Postharvest Biology and Technology 136 (2018) 66–73

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Effect of cold storage on stomatal functionality, water relations and flower performance in cut roses

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ARTICLE INFO

Keywords:
Cold storage
Cut flowers
Rose
Stomatal functionality
Water relations

ABSTRACT

Symptoms of water stress are the most frequent cause for the "end of vase life" in prior stored roses. It was hypothesized that dark storage may alter the stomatal functionality and may cause water balance problems during the subsequent vase life period. The effect of short- and long-term storage on functionality of stomatal and subsequent flower performance was investigated in two rose cultivars (cvs) ('Akito' and 'Grand Prix') with presumed different sensitivity for development of water stress symptoms during the vase life.

Compared to no storage, both short term storage (2.3 d at 6 °C) and long term storage (28 d at 0.5 °C) negatively affected the stomatal functionality in cultivar (cv) Akito. Stomatal functionality parameters such as the rapidity of the closing response upon dehydration and the relative water content at which stomata are fully closed showed good correlations with flower performance parameters (flower weight changes and vase life). This indicates that in cv Akito, the decreased stomatal functionality is one of the factors involved in the poor vase life of prior stored flowers. In cv Grand Prix, however, storage did not greatly affect the stomatal functionality but storage negatively affected flower performance in a comparable way as in cv Akito.

A pre-treatment with abscisic acid prior to storage slightly improved stomatal functionality in both cvs, but no clear effect on flower performance was observed. Addition of the bactericide 8-HQC to the vase water improved flower performance in both cvs but could not alleviate the negative effect of cold storage on flower performance.

Results show that in roses cold storage may, depending on the cultivar, negatively affect stomatal functionality and this may contribute to water stress and ultimately flower failure. In addition, cold storage may negatively affect xylem water conducting properties through processes not related to bacterial contamination.

1. Introduction

The flower auction "FloraHolland" in the Netherlands, does regular vase life tests on samples of the flowers that are supplied by the associated growers and from other parties. These flowers may be from batches of flowers that were grown in the Netherlands or from imported flowers. It was found that symptoms of water stress are the most frequent cause for the "end of vase life" in the majority of the tested rose cultivars. Second important cause of the "end of vase life" is botrytis and third cause is physiological senescence (Fanourakis et al., 2015). Most prominent symptoms of water stress are loss of turgor of the petals (flower wilting), bent neck and wilted leaves. Such symptoms are generally associated with a negative water balance, when water demand (transpiration) exceeds water uptake (van Doorn, 2012).

A negative water balance can be caused by either impaired water uptake, excessive transpiration or by a combination of both. Impaired uptake may be caused by an increased resistance of the xylem vessels to water flow due to e.g. bacterial growth, air embolism or physiological processes. Physiological blockage is related to the wound-induced production of polyphenolic compounds and has been described in e.g. *Chrysanthemum* and *Syringa*, but not in roses (van Doorn, 1997). Excessive transpiration may be caused by impaired functionality of the stomata. If stomata do not adequately respond to water stress signals or when stomata do not fully close e.g. when water uptake is limited, this quickly leads to development of a negative water balance (van Doorn, 2012).

When no adequate biocides are added to the vase solution, the accumulation of bacteria in the vase solution and in the vascular system will progressively limit the water uptake. In response to the developing water stress, stomata will close in order to maintain a favourable water balance. Even when the vase solution contains effective biocides and bacterial numbers are maintained at relatively low levels, the xylem vessels will develop an increasing resistance to water uptake due to cavitation of vessel elements and possible physiological reactions

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http://dx.doi.org/10.1016/j.postharvbio.2017.10.009
Received 2 August 2017; Received in revised form 20 October 2017; Accepted 22 October 2017

caused by wounding of the stem (Bleeksma and Van Doorn, 2003). As most water loss in rose flowers is through the leaves and minor part through the flower petals, an adequate response of the stomata to water stress is of vital importance for maintaining a favourable water balance especially when resistance of the xylem vessels to water flow develops. When stomata are fully functional, up to two thirds of the vessels may be blocked before the water balance becomes unfavourable and flowers show symptoms of water stress (van Doorn, 1997).

Pre harvest conditions have been shown to affect the functionality of stomata. Conditions of high humidity (low evaporative demand) during cultivation tend to render stomata less responsive to water stress and e.g. pre-harvest treatments with abscisic acid (ABA) may reverse this effect (Fanourakis et al., 2012, 2013a,b; Nejad and van Meeteren, 2007). Similarly, continuous lighting to increase productivity, negatively affects stomatal functionality. It has been shown that roses grown at relatively high humidity and from continuous lighting have a considerably reduced vase life (Fanourakis et al., 2013a).

Cold storage and handling of roses after harvest often negatively affects the vase life and this effect is more pronounced after long storage times (van Doorn, 1997). Currently there is a trend toward shipping flowers in refrigerated 40-feet containers (Reefer) over sea instead of transport by airplane. According to a recent study of Rabobank (van Rijswick, 2015), the most prominent cut flower Reefer transport routes are from Colombia to UK (approximately 700 containers per year); from Israel to EU (300 containers per year) and from Vietnam to Japan (400 containers per year). Although the journey by ship takes much longer than by airplane (2–4 weeks by ship versus 2–4 d by airplane), much better control of the storage conditions is possible in the Reefer. Due to the higher energy efficiency of the Reefer on a ship compared to an airplane, both the transport costs and CO_2 footprint of Reefer transported product are considerably lower.

During the extended transport period in Reefer containers, the product is held at a very low temperature (0.5–2 °C), densely packed in boxes, sleeved or within plastic liners creating a high relative humidity (> 95%). As symptoms of water stress are the most frequent cause for the “end of vase life” of these roses, we hypothesized these storage conditions may alter the stomatal functionally and in this way may cause water balance problems during the subsequent vase life. Here we report on the effect of short- and long term storage on functionality of stomata in two rose cultivars (‘Akito’ and ‘Grand Prix’) with different sensitivity for development of water stress symptoms during the vase life. In addition, the effect of a pre-storage treatment with abscisic acid (ABA) was investigated.

We show that in cv Akito the storage negatively affects stomatal functionality (measured at start of the vase life) and that loss of stomatal functionality correlates with a lesser performance of the stored roses. In cv Grand Prix, however, storage did not affect stomatal functionality but stored roses did show a lesser performance than non-stored roses. This indicates that apart from stomatal functionality additional factors play a role in the lesser performance of stored roses.

2. Materials and methods

2.1. Plant material and experimental design

Cut roses of cvs Akito and Grand Prix were obtained from commercial growers in the Netherlands. Flowers were transported dry at 4 °C to the laboratory within 3 h after harvest. Roses were graded for homogeneity, recut (at least 5 cm) and placed overnight in either water or water + 0.1 mM abscisic acid (Sigma-Aldrich, St Louis, MI, USA) at 4 °C and 90% relative humidity (RH). The last 6 h of treatment was in the light (8–10 μmol m^{-2} s^{-1}). Following the pre-treatment, stem length was shortened to 45 cm and roses were placed in a climate room (20 °C, 60% RH, 12 h light (13 μmol m^{-2} s^{-1}) and 12 h dark) to study the flower life and to perform measurements of physiological parameters. Remaining roses were stored dry, either at 6 °C and 90% RH for 2.3 d or at 0.5 °C and 90% RH for 28 d, simulating transport conditions by plane and by ship, respectively. Storage times were chosen in such a way that for both temperatures the total number of degrees-days was 14. Bunches of 10 flower stems were packed in poly-propylene sleeve and placed in the storage rooms. Flower weight loss during the storage period was monitored by weighing unpacked bunched before and just after storage.

Following storage, flower stems were re-cut (3–5 cm) and rehydrated overnight in tap water at 4 °C and 90% RH. The last 6 h of treatment were carried out under light (8–10 μmol m^{-2} s^{-1}). Thereafter, the flowers were recut to 45 cm and placed in the climate room to study the flower life and to perform measurements of physiological parameters.

2.2. Flower weight, water uptake and transpiration

Following the pre-treatment (in non-stored flowers) or following the rehydration treatment (in stored flowers), stems were recut to 45 cm and leaves from the lower end of the stem were removed (leaving about 4 fully grown leaves on the stem). Flowers were placed individually in flasks containing water or water + 150 mg L^{-1} 8-hydroxy quinoline citrate (8-HQC; Sigma-Aldrich, St Louis, MI, USA) (5 flowers per treatment). Flasks were randomly placed in the climate room. Flower weight and water uptake of flowers was monitored by daily measurement of the weight of the flowers and that of the flasks. This allowed to calculate the effects of the treatments on flower performance (flower weight change curves) and flower water balance parameters such as transpiration and uptake. Flower transpiration was calculated as the water uptake minus the flower weight change over a certain period of time (typically 1 d).

At the end of the experiment total leaf area of each flower was measured using LI-3100C Area meter (Li-Cor, Lincoln, NE, USA). In order to be able to compare the transpiration rate and the cumulative transpiration between the storage treatment, the results were corrected to the specific leave area and expressed per 100 cm^2 leave area. The flower performance was established on basis of the flower weight gain and loss. The daily flower weight was expressed in a percentage of the initial flower weight (100% on day 0). Flower performance parameters are derived from the flower weight curves i.e. the area under the curve and the number of days where the flower weight is above its initial weight (above 100% Calculated vase life).

2.3. Stomatal characteristics

Measurements of leaf stomatal characteristics were performed immediately following the pre-treatment (in non-stored flowers) or immediately following the rehydration treatment (in stored flowers). For determination of stomatal characteristics, the first two fully grown leaves under the flower head were removed from the stem (5 samples per treatment were measured; each sample consisted of 4 leaves excised from 2 stems). Leaves were weighed (measurement of fully hydrated state) and placed abaxial side up on the table in the climate room (20 °C and 60% relative humidity in light 13 μmol m^{-2} s^{-1}). Thereafter, leaf samples were weighed every 15 min till they had lost > 50% of their initial weight. At the end of the experiment, leaf dry weight was determined (drying at 70 °C for at least 24 h). This allowed to express the water loss as a function of the relative water content (RWC).

RWC was calculated according to Formula (1) (Fanourakis et al., 2013b).

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Sturated Fresh Weight} - \text{Dry weight}} \times 100 \quad (1)$$

Different stomatal characteristics were determined from curves depicting the water loss as a function of the RWC (described in Section 3.4).