Role of Sucrose in Gamma-irradiated Chrysanthemum Cut Flowers

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Vase solution containing 2% sucrose prevented the deterioration of chrysanthemum (Dendranthema grandiflorum Kitamura) cut flowers induced by gamma-rays at 750 Gy. Glucose, fructose, and sucrose in florists and leaves of irradiated chrysanthemums decreased more rapidly than those of unirradiated ones, when the cut chrysanthemums were held in a vase solution without sucrose. The sugar contents of florists and leaves and the respiratory rate of irradiated chrysanthemums held with sucrose remained at higher levels than those of unirradiated ones. Incorporation of 14C from [14C]sucrose into CO₂ was increased by irradiation. Incorporation of [α-32P]dTTP into thymidinonic acid (TCA) insoluble substances in florists was increased by irradiation and by exogenous sucrose supply. These results suggest that sucrose in a vase solution was used as a respiratory substrate and facilitated the repair of radiation-induced damage, resulting in the extension of longevity of irradiated chrysanthemums.

Key words: chrysanthemum; gamma-ray; irradiation; sucrose

Imported cut flowers contaminated with insect pests are fumigated with methyl bromide or cyanide for quarantine purposes. Fumigation, however, is not desirable from the viewpoints of both human health and environmental destruction. For example, the use of methyl bromide is going to be phased out globally, because of its ozone-depleting effect.1-3 One alternative treatment to fumigation is radiation disinfection.3 Radiation damages not only pests but host commodities. Tolerance to radiation varies with species of flowers4-5 and chrysanthemums are highly intolerant to radiation.5,8 We have reported that solutions of floral preservatives and sugars prevent the deterioration of chrysanthemum cut flowers induced by irradiation at doses up to 750 Gy and extend their longevity.9-12 In this study, we investigated the effects of exogenous sucrose on the longevity, sugar contents, respiratory rate, and DNA synthesis of irradiated chrysanthemum cut flowers to discover the role of sucrose.

Materials and Methods

Flower irradiation and storage. Flowers of chrysanthemum (Dendranthema grandiflorum Kitamura, cv. Seishu) grown in Ibaraki, Japan and harvested at the bud stage (diameter approximately 2 cm and fresh weight approximately 2.5 g) were purchased from a local florist in Tsukuba, Ibaraki, Japan. The chrysanthemums had not been treated with any chemicals since the harvest.

The chrysanthemum stems were recut to 20 cm with one flower and five leaves, placed in tap water for 2 h, and irradiated at 750 Gy with a Gammacell 220 (4.7 × 10⁶ Gy/h, 2.1 × 10⁶ TBq of ⁶⁰Co, Nordion International Inc., Canada) at 25°C while standing in tap water and in darkness. Irradiated chrysanthemum stems were held in a solution containing 0.02%, 8-hydroxyquinoline sulfate, 0.001% ampicillin, and 0.001% streptomycin sulfate with or without 2% sucrose at 25°C. The humidity and light were not controlled; indirect sunlight was introduced into the storage room through windows in daytime (about 8,000 lux for 14 h).

Determination of soluble sugars. Plant tissues (3.0 g) from florists or leaves were homogenized in 25 ml of 80% ethanol with a Polytron homogenizer (Kinematica GmbH, Switzerland) for 3 min at the maximum output and then refluxed at 85°C for 10 min. The homogenate was filtered and the residue was refluxed in 20 ml of 80% ethanol 3 times. The extract was dried in vacuo and dissolved in 3 ml of distilled water. The solution was neutralized by addition of 0.3 g of Amberlite MB-3 and filtered with a nitrocellulose membrane (0.45 μm). Sucrose and fructose were measured by colorimetry as described by Ashwell (1957).13 Glucose was measured enzymatically with a Glucose CII Test (Wako Pure Chemicals Ind., Ltd., Japan).

Measurement of respiratory rate. The respiratory rate was measured by the evolved CO₂ by gaschromatography as follows. A chrysanthemum stem with one flower and 5 leaves was placed in 10 ml of the vase solution with or without 2% sucrose, which was incubated in a sealed bottle (1000 ml) at 25°C for 6 h in darkness. Two ml of the sample gas was taken from the bottle through a rubber Septum with a syringe. The gas was analyzed with a Shimadzu Gaschromatograph GC-14B (Shimadzu Seisakusho Ltd., Japan) with a Porapack Q column (3 mm × 2 m) at 75°C and a thermal conductivity detector at 80°C to measure CO₂ evolved by

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the chrysanthemum stem. Helium gas at 45 ml min⁻¹ was used as a carrier gas.

The radioactivity incorporated into CO₂ from [¹⁴C]sucrose was measured as well. Immediately after irradiation, a chrysanthemum stem with one flower and 5 leaves held in 10 ml of vase solution containing 0.56 kBq/ml [U-¹⁴C] sucrose (23.2 GBq/mmol, Amersham International, USA) and 2% sucrose was incubated at 25°C for 15 h in an Erlenmeyer flask (300 ml) with an airtight rubber stopper, in darkness. The gas phase was pumped out and bubbled in 2 ml of 0.1 n KOH. One ml of the alkaline solution and 12 ml of Clear-sol II (Nacalai Tesque Inc., Japan) were mixed and neutralized by addition of 0.2 ml of 0.5 n HCl, the radioactivity of which was measured with a liquid scintillation analyzer (TRI-CARB 1900TR, Packard, USA).

Measurement of rate of translocation of sucrose. Rate of translocation of sucrose was measured by the radioactivities of leaves and florets of chrysanthemum stems pulse labelled with radiolabelled sucrose, as follows. One hour after irradiation, chrysanthemum stems were pulse labelled for 1 h at 25°C with a vase solution containing 7.4 kBq/ml [U-¹⁴C] sucrose (23.2 GBq/mmol, Amersham International, USA) and 2% sucrose (unlabelled), after which the stems were transferred to the vase solution with 2% sucrose (unlabelled) and incubated for 4 or 18 h at 25°C. Plant tissues (0.25–0.54 g) from florets or leaves were mixed with 12 ml of Clear-sol II. After 3 cycles of freeze-thaw treatment, the radioactivity was measured with a Tri-Carb 1900TR.

Measurement of incorporation of [³²P]dTTP into TCA insoluble substances. Immediately after irradiation, chrysanthemum stems were incubated for 1 h at 25°C standing in 10 ml of vase solution containing 37 kBq/ml [α-³²P]dTTP (110 TBq/mmol, Amersham International, USA) with or without 2% sucrose (unlabelled). The plant tissues (0.5 g) from florets were homogenized in 2 ml of 10% TCA, and the homogenate was centrifuged at 10,000 x g for 10 min. The precipitate was washed with 2 ml of 10% TCA 3 times. The radioactivity of the precipitate suspended in 12 ml of Clear-sol was measured with a Tri-Carb 1900TR.

Results
Effects of irradiation and exogenous sucrose supply on longevity and flower weight
Unirradiated chrysanthemums did not wilt for more than 21 days, irrespective of the presence of sucrose in a vase solution. Irradiated chrysanthemums held in the vase solution without sucrose showed the onsets of flower browning and leaf yellowing 9 days and 7 days after irradiation, respectively, while those held with sucrose showed the same longevity as unirradiated ones with and without sucrose. All the florets and leaves of irradiated chrysanthemums wilted about 15 days after irradiation, when they were held without sucrose. The weights of flowers irradiated at 750 Gy and held for 21 days with sucrose were about 14 g, while those without sucrose were about 3 g. The weights of unirradiated flow-

ers were about 15 g and 10 g after storage for 21 days with and without sucrose, respectively.

Sugar contents
The contents of glucose, fructose, and sucrose in the florets of both unirradiated and irradiated chrysanthemums held without sucrose continued to decrease during storage at 25°C (Fig. 1). The sugar contents of irradiated florets held without sucrose decreased more rapidly than those of unirradiated ones and reached undetectable levels 14 days after irradiation. Exogenous sucrose supply from the vase solution increased the levels of glucose and fructose and prevented the loss of su-

![Fig. 1. Sugar Contents of Florets of Chrysanthemums during Storage at 25°C after Irradiation.](attachment:image.png)

Δ: unirradiated chrysanthemum held in a vase solution without sucrose. △: unirradiated chrysanthemum held in a vase solution with 2% sucrose. ○: irradiated chrysanthemum held in a vase solution without sucrose. ●: irradiated chrysanthemum held in a vase solution with 2% sucrose.

Data show the mean and standard deviation of triplicate measurements.
Sucrose in Irradiated Chrysanthemum

crose in florets, irrespective of irradiation treatment. The sugar contents of the florets of irradiated chrysanthemums were always higher than those of unirradiated ones, when the stems were held in the vase solution with sucrone, irrespective of irradiation. The sugar contents of leaves of irradiated chrysanthemums were always higher than those of unirradiated ones, when the stems were held in the vase solution with sucrose.

The contents of the three sugars in the leaves of irradiated chrysanthemums held in the vase solution without sucrose increased for several days after irradiation and then decreased to undetectable levels within 14 days (Fig. 2). The sugar contents of the leaves of chrysanthemums held with sucrose increased for 7–14 days at 25°C and were higher than those without su-

**Respiratory rate**

The respiratory rate of chrysanthemums increased immediately after irradiation followed by a rapid decrease to almost the same levels as unirradiated ones, when they were held in the vase solution without sucrose (Fig. 3). Exogenous sucrose accelerated respiration, irrespective of irradiation. The respiratory rate of irradiated chrysanthemums was always higher than that of unirradiated ones, when the stems were held in the vase solution with sucrose. Incorporation of 14C from radiolabelled sucrose into CO2 evolved from irradiated and unirradiated stems were 85.5±0.7 Bq/g and 40.2±0.5 Bq/g, respectively.

**Translocation of [14C]sucrose**

The [14C]sucrose in a vase solution was transferred to leaves and then to florets (Fig. 4). The rate of translocation of the radioactivity from vase solution to florets through leaves was decreased by irradiation.

**Incorporation of [α-32P]dTTP into TCA insoluble substances**

Gamma-irradiation and exogenous sucrose supply increased the incorporation of 32P into TCA insoluble sub-

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**Fig. 2.** Sugar Contents of Leaves of Chrysanthemums during Storage at 25°C after Irradiation.

- △: unirradiated chrysanthemum held in a vase solution without sucrose.
- ▲: unirradiated chrysanthemum held in a vase solution with 2% sucrose.
- ○: irradiated chrysanthemum held in a vase solution without sucrose.
- ●: irradiated chrysanthemum held in a vase solution with 2% sucrose.

Data show the mean and standard deviation of triplicate measurements.

**Fig. 3.** Respiratory Rate of Chrysanthemums during storage at 25°C after Irradiation.

- △: unirradiated chrysanthemum held in a vase solution without sucrose.
- ▲: unirradiated chrysanthemum held in a vase solution with 2% sucrose.
- ○: irradiated chrysanthemum held in a vase solution without sucrose.
- ●: irradiated chrysanthemum held in a vase solution with 2% sucrose.

Data show the mean and standard deviation of triplicate measurements.
in preventing the detrimental effects of radiation but lactose is not effective.\textsuperscript{11} The results in our previous study suggested that sucrose inhibited the physiological deterioration that took place during storage following irradiation.\textsuperscript{10,11}

Ionizing radiation causes damage in a lot of important macromolecules including chromosomal DNA, and cells respond to such critical damage on DNA through a variety of repair reactions that require energy.\textsuperscript{17} Some of the energy-requiring reactions such as protein and polynucleotide syntheses would be accelerated in gamma-irradiated chrysanthemums, as shown by the increased incorporation of \(^{32}\text{P}\) into TCA insoluble fraction from \([\alpha-^{32}\text{P}]\text{dTTP}\) (Table I). Thus, gamma-irradiation accelerated the respiration of chrysanthemums (Fig. 3), resulting in a rapid consumption of endogenous carbohydrate in this study (Fig. 1). The accelerated respiration used exogenous sucrose as a substrate, as suggested by the increased incorporation of \(^{14}\text{C}\) into CO\(_2\) from \([\text{^{14}C}]\text{sucrose}\). All the florets and leaves wilted, when the endogenous sugars were used up (Figs. 1 and 2). Exogenous sucrose supply maintained high levels of sugar contents in florets and leaves of irradiated chrysanthemums. The higher concentration of sugars in each organ would allow such a high respiratory rate. Sucrose content is increased by irradiation in various plants such as potato,\textsuperscript{18} sweet potato,\textsuperscript{18,19} chestnut,\textsuperscript{20} and apple.\textsuperscript{21} It has been reported that the activities of phosphorylase, UDPG-pyrophosphorylase, sucrose-phosphate synthase, and sucrose synthase, which are responsible for starch-sugar interconversion, are increased by irradiation in gamma-irradiated potato tubers.\textsuperscript{22,23} Some of the enzymes in chrysanthemums responsible for starch-sugar interconversion would be enhanced by irradiation.

However, the rate of translocation of sucrose was lowered by irradiation (Fig. 4). Changes in membrane structure and function such as inhibition of ATPase activity, alteration of lipid composition and acceleration of electrolyte leakage have been observed in various irradiated plant materials.\textsuperscript{24-30} Irradiated chrysanthemums would undergo some of such membrane-associated disorders, which would adversely affect the translocation of metabolites such as sucrose. Leaves would play a role as a pool of sugars to be used as substrates for respiration at various organs. A higher concentration of sucrose in leaves would be required to provide all organs with enough respiratory substrate under such reduced translocation in irradiated chrysanthemums. A continuous supply of sucrose from a vase solution would maintain high levels of sugar contents in leaves and flowers and respiration in all organs of irradiated chrysanthemums, contributing to the repair of radiation-induced damage and resulting in the extension of longevity of irradiated chrysanthemums.

### Table I. Uptake of \([\alpha-^{32}\text{P}]\text{dTTP}\) into TCA Insoluble Substances in Florets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Radioactivity (Bq/g fresh weight)</th>
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<tbody>
<tr>
<td></td>
<td>+ sucrose</td>
</tr>
<tr>
<td>Irradiated</td>
<td>23.6 ± 1.09</td>
</tr>
<tr>
<td>Unirradiated</td>
<td>5.4 ± 0.68</td>
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</tbody>
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Data show the mean and standard deviation of triplicate measurements.

Discussion

The results on the longevity of irradiated chrysanthemums were consistent with our previous reports,\textsuperscript{9-12} in that the exogenous sucrose supply extended the longevity of irradiated chrysanthemum cut flowers. Sucrose exogenously supplied for unirradiated cut flowers prolongs the life and promotes florescence.\textsuperscript{14-16} Sucrose has been reported to maintain the pool of respiratory substrate, the structure and function of mitochondria, and the integrity of membranes, and to improve the osmotic potential of flowers, resulting in the prolonged life of unirradiated cut flowers.\textsuperscript{14-16} We have reported that sucrose, glucose, and fructose are equally effective

References

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