South African Journal of Botany 94 (2014) 140–148

Contents lists available at ScienceDirect

South African Journal of Botany

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

Physiological and biochemical responses of six herbaceous peony cultivars to cold stress

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ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form 10 March 2014
Accepted 13 May 2014
Available online 11 July 2014

Edited by JM Farrant

Keywords:
Relative electric conductivity
Soluble sugar
Soluble protein
Malondialdehyde
Proline
Superoxide dismutase

ABSTRACT

Although herbaceous peony cultivars have been introduced and promoted widely, a key limitation to their use is their level of cold hardiness. In this study, the physiological and biochemical responses of six herbaceous peony cultivars to different temperatures and periods of exposure to freezing temperatures were assessed by analyzing changes in six indices. Under our experimental conditions, relative electric conductivity, soluble sugar concentration, soluble protein concentration, and malondialdehyde concentration generally increased as temperature declined, while proline content decreased. Superoxide dismutase activity fluctuated. After the same indicators were used to assess cold resistance over time at −20 °C, the six cultivars could be ranked, in decreasing levels of cold resistance, as: 'Da Fu Gui', 'Fen Yu Nu', 'Kansas', 'Monsieur Jules Elie', 'Taff', and 'Pink Hawaiian Coral'.

1. Introduction

Due to the sessile nature of plants, they may be subjected to injury by extreme environmental conditions. Plant hardiness is a genetic characteristic that originates from the long-term adaptation to low temperature environmental changes (Chen and Liu, 2003). The expression of plant cold-resistance genes is closely related to environmental conditions and plant physiological activities (Sakai, 1987; Hughes and Dunn, 1996; Thomashow, 1999). Conversely, many physiological and biochemical processes can reflect a plant's response to environmental stresses (Lambers et al., 1998). An understanding of the physiological and biochemical mechanisms of cold hardiness is a prerequisite for efficient breeding as well as the introduction and management of plants into cold or freezing environments.

The peony, a perennial herb, has a cultivation history of more than 3900 years (Li, 1999; Shen et al., 2012) and is currently a sought-after cut flower in international markets. Peony cultivars used for cut flowers are divided into three groups: Lactiflora, Hybrid or Intersectional group (Qin, 2004; Yao, 2009). Most herbaceous peony cultivars are in the Lactiflora group which is derived from the Chinese species *Paeonia lactiflora* Pallas (Kamenetsky et al., 2003). More recently the focus of much herbaceous peony breeding has been shifting to the Hybrid group which is a cultivar series formed by a plurality of species with many advantages such as unique flower patterns and bright saturated colors. The six herbaceous peony cultivars used in this study are from the Lactiflora and Hybrid groups. As with many other perennial plants in temperate climates, herbaceous peonies have a perennial crown (metamorphosed underground shoot) which serves to accumulate storage products for plant renewal in the new growth season (Kapinos and Dubrov, 1993; Kamenetsky et al., 2003).

Although there has been some research on the physiological and biochemical responses of herbaceous peonies to heat stress (Lü and Liu, 2008), heat and humidity tolerance (Liu, 2008) and soil drought stress (Guo, 2009), there has been little research on cold stress. In contrast, tree peonies have been evaluated for their relative cold hardiness in northern China (Inner Mongolia and Harbin). This work showed differences among species (*Paeonia rockii* T. Hong et J.J. Li, *Paeonia suffruticosa* Andr.) and cultivars within species (Li, 2009; Ren, 2009; Zhao et al., 2011). Ju (2011), studying the ecological adaptability and cold resistance of 12 tree peony cultivars in Northeast China, found

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http://dx.doi.org/10.1016/j.sajb.2014.05.012
0254-6299/

that proline concentration and soluble polysaccharide concentration in shoots during winter as well as leaves during the growing season could be used to assess tree peony cold hardiness.

Membrane stability is closely related to plant cold hardiness (Peter, 1984). As early as 1912, Maximov (1912) suggested that disruption of the plasma membrane was the primary cause of freezing injury. In 1932, Dexter et al. (1932) developed a conductivity method for measuring cold hardiness of plants which has long been used as one of the main methods for identification of plant cold resistance. Underground buds and roots of herbaceous peonies are the only two remaining organs in the winter. However, there are more errors in the method of relative electric conductivity (REC) for root measurement (Li et al., 1993). In this experiment, the underground buds were used to measure REC because these organs are less affected by the thickness of slices and other factors, and the roots were used to measure other indices since they are easily obtained and closely related to the growth of herbaceous peonies in the next year. The basic assumption of this method is that the greater the injury of the living tissue, the greater the efflux of ions from the thawed cells (Jiwan et al., 1977). Freezing tolerance is a result of several cryoprotective mechanisms operating concurrently (Sakai, 1987). Because compatible solutes accumulate during cold acclimation, it is thought that this accumulation is a cryoprotective mechanism in some plants (Alberdi and Corcuera, 1991; Livingston, 1996). Soluble carbohydrates, protein and free proline may be involved in freezing point depression of cell sap and prevention of plasmolysis during cell dehydration caused by freezing (Sakai, 1987; Santarius, 1992). A cryoprotective function of proline, which accumulates in response to osmotic stresses such as drought, salt and cold in many plant species, has been demonstrated (Bajguz, 2009). The accumulation of malondialdehyde (MDA), which acts as an end product of lipid peroxidation, is considered to reflect the physiological state of plant membrane lipids under cold stress (Imahori et al., 2008). Superoxide dismutase (SOD) and peroxidase (POD) play a definitive role in low-temperature stress (Li et al., 2000; Lin et al., 2005; Xu et al., 2011; Mansour et al., 2012). SOD and POD enhance the protection of membrane structures, through increased activity, and are thus considered to be the main anti-oxidizing agents in plants. SOD is a metalloprotein catalyzing the dismutation of the superoxide free radical ($O_2^{\bullet-}$) to molecular oxygen and H_2O_2 (Constantine and Stanley, 1977; Cervilla et al., 2007).

The objective of this study was to determine how changing temperatures and the length of exposure to low (freezing or below-freezing) temperatures affect the physiology and biochemistry of six herbaceous peonies. Using these indices and the relative cold hardiness, a theoretical basis was established for the future introduction of these cultivars into cold environments.

2. Materials and methods

2.1. Plant material and experimental treatments

The six herbaceous peony cultivars (Table 1) were collected from nurseries in Jiufeng and Xiaotangshan, Beijing, all of which had been divided three years previously.

On October 10th, 2011, the dormant peonies were pruned to the ground, the roots lifted out of the ground and washed, and then with a sharp sterilized knife cut into divisions containing five to eight strong buds and fleshy root. The roots were shortened to 15 to 20 cm and the smaller, unhealthy roots were removed.

Fifteen plants of each cultivar that had been divided as indicated above and with root diameters between 1.2 cm and 1.8 cm were planted with the same mass of substrate (peat:perlite:vermiculite = 3:1:1, v/v) in the same plot of a nursery in Xiaotangshan, Beijing. Pit depth was between 25 and 30 cm. Plants were exposed to natural cold conditions until mid-December (average temperature was approximately 0 °C). From the initial 15 plants, 8 were randomly selected and dug out carefully on December 12th. From each, three underground buds were cut from the base. In addition, a 5-cm root (measuring from the root tip backwards) was cut, ensuring that the maximum diameter of each root was 1.5 cm. Three roots were removed from each plant so that 24 buds and roots were obtained per cultivar. All experimental materials were wrapped separately in aluminum foil and taken to the lab immediately for analyses. In the laboratory, the underground buds and roots were washed thoroughly with running tap water, rinsed three times with deionized water, then blotted dry on filter paper. Twenty-one buds and roots were selected randomly from the above 24 buds and roots, and the experimental materials were divided into seven groups (so that there were three buds and three roots in each part) and placed in separate sealed plastic bags for the different low temperature treatments.

A programmed cooling device (Model: IceCube 14S; SY-LAB Corp., Neupurkersdorf, Austria) was used to drop the temperature in a controlled manner. All experimental materials were placed into the device when its internal temperature had dropped from room temperature to 4 °C. Thereafter, the temperature was dropped at a rate of 0.02 °C·min^{-1} to 0 °C. After 5 h, one sample of each cultivar was removed. The same protocol was applied every 5 h for the following temperatures: 0, −6, −12, −18, −24, −30 and −36 °C. The underground buds of each cultivar that had been removed from the device were placed in a refrigerator at 4 °C and thawed for 8 h, and then the three underground buds were chopped and mixed evenly. The corresponding experimental materials were weighed equally to determine REC. In contrast, the three roots of each cultivar were stored in liquid nitrogen prior to determination of physiological indices (soluble sugar concentration, soluble protein concentration, proline concentration, MDA concentration and SOD activity). Before determining the indices, the roots were also chopped, mixed and weighed as described for the underground buds.

For the second experiment, five plants of each cultivar were randomly chosen from the remaining stock plants and the same method was used to collect underground buds and roots. This set of experimental materials was divided into four parts for each cultivar such that 24 samples were placed into the programmed cooling device at 4 °C. Temperature was dropped at a rate of 0.02 °C·min^{-1}. When −8 °C was reached, this temperature was maintained for 5 h, and then dropped at 0.02 °C·min^{-1} to −20 °C. After 5, 15, 25 and 35 h at this temperature, one sample (containing underground buds and roots) of each

Table 1
Characteristics of the six herbaceous peony cultivars used in this study.

Cultivar	Location developed	Group[a]	Flower color	Flower diameter (cm)	Flower type[b]	Plant height (cm)
'Da Fu Gui'	China	Lactiflora	Red	15	D	85
'Fen Yu Nu'	China	Lactiflora	Pinkish purple	12	S	68
'Monsieur Jules Elie'	France	Lactiflora	Pink	13	D	86
'Kansas'	U.S.A.	Lactiflora	Deep red	13	D	86
'Taff'	U.S.A.	Lactiflora	Pink	12	D	75
'Pink Hawaiian Coral'	U.S.A.	Hybrid	Coral pink	12	SD	90

Note: Information based on Yu et al. (2011a) and http://www.paeo.de (Carsten Burkhardt's Web Project Paeonia, n.d.).
[a] Lactiflora — intercultivar hybrid, hybrid — interspecific hybrid.
[b] S — single, SD — semi-double, D — double.