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THE RESPONSE OF BULGARIAN SPRAY-CARNATION (*D. CARYOPHYLLUS* F. *SPRAY,* HORT.) CV. 'RUSALKA' TO DROUGHT - *IN VITRO* INDUCED BY DIFFERENT PEG CONCENTRATIONS

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Abstract

In our study, to simulate water deficit induced by osmotic stress, different concentrations of polyethylene glycol (PEG-6000) were used: 10%, 20%, 30% and 40% at different durations of treatment (1, 3 and 6 days) in vitro conditions. The model plant was Bulgarian spray-carnation (D. caryophyllus f. spray, Hort.) flowers, cv. 'Rusalka'. The response to drought stress was studied based on the following end-points: plant growth reactions, relative water content (RWC %), and electrolyte leakage (conductivity). The water deficit varied from 16% (control) to 75% (40% PEG-6 days). 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 relative water content of the plant tissues decreased depending on PEG quantity, the lowest values - 25.16 ± 2.06% being reported at 40% PEG concentration on the 6th day. The highest values of electrolyte leakage up to 1712 µS/g fresh weight were reported on the 6th day at 40% PEG concentration.

Key words*: spray-carnation, drought, polyethylene glycol (PEG), water deficit, growth.*

INTRODUCTION

The ongoing worldwide climate changes enforced multiple studies of how the plants react towards them. One of the main components of the drought is the water deficit, an abiotic factor that causes multiple morphological and physiological changes in the plants that reduce their quality and economic value.

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 et al., 2000; Alexieva et al., 2003).

The drought simulation was done by means of an osmotic agent with high molecular weight (>3000) such as polyethylene glycol (PEG) (Murillo Amador et al., 2002). The use of PEG in liquid media allowed achieving precisely and recreating the necessary osmotic potential of the environment (Song et al., 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 et al., 2013)

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

MATERIALS AND METHODS

For the purpose of the experiment, the plant material of the *D. caryophyllus* f. *spray,* Hort.*)* cv. 'Rusalka' was reproduced *in vitro* on MS nutritive medium with added sacharose - 30 g/l and agar 6 g/l at pH = 5.7-5.8 prior to autoclaving (Murashige and Skoog, 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 µmol 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 micro explants' 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) × 100 - according to Turner's method (Turner, 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 AND DISCUSSIONS

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 102.8 ± 5.8 (Figure 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%), 58.94 ± 4.4 and 59.96 ± 2.5, respectively, with significance rate $P<0.0001$. Following short-term (one-day) osmotic stress, the explants showed little growth demonstrated by its gain in fresh weight (Figure 1).

The results were statistically significantly different at $P<0.05$ (*), $P<0.01$ (**), $P<0.0001$ (***), respectively, as compared to the control.

The reported growth values were 33.78 ± 6% at 30% PEG and 30.32 ± 2.7% at 40% PEG in the extended treatment periods (3 and 6 days) (Figure 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. At higher PEG concentrations and longer exposure times (3-6 days), a progressive decrease in the growth was observed: down to 50% below the control at 30% and 40% PEG. The control plants were fresh, green and with normal turgor on day 6 while the plants exposed to 10% and 20% PEG showed withering signs which were even more notable in the 30% and 40% PEG plants.

The suppression of growth was related to the reduced capacity of the plant to uptake water (Shabani et al., 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, 1980; Heyser and Nabor, 1981). Due to the low turgor pressure cell expansion and cell growth suppresses under water stress (Jaleel et al., 2009). 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 and Nencheva, 2013).

In Bulgarian spray carnation (*D. caryophyllus* f. *spray,* Hort.) flowers, cv IRA a progressive decrease in the growth was observed: down to 50% below the control at 30% and 40% PEG (Zapryanova et al., 2015).

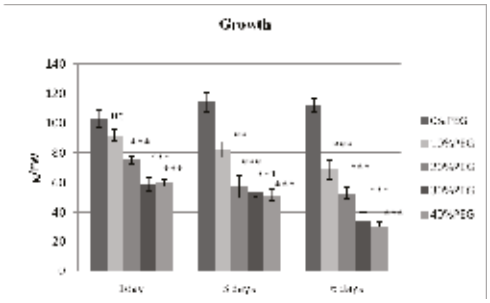

Fig. 1. Growth (gain in fresh weight) of *spray-carnation* explants cv. 'Rusalka' *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

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% (Figure 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 44.62 ± 2.3, 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 (29.25 ± 2.3) and 6th days (25.16 ± 2.06) lowest results for PEG. The results for all PEG concentrations vs. the control were statistically significant at P<0.05 and P<0.0001 (Figure 2).

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 et al., 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 and Nencheva, 2013).

In experiments with Bulgarian spray carnation (*D. caryophyllus* f. *spray,* Hort.) flowers, cv. 'IRA', the control explants (without PEG) maintained high RWC in their tissues: about 80%, regardless of the cultivation time (1, 3 or 6 days). The results clearly indicate that PEG-induced stress led to a gradual decrease in the explants' RWC. The lowest RWC values (28.52 ± 5.2) were obtained in the 40% PEG variant on day 6. Prolonged drought resulted in low RWC of cells and in low tissue turgor and irreversible withering, especially at higher concentrations (Zapryanova et al., 2015).

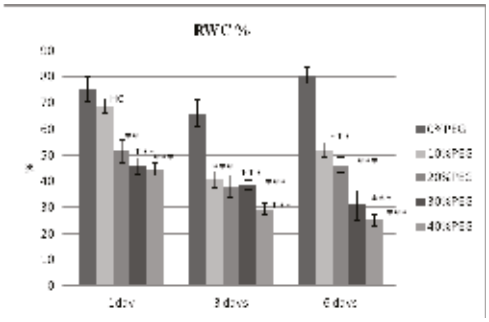

Fig. 2. Relative water content (RWC) of *spray-carnation* explants cv. 'Rusalka' *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

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 (μS/g fresh weight): 396.78 ± 54.8 - 1st day, 226.53 ± 27.9 - 3rd day and 292.478 ± 37.58 - 6th day 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 (Figure 3). The highest values – 1712 ± 363 μS/g fresh weights were reported at 40% PEG concentration - 6th day (Figure 3). The values of the electrolyte leakage at different PEG concentrations during the 3 and 6 days 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 (Figure 3).

Electrolyte leakage in the Bulgarian spray carnation (*D. caryophyllus* f. *spray,* Hort.) flowers, cv IRA - control plants was very low at day 1 and remained under 200 μS g^{-1} during the whole experiment, which is in good

correspondence with the expected normal values for the chosen plant material. The highest values 2633 ± 521 µS/g fresh weight were reported at 40% PEG concentration (Zapryanova et al., 2015).

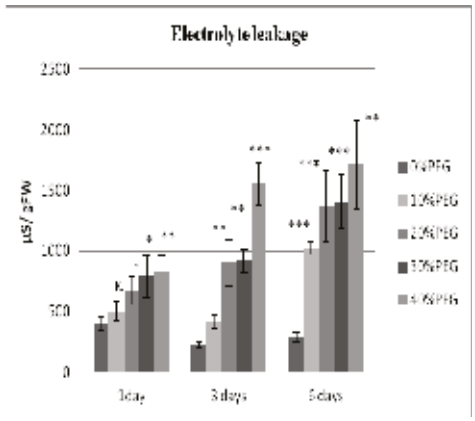

Fig. 3. Electrolyte leakage from *spray-carnation* explants cv. 'Rusalka' *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

The highest values of water deficit (WD %) were reported during the long-term stress (6 days period), namely 69% and 76% at 30% and 40% PEG (Figure 4). These results indicate that the drought stress induced in our experiments was of a moderate degree but developed rapidly. 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.

A similar response was observed in experiments with Bulgarian *spray carnation* (*D. caryophyllus* f. *spray,* Hort.) flowers, cv. 'IRA'. Dehydration of plant tissues to 69% for the PEG 30% variant and to 71% for the PEG 40% variant on day 6th was observed, whereas the values on day 1 were 46.2% and 63.62%, respectively (Zapryanova et al., 2015).

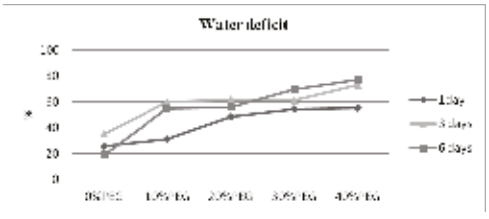

Figure 4. Water deficit (%) in the plant tissues of *spray-carnation* explants cv. 'Rusalka' *in vitro* at different PEG concentrations and stress duration (1, 3 and 6 days)

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: 100% for 10% and 20% PEG; 75% for 30% PEG and 60% rooting - for 40% PEG.

The plants recovered 100% after the medium - term stress (3 days) at PEG concentrations - 10%, 20% and 30%, while only 90% recovered at 40% PEG concentration. The best manifestation of the rooting - 90% - was at 10% PEG, where it was expressed to a smaller degree at the higher PEG concentrations of 30% - 50% rooting and 40% - 30% rooting.

The long-term 6 days stress of PEG showed 100% recovery of the plants at 10%, 20% and 30% PEG concentrations but the percentage of rooted plants went down to 50. The recovery at higher PEG concentrations of 40% was 30% and rooting only 10%.

The explants of *spray-carnation* 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 (Zapryanova et al., 2015).

In conditions of moderate PEG-induced drought stress- *in vitro*, the explants of Bulgarian *chrysanthemum* cv. 'Zhoro' showed an adaptive response, rather than stress-induced damage, as inferred from the relatively high recovery rate (80% and 60%) following 30% and 40% PEG treatment (Zapryanova and Nencheva, 2013).

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. 'Rusalka'. The highest values of electrolyte leakage up to 1712 ± 363 µS/g fresh weight were reported on the 6th day at 40% PEG concentration.

The relative water content of the plant tissues decreased depending on PEG quantity, the lowest values – 25.16 ± 2.06% being reported at 40% PEG concentration on the 6th day.

The water deficit varied within 18% - 76% depending on PEG concentration and durations of treatment.

The explants of *spray-carnation* cv. Rusalka showed a good adaptive response that was confirmed by the high recovery percentage - 100% and 30 %, reported for the high PEG concentrations - 30% and 40%, respectively on the 6th day.

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