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EFFECTS OF PEG 6000 STRESS ON STRAWBERRY (*FRAGARIA* × *ANANASSA* DUCH.) *IN VITRO* PROPAGATION

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Abstract

The aim of the study was to assess the drought tolerance of two varieties of strawberry, Tecla and Hecker, according to their reaction by morphometric characteristics to the presence of PEG 6000 in different concentrations in culture media (0 g/L - control, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L). The results show that cv. Hecker exhibited the lowest value of dry weight (66.08 ± 12.17) at 50 g/L PEG concentration. The highest concentrations of PEG 6000 (40 g/L and 50 g/L) considerably decreased the DW in the plantlets in both Tecla and Hecker cvs. High concentrations of PEG 6000 decreased significantly the plantlets' length and proliferation rate in both cultivars. A dramatic decrease (71.77%) in average height was recorded in cv. Hecker at the highest concentration of PEG 6000 (50 g/L) when compared to the control, from 8.68 ± 0.13 cm to 2.45 ± 0.19 cm (PEG 6000 50 g/L). These results suggest that PEG 6000 disrupted the water metabolism in both strawberry cultivars.

Key words*: drought stress, polyethylene glycol, proliferation rate, strawberry.*

INTRODUCTION

Due to the climatic changes, plants are exposed to various abiotic stresses: water deficit, excess water, low temperatures, high temperatures, high light intensity, salinity and heavy metals (Sah et al., 2020; Albergaria et al., 2020; Zhang et al., 2020, Hassan et al., 2020; Arteaga et al., 2020). As a consequence of global warming, some plant species face new environmental conditions for which they are not adapted (Vuksanović et al., 2019). Drought is one of the main factors of abiotic stress that limits plant growth and productivity (Nadeem et al., 2019).

Drought stress imposes changes in important plant growth and development processes, including germination, plant size, dry matter production and distribution, flower and fruit development and plant maturity (Anjum et al., 2017).

In vitro cultures have been used to assess drought tolerance for different species: olive (*Olea europaea*) (Baccari et al., 2016), strawberry (*Fragaria ananasa*) (Salma et al., 2016), guava (*Psidium guajava* L.) (Youssef et al., 2016), chickpeas (*Cicer arietinum* L.) (Hussein et al., 2017) quinoa (*Chenopodium Quinoa* Willd.) (Telahigue and Toumi, 2017).

Plant response to hydric stress is a complex phenomenon and *in vitro* culture could be used to study the numerous physiological and biochemical changes in all plant organs, as a consequence of the reduced availability of water (Gupta et al., 2016; Peiro et al., 2020).

In vitro simulation of drought stress using chemical reagents, such as polyethylene glycols (PEG), constitutes a convenient way to assess, in controlled conditions, the effects of drought on plant growth and development (Khayatnezhad et al., 2010).

Polyethylene glycol PEG 6000 is a polyether compound derived from petroleum that is usually used to simulate drought stress in *in vitro* culture conditions (Vuksanović, 2019). The PEG 6000 is a non-penetrating and nontoxic osmotic substance, and the addition of this selective agent to the *in vitro* culture media determines variations of the water potential of plants (Sen et al., 2013; Gupta et al., 2016; Beyaz, 2019).

Genotypic differences in drought tolerance have been evaluated for various crop species (Bota et al., 2001; Herralde et al., 2001; Zhong et al., 2018) including strawberry (Hussein et al., 2017). However, there is still a lack of

information about morpho-physiological changes in different *in vitro* cultured strawberry cultivars under limited water availability (Adak and Kaynak, 2020).
Therefore, the main objective of this study was to evaluate the drought tolerance of two *in vitro* cultured varieties of *Fragaria* × *ananassa* Duch. The addition of PEG in different concentrations into the culture media determined plant morphological changes which have been quantified by morphometric analysis.

MATERIALS AND METHODS

Two varieties of strawberry were tested: Tecla and Hecker. Five shoot tips were cultured per 720 ml glass jar with 100 ml of Murashige & Skoog (1962) (MS) medium supplemented with 0.2 mg/L N6-benzyladenine (BA), 4 g/L Plant Agar and 30 g/L sugar.
The experimental design included six treatments/concentrations of PEG 6000: 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L and control with three repetitions/jar per treatment and cultivar. The PEG 6000 was added to the culture media before adjusting the pH and before autoclavation. The pH of the media was adjusted to 5.8. The media were autoclaved at 120°C for 20 minutes. After inoculation, the culture vessels were incubated in the growth room with a controlled environment (23 ± 3°C, 27-32.4 mmol·m^{-2}·s^{-1}, 16 h photoperiod). After 10 weeks of *in vitro* culture, fresh weight, dry weight of plantlets and plantlet heights were measured and the plantlets resulted by axillary shoot proliferation at the end of the culture cycle were counted and proliferation rates were calculated.
To determine the dry wheight (DW) of plantlets of each experimental group and the control plantlets, first the fresh weights (FW) of samples were measured. Next, samples were dried at 45°C for 48 h and their DW were determined.
Data Analysis
To analyse the data, ANOVA analysis was performed first to check the differences among the means. When the null hypothesis was rejected, Tukey's HSD test (P≤0.05) was performed to determine the means that were significantly different from each other. Values shown are means ± SE.

RESULTS

In this study, the *in vitro* studies for drought tolerance assessment was achieved using two strawberry cultivars and five concentrations (i.e. 10, 20, 30, 40, and 50 g/L) of polyethylene glycol (PEG).
Results showed that a high PEG concentration in the culture media had a negative effect on the growth and development of the plantlets during the *in vitro* multiplication phase on the MS medium supplemented with 0.2 mg/L BA.
The results of Tukey's HSD test (P≤0.05) show that there was a statistically significant difference in the dry weight of plantlets grown on culture media supplemented with different concentrations of PEG versus control (without PEG) in both strawberry cultivars and the DW was PEG 6000 concentration dependent.
Our results show that cv. Hecker exhibited the lowest value of dry weight (66.08 ± 12.17) at 50 g/L PEG concentration. Furthermore, the highest concentrations of PEG 6000 (40 g/L and 50 g/L) considerably decreased the DW in both cv. Tecla and cv. Hecker plantlets (Figure 1).

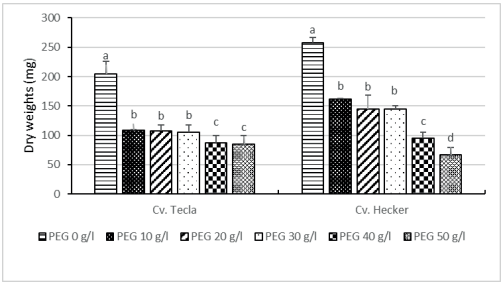

Figure 1. Effect of PEG 6000 on *in vitro* cultured strawberry cv Tecla and cv Hecker dry weights (PEG 0 g/L: MS+0.2 mg/L BA without PEG 6000 - control; PEG 10 g/L: MS+0.2 mg/L BA+10 g/L PEG 6000; PEG 20 g/L: MS+0.2 mg/L BA+20 g/L PEG 6000; PEG 30 g/L: MS+0.2 mg/L BA+30 g/L PEG 6000; PEG 40 g/L: MS+0.2 mg/L BA+40 g/L PEG 6000; PEG 50 g/L: MS+0.2 mg/L BA+50 g/L PEG 6000). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test (p≤ 0.05)

Regarding the number of plantlets it was observed that the addition of PEG 6000 into the culture media variants used for the *in vitro* plant multiplication phase led to a decrease in the number of plantlets/culture jar.

The average number of plantlets /culture jar decreased from 52.6 ± 0.29 (without PEG) to 38 ± 0.51 (50 g/L PEG) at cv. Tecla and from 38 ± 0.25 (without PEG) to 19.66 ± 0.29 (50 g/L PEG) in case of cv. Hecker. The differences between the control variant (0 g/L PEG) and the other treatments with different PEG concentrations (10-30 g/L) were statistically significant in both Tecla and Hecker cultivars (Figure 2). In the case of both cultivars, there was no statistically significant difference regarding the average number of plantlets /culture jar recorded in the variants supplemented with 40 g/L PEG and 50 g/L PEG, respectively.

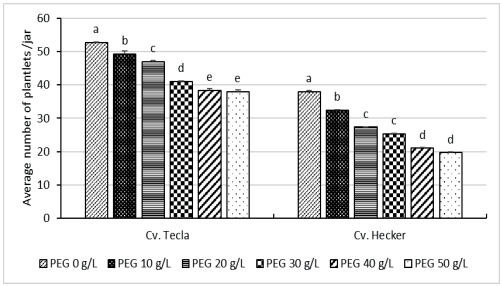

Figure 2. Effect of PEG 6000 on *in vitro* cultured strawberry cv Tecla and cv Hecker average number of plantlets/culture jar (PEG 0 g/L: MS+0.2 mg/L BA without PEG 6000 - control; PEG 10 g/L: MS+0.2 mg/L BA+10 g/L PEG 6000; PEG 20 g/L: MS+0.2 mg/L BA+20 g/L PEG 6000; PEG 30 g/L: MS+0.2 mg/L BA+30 g/L PEG 6000; PEG 40 g/L: MS+0.2 mg/L BA+40 g/L PEG 6000; PEG 50 g/L: MS+0.2 mg/L BA+50 g/L PEG 6000). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

The same decreasing trends of the values were registered at the proliferation rate/ inoculum, compared to the increase of the PEG concentrations. The proliferation rate decreased from 10.53 ± 0.99 (without PEG) to 7.60 ± 0.64 (PEG 50 g/L) in case of cv. Tecla. Hecker's results show values from 7.6 ± 0.51 (control variant) to 3.9 ± 0.26 (PEG 50 g/L) (Figure 3). The increase in PEG concentration in the culture media was accompanied by a general decrease in the plantlets' height in both Tecla and Hecker plantlets (Figure 4). It is worth mentioning that on MS supplemented with 0.2 mg/L BA Tecla regenerants recorded higher values of proliferation rate than Hecker, but the average height of the plantlets was smaller, with an average of 5.48 ± 0.15 cm compared to 8.68 ± 0.13 cm on Hecker cultivar (Figure 5).

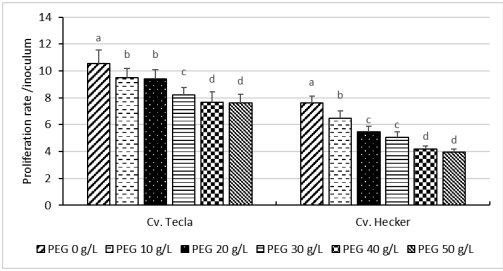

Figure 3. Effect of PEG 6000 on *in vitro* cultured strawberry cv Tecla and cv Hecker proliferation rate/ inoculum (PEG 0 g/L: MS+0.2 mg/L BA without PEG 6000 - control; PEG 10 g/L: MS+0.2 mg/L BA+10 g/L PEG 6000; PEG 20 g/L: MS+0.2 mg/L BA+20 g/L PEG 6000; PEG 30 g/L: MS+0.2 mg/L BA+30 g/L PEG 6000; PEG 40 g/L: MS+0.2 mg/L BA+40 g/L PEG 6000; PEG 50 g/L: MS+0.2 mg/L BA+50 g/L PEG 6000). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

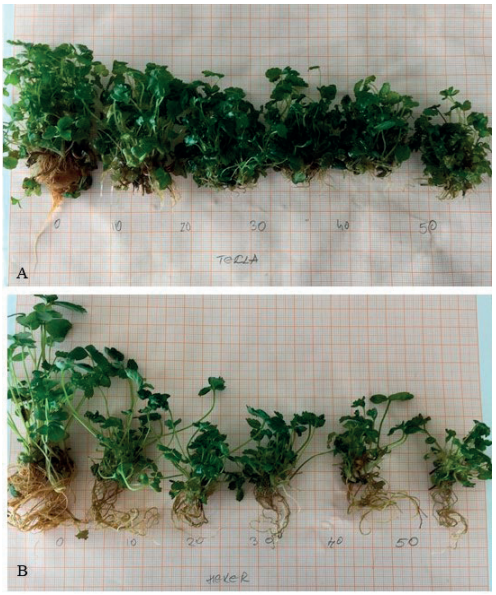

Figure 4. *In vitro* culture of strawberry with different PEG 6000 concentrations: A - cv. Tecla and B - cv. Hecker

There were differences between the analyzed strawberry genotypes referring to their reaction to the used PEG concentrations. In both cases, there was an inverse correlation between the PEG concentration and the height of the plantlets. Our results show that high

concentrations of PEG (30, 40, and 50 g/L) strongly inhibited the growth of Hecker plantlets but there was no statistically significant difference between the length of plantlets from Hecker and also Tecla cultivars (Figure 5).

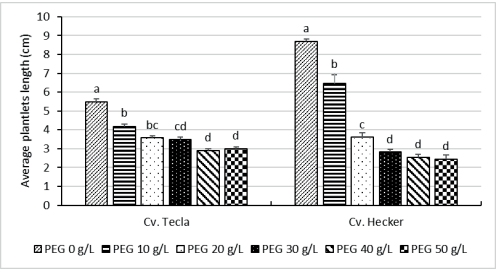

Figure. 5. Effect of PEG 6000 on *in vitro* cultured strawberry cv Tecla and cv Hecker: average plantlet height (PEG 0 g/L: MS+0.2 mg/L BA without PEG 6000 - control; PEG 10 g/L: MS+0.2 mg/L BA+ 10 g/L PEG 6000; PEG 20 g/L: MS+0.2 mg/L BA+ 20 g/L PEG 6000; PEG 30 g/L: MS+0.2 mg/L BA+ 30 g/L PEG 6000; PEG 40 g/L: MS+0.2 mg/L BA+ 40 g/L PEG 6000; PEG 50 g/L: MS+0.2 mg/L BA+ 50 g/L PEG 6000). The values shown are means ± SE. Different lowercase letters above the bars indicate significant differences between the means of different treatments according to Tukey's HSD test ($p \leq 0.05$)

In this study, the average length of Tecla plantlets grown on culture media supplemented with 50 g/L was reduced to 42.76% compared to the control variant (without PEG), while the Hecker plantlets recorded only 28.26% of the plantlets length of control variant, as can be observed in Figure 5.

DISCUSSIONS

Strawberry (*Fragaria×ananassa* Duch) is a small fruit crop of prime importance all over the world (Thokchom et al., 2019). However, like other horticultural crops, the performance of strawberry is negatively affected by environmental stress, especially water stress (Mozafari et al., 2019; Yosefi et al., 2020). The results obtained in this study were in agreement with those reported by Erdogan et al. (2016) who reported that diminishing water supply caused a gradual decrease in strawberry plant growth under water deficit stress.

Our results show that water stress, induced by the incorporation of PEG 6000 in culture media, can adversely affect the development of *in vitro* strawberry plantlets, results confirmed in previous studies performed on other plant species (Kacem et al., 2017; Islam et al., 2019). *In vitro* screening techniques are highly recommended in many studies for minimizing the impact of the changing external experimental conditions (Piwowarczyk et al., 2014). The simulation of drought conditions is relatively easy since non-penetrating and inert polyethylene glycol (PEG) induces water stress in plants (Rai et al., 2011). Until now, *in vitro* studies on the influence of water stress on the regeneration ability of cultivars from the genus *Fragaria* have received less attention (Hussein et al., 2016; Thokchom et al., 2019).

In general, the findings that PEG reduced the multiplication rate and plantlet vigour of strawberry cultivars are similar to those reported for *in vitro* drought screening of other plant species (Rai et al., 2011; Piwowarczyk et al., 2014; Ahmad et al., 2020). Our results suggest that the interaction between PEG and varieties leads to decreased plantlets length in Tecla and Hecker cultivars (Figure 5) and more profound decrease was observed in cv Hecker as compared to cv Tecla.

It is interesting to note that the intensity of multiplication rate and plantlets vigour reduction under certain levels of PEG treatment (40 and 50 g/L) was not genotype dependent, and this is in contrast with the results reported by Thokchom et al. (2019). With increasing concentration of polyethylene glycol, the plantlets' vigour declined. According to Jaleel et al. (2009) this may be a consequence of greatly suppressed cell elongation as a result of the low turgor pressure due to the low hydraulic conductivity in plant cells.

CONCLUSIONS

The results of the present study showed that PEG-drought stress as well as difference in varietal traits leads to alter different morpho-physiological characteristics of strawberry plantlets.

Different levels of PEG treatments and varieties cause certain changes in the growth attributes as well as in the DW of strawberry plantlets.

The treatments with concentrations of 20 and 30 g/L PEG 6000 had lower effects than 40 and

50 g/L PEG in all the analysed morphological parameters. Also, our study showed that Tecla showed better performance in most of the morphophysiological parameters as compared to Hecker variety.

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