EFFECT OF WATER DEFICIT INDUCED BY OSMOTIC STRESS ON BULGARIAN SPRAY-CARNATION *(D. CARYOPHYLLUS F. SPRAY HORT.)* CV. IRA *IN VITRO*

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Abstract

Drought is one of the main unfavourable environmental factors which affect the quality and productivity of ornamental plants. That is why, it is important to study soil drought as a stress factor. Plant responses to the harmful effect of stress factors (drought) are not yet fully understood, as the plant organism employs a large array of adaptive defense reactions to respond to a stress stimulus. By studying the physiological mechanisms of plant resistance in laboratory conditions, the specific responses of the plant culture to a single stress factor can be observed. In our study, to simulate water deficit induced by osmotic stress, different concentrations of polyethylene glycol (PEG) were used: 10%, 20%, 30% and 40%. The model plant was Bulgarian spray-carnation cultivar cv. Ira *in vitro*. The response to drought stress was studied based on the following end-points: plant growth reactions, relative water content (RWC %), and electrolyte leakage (conductivity).

Introduction

Drought is one of the major abiotic factors that suppress the growth and development of cultivated plants and considerably reduce their productivity. Water deficit is its basic component that causes a number of morphological and physiological changes in the plants.

According to the climatic forecasts, rainfalls in Southeast Europe, including the Balkan Peninsula, are expected to increase in winter and decrease during the warm months of the year in the 21st century. The high air temperatures, combined with the rainfall deficit during the summer season will result in higher transpiration and evapotranspiration values. This will increase the risk of all kinds of drought – atmospheric, soil, soil-atmospheric and hydrological (Alexandrov, Genev, 2004).

The research on the physiological mechanisms of plant resistance in laboratory conditions gives an opportunity to monitor the specific response of the plants to one of the impact factors (Yordanov I., Velikova V., Tsonev T. 2000 Alexieva V., Ivanov S., Sergiev I., Karanov E. 2003). The advantage of the controlled conditions is in the recreation and opportunity to study a specific limiting factor in isolation, which would be impossible in natural environment.

The drought simulation was done by means of an osmotic agent with high molecular weight (>3000) such as polyethylene glycol (PEG) (Murillo Amador B., Lopez-Aguilar, Kaya R., Larrinaga-Mayoral J., Flores-Hernandez A. 2002). The osmotically induced water deficit by PEG gave the opportunity to achieve plant dehydration in a wide range, i.e. the closest possible to the effect of the real soil drought. The use of PEG in liquid media allowed achieving precisely and recreating the necessary osmotic potential of the environment (Song, H., Seo, Y., Jeong, M., Kim, H., Im, H., Cho, H., & Choi, M. 2013).

The response of the *in vitro* cultures to induced stress enabled the selection of water deficit tolerant plants at an early stage. This was made possible by the existing correlation in the response of the plants to stress on cellular level – *in vitro* and *in vivo* (Song, H., Seo, Y., Jeong, M., Kim, H., Im, H., Cho, H., & Choi, M. 2013)

The purpose of the investigation was to establish the physiological and adaptive response of the Bulgarian spray-carnation cultivar to drought.

Methodic/Methodogy

For the purpose of the experiment, the plant material of the cv. IRA was reproduced *in vitro* on MS nutritive medium with added saccharose – 30 g/l and agar 6 g/l at pH = 5.7 - 5.8 prior to autoclaving (Murashige T. and Skoog F. 1962). Then it was grown in a phytostatic room at a temperature of 22°C, photoperiod of 16:8 (day:night) hours and light intensity 30 $\mu mol.m^{-2}.s^{-1}$.

For the induction of the experiment, an MS medium was used containing salts and vitamins, saccharose – 30 g/l and polyethylene glycol (PEG 6000) in the following concentrations – 10%, 20%, 30% and 40% with pH = 5.7 – 5.8 prior to autoclaving.

The explants were placed in test-tubes on control (0) and stress inducing liquid medium (PEG – 10%, 20%, 30% and 40%) on filter paper bridges, 10 for each concentration and the control in 3 replications. The duration of stress impact was short (one day), medium (three days) and long-term (six days).

The explants used were 2-3 cm long and had a weight of 100-300 mg, measured in sterile conditions prior to the initiation of the stress inducing medium.

In order to establish the effect of water deficit on plant tissues, we measured the explants' growth and rooting, the rate of cell membranes damage and the relative water content.

In *in vitro* research, growth was expressed as the percentage of microexplants' weight increase after being cultivated for a certain period of time (1, 3 and 6 days) on 10%, 20%, 30% and 40% PEG, compared to the initial weight.

The rate of the membrane damage was defined by the electrolyte leakage from the leaves with accounting for conductivity only after stress and was expressed as μS/g fresh weight.

The relative water content (RWC) was measured simultaneously with electrolyte leakage and calculated by the following formula:

RWC % = (fresh weight – dry weight) / (turgor weight – dry weight) X 100 – according to Turner's method (Turner N.C.1981).

The water deficit (WD) was expressed by the following formula:

WD % = 1 – RWC.

Following the stress period, the explants were transferred to an MS medium without a stress agent (PEG) in order to establish their capacity for recovery and report the rooting percentage.

The data on the figures below were expressed as an average value ± SE of two independent experiments, carried out in 10 replications per variant. They were analyzed for significance by means of the t-test of the GraphPad Prizm software. The results were statistically significantly different at $P<0.05$ (*), $P<0.01$ (**), $P<0.0001$ (***), respectively, as compared to the control.

Results

Following the exertion of a short-term osmotic stress (1 day), the explants, grown on the control medium, showed a slight growth and reached values up to 109.1 ± 2.1 (Fig. 1). The

growth decreased proportionally to the increase of PEG concentration in the nutritive medium, it was less than 50% vs. the control in the high PEG concentrations (30% and 40%), 32.02 ± 1.97 and 33.55 ± 2.8, respectively, with significance rate P<0.0001. The suppression of growth was related to the reduced capacity of the plant to uptake water (Shabani A, Sepaskhah AR, Kamgar-Haghighi AA. 2013). The effect of the osmotic stress on cellular level is expressed by slowing down cell division, they lose their turgor and this leads to weight loss (Levitt, J. 1980; Heyser, J., Nabors, M. 1981). In chrysanthemum, the values of the fresh weight decreased up to 50% vs. the control. The plants were wilting in low PEG concentrations (10% and 20%), while 20% of the trial explants became necrotic in addition to wilting in the high concentrations (30% and 40 %) (Zapryanova, Nencheva, 2013).

The reported growth values vs. the control were 56.2% at 30% PEG and 66.0% at 40% PEG in the extended treatment periods (3 and 6 days) (Fig. 1). The control plants were fresh, green and in normal turgor condition and showed initial rooting on the 6th day. The plants placed on 10% and 20% PEG concentration showed slight wilting, compared to the higher wilting rate on 30% and 40 % and no explants necrosis.

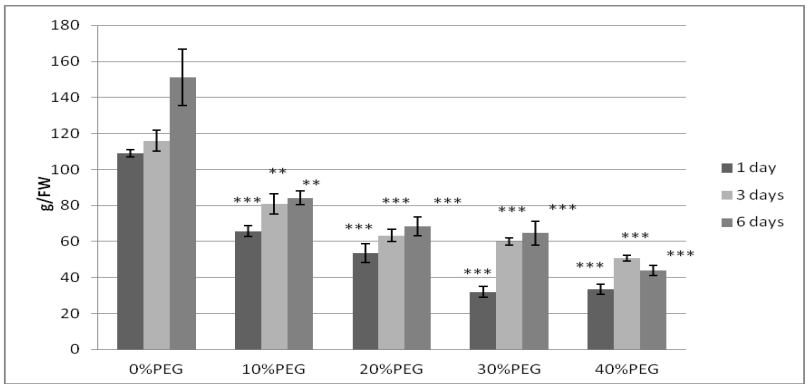

Fig. 1. Growth (gain in fresh weight) of spray-carnation explants cv. IRA *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The index of the relative water content (RWC) is often used to define the water status. It decreases with the increase of the PEG concentrations in plant tissues. The control plants maintained a high percent of water content in their tissues – about 80% (Fig. 2). The results of day 1 showed that the exerted PEG stress lead to the gradual decrease of plant water content, the lowest values of 28.52 ± 5.2 being reported at 40% PEG concentration. The difference between the separate variants and the control were statistically significant at P<0.0001. That tendency was maintained on both the 3rd and 6th days. The results for all PEG concentrations vs. the control were statistically significant at P<0.05 and P<0.0001.

A similar response was observed in callus culture of *Carthamus tinctorius* L. – the lowest growth values and RWC percentage were reported for 40% PEG concentration (Kakaei, M., Mansouri, M., Abdollahi, M. R., Moradi, F. 2013).

The long-term drought stress in chrysanthemum strongly reduced the water potential of the cell, lead to the decrease of tissue turgor and final wilting of the plants, especially in the higher PEG concentration. Whereas the values of the control plants were constant at about 70%, they reached 30% on the 6th day at 40% PEG concentration (Zapryanova, Nencheva, 2013).

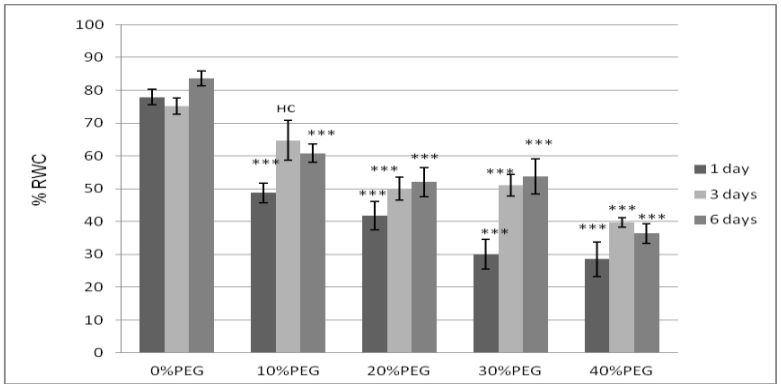

Fig. 2. Relative water content (RWC) of spray-carnation explants cv. IRA *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The damage of cell membrane organization and content is one of the first responses of the plant organism to the impact of the stress agent. Electrolyte leakage in control plants was observed in low rates on the 1st day after trial initiation and was maintained throughout the trial duration – below 200 µS/g fresh weight that corresponded to the normally expected response of the studied material.

The simulated experimental drought showed a sharp increase of electrolyte leakage during short-term stress (1 day) in all the used PEG concentrations (Fig. 3). The highest values – 2633±521 µS/g fresh weight were reported at 40% PEG concentration (Fig. 3). The values of the electrolyte leakage at different PEG concentrations during the 3- and 6-day stress were constantly higher than the control plants but lower in comparison to the results of the short-term stress. The results had a good statistical significance between the separate variants and the control at $P<0.05$ and $P<0.0001$.

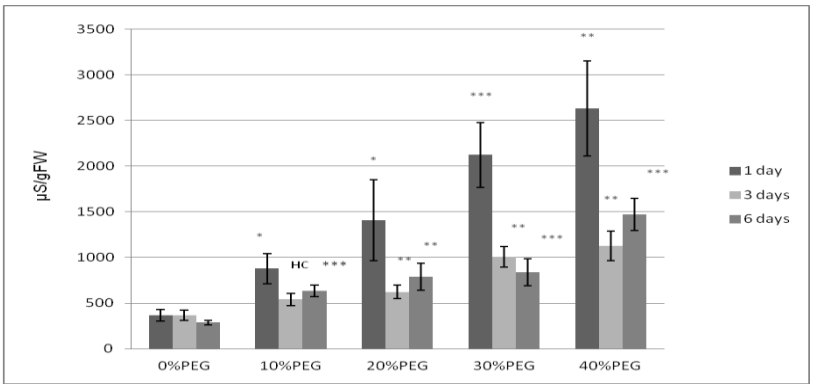

Fig. 3. Electrolyte leakage from spray-carnation explants cv. IRA *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The water deficit was about 20% in the control plants, whereas it varied between 30-70% at different PEG concentrations. According to the concept of Cornic G., Fresneau C. 2002, dehydration that causes up to 30% water deficit in plants, is assumed as mild or moderate stress. The increase of water deficit values up to 39.22% was reported as early as the 1st and 3rd days at the low PEG concentration, i.e. 10% (Fig. 4). The highest values of this index were observed during the long-term stress (6-day period), namely 69% and 71% at 30% and 40% PEG.

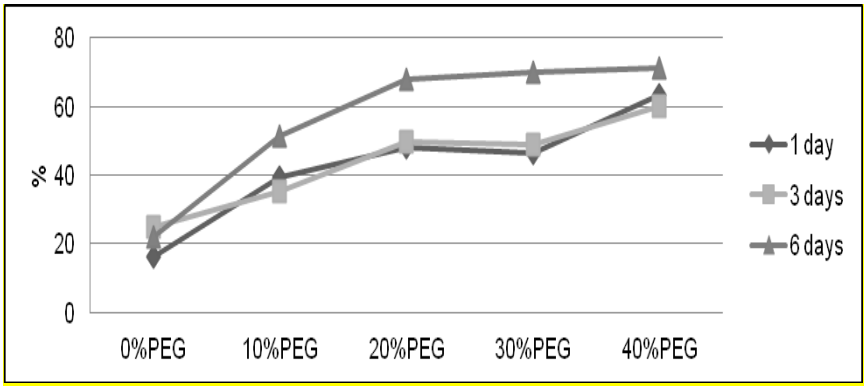

Fig. 4. Water deficit (%) in the plant tissues of spray-carnation explants cv. IRA *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The spray-carnation explants were placed on MS medium after the different stress periods for recovery. The results showed that the recovery is 100% after the short-term stress (1 day). Rooting was reported for all the concentrations, regardless of PEG quantity.

The plants recovered 100% after the medium-term stress (3 days) at PEG concentrations – 10%, 20% and 30%, while only 80% recovered at 40% PEG concentration. The best manifestation of the rooting – 80% – was at 10% PEG, whereas it was expressed to a smaller degree – only 10% – at the higher PEG concentrations of 30% and 40 %.

The long-term 6-day stress of PEG showed 100% recovery of the plants at 10% and 20 % PEG concentrations but the percentage of rooted plants went down to 50. The recovery at higher PEG concentrations of 30% and 40 % was 60% and 40 %, respectively, but there was no record of rooting.

Conclusions

The growth of the explants proportionally decreased with the increase of polyethylene glycol concentration from 10% to 40% and the fresh weight was below 50% vs. the control at 30% and 40% PEG.

The drought, simulated by means of different polyethylene glycol concentrations, caused changes in the cell membranes of spray-carnation cv. IRA. The highest values of electrolyte leakage up to 2633 ± 521 µS/g fresh weight were reported on the 1st day at 40% PEG concentration.

The relative water content of the plant tissues decreased depending on PEG quantity, the lowest values – 28.52 ± 5.2 being reported at 40% PEG concentration on the 6th day.

The water deficit varied within 30% - 70% depending on PEGN concentration.

The explants of cv. IRA showed a good adaptive response that was confirmed by the high recovery percentage - 60% and 40 %, reported for the high PEG concentrations - 30% and 40%, respectively.

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