, 1992; D e l f i n e et al., 1998; 1999; C e n t r i t t o et al., 2003). According to some authors (B o n g i and L o r e t o, 1989), stomatal limitation seems to prevail at intermediate salinity levels, while the non-stomatal limitations predominate under severe salt stress conditions. As a consequence of decreased photosynthetic rate (Pn), the plants are exposed to excess light energy, which, if not safely dissipated, may be harmful to photosystem II (PSII) due to over-reduction of reaction centers. It has been previously reported that the salt stress can predispose plants to photoinhibition and photodamage of PSII (B e l k h o d j a et al., 1994). Furthermore, salinity can cause progressive loss of chlorophyll content, leading to a corresponding reduction of light absorption by leaves (E v a n s , 1996). However, salt-tolerant plant species are thought to have mechanisms that allow them to maintain photosynthesis in the presence of high salt levels.

Centaurium erythraea Rafn. (common centaury) is an annual or biennial medicinal plant belonging to the *Gentianaceae* family. It is distributed throughout Europe, the Caucasus region and Persia in Asia, North Africa, and North America (where it grows as a naturalized form). The species inhabits dry grassland, scrub, and mountain

slopes (M e l d e r i s, 1972), but can also be found in saline soils (K n e ž e v i ć, 1994; B u d a k, 1998). Previous phytochemical investigations of *C. erythraea* resulted in detection of a variety of secondary plant metabolites. The pharmacological activities of common centaury are ascribed to seciridoid glucosides such as gentiopicrin, sweroside, and swertiamarin. The herb is officially listed in the pharmacopoeias of many European and American countries.

The *in vitro* propagation of common centaury and the production of secondary metabolites under culture conditions has been previously reported (V á g n e r o v a, 1992; B e e r h u e s, 1996; J a n k o v i ć et al., 2000; S u b o t i ć et al., 2003/4; P i a t z a k et al., 2005, 2006). Our objective was to investigate how, under *in vitro* conditions, common centaury responds to salinity in terms of growth, photosynthetic rate, photosystem II efficiency, and chlorophyll content. This information may provide a background for cultivation of common centaury in saline soils, where the growth of many species is markedly reduced.

MATERIAL AND METHODS

Plant material

Seeds of *C. erythraea* were collected in August of 2001 in the area of Lake Vlasina (Serbia). They were stored in glass jars at room temperature until use.

Seed sterilization and germination

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 and aseptically transferred to half-strength MS medium (M u r a s h i g e and S k o o g, 1962) supplemented with 100 mg dm^{-3} *myo*-inositol, 30 g dm^{-3} sucrose, and 7 g dm^{-3} agar (Torlak, Belgrade, Serbia). The pH of the medium was adjusted to 5.8 before sterilizing by autoclaving at 114 °C for 25 min.

Experimental design and culture conditions

Two month-old seedlings were used in experiments to determine the effects of different salt concentrations on *in vitro* growth, morphogenesis, and photosynthetic activity of *C. erythraea*. The roots were cut off from seedlings and the explants were transferred to half-strength MS media supplemented with NaCl in concentration ranging from 0 to 400 mM. After eight weeks in culture, the efficacy of each medium composition was determined by recording the length and fresh weight of shoots and roots. All treatments were repeated twice, with 30 explants per treatment.

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

Photosynthetic efficiency

The net photosynthetic rate was determined as the measure of CO_2 influx using a Li-6200 closed photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). Measurements were conducted with leaf chamber CO_2 concentration of 350 ppm at chamber temperature of 20 ºC, relative humidity of 55% and PPFD above 850 μmol photons m^{-2} s^{-1}. The net photosynthetic rate is expressed in relation to leaf area as determined using Areameter software (K a r a d ž i ć et al., 1999).

Steady-state fluorescence was determined with a Plant Stress Meter (Polartech, Umea, Sweden) by the method of induced fluorometry (O q u i s t and W a s s, 1988). Photosynthetic function was assessed as the rate of basic chlorophyll (Chl) fluorescence, i.e., the ratio of variable to maximal fluorescence (Fv/Fm). The plants were dark-adapted for 10 min before measuring maximum photosynthetic efficiency of PSII (Fv/Fm). Experiments were performed with 10 replicates each.

Chl contents

After 8 weeks of cultivation on media with different NaCl concentrations, plants were harvested, frozen in liquid nitrogen, and stored at -70ºC until use.

About 0.2 g of tissue (stem + leaves) was used for each extraction. Tissue was homogenized in liquid nitrogen and total pigments extracted in 6 ml of 80% acetone for 24 h. Extracts were then centrifuged at 10000g for 10 min and the absorbance of the supernatant was measured at 470, 646.8, and 663.2 nm (using a Shimadzu UV-2501PC instrument). Pigment concentrations were calculated according to L i c h t e n t h a l e r (1987). All ex-

tractions and measurements were performed in triplicate or quadruplicate.

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=0.05.

RESULTS AND DISCUSSION

Numerous works comparing general responses of some plant species to different salinity levels report growth reduction under salt stress conditions (A k h t a r et al., 2003; M u s c o l o et al., 2003). Under our experimental conditions, growth of *C. erythraea* roots was found to be affected more adversely by an increasing supply of NaCl than was that of shoots. Both root length (Fig. 1A) and the mean number of rooted shoots (data not shown) decreased with increasing salt concentration in the culture medium. These results are in agreement with results obtained previously, which also indicated roots to be among the first plant organs affected by salt stress and the most sensitive ones (Z i d a n et al., 1990; M u s c o l o et al., 2003). According to N e u m a n n (1997), salinity can rapidly inhibit root growth and hence the capacity for uptake of water and essential mineral nutrients from the soil. In culture conditions, *C. erythraea* keeps the rosette form, and elevated NaCl concentrations in our experimental conditions showed no inhibitory effect on shoot growth (Fig. 1A). With increasing salt concentrations up to an optimum of 100 mM NaCl, the fresh weight (FW) of shoots and roots increased (Fig. 1B). However, the FW of shoots and roots decreased with further increase of salt. The increased value of the shoot/root FW ratio at and above 100 mM NaCl (data not shown) also indicates that roots were affected more by salinity than were shoots. Stimulation of axillary and adventitious bud formation was observed at high NaCl concentrations. Both the frequency of explants forming buds (Fig. 2A) and the mean number of buds per explant (Fig. 2B) were

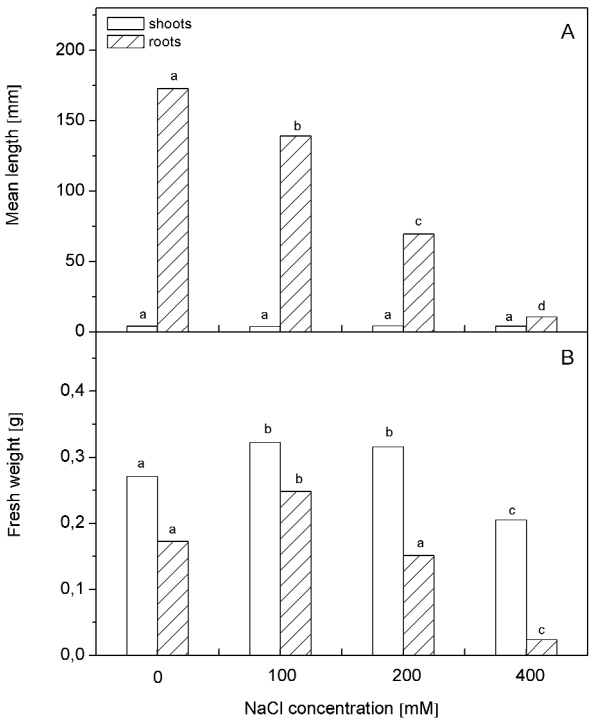

Fig. 1. Effect of salt on *in vitro* growth of *C. erythraea*: A) Mean length of shoots and roots; B) Fresh weight of shoots and roots. Within each parameter, values with the same letter are not significantly different at the p=0.05 level according to the LSD test.

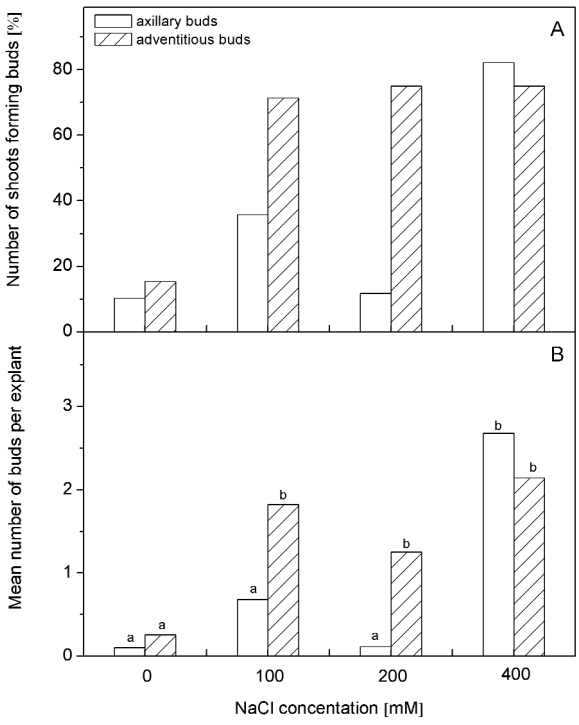

Fig. 2. Formation of axillary and adventitious buds on *C. erythraea* shoots grown on media with different NaCl concentrations: A) Number of shoots forming buds; B) Mean number of buds per explant. Within each parameter, means followed by the same letter were not statistically different at the p=0.05 level according to the LSD test.

increased. The most efficient NaCl concentration was 400 mM.

The decline in growth observed in many plants subjected to salinity stress is often associated with a decrease in their photosynthetic capacity (Q i u and L u, 2003). Decrease in Pn under salinity has been previously reported for several plant species (D e l f i n e et al., 1999; L o r e t o et al., 2003; Q a s i m et al., 2003). The Pn of *C. erythraea* at moderate salinity (50-200 mM NaCl) was increased as compared to the control (Fig. 3). The application of high salt concentration (400 mM NaCl) significantly reduced the Pn.

The range of Fv/Fm for various plant species under non-stress conditions varies from 0.80 early in the growing season to 0.60 or less in the latter part of the growing season (F i g u e r o a et al., 1997). Maximal efficiency of PSII photochemistry, i.e., the Fv/Fm ratio, has been shown to be highly resistant to salt stress (M o r a l e s et al., 1992) and is widely used as an indicator of photoinhibition (K r a u s e and W e i s, 1991). It has been reported for some salt-tolerant species that the Fv/Fm ratio was unaffected by NaCl (B r u g n o l i and B j o r k m a n, 1992; J u n g k l a n g et al., 2003). In salt-sensitive species, strong salt stress severely reduces the potential of electron transport in PSII (J u n g k l a n g et al., 2003). In our experiments, high levels of Fv/Fm (0.6-0.75 after salinity treatments) indicate that *C. erythraea* was able to maintain maximum photochemical efficiency of PSII at high salinity levels and demonstrate a relatively high degree of salinity tolerance. NaCl concentrations in the range of 100 to 200 mM NaCl increased the values of Fv/Fm (Fig. 3). When 400 mM NaCl was applied, the values of Fv/Fm were slightly below the control values.

Reductions of chlorophyll content under elevated salinity conditions were observed for some salt-sensitive plant species (D e l f i n e et al., 1999; A s h r a f et al., 2000; J u n g k l a n g et al., 2003; L e e et al., 2004). The decrease of chlorophyll content was dependent on the salinity level, the time of exposure to salts and the species. In contrast, Chl content in salt-tolerant plants either does not decline or else rises with increasing salinity (B r u g n o l i and B j ö r k m a n, 1992; Q u i et al., 2003). According to Y e o and F l o w e r s (1983), chlorophyll concentration can be used as a sensitive indicator of the cellular metabolic state; thus, its decrease signifies toxicity in tissues due to accumulation of ions. In our experiments, chlorophyll a and b contents and total chlorophyll decreased with increasing NaCl supply (Fig. 4A). The Chl a/b ratio also slightly decreased with increased salt concentration (Fig. 4B), suggesting that the ratio between PSII content and PSI content changes in stressed leaves.

In conclusion, our results demonstrate that *C. erythraea* is tolerant to salinity (up to 200 mM), which is indicated by the fact that there were no reductions of root and shoot growth, biomass production, Pn, Fv/Fm, and chlorophyll content between control and salt-treated plants up to 200 mM. Thus, our results suggest that common centaury could be successfully cultivated in saline soils without any loss in growth or productivity. Commercial culti-

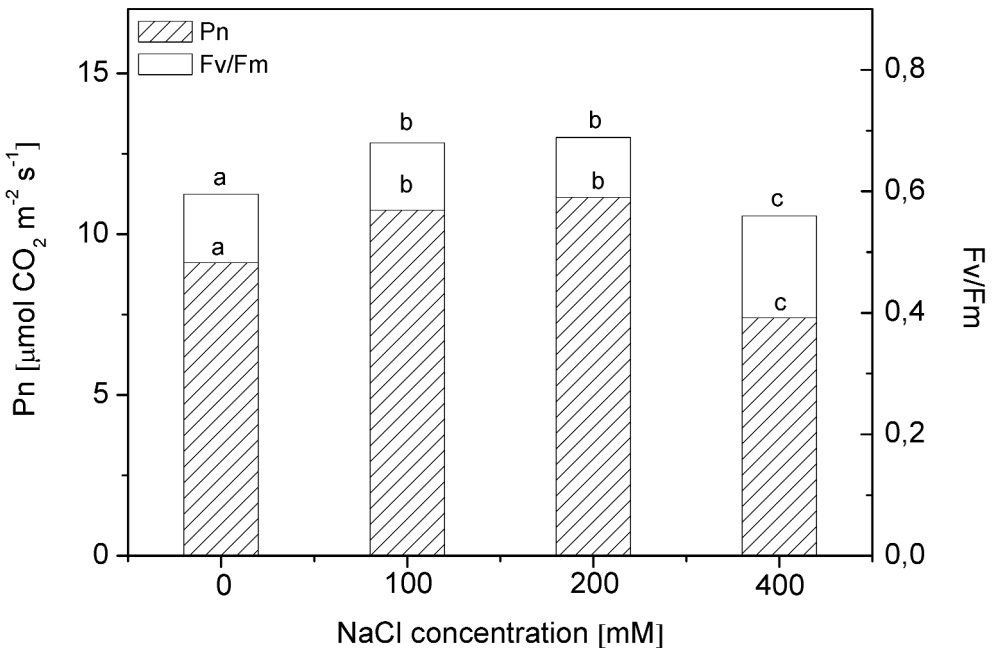

Fig. 3. Photosynthetic efficiency (Pn) and the maximal efficiency of PSII photochemistry (Fv/Fm) of *C. erythraea* shoots at different salinity levels. Within each parameter, values with the same letter are not significantly different at the p=0.05 level according to the LSD test.

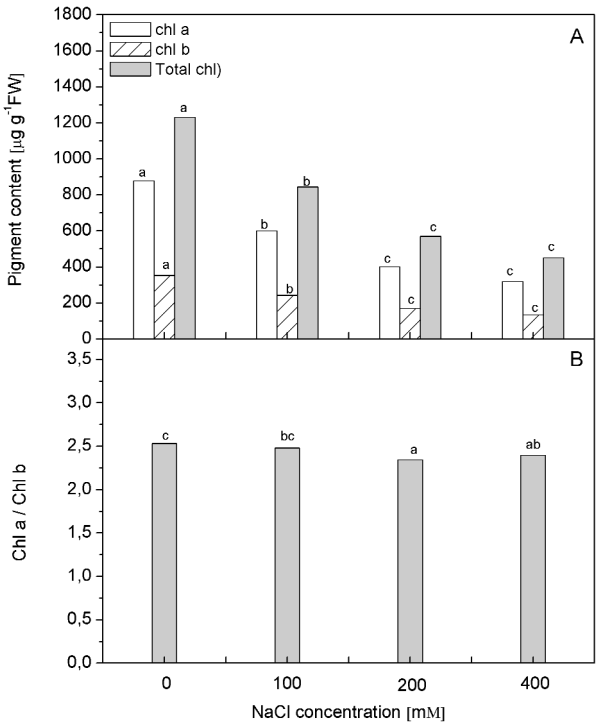

Fig. 4. Photosynthetic pigment composition (A) and Chl a/Chl b ratio in shoots of common centaury under conditions of different salinity treatments. Within each parameter, means followed by the same letter were not statistically different at the p=0.05 level according to multiple range tests.

vation of *C. erythraea* in saline soils, where the growth of many species is markedly reduced, is a good alternative for large-scale production of secoiridoid compounds, which are widely used in the pharmaceutical and food industries.

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ЕФЕКАТ САЛИНИТЕТА НА РАСТ И ФОТОСИНТЕЗУ КИЧИЦЕ (*CENTAURIUM ERYTHRAEA* RAFN.) У *IN VITRO* УСЛОВИМА

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Праћен је ефекат NaCl (у распону концентрација од 0 до 400 mM) на раст, морфогенезу, интензитет фотосинтезе (Pn), ефикасност фотосистема II (Fv/Fm) и садржај хлорофила a и b код врсте *Centaurium erythraea* Rafn. у *in vitro* условима. Утврђено је да су корени кичице осетљивији на високе концентрације соли у хранљивој подлози у поређењу са изданцима. Високе концентрације соли су се показале ефикасним у погледу индукције формирања аксиларних и адвентивних пупољака на изданцима. У условима умереног стреса (100 и 200 mM NaCl), вредности Pn и Fv/Fm расту у поређену са вредностима добијеним код контролне групе биљака. Примена 400 mM NaCl довела је до опадања вредности Pn и Fv/Fm испод контролних вредности. Садржај хлорофила a и b, као и укупног хлорофила опда са порастом садржаја соли у хранљивој подлози. Однос Chl a/b такође опада. Може се закључити да је *Centaurium erythraea* Rafn врста која је отпорна на повећани салинитет подлоге у којој расте. Према томе, може се претпоставити да је комерцијално гајење ове врсте кичице остваривo на заслањеним теренима.