Scientia Horticulturae 229 (2018) 167–174

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

Controlled drought stress affects the chilling-hardening capacity of tomato seedlings as indicated by changes in phenol metabolisms, antioxidant enzymes activity, osmolytes concentration and abscisic acid accumulation

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ARTICLE INFO

Keywords:
Abscisic acid
Anthocyanin
Antioxidant enzymes
Hardening
Hydrogen peroxide

ABSTRACT

To elucidate the physiological, biochemical and hormonal mechanisms of chilling stress mitigated by drought pretreatment, tomato seedlings (*Lycopersicon esculentum* cv. C.H Falat) were pretreated by 0, 10 and 20% Polyethylene glycol (PEG) for 7 days followed by subjecting to chilling stress at 3 °C for 6 days and 6 h per day. Results showed that PEG-induced drought improved growth rate of tomato seedling subjected to chilling stress and enhanced their antioxidant enzyme activity, abscisic acid (ABA), anthocyanin accumulation, potassium (K^+) and proline content compared with the control (0% PEG) at the end of chilling stress period. PEG pretreatment provided significant protection against chilling stress and reduced poly phenol oxidase (PPO) activity, electrolyte leakage (EL) and H_2O_2 content in root and leaf of chilled seedlings. Moreover, when PEG pretreatment was applied with chilling stress, we observed an alleviation in the growth impeding and a decrease in chilling symptoms as compared to control seedlings. The highest chilling tolerance was induced by application 20% of PEG. In general, the results indicate that PEG-induced drought, by altering in some tolerant responses, could be effectively used to protect tomato's seedling from the adverse effects of low temperatures stress.

1. Introduction

The low temperature is considered as one of the environmental factors influencing crop production, geographical distribution and the growth and development of many plants (Allen and Ort, 2001; Saltveit, 2001). During seed germination and early stage of plant growth, chilling can remarkably affect crop production and cause some physiological and biochemical characteristics through damaging to cellular membranes integrity, generating Reactive Oxygen Species (ROS), destroying protein and accumulating toxic compounds (Allen and Ort, 2001; Kuk et al., 2003; Nayyar et al., 2005).

Due to its low level of calories and being as an excellent source of C and A vitamins as well as containing an antioxidant called lycopene, tomato among other plants is extensively consumed in the world (Rai et al., 2013). Similar to other tropical crops, tomato is sensitive to chilling stress and easily damages at temperatures below 10 °C (Malekzadeh et al., 2014; Pardossi et al., 1988; Spicher et al., 2016). Although the environmental condition of Iran is suitable for tomato cultivation, chilling stress usually takes place at early stage of growth season and causes to delay its harvest, and in case of aggravating condition it even leads to destroy this plant.

Applying different environmental conditions and cultivation techniques within greenhouse can mitigate the chilling stress injuries of seedlings (Dong et al., 2013; Krasensky and Jonak, 2012; Li et al., 2013). For example, it has been revealed that water deficit can improve some morphological and physiological characteristics such as osmoregulation, change at plant hormone levels and stomata conductance, reduction in the growth of aerial parts of plants, and elevation in root growth and induction of plants' tolerance against environmental stresses (Bahrun et al., 2002; Banon et al., 2003; De Juan Javier et al., 1997; Hoffman et al., 2012) by which the tolerance of plants may be reinforced in coming stresses. This phenomenon is so-called cross-resistance or cross-adaptation and it means that exposing plants to stressful conditions can induce plant tolerance to upcoming stresses (Cayuela et al., 2007; Gong et al., 2001). Li et al. (2015) stated that under chilling condition, the drought-pretreated wheat plants possessed higher antioxidant activity, and hence reduced the oxidative damage to the plants, resulting in higher grain yield. Amundson et al. (1993) reported that moderate drought stress, through altering the rate of carbohydrate accumulation, induced better chilling tolerance in red spruce

Abbreviations: ABA, abscisic acid; CAT, catalase; CI, chilling injury index; Chl, chlorophyll; EC, electrical conductivity; EL, electrolyte leakage; H_2O_2, hydrogen peroxide; POD, peroxidase; PEG, polyethylene glycol; PPO, polyphenol oxidase; ROS, reactive oxygen species; RDW, root dry weight; SDW, shoot dry weight; SOD, superoxide dismutase

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http://dx.doi.org/10.1016/j.scienta.2017.10.009
Received 22 December 2016; Received in revised form 9 September 2017; Accepted 10 October 2017

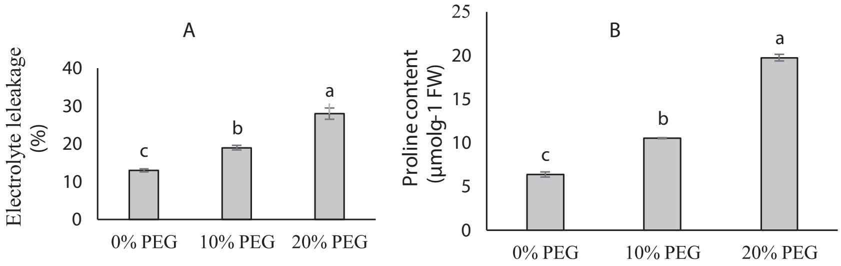

Fig. 1. The effect of drought pretreatment by PEG on electrolyte leakage (EL) (A) and proline content (B) of tomato seedlings before chilling treatment. Bars represent standard errors of four replication. At each treatment, values with the different letters are significantly different according to Duncan's Mul¬tiple Range Test at $P < 0.05$.

foliage. In this regard, Dong et al. (2013) found that cucumber seedlings experiencing drought stress enhanced their antioxidant content, and hence they protected their cell membrane integrity after exposure to chilling stress. Also, there are some reports documenting the relationship between drought stresses and inducing chilling tolerance in perennial ryegrass (Hoffman et al., 2012). This mechanism enabled seedlings to cope with subsequent environmental stresses after their transplanting (Li et al., 2013; Noctor et al., 2014). In this regard, the degree of water stress will be crucial. Depending on severity and time of experiencing drought condition under chilling stress, the rate of wheat's yield reduction will be different in the sense that severe draught in contrast to mild ones significantly intensifies the negative effect of low temperature on yield reduction (Li et al., 2014).

Maintaining of water potential on root media culture is difficult, therefore, simulating drought-stress condition with different osmotic substances is one of the most important methods to study the effect of drought stress on plants. Among various osmotic substances, PEG is generally used in owing to its same effects as natural drought stress on plant (Ibrahim et al., 2001). PEG, due to high molecular mass, cannot cross cellular wall and thereby being mainly employed in order for adjusting water potential as well as creating drought condition in plants (Dong et al., 2013). Comparing to other osmolytes like mannitol, sugar, and salt used to simulate drought stress in plants, PEG is a suitable osmolyte due to its stagnancy, non-ionic, and non-toxicity as well as absence of ability to cross tissues (Whalley et al., 1998). Application of PEG has been used to simulate drought stress condition on plants, and its effects on emerging drought stress were illustrated by many researchers (Whalley et al., 1998; Alexieva et al., 2001; Ibrahim et al., 2001; Dong et al., 2013; Shi et al., 2016).

To the best of our knowledge, there is not sufficient information about the effects of drought stress simulated by PEG on tomato seedlings chilling tolerance. Therefore, the goal of our study was to evaluate the beneficial effects of PEG pretreatment on enhancing antioxidant activities, H_2O_2 content, ABA accumulation, pigments and proline content of tomato seedlings subjected to chilling stress. This study was undertaken to evaluate the effects of PEG-induced drought on chilling-hardening capacity of tomato seedlings and to elucidate the underlying mechanism by which PEG stimulated-drought stress alleviate the damages caused by low temperature stress.

2. Material and methods

2.1. Plant material and growth conditions

This experiment was conducted in greenhouse and research laboratories of agricultural college of Bu Ali Sina University. First of all, the seeds of tomato cv. C.H Falat, being one of the most important cultivars grown in Iran, were planted into 6.2 cm-deep cells (48-cell plug trays, 148.8 cm^3 volume for each cell) filled with 40 g of perlite and vermiculite (ratio 2:1). Seedlings were grown in an environmentally controlled greenhouse at 25 ± 2 °C/18 ± 2 °C (day/night) under sunlight condition (light intensity between 943 and 1886 $\mu mol\ m^{-2}s^{-1}$). Greenhouse temperature was adjusted by a pad-and-fan cooling system during the experiment. In order to meet the nutritional needs for extending their growth and development, seedlings were fertigated by full PHOSAMCO BIO™ fertilizer (containing: 100 gl^{-1} N, 40 gl^{-1} P_2O_5, 70 gl^{-1} K_2O + trace elements) at concentration of 1/1000 (V/V).

2.2. Treatments

At four-leaf fully development stage, seedlings were subjected to 7 d drought simulated with PEG 6000 at three levels: Control (0% PEG), mild stress (10% PEG equaling to 0.18 Mpa osmotic potential) and severe stress (20% PEG equaling to 0.57 Mpa osmotic potential). To avoid unexpected shock, seedlings received PEG treatments for 3 d in the way that 1/3 of PEG was gradually employed each day. At the end of drought period, the rate of proline and EL were measured as typical indicators of stress (Fig. 1). Then, to remove PEG residues fully on media cultures, they were rinsed by water several times. Then, the seedlings were divided into two groups (144 seedlings for each): non-chilled (control) and chilled seedlings. Controls were kept in greenhouse under determined condition. For imposing chilling stress, second group of seedlings were subjected to low temperature 6 h/day at 3 °C for 6 days in growth chamber equipped with suitable aeration. In order for receiving identical chilling treatments, the position of trays in growth chamber were replaced. After receiving chilling treatment, seedlings were transferred to the greenhouse. They received chilling treatment at 11 pm to 5 am under dark condition As chilling damage often occurs nightly in early spring on local regions of growing tomato in Iran, this time was chosen to apply chilling treatments. All seedlings were assessed 72 h after the end of chilling stress to determine the extent of chilling injury and data were collected (Baninasab, 2009).

2.3. Measurements

2.3.1. Electrolyte leakage

In order to measuring electrolyte leakage, 0.3 g of middle layers of leaves and roots were taken and electrolyte leakage was calculated based on Baninasab (2009) method.

2.3.2. Chlorophyll and carotenoid

In order to measure the rate of chlorophyll (Chl) and carotenoid content, 0.1 g of fresh leaf tissue was grinded with 5 ml acetone 80% until producing homogenized product. After centrifuging solution, the supernatant was taken and its absorbance was read at 663, 645 and 470 nm by spectrophotometer. According to the following formula the rate of Chl and carotenoid were determined and reported as $mgg^{-1}FW$ (Mousa et al., 2007).

$$Chl\ a = 12.7(A663) - 2.69(A645)$$

$$Chl\ b = 25.8(A645) - 4.68(A663)$$

$$Chl\ total = 20.21(A645) + 8.02(A663)$$

$$Carotenoids = (1000(A470) - 2.27(Chl\ a) - 81.4(Chl\ b))/227$$

2.3.3. Chilling injury index

Wilting and necrosis of shoots were considered as the indictors of