Changes in Carbohydrate Content in Cut Chrysanthemum [Dendranthema × grandiflorum (Ramat.) Kitamura] ‘Shuho-no-chikara’ Stems Kept at Different Temperatures during Anthesis and Senescence

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Summary

Changes in carbohydrate content in capitulum, upper stems, middle stems, lower stems, upper leaves, and middle leaves in cut chrysanthemum stalks placed in vases at 20, 25 and 30 °C were investigated for 3 weeks. The capitula showed no apparent senescence symptoms within 3 weeks at 20 °C and 25 °C, but senesced rapidly at 30 °C. The leaves wilted earlier than did the capitula. Fructans were found at all plant parts, particularly, in the stems. During capitulum development, fructan content in the upper and middle stems rapidly declined to a low level which suggests that fructans in these stems were utilized for capitulum development. After the anthesis, leaves and stems seemed to serve as a carbohydrate source, but we could not elucidate whether the quantity of carbohydrates in the leaves and stems is related to the longevity of cut chrysanthemums.

Key Words: cut flower, carbohydrate content, longevity, chrysanthemum [Dendranthema × grandiflorum (Ramat.) Kitamura], temperature.

Introduction

Cut chrysanthemum of some cultivars placed in interior room can keep their fresh quality for 20 or to 30 days (Funakoshi, 1984); in contrast to carnation (Eze et al., 1986; Nichols and Ho, 1975), hibiscus (Woodson and Handa, 1987), and morning glory (Kende and Baumgartner, 1974; Mayak and Harev, 1980), which senesces rapidly and wilt within several hours after the opening of their flowers.

Nichols (1980) and Reid (1989) suggested that chrysanthemums are insensitive to ethylene during the senescence process, whereas Serek et al. (1994) found that in gladiolus flowers, which is insensitive to ethylene, senescence was related to the change in carbohydrate content. Chrysanthemums and other many species in the Compositae family store fructans in their stems and roots (Frehner et al., 1984). Trusty and Miller (1991) reported that potted chrysanthemums accumulate sufficient reserve fructans in the inflorescences and leaves as substrate for petal expansion. Thus, fructans are used for the development and maintenance of capitulum during the long vase life of cut chrysanthemums. To confirm this, changes in carbohydrate content during anthesis and senescence of cut chrysanthemums were investigated at three different temperatures, 20, 25, or 30 °C, because there is no published data about the effects of high temperature on carbohydrate status and senescence in cut flowers during their vase life.

Materials and Methods

Plant material and treatments

Cut chrysanthemums, ‘Shuho-no-chikara’, were obtained from Aichi Prefecture, where they were harvested on March 5th in 1991, and transported to the laboratory the next day. The diameters of the capitula was about 8 cm, which was two-thirds of fully opened ones. Subsequently, the stems were cut to 60 cm lengths and placed in 200 ml glass jars containing 100 ml of deionized water. The stalks were divided into three groups of 40 shoots each and placed in a growth room controlled at 20, 25, or 30 °C and 60-70% RH with continuous light. To simulate indoor conditions, light intensity was adjusted to 2,000-2,300 lx at the shoot tips. Each cut plant was placed in a 15 × 20 cm plot in a room and tagged. The samples kept at 20 and 25 °C were collected after 0, 1, 3, 6, 9, 12, 15, 18, and 21 days, or after 0, 1, 3, 5, 6, 7, 9, 12, and 15 days with those kept at 30 °C. Five shoots from each room were sampled daily at random using a table of random numbers.

As indexes in a three dimensional development of capitulum, the diameter of the capitulum (the mean value of the maximum diameter and its rectangular diameter) and the developmental angle of the florets (the angle between a theoretical line from the centre of the

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involved to the outermost ray floret and a vertical line) were measured. The plants were then divided to capitula, upper leaves, middle leaves, upper stems, middle stems, and lower stems. Involutecs were not used for the measurements since their volumes and weights were too small. After the fresh weight (FW) was measured, the samples were freeze-dried and the dry weight (DW) measured.

**Carbohydrate analysis**

The dried plant materials were ground into fine powder by a Chinese medical mill and a 0.1g subsample was extracted in 80% ethanol at 80 °C for 2 hours. The extract was evaporated to dryness in a rotary evaporator and re-dissolved in 250 μl water and a small amount of dichloromethane. The solution was deionized by passing it through a small column of ion-exchange resin (MB3, Amberlite). The eluate was centrifuged at 15,000g for 5 minutes, then an aliquot of 200 μl of the supernatant was combined with an equal volume of acetonitrile. The mixture was subjected to HPLC analysis on 6A system (Shimadzu, Kyoto), equipped with a refractive index detector (Shimamura Instrument, Tokyo). Separation was carried out on a Cosmosil-5NH2 column (Nakarai Tesque, Kyoto) eluted with acetonitrile:water (70:30 v/v) at 35 °C. Standard fructans for peak identification were prepared by partially acid-hydrolysing purified inulin with 0.01N oxalic acid for 15 minutes, which was then neutralised with 0.01N NaOH. The concentrations of fructans in the samples were calculated by comparing the peak areas for fructans of DP (degree of polymerization)3-10 or the heights for DP1-2 sugars (glucose, fructose and sucrose) with those of 2mg·ml⁻¹ melezitose as a standard. Concentrations of DP1-2 sugars, DP3-4 fructans, and DP5-10 fructans were calculated on a dry weight basis.

As fructose, glucose, and sucrose were not clearly separated on HPLC, the fructose and glucose concentrations were measured by an enzymatic method (F-kit glucose fructose, Boehringer-Mannheim).

**Results**

**Effects of temperature on flower opening and senescence**

The diameter of the capitulum reached its maximum as the developmental angle of the florets arrived at 90°, when the capitulum was regarded as fully open. The capitula, which were half-open at the start of the experiment became fully open after 4.4, 4.2, and 3.3 days at 20 °C, 25 °C and 30 °C, respectively. After the capitula were fully opened, they changed from white to brown and wilted rapidly as shown by 1) decrease in the diameter of the capitulum, 2) increase in the developmental angle of the florets and 3) wrinkling of the corollas at 30 °C. However, these senescent symptoms were seldom observed at 20 °C and 25 °C. The rapid wilting and yellowing of leaves occurred at all temperatures in 5 or 6 days after the start of the experiment.

**Changes in FW and DW**

FW of the capitula (Fig. 1a) gradually increased for 1 to 3 days at all temperatures, after which, the FW remained constant at 20 °C but decreased rapidly at 30 °C; the change at 25 °C occurred at an intermediate rate. In contrast to FW, DW in the capitula (Fig. 1b) gradually increased, followed by no decrease at 20 °C and 25 °C and a small decrease at 30 °C.

FW of stems (Figs. 2a, c, e) started to decrease after 1 to 3 days at all temperatures, especially in the upper stems. In the upper stems, DW decreased rapidly at first and then remained constant at 25 °C and 30 °C, whereas no such changes occurred at 20 °C (Fig.2b). In the middle and lower stems, DW changes throughout the experiment were small (Figs. 2d, f).

FW of the upper (Fig. 3a) and middle (Fig. 3c) leaves

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**Fig. 1.** Changes in fresh weight (a), dry weight (b), and DP1-2 sugars (c) in capitula of cut chrysanthemum 'Shuho-no-chikara' plants placed at 20 °C (o), 25 °C (▲) and 30 °C (■). Means of 3 to 5 replications ± SE.
Fig. 2. Changes in fresh weight (a, c, e) and dry weight (b, d, f) in upper (a, b), middle (c, d), and lower (e, f) stems of cut chrysanthemum 'Shuho-no-chikara' plants placed at 20 °C ( ), 25 °C ( ▲ ) and 30 °C ( ■ ). Means of 3 to 5 replications ± SE.

Fig. 3. Changes in fresh weight (a, c) and dry weight (b, d) in upper (a, b) and middle (c, d) leaves of cut chrysanthemum 'Shuho-no-chikara' plants placed at 20 °C ( ), 25 °C ( ▲ ) and 30 °C ( ■ ). Means of 3 to 5 replications ± SE.
gradually decreased after 1 to 6 days, particularly, at 30 °C. The changes in DW (Figs. 3b, d) were small as compared with FW and the decrease occurred during the later period of the experiment at 30 °C.

Changes in carbohydrates

Ethanol soluble extracts separated on HPLC revealed several oligosaccharides with DP larger than 2. After acid hydrolysis, the extracts contained predominantly fructose and a small amount of glucose, indicating that the oligosaccharides were fructans. Fructans were detected in all parts of chrysanthemum plants. There were negligible levels of fructans above DP5 in the capitulum and the leaves.

Concentration of DP1-2 sugars in the capitula continuously increased at 20 °C, increased for 3 days but remained constant thereafter at 25 °C; it increased markedly for the initial 3 days and then decreased at 30 °C (Fig. 1c).

In the upper and middle stems, DP5-10 fructan concentrations decreased for the initial 6 days to a barely detectable level (Figs. 4a, d). In the lower stems, DP5-10 fructans gradually decreased at 20 and 25 °C, but they fluctuated at 30 °C (Fig. 4g). The concentrations of DP3-4 fructans (Figs. 4b, e, h) and of DP1-2 sugars (Figs. 4c, f, i) increased for the initial 3 days, then they decreased in the next 3 days; the changes were the largest at 30 °C. The decrease continued throughout the experimental period in the middle stems. Total amount of fructans of DP3-10 per whole stem also gradually decreased after the anthesis, notably at 30 °C (data not shown).

Concentration of DP1-2 sugars in the upper leaves remained constant, particularly after 6 days, at 20 °C and 25 °C; but the concentration increased at 30 °C (Fig. 5a). In the middle leaves, the concentration increased, then decreased at 20 °C, remained constant at 25 °C, and markedly increased after 9 days at 30 °C (Fig. 5b).

To determine the concentrations of fructose and glucose, independently, enzymatic analysis was also carried out for a part of the samples. Fructose was the predominant hexose in the capitula (Fig. 6), the upper leaves (Fig. 7a), and the upper stems (Fig. 7b). The changes in fructose and glucose concentrations (Fig. 6) were similar to those of DP1-2 sugars (Fig. 1c) at each temperature. In the upper leaves kept at 20 °C, fructose and glucose concentrations increased from 0 to 3 days, then decreased, and remained constant (Fig. 7a). In the

![Fig. 4. Changes in concentrations of DP 5-10 fructans (a, d, g), DP 3-4 fructans (b, e, h) and DP1-2 sugars (c, f, i) in upper (a, b, c), middle (d, e, f) and lower stems (g, h, i) of cut chrysanthemum 'Shuho-no-chikara' plants placed at 20 °C (●), 25 °C (▲), and 30 °C (■). Means of 3 to 5 replications ± SE.](image-url)

<table>
<thead>
<tr>
<th>Days after start of treatment</th>
<th>Concentration (mg · g⁻¹ DW)</th>
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<tr>
<td>DP5-10</td>
<td>20</td>
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<tr>
<td>DP3-4</td>
<td>15</td>
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<td>DP1-2</td>
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upper stems kept at 20 °C, fructose concentration initially increased from 0 to 3 days, then rapidly decreased to the 6th day, followed by a gradual decrease, whereas the glucose concentration, which was much lower, slightly decreased during the experiment (Fig. 7b). Starch was not detected by enzymatic analysis in any part during the experimental period.

Discussion

Vase life of cut flowers is divided into two phases, anthesis and senescence. Developmental changes in FW, DW, and content of sugars in flowers are characterized by an increase during flower opening and a decrease during senescence (Bieleski, 1993; Mayak and Halevy,
Anthesis was observed at all temperatures in this study, whereas senescence phase was observed only at 30°C. After the full opening, FW (Fig. 1a), DW (Fig. 1b), and concentration of DP1-2 sugars (Figs. 1c, 6) in the capitula increased as they opened, and reached a maximum from 3 to 6 days after the start of the treatment when petals became fully unfolded. After the full expansion, the weights and sugar concentration decreased as wilting and browning of petals proceeded at 30°C; there were no visible senescence symptoms for 3 weeks at 20°C and 25°C. Kofoane and Reid (1983) reported that temperatures in the 15 to 21°C range gave optimal opening time and flower quality to cut chrysanthemums. The decrease of FW (Fig. 1a), DW (Fig. 1b), and sugars (Fig. 1c) in the capitula was least at 20°C, which is within the optimum temperatures described above.

The increase in DW (Fig. 1b) and soluble sugars of DP1-2 (Fig. 1c) in the capitula during anthesis indicates that the carbohydrate supply to the capitula came from other parts. The HPLC and enzymatic analysis showed that the stems contained fructose, glucose, sucrose, and fructans of DP3-10, whereas the leaves contained the same sugars and fructans of DP3-4; no starch was detectable. During anthesis, fructans of DP5-10 (Figs. 4a, d, g) decreased in the upper (Fig. 4a) and middle (Fig. 4d) stem; fructans of DP5-4 (Figs. 4b, e, h), sucrose, and fructose (Figs. 4c, f, i) increased in these stems.

These observations suggest that DP5-10 fructans in the upper stems were mobilized by hydrolysis, and provided necessary sugars for the development of the capitula. The increase in fructose concentration in the stem parts is attributed to fructan hydrolysis, because the sucrose level was consistently lower than that of fructose (Fig. 7b). In wheat, fructans are supplied from stems to ears during starch accumulation in the grain (Dubois et al., 1990). The decrease in DP5-10 fructans was larger in the upper and middle stems than in the lower stems, suggesting that upper and middle stems are a larger source of carbohydrates for the flowers than are lower stems in cut chrysanthemums. In particular, the middle stems could be larger reserve, because DP1-2 sugars and DP3-4 fructans gradually decreased during the experiment.

In potted chrysanthemum, 10-100 mg·g⁻¹ DW of starch were detected in the capitula, leaves, and stems (Trusty and Miller, 1991; Rajapakse and Kelly, 1995a, b; Rajapakse et al., 1996). They proposed that photosynthates which accumulate as starch is used for the development and maintenance of the capitula. In this study, however, starch was not detected in any part of a stalk, possibly because it was hydrolyzed to mono- and di-saccharides during the transport to the laboratory after harvest.

After the capitula became fully open, DW in the whole cut plants as well as FW in the capitula decreased at 30°C, which indicates that high temperature accelerates the consumption of carbohydrates and senescing flowers because of the increased respiratory activity (Maxie et al., 1973). At 30°C, the decrease in DW and sugars in the capitula (Fig. 1) accompanied the decrease in DW in the upper and middle leaves (Fig. 3); such decreases were not observed in the stem parts (Fig. 2). Leaves likely serve as a source of respiratory substrates for the capitula after their full opening. The rapid increase in the concentration of sugars in the leaves at 30°C may suggest that carbohydrates in leaves are mobilized and provided for the capitula. Leaves serve as a carbohydrate source for flowers of roses (Ho and Nichols, 1977), protea (Dai and Paull, 1995; McConchie et al., 1991; McConchie and Lang, 1993) under postharvest environments. Moreover, leaves produce photosynthates after cut flowers are placed in vases (Dai and Paull, 1995; McConchie et al., 1991; McConchie and Lang, 1993). Trusty and Miller (1991) regarded stems and leaves as main sources of carbohydrates during anthesis and after full opening, respectively, in potted chrysanthemums, which agrees with the results of this study.

In our study, however, yellowing and wilting occurred earlier in the leaves than in the capitula, indicating that the rate of photosynthesis and carbohydrate supply were limiting. Although senescence in the leaves proceeded, the capitula maintained their quality for 3 weeks. We think that sugars in the stems may also provide carbohydrates to the capitula after the full opening, because dry weight (Fig. 2) and sugars (Fig. 4) gradually decreased in the middle stems and did fructose in the upper stems (Fig. 7b).

This study showed that fructans in the stem, particularly, in the middle position, are used for the development of capitula in cut chrysanthemums. After the full opening, FW, DW, and sugar concentration in the capitula remained at constant levels and senescent symptoms were not observed at 20°C and 25°C, whereas, at 30°C, the weights and sugar concentration rapidly decreased during senescence. Leaves as well as stems seemed to serve as a carbohydrate source after anthesis. However, it is not clear whether carbohydrates in stems and leaves are involved in extending the longevity of chrysanthemums capitula, because their concentration did not decrease noticeably. Since the wilting of petals was evident and FW of the capitula decreased while DW unchanged, water loss may be the main cause for the acceleration of their senescence. The small drop in DW compared with FW, which was attributed to a slow respiratory loss or export of soluble carbohydrates from capitula to stems or leaves, may account for the slow start of senescence phase and a long vase life at 20 and 25°C.

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**Literature Cited**


収穫後のキク切り花‘秀芳の力’における開花と老化に伴う炭水化物含量の変化と温度の影響

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摘 要

5分咲きのキク切り花を20、25および30℃に置き、頭状花序、上位茎、中位茎、下位茎、上位葉および中位葉の各部位の炭水化物含量の変化を測定した。3週間の実験期間中、20および25℃の頭花に老化の兆候は認められなかったが、30℃の頭花は急速に開花した後急速に老化した。どの温度においても葉は頭花より早くしおれ黄化した。フルクタンは全部位で検出されたが、特に茎の含量が多かった。上位茎および中位茎のフルクタンは開花にともなって急速に減少し、開花後にはほとんど認められなかった。従って、茎のフルクタンが頭花の生長のために供給されたと考えられた。開花後ににおいても茎および葉の炭水化物が頭花の維持のために供給された可能性があったが、それらの炭水化物がキク切り花の日持ちの延長に関係しているかどうかは明確でなかった。

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