Scientia Horticulturae 245 (2019) 12–18

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Three-year screening for cold hardiness of garden roses

Lin Ouyang[a], Leen Leus[b], Marie-Christine Van Labeke[a,*]

[a] Department of Plants and Crops, Ghent University, Coupure links 653, 9000, Gent, Belgium
[b] Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Caritasstraat 39, 9090, Melle, Belgium

ARTICLE INFO

Keywords:
Index of injury
Total soluble sugars
Sucrose
Oligosaccharides
USDA plant hardiness zone

ABSTRACT

Garden roses have a high ornamental and economic value. They are usually produced in one climatic zone, then sold to another climatic zone. Cold stress can severely inhibit their regrowth and ornamental quality. Knowledge of cold hardiness of specific genotypes is therefore necessary before commercialization to colder regions. 17 roses with diverse genetic backgrounds and belonging to different USDA (United States Department of Agriculture) plant hardiness zones were chosen for a three-year cold hardiness screening based on the index of injury method. Stems were sampled on 3rd February 2015, 20th January 2016 and 23rd January 2017 after a freezing period of six successive days. Cold hardiness levels were determined by controlled freezing to −20 °C. Genotypes with the highest and lowest levels of cold hardiness were confirmed during these three years. Soluble carbohydrates were analyzed. The results showed that both sucrose and oligosaccharides contributed to cold hardiness in the studied roses. When genotypes with known cold hardiness are included in screening programs, this approach can lead to rapid screening of new rose genotypes.

1. Introduction

Rose is one of the most economically important ornamental flowers worldwide. Each year, approximately 10 billion cut roses (AIPH, 2018), 220 million garden roses and 80 million potted roses are sold (Roberts and Gudin, 2003). Garden roses, which have a long cultivation history and a large number of varieties, are one of the most popular flowering garden plants worldwide. They are applied in the private garden as well as in public spaces as landscaping plants, hedges, screens and groundcover. Some fragrant genotypes (*R. x damascena*, *R. gallica* and *R. rugosa,* etc.) are used in rose oil production (Kovacheva et al., 2010). Some genotypes (*R. canina*, *R. rugosa and R. cinnamomea*, etc.) are cultivated for hip production as a rich source of vitamin C (Türkben et al., 2005).

Numerous breeding efforts in rose have focused on the demand for novelties such as flower color, shape and fragrance. Until recently, little attention was given to resistance breeding to biotic stresses such as black spot and powdery mildew (Debener and Byrne, 2014; Moghaddam et al., 2012) and abiotic stresses such as low temperature (Zhang et al., 2016), heat (Liang et al., 2017), salinity (Cabrera et al., 2009) and drought (Genhua and Rodriguez, 2009).

In northern climates, the freezing temperature in winter can seriously affect the survival, regrowth and commercial quality of garden roses. Good cold hardiness is an important breeding target and was introduced in roses through hybridization with wild species such as *R. rugosa, R. arkansana* and *R. wichurana* (Smulders et al., 2011; Vukosavljev et al., 2013). *R. rugosa* is well known for its high resistance to environmental stress and especially to sub-zero temperatures (Hakam et al., 2000). *R. wichurana,* which originated from temperate regions in China, can experience cane dieback in winter but is able to recover in spring. Breeders in Canada, North America and Germany have made great efforts to achieve cold hardy roses. From the late 1960s through the 1990s, a large set of Canadian garden roses (Canadian Explore series and Canadian Portland series) with strong cold hardiness are developed by crossing several cultivars with *R. x kordesii* and *R. rugosa* hybrids (Ogilvie et al., 1999; Svejda, 1979, 1977). *R. x kordesii* is thought to be an amphidiploid containing *R. rugosa* and *R. wichurana* in its background. This further emphasizes *R. rugosa* and *R. wichurana* as a key source of hardiness.

Cold hardiness might be influenced by the interaction between rootstock and scion, as not all roses are propagated by cuttings. Koepke and Dhingra (2013) reviewed this interaction for woody plants in general but few interactions were found for cold hardiness and this review does not include data on roses. In roses, a positive effect of rootstock upon scion on winter hardiness was only observed if *R. canina* is used as rootstock but no effects were found in scions when using other hardy rootstocks (Buck, 1964; De Vries, 1993). Buck (1964) suggests that this positive effect might due to the incomplete

* Corresponding author.
E-mail address: MarieChristine.VanLabeke@UGent.be (M.-C. Van Labeke).

https://doi.org/10.1016/j.scienta.2018.10.003
Received 10 July 2018; Received in revised form 11 September 2018; Accepted 2 October 2018

Table 1
Background information of the selected genotypes ranked according their USDA cold hardiness zone.

Name	Synonym	Rose type[1]	Breeder / Author[1]	Coldest USDA Zone[2]	Temperature range (°C)[3]	Ploidy level[4]	Propagation method
'John Cabot'		Hybrid Kordesii	Felicitas Svejda	2b	−42.8 ~ −40.0	4x	Cutting
'J.P. Connell'		shrub	Felicitas Svejda	2b	−42.8 ~ −40.0	3x	Cutting
'Henry Kelsey'		Hybrid Kordesii	Felicitas Svejda	3a	−40 ~ −37.2	4x	Cutting
'Dagmar Hastrup'	'Frau Dagmar Hastrup'; 'Frau Dagmar Hastopp'	Hybrid Rugosa	Knud Julianus Hastrup	3b	−37.2 ~ −34.4	2x	Cutting
'Moje Hammarberg'		Hybrid Rugosa	Hammarberg	3b	−37.2 ~ −34.4	2x	Cutting
'Modern Centennial'		Shrub	Henry H. Marshall	3b	−37.2 ~ −34.4	4x	Cutting
'Prairie Joy'		Shrub	Lynn M. Cullicut	3b	−37.2 ~ -34.4	4x	Budding on *R.corymbifera* 'Laxa'
'Hope for Humanity'		Shrub	Lynn M. Cullicut	3b	−37.2 ~ −34.4	4x	Cutting
'Yesterday'		Polyantha	Harkness	4b	−31.7 ~ −28.9	2x	Grafted on *R. canina* 'Pfänder'
'The Fairy'		Polyantha	Bentall	4b	−31.7 ~ −28.9	2x	Budding on *R. corymbifera* 'Laxa'
'Milly Winterjewel'	'BOZmillwin'	Shrub	Biljana Bozanic Tanjga	5b	−26.1 ~ −23.3	4x	Budding on *R. corymbifera* 'Laxa'
'Abraham Darby'	'AUScot'	Shrub	David Austin	5b	−26.1 ~ −23.3	4x	Budding on *R. corymbifera* 'Laxa'
'Compassion'		Hybrid Tea	Harkness	5b	−26.1 ~ −23.3	4x	Budding on *R. corymbifera* 'Laxa'
R. wichurana		Species	Crépin	5b	−26.1 ~ −23.3	2x	Cutting
'Snow Ballet'		Shrub	Clayworth	6b	−20.6 ~ −17.8	3x	Budding on *R. corymbifera* 'Laxa'
'Chandos Beauty'	'HARmisty'; 'Sweet Love'	Hybrid Tea	Harkness	6b	−20.6 ~ −17.8	4x	Budding on *R.* Laxa
'Red New Dawn'	'Etandard'	Hybrid Tea	Robichon	6b	−20.6 ~ −17.8	4x	Budding on *R.* Laxa

[1]The information of 'Milly Winterjewel' is provided by Pheno Geno Roses B.V. (The Netherlands); information of other genotypes came from Cairns (2000).
[2]The coldest USDA zone was obtained based on the website http://www.helpmefind.com/rose/index.php.
[3]Temperature range corresponding to coldest USDA zone, which is based on the average annual extreme minimum temperature for the period 1976-2005.
[4]The ploidy levels of 17 roses were determined at ILVO. Analysis of ploidy levels was done using flow cytometry according to Denaeghel et al. (2018).

compatibility between the *R. canina*-type rootstock and the scion, which reduces the transport of starch of the cane to the rootstock, which leads to greater maturity and thus enhances cold hardiness.

Due to a wide range of genetic variation within and between rose genotypes in response to low temperatures, effective selection for their cold hardiness is needed. Field trials are still the main method to assess for cold hardiness in roses. Cold hardiness is evaluated by several criteria varying from mortality, scoring of the overall winter injury in plants, cane die-back, regrowth and cane length increase in the following growing season (Anderson, 1990; Carlson-Nilsson and Davidson, 2009; Zlesak et al., 2017). Field trials are the most direct way to test but they are time-consuming, as winter conditions vary over time and many years of observation are required (Karam and Sullivan, 1991). To replace time-consuming field selection, researchers are trying to develop a quick and reliable laboratory test for cold hardiness in roses. Bud/shoot survival as a proxy for cold hardiness is conducted by measuring the budburst rate of stem segments after treating them with freezing temperatures (Ercisli, 2003; Karam and Sullivan, 1991). Hakam et al. (2000) measured the stability of chlorophyll fluorescence under low temperature to screen rose genotypes for cold hardiness. Because the formation of extra-cellular ice under freezing temperature can cause living bark to shrink, Ameglio et al. (2003) introduced a simple method called Gelista™ by measuring the stem diameter changes under freeze-thaw cycles in a temperature controlled chamber. However, the most commonly reported method for the determination of cold hardiness in plants is the electrolyte leakage method (Lintunen et al., 2015; Pagter et al., 2011; Sutinen et al., 1992). Indeed, cold stress can induce a membrane transition from a fluid state to a rigid gel phase resulting in leakage of electrolytes from the symplast to the apoplast. The degree of electrolyte leakage differs significantly across genotypes and is associated with the freezing temperature and the cold hardiness of plants. Flint et al. (1967) improved the calculation of the electrolyte leakage and developed the index of injury, which transforms the electrolyte leakage as a relative electrolyte leakage by rescaling with the non-freezing samples (0%) and heat-killed samples (100%). This approach was used to evaluate cellular damage due to freezing temperatures of stems of several ornamental shrubs, strawberry crowns and apple roots (Flint et al., 1967). Burr et al. (1990) introduced an index LT_{50} which is the temperature when the index of injury reached 50%. Frost injury in cells determined by either the index of injury or LT_{50} based on a controlled freezing test became a rapid method for assessment of cold hardiness.

Woody plants have developed the ability to develop cold hardiness under freezing temperatures. In late autumn/early winter, induced by the decline of photoperiod, light intensity and temperature, woody plants gradually increase their cold hardiness level (Arora et al., 1994; Li et al., 2003; Welling et al., 1997). In mid-winter, cold hardiness reaches its maximum level and decreases towards spring when temperature and day-length rise again. Cold hardiness is thus a dynamic process including three stages: acclimation, mid-winter hardiness and deacclimation.

To cope with cold stress in winter, numerous changings in metabolism are involved in the cold acclimation period. Among those contributing factors, soluble carbohydrates as osmolytes play an important role for protecting the cell membrane and vital macromolecules against freezing induced dehydration. Soluble sugars can also reduce osmotic potential to depress freezing point and prevent the formation of intracellular ice crystals (Guy, 1990; Li et al., 2004; Xin and Browse, 2000). The increase in cold hardiness was found to be associated with the accumulation of certain soluble sugars in many species (Morin et al., 2007; Palma et al., 2014; Shin et al., 2015). The accumulation of sucrose and oligosaccharides in response to cold stress are most frequently reported (Hinesley et al., 1992; Ögren, 1999; Pagter et al., 2008). Besides, the reduction in sugars, the concentration of glucose and fructose and the ratio of sucrose : (glucose + fructose) were also reported to be positively correlated with cold hardiness (Sasaki et al.,