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Estimation on photosynthetic efficiency in three populations of *Nepeta rtanjensis* Diklić & Milojević

Abstract

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The causes of limited number and distribution of critically endangered *Nepeta rtanjensis* were investigated in the sense of ecophysiological status of this species. Three populations of *N. rtanjensis* were subjected to analysis of photosynthetic efficiency: natural population (growing at Rtanj Mt.), re-introduced population (the population established by planting of seedlings near the natural site) and the cultivated population (growing in controlled conditions). The seedlings for planting were obtained by the method of in vitro micropropagation and pre-adapted for ex vitro planting. The aim of this study was to achieve data on ecophysiological adaptation of in vitro propagated seedlings. Results showed that the favorable photosynthetic efficiency of these plants was related to their successful adaptation at natural site. Such adaptive response is in favor of further propagation and re-introduction of this rare species.

Introduction

Nepeta rtanjensis Diklić & Milojević (fam. *Lamiaceae*) is a rare endemorelict species with limited distribution at Rtanj Mt. (SE Serbia). Significant distance and distinctness of this species' areal related to the distributional center of Mediterranean complex of *Nepeta sibthorpii* points out to its relict character. It is preserved only on few places, on the open calcareous stony grounds in the zone of the oak forest ass. *Quercetum frainetto-cerridis* Rudski (Tomić 2006), at 650-850 m asl. According to IUCN categorization *N. rtanjensis* is critically endangered (CR B_{2c}) species in Serbia (Diklić 1999). The number of individuals of *N. rtanjensis* at Rtanj Mt. is about 500-700. It has been postulated that the fungal infection is the main cause that affects adversely upon the seed viability (Rančić & al. 2002; Ljaljević-Grbić 2006). *N. rtanjensis*, like the majority of *Nepeta* species, is a plant with some medical properties. Nepetalactone is the main component of essential oil of this species (Chalchar & al. 2000; Mišić 2003), so the individuals of this species may be produced in pharmacological purposes. The cultivation of *N. rtanjensis* could be a way to prevent its further disappearing because of uncontrolled gathering in natural sites. The ecophysiological investigations presented in this study were the part of the greater experiment performed in order to evaluate the establishment of planted individuals of *N. rtanjensis*.

Material and methods

The estimation of photosynthetic efficiency was performed on three populations of *N. rtanjensis*. The natural population growing at limited area at Rtanj Mt. was observed to investigate the ecophysiological status of this plant in natural habitat (with extreme high temperatures and irradiation, and low humidity). A number of individuals of *N. rtanjensis* that were obtained in the micropropagation process (Mišić 2003) represented an initial material establishing other two populations. These plants were growing in laboratory conditions for 6 to 12 months, pre-adapted to *ex vitro* conditions in glass house and then they were planted on the garden plots in March 2004. A thousand individuals from garden plots were planted at Rtanj Mt. in April 2004. The site for planting was open, calcareous and similar to original species' site. This group of individuals was considered as 're-introduced' and observed in order to evaluate its adaptive response. The third population was planted at experimental plots at the Institute for Biological Research in Belgrade, where they were subjected to regular watering. This 'cultivated' population was considered as control. Photosynthetic efficiency of reintroduced and cultivated plants has been evaluated based on the chlorophyll fluorescence, using an induction fluorometer (Plant Stress Meter, Umea, Sweden). Photosynthetic efficiency is known to be one of the most sensitive processes and it can be inhibited by high temperature and intensive irradiation, before other symptoms of stress are detected (Krause & Weis 1984; Öquist & Wass 1988; Scheriber & al. 1994).

All measurements in natural site were performed on a sunny summer day, July 1st, 2004 (at 32ºC). Individuals from garden plots were measured on July 4th (at 30ºC). All plants were dark adapted for at least 20 min before measuring. The actinic photon flux density was 200 µmol $m^{-2}s^{-1}$. Data analysis was carried out using the analytical tool of the Statistica for Windows program package. All correlation coefficients r are Pearson coefficients for all correlations. Relationships are called significant if $p < 0.05$. Means of F_v/F_m and $t_{1/2}$ were compared using t-test.

Results and discussion

In the present study, we monitored the photosynthetic efficiency of planted individuals of *N. rtanjensis*, in order to evaluate their establishment through the ecophysiological aspect. Measuring of chlorophyll fluorescence during the actual exposure to direct sunlight in the summer period allowed a direct determination of the degree of environmental stress experienced by individuals of this sun-adapted species. A high percentage of light absorbed in PS II antennae that is utilized in PS II photochemistry corresponds to a high level of excess light, and the ratio of variable fluorescence and maximal fluorescence (F_v/F_m) in photoinhibited, intact leaves correlates very well with the quantum yield of net photosynthesis (Ögren 1988). It was documented that decrease in fluorescence parameters (F_v/F_m) from optimal values (0.849, Björkman & Demming 1987) implies decreased photochemical conversion efficiency of PS II, which could be considered as an indicator of the stress conditions.

On the basis of differences in environmental conditions that planted individuals have experienced on garden plots (‘control’) and on the natural site (‘re-introduced’), it was possible to expect differences between them related to functioning of PS II. Contrary to the expectation, we haven’t found a significant difference between all investigated groups according to values of F_v/F_m, as shown of Figure 1. However, values for half rise time were significantly higher for plants from natural population, in comparison with reintroduced individuals on the same site ($t = 2.12$, $p = 0.049$), and in comparison with individuals that were cultivated on garden plots ($t = 2.77$, $p = 0.011$). In natural population $t_{1/2}$ had the highest mean value (437.78±187.77 ms), and only in this group we found a significant relation ($r = -0.75$) between the suppression of photosynthetic efficiency and prolonged half rise time (Fig. 2). Mean value for $t_{1/2}$ in reintroduced individuals was 301.10±75.78 ms, and the lowest values were registered in cultivated group of plants 280.50±81.99 ms.

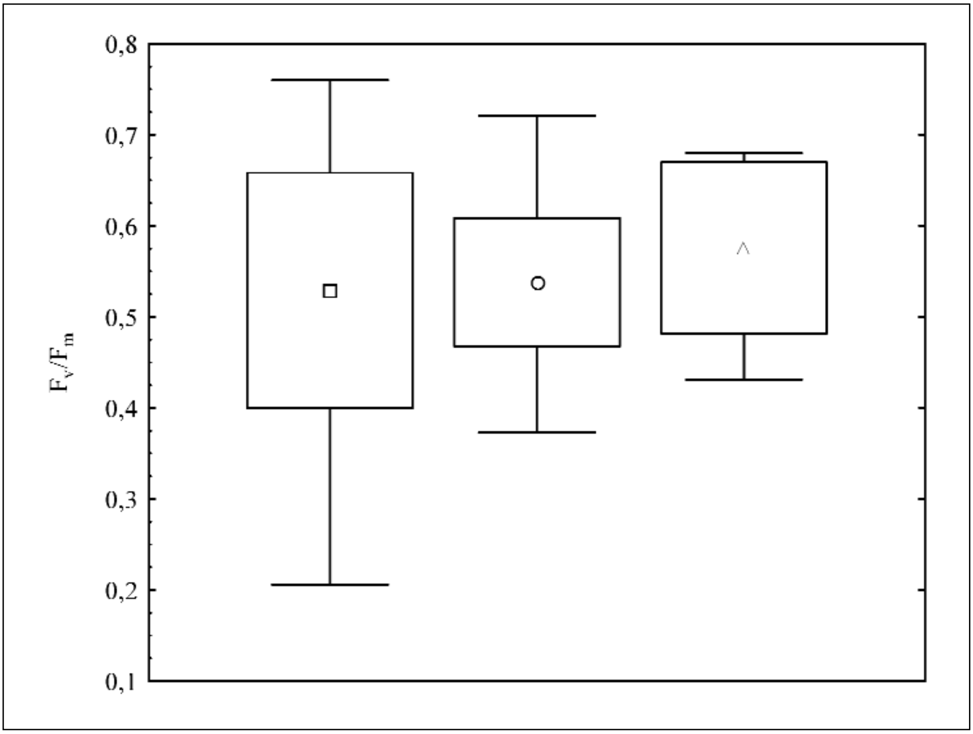

Fig. 1: The ratio of variable fluorescence to maximal fluorescence (F_v/F_m) for individuals from natural population (square symbol), reintroduced (circle) and cultivated individuals (triangle). Means±S.D.

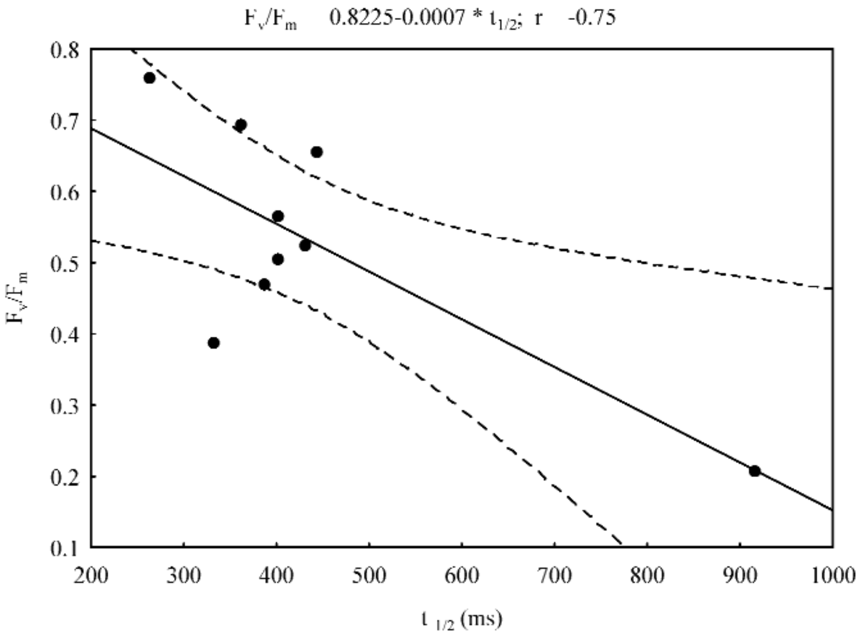

Fig. 2: The relationship between the photosynthetic efficiency (F_v/F_m) and half-rise time ($t_{1/2}$) of individuals from natural population (F_m/F_v = 0.225-0.0007* $t_{1/2}$; r = -0.75).

On the basis of measured parameters of photosynthetic efficiency we could conclude that plant material obtained in micropropagation process is a good source for planting with the purpose of reintroduction. In addition, such similarity in values for F_v/F_m could be a good predictor for successful adaptation of planted individuals, which was proved with a remarkable percent of established individuals in reintroduction experiment (> 99%, Mijović & al. 2005).

Conclusion

An analysis of photosynthetic efficiency showed that the individuals of *N. rtanjensis* obtained in the micropropagation process appear as suitable for planting at the natural habitat and for the cultivation purposes as well. Moreover, we haven't found a significant difference between all investigated groups according to the ratio of variable and maximum fluorescence (F_v/F_m). According to our estimating, an endangered status of this species is not a consequent of decreased photosynthetic efficiency, i.e. low photosynthetic capacity in the means of plant productivity.

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