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Changes in Soluble Carbohydrates Content and Ethylene Production during Postharvest Period of Cut Rose Flowers

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ABSTRACT

Background: Cut flowers rely on stored carbohydrate reserves for flower opening and maintenance and the postharvest life of many cut flowers can be extended by adding sucrose to the vase solution. **Objective:** To study the role of soluble carbohydrates on the longevity and to understand the physiological basis of sucrose on the vase life, we investigated the changes in soluble carbohydrate pools and ethylene production during the postharvest phase of cut roses (*Rosa hybrida* L. cvs. 'Audio' and 'Black Magic') treated with 8-hydroxyquinoline citrate (HQC) or sucrose plus HQC. Cut rose stems were placed in distilled water, 200 mg L^{-1} HQC, or 20 g L^{-1} sucrose plus 200 mg L^{-1} HQC solutions, and kept at 23 ◦C for evaluation. **Results:** The vase life of 'Audio' was 11.3 d whereas that of 'Black Magic' was 5.7 d in distilled water. HQC and sucrose plus HQC treatments increased 'Audio' and 'Black Magic' flower longevity by 2-5 days. 'Black Magic' flowers produced higher ethylene rate than 'Audio' flowers during postharvest period and petals abscised at a quicker rate. Treatment with sucrose plus HQC delayed in the utilization of the glucose, fructose and sucrose in petals and stems and delayed ethylene production in both cultivars. Concentrations of soluble carbohydrates in 'Audio' petals and stems were higher than those in 'Black Magic' flowers throughout the experimental period. Fructose was the main carbohydrate in petals of both cultivars, followed by glucose and sucrose. **Conclusion:** these results indicate that carbohydrate supply and ethylene production rate may determine the length of the vase life of these cut rose flowers.

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INTRODUCTION

Flower crops used commercially in the cut flower industry have to survive harvest, packing and distribution, and still have acceptable quality for the consumer. Two important characteristics of cut flowers are the ability of flower buds to open after the stems have been harvested, and for open flowers to have a reasonable vase life. Cut flowers rely on stored carbohydrate reserves for flower opening and maintenance, as their carbohydrate supply from the rest of the plant ceases at harvest, and as cut flowers are often placed in low light conditions, there is little or no net carbon gain from photosynthesis (Halevy and Mayak, 1979). The vase life of cut rose flowers is often short. The cut flowers wilt and the floral axis becomes bent just below the flower head, a phenomenon known as "bent neck". Development of "bent neck" is thought to result from vascular occlusion, which inhibits water supply to the flowers (Mayak *et al*., 1974; de Stigter, 1980). Van Doorn and D'hont (1994) reported that resistance to bacteria and dry storage is involved in cultivar variation in the vase life of cut roses. Dehydration and wilting, caused by either air embolisms in the stem xylem (at the time of cutting) or bacteria entering the stem from the vase water and blocking water uptake (Van Doorn, 1997), can also affect both the ability of flower buds to open and their vase life. Developing flowers in cut flower stems are active sink organs that require a continuous supply of carbohydrates as an energy source, as building blocks for cell wall synthesis and to maintain osmotic potential. Photosynthesis in leaves of cut flowers under low light levels in interior environments is very limited and does not provide enough carbohydrate for proper opening and longevity of flowers. Thus, an exogenous supply of carbohydrates is usually beneficial in maintaining quality and longevity of cut flower stems. Common commercial preservative solutions typically contain a soluble carbohydrate source in addition to an acidifying agent (to improve water uptake), and a germicide (to prevent microbial growth). The beneficial effects of exogenous sucrose have been documented in many cut flower species. These effects range from increased flower bud or floret (in the case of inflorescences) opening (Han, 1992; Kuiper *et al*., 1995;

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Ichimura and Hisamatsu, 1999; Su *et al*., 2001), delayed senescence of individual flowers or florets (Eason *et al*., 1997; Ichimura and Suto, 1999; Liao *et al*., 2000), increased flower size (Eason *et al*., 1997), increased intensity of petal color (Eason *et al*., 1997; Han, 2003), and suppression of ethylene production (Ichimura and Hisamatsu, 1999; Ichimura and Suto, 1999). For ethylene-sensitive species, such as rose and *Curcuma alismatifolia* ethylene-induced senescence, expressed as premature petal drop or leaf yellowing, can markedly decrease vase life (Ichimura *et al*., 2005; Bunya-atichart *et al*., 2004). We would like to be able to manipulate the carbon dynamics during production to maximize the postharvest performance of cut rose flowers. Consequently, the aims of the current study were three fold: (i) to compare the vase lives of two cut rose cultivars, (ii) to compare the carbohydrate dynamics of cut roses during their vase life, (iii) to compare the effect of exogenous sucrose in metabolism of carbohydrates and ethylene production.

Methodology:

Plant materials:

Cut rose (*Rosa hybrida* L. cvs. ‘Black magic’ and ‘Audio’) flowers, harvested at normal harvest maturity, were obtained from a commercial grower in Karaj, Iran. After harvest, the cut ends of flower stems were immersed in tap water within 1h. The cut flowers were then transported to the laboratory and used for experiments within 2 h.

Postharvest treatments and evaluation:

Flower stems were trimmed to 40 cm, and all five-leaflet leaves except for upper three were removed. Three cut flowers were placed in each 500 ml jars containing 400 ml distilled water (control), 200 mg l^{-1} HQC, or 20 g l^{-1} sucrose plus 200 mg l^{-1} HQC. Nine flowers were used for each treatment. Stems were kept in a postharvest evaluation room maintained at 23 ˚C under 70% RH with 15µmol m^{-2} s^{-1} of light from cool white florescent lamps (12 h day/night cycle). Vase life was the period between the time when either the petals lost turgor or at least one petal abscised. The diameter of each cut flower, the fresh weight and the amount of water uptake were measured daily. The amounts of water uptake were corrected by subtracting evaporation of water from a jar without cut flowers. The amount of water loss was calculated by subtracting the increase in fresh weight from the amount of water uptake.

Carbohydrate extraction and analysis:

Petals from the third and fourth whorl and stems (1 g each) were obtained from three cut flowers and immersed separately in 10 ml of 80% ethanol at 75 ˚C for 30 min. after cooling, 100 µl 25 g l^{-1} mannitol was added to each extraction solution, as an internal standard. The tissues were then homogenized and centrifuged at 3000 × g for 10 min. The three supernatants were combined and concentrated *in vacuo* below 50 ˚C. The residue was then dissolved in 1 ml distilled water and passed through a Sep-Pak C18 cartridge (Millipore, Milford, MA, USA). Aliquots of the eluate were separated using an HPLC system (Jasco HPLC, Tokyo, Japan) equipped with a refractive index detector and a Shodex SUGAR SP0810 column (Showa Denko, Tokyo). The amount of each sample was calculated as described previously (Ichimura *et al*., 2000).

Starch was extracted from petals and stems on the day of harvest and the concentrations determined as described previously (Ichimura *et al*., 1999).

Ethylene measurement:

Each flower was sealed in a 3 L glass vessel and kept at 20˚C. After 2 h a 1 ml gas sample was withdrawn into a syringe and the ethylene concentration determined by a Shimadzu gas-chromatograph (Model GC-7A) equipped with an activated alumina column fitted with a flame ionization detector. Ethylene concentrations were calculated and expressed in nL g^{-1} h^{-1}.

Statistical Analyses:

Data were taken from three independent experiments, and mean and SE values were determined. Analysis of variance was performed to detect treatment effects, and Duncan’s Multiple Range test was performed for mean comparison using SAS software.

Results:

Postharvest performance:

For the distilled water controls, the vase life of ‘Audio’ flowers was much longer than that of ‘Black Magic’ flowers (Table 1). The longevity of flowers was improved by HQC (18–28% increase) and sucrose plus HQC (45–48% increase, Table 1). The effect of sucrose plus HQC on flower longevity was more pronounced compared to HQC on its one.

Flower diameter in sucrose plus HQC-treated flowers was much greater than in control or HQC-only treated flowers (Table 1). Although sucrose plus HQC treatment significantly increased flower diameter in both cultivars, the increase was greater in 'Audio' than in 'Black Magic'.

Fresh weight in control stems initially increased from day 1 to day 3 or 5, and then decreased thereafter (Fig 1). Treatment with HQC delayed the time when the fresh weight started to decrease and increased the maximum fresh weight of both cultivars. HQC enhanced the fresh weight of 'Audio' flower better than sucrose plus HQC whereas in 'Black Magic' flower treatment with sucrose plus HQC had the best result.

Water uptake of control group initially increased and then decreased very fast but treatment with HQC or sucrose plus HQC delayed the time when the water uptake started to decrease in both cultivars (Fig 2).

Carbohydrate dynamics in the stems and petals:

Glucose, fructose and sucrose were the major soluble sugars detected in rose petals and stems. Starch concentrations compared to soluble carbohydrates did not change significantly throughout the experiment in any tissue in any treatment (data not shown). However, in petals, the starch concentration in 'Black Magic' was twice as high as in 'Audio' at the beginning of experiment (Table 2). In stems, however, starch concentration in 'Audio' was higher than it in 'Black Magic'.

At the beginning of the experiment, in stems, sucrose was the main carbohydrate in 'Audio' flowers at a concentration of about 8.1 mg g^{-1}, followed by fructose (6.5 mg g^{-1}) and glucose (3.2 mg g^{-1}, Fig. 3). In contrast, fructose was the main carbohydrate in 'Black Magic' flowers at a concentration of about 6.2 mg g^{-1}, followed by fructose (5 mg g^{-1}) and glucose (2.9 mg g^{-1}). In total, concentrations of starch and soluble carbohydrates in stems of 'Audio' and 'Black Magic' flowers were approximately 18.9 mg g^{-1} and 14.7 mg g^{-1} of fresh weight, respectively.

At the beginning of the experiment, the concentrations of fructose, glucose and sucrose in petals of 'Audio' were almost 1.75, 1.88 and 1.69 times higher than those in 'Black Magic', respectively (Fig 3). Fructose was the main carbohydrate in petals of both cultivars, followed by glucose and sucrose. The concentration of sucrose in petals was approximately half as low as in stems of both cultivars, whereas, glucose and fructose concentrations in petals were higher than in stems. During the postharvest phase in control stems and petals in both cultivars, the concentration of glucose and fructose decreased gradually reaching less than 50% of its original level after 7 days. However, exogenous sucrose plus HQC treatment delayed in the utilization of the glucose, fructose and sucrose in petals and stems of both cultivars. Utilization of carbohydrates in petals of 'Audio' flowers was higher than in 'Black Magic' flowers. Glucose and fructose concentrations in sucrose plus HQC-treated flowers remained constant in 'Black Magic' flowers, whereas sucrose increased and peaked at day 5 and declined thereafter.

Ethylene production:

During the postharvest phase in 'Audio' and 'Black Magic' flowers the concentration of ethylene production increased steadily up to day 5 but decreased thereafter (Fig 4). Higher ethylene production was attributed to 'Black Magic' flowers during vase life in all treatments. Exogenous sucrose plus HQC treatment decreased the rate of ethylene production in both cultivars.

Discussion:

The vase life of cut roses was found to vary markedly between two cultivars; the vase life of 'Black Magic' was shorter than in 'Audio' (Table 1). Ichimura *et al*. (2002) also reported that there were marked variations in the vase life among 25 cultivars. The vase life of cut roses is affected by various factors. HQC and sucrose plus HQC treatments increased 'Audio' and 'Black Magic' flower longevity by 2-5 days. However, there was advantage of sucrose plus HQC over HQC on its one, which agrees with previous reports by Ichimura *et al*. (2005). A major cause of deterioration in cut flowers is blockage of xylem vessels by microorganisms that accumulate in the vase solution or in the vessels themselves. Van Doorn and D'hont (1994) reported that 'Frisco' rose is resistant to bacteria, which is considered to cause vascular occlusion and shorten the vase life of this cultivar. In this study, treatment with HQC significantly extended the vase life of both cultivars. Thus, vascular occlusion is likely to be a cause of the short vase life in these cultivars. The vase life of these cultivars was extended by treatment with sucrose plus HQC (Table 1). HQC inhibits vascular occlusion and sucrose supplies soluble carbohydrates. Therefore, shortage of soluble carbohydrate is a greater factor in determining a shorter vase life. Cut roses are usually harvested at the bud stage, and therefore, a large amount of soluble carbohydrates is required for flower opening. Petal growth associated with flower bud opening results from cell expansion (Kenis *et al*., 1985), which requires the influx of water and osmolyte such as carbohydrates into petal cells (Evans and Reid, 1988). Thus treatment with sucrose is considered to satisfy the supply of such soluble carbohydrates. In this study, the increase in flower diameter was significantly promoted by sucrose plus HQC-treated flowers and as a result these flowers opened faster than in control flowers (Table 1). This is in accordance with the findings of Halevy and Mayak (1979) who reported opening of cut flowers is often promoted by addition of carbohydrate to the vase water. In roses, carbohydrates are required as an energy source for flower opening (Ho and Nichols, 1977; Hammond, 1982; Evans and Reid, 1988; Van Doorn *et al*., 1991; Kuiper *et al*., 1995; Marissen and La Brijin, 1995). Petal growth associated with flower opening is the result of cell expansion (Kenis *et al*., 1985), which requires the influx of water and osmolytes into the vacuole (Evans and Reid, 1988). The active transport system, including a specific carrier, is required to transport osmolytes both from free space into the cytoplasm and from cytoplasm to vacuole (Bush, 1993; Tanner and Caspari, 1996). Therefore, to utilize applied carbohydrates as osmolytes, active transport systems as well as their metabolic enzymes are required. This view is supported by the finding that 20 g l^{-1} sucrose, markedly increased flower diameter (Table 1), accompanied by an increase in carbohydrate concentrations (Fig 3).

In control ‘Black Magic’ flowers, vase life was relatively short, and the opening of flower delayed. Treatment with sucrose plus HQC increased opening of flowers and extended vase life (Table 1). This treatment markedly increased soluble carbohydrate levels in rose petals (Ichimura *et al*., 1999). Thus, a shortage of soluble carbohydrates probably shortens vase life, and leads to delayed opening, in cut ‘Black Magic’ flowers. On the other hand, the vase life of ‘Audio’ flowers was longer than that of ‘Black Magic’ flowers, and ‘Audio’ flowers opened faster. Concentrations of soluble carbohydrates in ‘Audio’ petals and stems were higher than those in ‘Black Magic’ flowers throughout the experimental period (Fig 3). Therefore, long vase life and faster opening in cut ‘Audio’ flowers can be attributed, in part, to the higher concentrations of soluble carbohydrates in their petals and stems.

Starch has been shown to be an important reserve carbohydrate in rose flowers (Evans and Reid, 1988; Shellabear *et al*., 1993). In this study, starch concentrations were higher in ‘Black Magic’ than in ‘Audio’ petals, whereas starch concentrations in stems were higher in ‘Audio’ than in ‘black Magic’ (Table 2). However, the overall concentrations of starch were much lower than those of total soluble carbohydrates in both cultivars. Thus, starch is probably not responsible for faster opening and longer vase life in ‘Audio’.

In roses, ethylene is produced as part of the senescence process and some rose cultivars are highly sensitive to external ethylene (Serek *et al*., 1996; Macnish *et al*., 2010). Ethylene symptoms in susceptible roses include yellowing and loss of buds, a reduction in flower opening, premature senescence, petal and leaf abscission, and leaf discoloration (Serek *et al*., 1996; Macnish *et al*., 2010). In this study, ‘Black Magic’ flowers produced higher ethylene rate than ‘Audio’ flowers during postharvest period and petals abscised at a quicker rate. Treatment with sucrose plus HQC delayed ethylene production in both cultivars (Fig 4), suggesting that the beneficial effect of sugars in decreasing in ethylene production by cut roses. Similar findings have been reported in *Gentiana* (Zhang and Leung, 2001) and *Delphinium* (Ichimura *et al*., 2000).

Table 1: Effects of HQC (200 mg L^{-1}) or sucrose (20 g L^{-1}) plus HQC (200 mg L^{-1}) on the vase life and flower diameter of cut ‘Audio’ and ‘Black Magic’ rose flowers. Data are mean ± standard errors (n = 3 analysis replicates). Values with different letters in the same list are statistically significant by Duncan’s multiple range test at 5% level.

Cultivar	Treatment	Vase life (days)	Flower diameter (mm)
‘Audio’	Control	11.3 ± 0.3 c	87.4 ± 1.3 c
	HQC	13.3 ± 0.3 b	91.7 ± 1.1 b
	Sucrose + HQC	16.7 ± 0.4 a	96.2 ± 1.9 a
‘Black Magic’	Control	5.7 ± 0.2 e	84.1 ± 1.4 d
	HQC	7.3 ± 0.3 d	87.1 ± 1.2 c
	Sucrose + HQC	8.3 ± 0.4 d	91.3 ± 1.5 b

Table 2: Starch concentrations in petals and stems of cut ‘Audio’ and ‘Black Magic’ rose flowers at the beginning of experiment. Data are mean ± standard errors (n = 3 analysis replicates). Values with different letters in the same list are statistically significant by Duncan’s multiple range test at 5% level.

	Starch concentration (mg g^{-1} FW)	
Cultivar	Petal	Stem
‘Audio’	3.2 ± 0.2 b	1.1 ± 0.1 a
‘Black Magic’	5.7 ± 0.4 a	0.6 ± 0.1 b

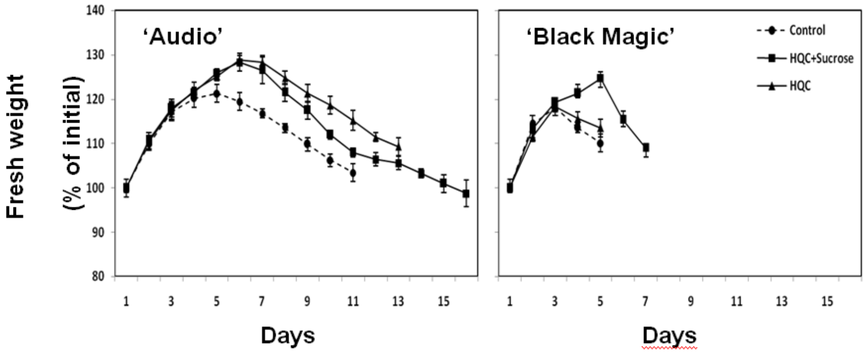

Fig. 1: Effects of HQC (200 mg L^{-1}) or sucrose (20 g L^{-1}) plus HQC (200 mg L^{-1}) on the fresh weight of cut 'Audio' and 'Black Magic' rose flowers. Vertical bars show standard errors of means (n = 3).

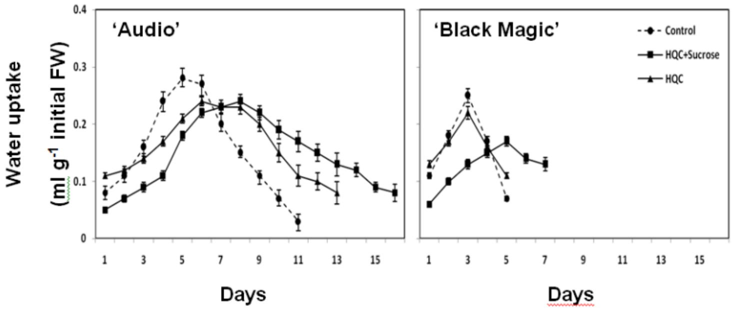

Fig. 2: Effects of HQC (200 mg L^{-1}) or sucrose (20 g L^{-1}) plus HQC (200 mg L^{-1}) on the water uptake of cut 'Audio' and 'Black Magic' rose flowers. Vertical bars show standard errors of means (n = 3).

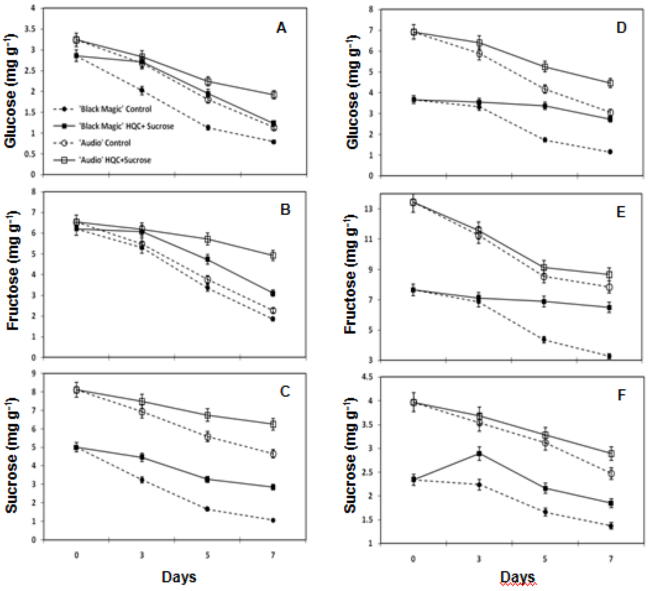

Fig. 3: Changes in the concentrations of soluble carbohydrates (glucose, fructose and sucrose) during postharvest period in stems (A, B and C) and petals (D, E and F) of cut 'Audio' and 'Black Magic' rose flowers supplied with sucrose (20 g L^{-1}) plus HQC (200 mg L^{-1}) in the vase solution. Vertical bars show standard errors of means (n = 3).

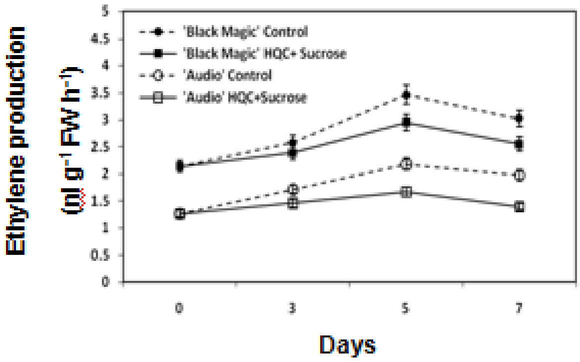

Fig. 4: Ethylene production during postharvest period of cut 'Audio' and 'Black Magic' rose flowers supplied with sucrose (20 g L^{-1}) plus HQC (200 mg L^{-1}) in the vase solution. Vertical bars show standard errors of means (n = 3).

Conclusion:

The vase life of cut ‘Audio’ flowers was longer than that of ‘Black Magic’ flowers. Carbohydrate concentrations in petals and stems of ‘Audio’ were much higher than those in ‘Black Magic’. Therefore, carbohydrate supply may determine the length of the vase life of these cut rose flowers. Also, ethylene plays an important role in vase life of these cultivars and ethylene production in petals increases during natural flower senescence. In addition, exogenous sucrose in vase solution provides additional substrates for respiration, improves the water balance and decreases ethylene production.

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