Stress induced ethylene production, ethylene binding, and the response to the ethylene action inhibitor 1-MCP in miniature roses

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Abstract

In an interior environment, the display life of miniature potted roses (\textit{Rosa hybrida} L.) ‘Bronze’ was 7 days, while that of ‘Vanilla’ was 23 days. Although there was a substantial difference in ethylene production between flowers from two cultivars, there was no difference in binding site activity ($k_d = 0.14 \text{ nl l}^{-1}$). Pre-treatment with the ethylene action inhibitor 1-methylocyclopropene (1-MCP) did not have a clear effect on display life without preceding transport stress. When the plants were subjected to simulated transportation stress, post-harvest life of both cultivars was reduced (to 6 days for ‘Bronze’, and to 17 days for ‘Vanilla’). In ‘Bronze’ the stress resulted in a modest increase in ethylene production, but there was no effect of stress on the low ethylene production by ‘Vanilla’ flowers. Nevertheless, pretreatment with 1-MCP before transport simulation improved display life of both cultivars. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Stress promotes ethylene production in many plant species. Abeles (1973) introduced the term “stress ethylene” to refer to the accelerated biosynthesis of the gas associated with biotic or abiotic stresses in plants. When potted miniature...
roses are subjected to stress under transport conditions, like darkness, water deficit, or vibration, they often exhibit symptoms such as bud and flower drop, leaf abscission and accelerated rate of flower wilting (Serek, 1993; Reid et al., 1989a,b). Faragher et al. (1987) and Mor et al. (1989) showed that cut roses produce substantial amounts of ethylene in response to stresses such as cold storage. It can be assumed that in pot roses too, transport induces the production of stress ethylene.

In a previous study (Müller et al., 1998), a number of commercial miniature rose cultivars showed great variety in their display life in an ethylene free environment. Sensitivity to exogenous ethylene, which seems to be an important natural regulator of rose flower senescence, was different among the cultivars examined. Variation in post-harvest life is partly due to differences in sensitivity to exogenous ethylene and partly to differences in endogenous ethylene production. The cultivar ‘Bronze’ had the highest flower ethylene production and the shortest display life. This cultivar showed a clear climacteric rise in ethylene production during flower senescence, similar to that seen in climacteric flowers like carnations (Wu et al., 1991a,b). In contrast, other rose cultivars only showed a moderate or very low ethylene production. In some of the miniature roses investigated, variation in longevity among cultivars could be understood by differences in the ethylene production of the flowers. However, flowers of the cultivar ‘Vanilla’ had long post-harvest life despite having relatively high ethylene production (approx. 2.0 nl gf w \(^{-1}\) h in senescing flowers) than would be consistent with this explanation. We chose ‘Bronze’ and ‘Vanilla’, which have quite different flower longevity despite both exhibiting a climacteric rise in ethylene production during flower senescence, as model plants for further investigations. We hypothesized that differences in flower life of these two miniature rose cultivars might result from differences in ethylene binding activity. As a tool to test this hypothesis, we examined their flower longevity response to simulated transportation stress and its presumed increase in ethylene production.

2. Materials and methods

2.1. Plant materials

The cultivar *Rosa hybrida* Kordana ‘Vanilla’ originates from Rosen Kordes, Sparrieshoop, Germany and *R. hybrida* Parade ‘Bronze’ from Poulsen Roser, Fredensborg, Denmark. Both cultivars were produced in the same commercial nursery in Denmark under equal preharvest conditions, and sent to the university within 24 h after packing at normal commercial maturity (2–5 open flowers).

Production time for both cultivars was 11–12 weeks, the plants were grown in 10 cm-pots (0.55 l) under standard commercial growing conditions at
19–20°/19°C (day/night temperature) and relative humidity of 60–85% (RH). Daylight was supplemented with 230 μmol m\(^{-2}\) s\(^{-1}\) (SON-T lamps) over a 19 h photoperiod. As retardant Paclobutrazol was used at a concentration of 0.5%.

2.2. Stress induced ethylene production

Transport stress was simulated by keeping the plants for 4 days in darkness with continuous shaking (105 ± 5 RPM) on a rotary lab shaker; temperature was 20 ± 1°C and relative humidity (RH) 60–85%. Control plants were placed at the same temperature and RH, but were exposed to 12 h light from fluorescent tubes providing 20 μmol m\(^{-2}\) s\(^{-1}\) PAR and irrigated from above with tap water as needed.

After this treatment open flowers without visible symptoms of senescence were excised just below the receptacle from stressed and control plants. Ethylene measurement for each treatment was conducted four times, for each measurement three replicate flowers from different plants were placed in 130 ml vials, sealed with a septum, and kept for 2 h at 20°C. A 1 ml aliquot of the air in the vial was withdrawn for measurement of ethylene, using a gas chromatograph (Photovac 10S plus, NY) fitted with a photoionization detector.

2.3. Effects of stress and 1-MCP on display life

Immediately after receipt, five potted roses per treatment were placed in sealed glass containers, at 20 ± 1°C and a light intensity of 20 μmol m\(^{-2}\) s\(^{-1}\). The plants were treated with 1-MCP by releasing it from a commercial powdered formulation (EthylBloc, Biotechnologies for Horticulture, SC), through addition of 10 ml water to 0.02 g of the chemical, which was expected to release 100 nl l\(^{-1}\) 1-MCP gas in the 160 l container. The plants were exposed to 1-MCP for 6 h, control plants were sealed in air in identical containers for 6 h. After treatment, the plants were exposed to a transport simulation (as described above), or placed in the controlled environment without preceding stress treatment. The miniature roses were exposed to 12 h light from fluorescent tubes providing 20 μmol m\(^{-2}\) s\(^{-1}\) PAR and irrigated by ebb/flood. Display life was followed by recording the number of “healthy flowers” every other day until the end of the display life of the two cultivars. “Healthy flowers” were defined as not abscised half-open and open flowers, without any senescence symptoms.

2.4. Ethylene binding

Ethylene binding to petal tissue was measured following the procedure of Sisler (1979). The labeled ethylene (50 mCi/mmole, American Radiolabeled Chemicals)
was trapped with mercuric perchlorate. Five replicate samples of a 4 g petals were placed in a 236 ml jar containing three smaller vials, one containing 15 µl of the ethylene mercuric complex, and two other vials with 0.5 ml of 10 N NaOH and 0.5 ml AgNO₃, to absorb CO₂ and reduce background. The ¹⁴C-labeled ethylene was released from the mercuric perchlorate complex by addition of 1 ml saturated LiCl. After addition of LiCl, the jar was closed with a lid with a rubber serum cap. The vial with the mercuric perchlorate contained a small magnet; after the LiCl was added, the magnetic stirrer was run for 5 min to aid the release of the radioactive ethylene. Then unlabeled ethylene was injected into eight replicate jars in aliquots so as to provide the following final ethylene concentrations: 0, 0.025, 0.05, 0.15, 0.25, 0.5, 1.5, 500 µl⁻¹.

The petals were allowed to stand 3 h in the presence of the labeled and unlabeled ethylene mixture. After 3 h the petals were removed from the jar, shaken in air for 30 s, and placed in a second 236 ml jar containing 1 ml mercuric perchlorate in a scintillation vial. A 2 cm piece of glass filter was included in the vial to increase surface area. The total time of removing, shaking and transfer was 60 s. The petals were allowed to stand 15–16 h to ensure collection of approximately 95% of the labeled ethylene from the petals. Then the scintillation vials were removed, filled with scintillation fluid and the radioactivity of the trapped ethylene was determined in a liquid scintillation counter (Packard liquid scintillation counter, TRI-CARB, California).

3. Results

3.1. Stress induced ethylene production

In open flowers of *R. hybrida* ‘Vanilla’ endogenous ethylene production was negligible (less than 0.5 nl l⁻¹ h⁻¹) and shaking in darkness for 4 days, did not substantially increase ethylene production (Fig. 1). In contrast, flowers of the rose cultivar ‘Bronze’ produced substantial amounts of ethylene (4.6 nl l⁻¹ h⁻¹) even without preceding transport simulation. In this cultivar, transport stress resulted in a significant increase of ethylene production of the flowers (to approx. 7.2 nl l⁻¹ h⁻¹).

3.2. Effects of stress and 1-MCP on display life

Post-harvest performance, expressed as percentage healthy flowers, differed distinctly between the two rose cultivars (Fig. 2). Control plants of ‘Vanilla’ finished flowering on 30th day (Fig. 2a); while ‘Bronze’ controls finished in 10 days (Fig. 2b). When display life is defined as days until 80% of the flowers had
faded, ‘Vanilla’ lasted 23 days, while ‘Bronze’ exhibited only 7 days of post-harvest life. The percentage of healthy flowers in 1-MCP pre-treated plants of ‘Bronze’ was higher from day 8 until the end of the experiment (Fig. 2b). However, comparison of day 0 to day 14 of the observation period by regression analysis revealed that pre-treatment with 1-MCP without preceding transport stress did not show a significant difference between percentage healthy flowers of either cultivar (Fig. 2a and b).

Transport simulation reduced display life of both cultivars, to 6 days for ‘Bronze’ and to 17 days for ‘Vanilla’. Both cultivars responded markedly to pre-treatment with 1-MCP and subsequent transport stress; pre-treatment with 1-MCP improved display life of both cultivars to at least control level (Fig. 2). Regression analysis indicated that the pre-treatment resulted in a significantly improved post-harvest performance (as determined as % healthy flowers) comparing day 0 to day 14 in the cultivar ‘Bronze’ \( (P > 0.001) \), and day 10 to day 30 in ‘Vanilla’ \( (P > 0.01) \).

### 3.3. Ethylene binding

Incubation with different concentrations of unlabeled ethylene in the presence of \(^{14}\)C-ethylene binding yielded Scatchard plots, which were used to estimate \( k_d \). For both cultivars a \( k_d \) of 0.14 nl l\(^{-1}\) was obtained, the petal tissue affinity for ethylene was the same (Fig. 3).
Fig. 2. Post-harvest life, expressed as percentage healthy flowers, of *R. hybrida* ‘Vanilla’ (a) and ‘Bronze’ (b) after transport stress (darkness and shaking for 4 days) and 1-MCP treatment. Each point represents means ± SE for five replicate rose plants. ‘Vanilla’: without MCP, stress vs. MCP, stress — $L^{n.s} Q^{**}$; without MCP, no stress vs. MCP, no stress — $L^{n.s} Q^{n.s}$; ‘Bronze’: Without MCP, stress vs. MCP, stress — $L^{n.s} Q^{***}$ without MCP, no stress vs. MCP, no stress — $L^{n.s} Q^{n.s}$. 
4. Discussion

In a previous study we found significant differences in the longevity of a range of commercial cultivars of potted roses. This variation in display life partly appeared to be a result of differences in ethylene production or sensitivity to ethylene exposure (Müller et al., 1998). However, differences in miniature rose cultivars cannot in all cultivars totally be explained by differences in sensitivity to

Fig. 3. Scatchard plots of $^{14}\text{C}_2\text{H}_4$ displacement from petals of the miniature rose cultivars Vanilla (a) and Bronze (b) in the presence of different concentrations of unlabelled $^{14}\text{C}_2\text{H}_4$. Each point represents means ± SE for five measurements (a 4 g flower petals).
exogenous ethylene and flower’s ethylene production. We hypothesize that
differences in post-harvest life were due to differences in binding activity
between both cultivars. Wu et al. (1991b) found clear differences in ethylene
affinity of carnation cultivars with different post-harvest characteristics. However,
differences in post-harvest characteristics in the rose cultivars investigated here
could not be explained by differences in ethylene binding activity, which was
approximately identical for flowers of the long-lived ‘Vanilla’ and the short-lived
‘Bronze’.

The short flower life of ‘Bronze’ can be explained by a high natural ethylene
production during flower senescence, and furthermore reduction of display life in
this cultivar due to transportation seems to be related to enhanced ethylene
production. This result is consistent with the findings of Faragher et al. (1987)
and Mor et al. (1989), who showed substantial amounts of ethylene produced by
cut roses in response to cold storage stress. The reduced vase life of roses after
long storage was associated with an increase in their ethylene production
(Faragher and Mayak, 1984).

1-MCP pre-treatment did not have a significant effect on flower life of ‘Vanilla’
without preceding stress treatment (Fig. 2a). However, the analysis of data raises
some questions about the effect of 1-MCP on display life of ‘Bronze’. Comparison of day 0 to day 14 by regression analysis revealed that pre-treatment
with 1-MCP without preceding transport stress did not significantly improve
display life. Nevertheless, the percentage of good flowers in 1-MCP pre-treated
plants of ‘Bronze’ was higher from day 8 until the end of the experiment (Fig.
2b). A difference between 1-MCP treated and untreated control plants may
manifest only at late stages of flower development, where ethylene production is
high.

When the plants were subjected to simulated transportation stress, post-harvest
life of both cultivars was reduced. Pre-treatment with 1-MCP before transport
simulation improved display life of both cultivars to at least control levels. It is
clear that ethylene production in flowers only is one component of difference in
display life of these two very different cultivars. 1-MCP pre-treatment, which
effectively blocks the action of ethylene (Serek et al., 1994, 1996), did not extend
the life of ‘Bronze’ plants. On the other hand, this chemical improved display life
in stressed ‘Vanilla’, although ethylene production of flowers was not increased
by transport simulation. Perhaps stress increased ethylene binding site activity, or
influenced other steps of the ethylene signal transduction pathway in this cultivar.

In a previous study, we found differences in the expression for genes for an
ethylene receptor in senescing flowers of the two cultivars. ‘Bronze’ with short
flower life exhibited a higher expression of genes for an ethylene receptor than
the long-lasting ‘Vanilla’, suggesting that variation in flower life is partly due to
differences in receptor levels during flower development (Müller et al.,
unpublished). The curious results in this present study indicate the need to
examine the effects of stress on receptor activity and expression of the receptor genes.

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