

Susceptibility and proline content of *Chelidonium majus* L. under drought and salinity stress

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Introduction

Chelidonium majus L. or *Ch. majus* (greater celandine) is a well-known and widespread herbaceous perennial medicinal plant which is native to Europe, Northern Africa and Western Asia. The whole plant contains isoquinoline alkaloids (coptisine, allocryptopine, protopine, berberine, chelidonine, sanguinarine, chelerythrine, etc.). The herb is used for different purposes in traditional medicine in various countries. Nowadays it is a component in a few remedies (Maji and Banerji, 2015; Zielinska et al., 2018).

Drought and soil salinity have a disastrous effect on plant growth and development. They are becoming increasingly serious problems worldwide.

When plants are under the influence of stressful factors such as drought, soil salinity, extreme temperatures, heavy metals, etc., they accumulate different types of metabolites. Among them are amino acids, and especially proline which is overproduced in plants when they are under stress. Moreover, it contributes to stress tolerance (Verbruggen and Hermans, 2008).

The aim of this study is to reveal how different levels of PEG 6000 and NaCl affect plant survival and proline concentration in cultivated plants of *Ch. majus*. PEG and NaCl are used in order to resemble

drought and soil salinity.

Materials and methods

Seed origin

The seeds from *Ch. majus* were collected from plants grown in the village of Mramor, near Sofia, Bulgaria. They were pre-soaked in distilled water for 24 hours before sterilization.

Seed sterilization and germination

The seeds were soaked in 70% ethanol (2 minutes) and sterilized in 0.1% HgCl₂ (2 minutes), then rinsed once with sterile distilled water. Thereafter, the seeds were sterilized with commercial bleach (chlorine < 2.5%) half-diluted with sterile distilled water for 10 minutes and then rinsed three times with sterile distilled water.

The seeds germinated on filter paper moistened with distilled water at 8±2°C in dark for 7 days. Afterwards, they were placed at 23±3°C in dark for 14 days and then in light for 7 days.

Seedling cultivation and proline content

The seedlings were planted in plastic pots filled with 30 g perlite. Firstly, they were watered with liquid medium B5 (Gamborg et al., 1968) supplemented with three different concentrations of PEG 6000 (1%; 5%; 10%) or NaCl (50 mM; 100

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mM; 150 mM). Afterwards, the plants were watered only with liquid medium B5 without sucrose, adjusted to pH 5.5. The plants were cultivated in a growth camera (POL-EKO Aparatura) for 8 weeks. The surviving plants have been presented as a percentage.

Proline was determined according to the method of Bates et al. (1973).

Results and discussion

The highest percentage of surviving plants from the treated ones was observed in the variant with 1% PEG ($80.00 \pm 1.73\%$), which is close to the percentage of the control plants ($96.67 \pm 0.58\%$). The higher the PEG concentration, the lower the survival rate. The highest concentration of PEG (10%) accounted for $3.33 \pm 0.58\%$ surviving plants. The plants are more susceptible to NaCl influence. $73.33 \pm 0.57\%$ of the plants survived at the lowest concentration of NaCl (50 mM NaCl), where the survival rate sharply decreased to $6.67 \pm 0.51\%$ and 0.00% at 100 mM and 150 mM, respectively. The plants cultivated in perlite with PEG accumulated high content of proline. In the studied concentrations its content significantly increased in comparison with the control ($4.59 \pm 0.10 \mu\text{M/g}$) - $38.61 \pm 0.01 \mu\text{M/g}$ for 1%, and $31.46 \pm 0.02 \mu\text{M/g}$ for 5% PEG, where the highest content was observed in the variant with 10% PEG ($162.55 \pm 0.42 \mu\text{M/g}$). Plants cultivated with 50 mM NaCl didn't accumulated high content of proline and its percentage ($6.20 \pm 0.09 \mu\text{M/g}$) is close to that of the control. Because of the absence or low percentages of surviving plants in the other two concentrations proline content wasn't analysed in them.

Plants accumulated higher content of proline under stress, and a lot of research correlated the higher content of proline with adaptation to the stress conditions (Verbruggen and Hermans, 2008). Our study showed that the plants cultivated with PEG accumulated significantly more proline than the variant with NaCl.

According to the content of proline and plant survival rate we can assume that *Ch. majus* can possibly adapt more successfully to drought than salt stress. Significantly higher proline content in all PEG concentrations revealed the species' higher ability to adapt to these conditions.

Conclusion

Cultivated plants of *Ch. majus* displayed good survival rate at low concentrations of PEG and NaCl. Increase in the concentrations decreased the percentage of surviving plants. Proline content was significantly influenced by supplementation of PEG and slightly less by NaCl compared to the control group. In light of our results we could presume that *Ch. majus* is more resistant to drought than to salinity stress and further research is required.

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