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SUCROSE DELAYS MEMBRANE DETERIORATION OF CHRYSANTHEMUM FLOWERS INDUCED BY GAMMA-RAYS

O.K. KIKUCHI[1], S. TODORIKI AND T. HAYASHI

National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries
Kannondai, Tsukuba, Ibaraki 305, Japan

[1]Institute of Nuclear and Energy Research, National Commission of Nuclear Energy
Caixa Postal 11049, CEP 05499-970, São Paulo, SP, Brazil

ABSTRACT

Fluidity of the flower membranes of cut chrysanthemums (*Dendranthema grandiflorum* Kitamura) decreased soon after gamma-irradiation at 750Gy and continued to decrease during storage following irradiation. Holding chrysanthemum cut inflorescence in 2% sucrose suppressed the decrease. The results suggest that sugars reduce radiation-induced physiological deterioration of chrysanthemum flower membranes.

KEYWORDS

Chrysanthemum; fluidity; ionizing radiation; membranes; sucrose.

INTRODUCTION

Cut flower vase-life can be extended by holding in sucrose or preservative solutions, which delay the senescence process and hold up the deleterious effects caused by some physical factors. The sugar is an energy source and a substrate for respiration (Mayak and Halevy, 1986) and it has been reported that the exogenous sucrose supply holds the petal membranes microviscosity of rose (Goszczynka et al, 1990) and carnation (Adam et al, 1983). Besides, it has been reported that the sugar protects the structure and function of the cells submitted to a water stress (Crowne et al, 1988) and preserves the viability of the irradiated chrysanthemum cut inflorescences (Dohino and Hayashi, 1995; Kikuchi et al, 1995; Hayashi and Todoriki, 1996).

The modifications of the plant cell membranes caused by ionizing radiation have been investigated on cauliflower floret (Voisine et al, 1991, 1993), apple fruit (Dong et al, 1994), muskmelon fruit (Lester and Whitaker, 1996), potato tuber (Hayashi et al, 1992; Todoriki et al, 1994), *Chelidonium majus* leaf (Sato et al, 1995) and kidney bean hypocotyl (Pai and Gaur, 1983, 1987).

It was expected that sucrose influenced the membranes of irradiated chrysanthemum. We investigated the effects of gamma-rays and/or sucrose supply on chrysanthemum flower membranes.

MATERIALS AND METHODS

Plant material

White chrysanthemum cut inflorescences (*Dendranthema grandiflorum* Kitamura) were purchased in a local florist, in Tsukuba city, Japan. About 15hours before the irradiation the stems were cut and half of them soaked in preservative solution containing 0.02% (w/v) 8-hydroxyquinoline sulfate, 0.01% (w/v) ampicillin, 0.01% (w/v) streptomycin and 2% (w/v) sucrose. The other half was soaked in preservative solution without sucrose.

Flower irradiation and storage

The stems, with the inflorescence, were cut to about 20cm, just before the gamma-irradiation with a single dose of 750Gy in a Gammacell 220 ($4.9 \times 10^3 Gy.h^{-1}$, $2.1 \times 10^2 TBq$ of ^{60}Co; Nordion Intl., Ont., Canada). The samples were divided into four groups: C - control, non-irradiated and soaked in preservative solution without sucrose; I - irradiated and soaked in preservative solution without sucrose; S - non-irradiated and soaked in preservative solution with 2% sucrose, and; IS - irradiated and soaked in preservative solution with 2% sucrose. After irradiation the flowers were maintained at 20°C.

The sampling was carried out soon after the irradiation, in about 30minutes, and them one, two, seven, fourteen and twenty one days after the irradiation. The flowers of two inflorescences per group were processed .

Preparation of membrane fractions

The flowers of one inflorescence were picked out from the head, weighted (fresh weight) and homogenized in 40ml of cold grinding medium containing 0.5M mannitol, 75mM MOPS-KOH (pH 7.6), 5mM EDTA, 5mM EGTA, 10mM KF, 2mM PMSF, 0,1% BSA, 1.5% PVP, 5mM DTT and 0.001% BHT. The homogenate was filtered through four layers of gauze and centrifuged at 1,500 x g, for 10 minutes, at 4°C. The pellet was discarded and the supernatant was centrifuged at 10,000 x g, at 4°C, for 30 minutes. The pellet, corresponding to the mitochondrial fraction, was suspended in 1ml of cold suspending solution containing 10mM MES-Tris (pH 7.3), 0.25M mannitol, 1mM EGTA and 20% glycerol. The supernatant was ultracentrifuged at 145,000 x g for one hour, at 4°C, and the pellet, the microsomal membrane fraction, was suspended in 1ml of the same suspending solution used in the mithocondrial fraction, with the addition of 1mM DTT.

Membrane fluidity

The microviscosity was measured on the same day as the membrane preparation, using 100ul of the microsomal membranes solution (100-200ug protein.ml^{-1}), suspended in 2.5ml of suspending buffer containing 5mM K-phosphate (pH 7.3), 0.6M mannitol and 1mM EDTA. The DPH (1uM) was used as fluorescent probe (Borochov et al., 1978; Adam et al., 1983; Shinitzky and Barenholz, 1978). The polarization was measured in a fluorescence polarization spectrophotometer FS-501 A, Photal (Otsuka Electronics Co. Ltd., Japan), at 22°C, with continuous stirring. The fluidity was calculated according to Shinitzky and Barenholz (1978) and Adam et al. (1983).

Determination protein concentration

The protein concentration was evaluated by the method of Lowry et al., as modified by Peterson (1977), using bovine serum albumin as the standard.

Lipid extraction and determination of phospholipid and sterol contents

The microsomal membrane lipids were extracted by the method of Bligh and Dyer (1959). The phospholipid concentration was determined according to the method of Bartlett (1959) and the sterol content was determined using the F-kit Cholesterol (Boehringer Mannhein, GmbH, Germany).

RESULTS AND DISCUSSION

The microsomal membrane fluidity decreased (Fig.1) and the sterol:phospholipid ratio (Table 1) increased one day after the irradiation, in the irradiated (I) and irradiated and sucrose supplied (IS) flowers. However, while the fluidity of the irradiated flowers (I) continued to decrease, the irradiated and sucrose supplied ones (IS) stabilized after the second day (Fig. 1), indicating that the sucrose was effective to the maintenance of the membrane fluidity.

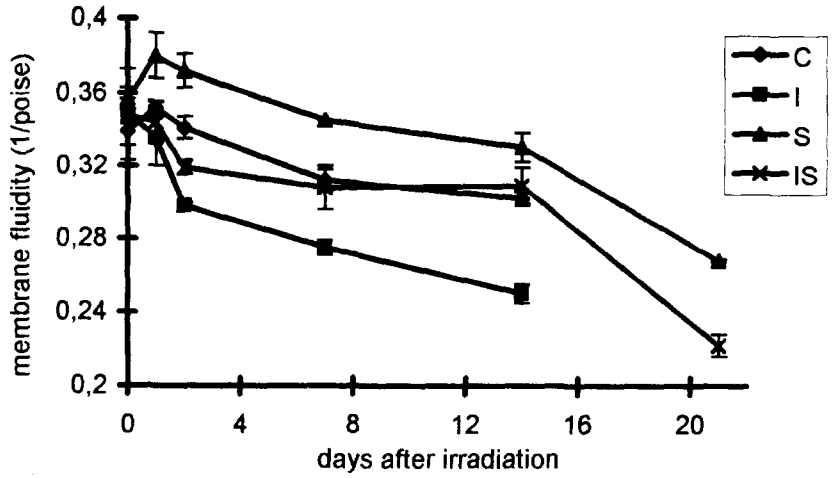

Figure 1. Fluidity of the chrysanthemum flower microsomal membranes after gamma-irradiation with 750Gy. C - control; I - irradiated; S - non-irradiated, supplied with sucrose; IS - irradiated, supplied with sucrose.

The increase of the sterol:phospholipid ratio at the beginning (Table 1) was attributed to the high sterol content, one and two days after the irradiation (Table 2). Besides, the sterol content continued to increase during the observation period in all samples, probably because the experiment was conducted during the flower growth, which comprises a series of biochemical changes. It has been observed that during the flower development of *Brassica campestris* there is an increase of campesterol, which seems to be involved in the petal formation (Hobbs et al., 1996) and about 80% of *Chrysanthemum parthenium* total sterol content has been identified in flowers as campesterol and sistosterol (Wilkomirski and Dubielecka, 1996). The phospholipid content decreased only on the 14th day, two days before the wilting of the control (C) and irradiated flowers (I) (Table 3), similarly to the membrane modifications that occur during the senescence process on other plants (Voisine et al, 1991, 1993; Borochov et al, 1978, 1990; Drory et al, 1992).

The flower fresh weight increased notably on the sucrose supplied samples (S) and the IS flowers also continued to develop as well as the control (C), while the development of the irradiated ones (I) were inhibited by 750Gy (Table 4). Sucrose supply extended the vase-life of the IS flowers more than the control (C). While C vase-life was about 16 days, the S and IS vase-life extended for more

Table 1. Sterol/phospholipid ratio of the microsomal membranes (mole ratio). SD values of two measurements are given in brackets.

days	C	I	S	IS
0	0.189 (0.047)	0.157 (0.037)	0.220 (0.049)	0.155 (0.003)
1	0.260 (0.001)	0.356 (0.069)	0.226 (0.040)	0.349 (0.007)
2	0.303 (0.010)	0.371 (0.045)	0.239 (0.000)	0.333 (0.044)
7	0.377 (0.048)	0.423 (0.018)	0.453 (0.069)	0.380 (0.008)
14	0.552 (0.040)	0.481 (0.031)	0.468 (0.026)	0.475 (0.010)
21	-	-	0.472 (0.035)	0.492 (0.067)

Table 2. Sterol content of the microsomal membranes (umole/mg protein)

days	C	I	S	IS
0	0.039 (0.013)	0.025 (0.001)	0.034 (0.002)	0.027 (0.001)
1	0.036 (0.007)	0.052 (0.002)	0.040 (0.003)	0.057 (0.002)
2	0.052 (0.000)	0.074 (0.004)	0.053 (0.000)	0.065 (0.000)
7	0.088 (0.001)	0.104 (0.013)	0.104 (0.000)	0.089 (0.013)
14	0.108 (0.014)	0.104 (0.012)	0.122 (0.006)	0.131 (0.008)
21	-	-	0.140 (0.016)	0.139 (0.021)

Table 3. Phospholipid content of the microsomal membranes (umole/mg protein)

days	C	I	S	IS
0	0.205 (0.015)	0.166 (0.049)	0.157 (0.025)	0.175 (0.004)
1	0.139 (0.025)	0.148 (0.022)	0.180 (0.018)	0.161 (0.010)
2	0.173 (0.006)	0.201 (0.013)	0.219 (0.000)	0.199 (0.026)
7	0.234 (0.028)	0.244 (0.022)	0.233 (0.035)	0.233 (0.030)
14	0.195 (0.011)	0.215 (0.011)	0.262 (0.025)	0.275 (0.013)
21	-	-	0.296 (0.013)	0.282 (0.004)

Table 4. Fresh weight (g) of the chrysanthemum flowers per inflorescence.

days	C	I	S	IS
0	7.42 (0.44)	8.27 (2.07)	7.27 (0.16)	7.79 (0.43)
1	8.33 (0.86)	7.43 (0.88)	9.78 (1.22)	8.87 (1.25)
2	7.97 (0.85)	8.95 (2.31)	9.19 (0.01)	10.98 (3.56)
7	10.89 (0.89)	8.16 (0.01)	21.44 (4.55)	11.65 (1.27)
14	11.74 (3.44)	7.62 (1.88)	22.13 (1.66)	15.35 (0.76)
21	-	-	21.56 (4.30)	16.51 (3.59)

than 21 days. The S flowers could be maintained viable for four weeks. The concentrations of the microsomal protein (Table 5) and the mitochondrial protein (Table 6) were not affected by irradiation.

The visual wilting symptoms in irradiated samples (I) appeared only at 7^{th} day after the treatment, besides of the strong inhibition of the inflorescence development, as can be seen by the fresh weight (Table 4).

Table 5. Microsomal membrane protein content per fresh weight (mg/g).

days	C	I	S	IS
0	0.874 (0.062)	0.926 (0.023)	0.950 (0.018)	0.887 (0.012)
1	0.977 (0.083)	0.939 (0.063)	0.937 (0.089)	0.862 (0.014)
2	0.730 (0.074)	0.692 (0.123)	0.762 (0.030)	0.646 (0.190)
7	0.705 (0.007)	0.711 (0.018)	0.800 (0.028)	0.720 (0.099)
14	0.700 (0.014)	0.716 (0.066)	0.670 (0.028)	0.660 (0.028)
21	-	-	0.650 (0.000)	0.645 (0.078)

Table 6. Mitochondria protein content per fresh weight (mg/g).

days	C	I	S	IS
0	2.074 (0.049)	1.998 (0.107)	2.134 (0.048)	1.965 (0.454)
1	1.884 (0.083)	2.035 (0.074)	1.828 (0.032)	2.259 (0.757)
2	1.644 (0.015)	1.607 (0.123)	1.546 (0.049)	1.494 (0.062)
7	1.650 (0.000)	1.704 (0.021)	1.660 (0.042)	1.530 (0.014)
14	1.415 (0.021)	1.475 (0.016)	1.220 (0.170)	1.370 (0.028)
21	-	-	1.300 (0.028)	1.270 (0.035)

CONCLUSION

We concluded that the sucrose supply during the vase-life preserved the membrane properties of the irradiated flowers, maintaining the viability and the development of the inflorescence. The membrane fluidity maintenance soon after the irradiation seemed to be one important requirement to the flower for overcoming the radiation stress.

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