

Proceedings of the Korean Nuclear Society Spring Meeting
Gyeong ju, Korea, May 2003

The Relationship between Carbohydrate Content and Gamma Irradiation during Rooting of Chrysanthemum Cuttings

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Abstract

The effect of gamma radiation on carbohydrate metabolism was studied in chrysanthemum cuttings. Total water-soluble carbohydrate, glucose, fructose, sucrose, and starch contents were measured in leaves and stems. Differences in the accumulation of carbohydrate associated with inhibition or stimulation in response to gamma irradiation. Sucrose levels increased significantly in leaves and stems until the 15th day, reaching maximum values on that day. Glucose contents declined rapidly until the 10th day and increased later, reaching maximum values on the 15th day. Fructose levels gradually increased, reaching maximum values at the 10th day, and then decreased again. Differences in the components of soluble carbohydrates were evident between rooting durations and doses. Soluble sugars were in the highest contents in the 20 Gy irradiated group. However, irradiation dose higher than 20 Gy resulted in an inhibitory effect.

1. Introduction

Natural and anthropogenic environmental forces keep all living things in a state of genomic flux. But, toxic environments are the key factors influencing the genome of every organism. Among the abiotic stresses, ionizing radiation constitutes one of the major hazards to agriculture [1]. Ionizing radiation is a potent mutagen, causing biochemical modifications of bases and double-strand breaks in DNA. The enhanced levels of radiation have induced damage to various process, resulting in lowered photosynthesis, inhibited growth [2], and decline in plant productivity [3]. Alteration in the level of compatible solutes, such as amino acids, polyamines, carbohydrates, etc., is claimed to be an effective stress tolerance mechanism[4-8]. Changes in carbohydrate content are of particular importance

because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration. Sucrose, glucose and fructose are the principal sugar component. Sucrose can act in water replacement to maintain membrane phospholipids in the liquidcrystalline phase and to prevent structural changes in soluble proteins. Reduction of the quantities of reducing sugars is a prerequisite for quiescence [9-10]. Glucose participates in crosslinking with protein by a complex glycosylation reaction between amino and carbonyl groups (known as the maillard reaction) [11-12]. Next to their role as plant carbohydrate reserve, fructans, as sucrose-derived oligosaccharides, also play a role in the stress-induced metabolic process [13]. Fructans are fructose polymers that are much smaller than the glucose polymers of starch [14]. Many metabolic reactions are known to be inhibited or stimulated by irradiation. The objective of this study is to present results on the relationship between radiation response and the accumulation of carbohydrates during rooting of chrysanthemum cuttings for exposure to gamma radiation.

2. Materials and methods

Plant Material and Irradiation

Unrooted cuttings of a garden chrysanthemum cultivar 'Emily' were obtained from 'Cheongju Flower Garden' in Chungcheongnam-Do. Each cutting was six cm in length with three real leaves and was irradiated with γ -ray of 20, 30, 50, 70 Gy from ^{60}Co source (150 TBq, Panoramic Irradiator. Atomic Energy of Canada Ltd.). Cuttings were inserted into plug tray of 172 cells filled with vermiculite. All cuttings were rooted under mist in a glasshouse. Air temperatures during rooting and plant growth were 29.7/21.5 and 31.5/22.0 (maximum temperature/minimum temperature).

Carbohydrate Analysis

For carbohydrate analysis, leaves and stems were separated, immediately frozen in liquid nitrogen, stored at -70°C until lyophilization. Dry weights were then recorded and leaf and stem tissues were ground in Wiley mill to pass through 20-mesh screen. Five hundred milligrams of ground tissue was extracted with 12 methanol : 5 chloroform : 3 water (MCW; by volume) for soluble sugar analysis as described by Miller and Langhans [15]. The extract was evaporated to dryness in vacuo at 40°C , and the residue was dissolved in 1.5 mL of HPLC grade water. Sucrose, glucose, and fructose were separated and detected using a Water HPLC system (Water 600E system controller, 700 WISP autosampler, 410 refractive index detector and 810 baseline workstation, water Associated, Milford,

Mass) with a Bio-Rad HPX-87C column maintained at 80 °C. Starch was determined using enzymatic hydrolysis of dried residue following soluble sugar extraction. The residue was with 80% ethanol, then suspended in 4ml of glassdistilled water and heated for 2hr in a boiling water bath while being shaker continuously to solubilize the starch. After solubilization, the suspension under test was cooled to room temperature, water lost by evaporation was replaced, and 1.5ml of buffer solution (1.2M sodium acetate-acetic acid, pH 4.6) and 0.5ml of a 1mg·ml⁻¹ solution of amyloglucosidase (sigma) was added. The suspension was incubated in a 37 °C water bath with continuous shaking for 36hr, and filtered. Filtered solution was measured by spectrophotometer at 470nm.

3. Results and Discussion

The relationship between carbohydrates and adventitious root formation remains controversial. Since Krause and Kraybill [16] hypothesized the importance of carbohydrate-to-nitrogen (C/N) ratio in plant growth and development, rooting ability of cuttings has been discussed in relation to carbohydrate content. The carbohydrate pools of sugars (soluble carbohydrates) and storage carbohydrates (starches or insoluble carbohydrates) are important to rooting as building blocks of complex macromolecules, structural elements, and energy sources [17, 18, 19]. The patterns of change in soluble carbohydrates of cuttings during rooting are shown in Fig. 1-3. In general, sucrose levels declined in leaves and stems until the 5th day, and increased later (Fig. 1). Differences in the components of soluble carbohydrates were evident between rooting durations and doses. Although a continuous sucrose accumulation was observed in all the treatments until the 15th day of rooting, in the 20 Gy irradiated group with a significantly higher (12% on average) value than in contents among the others, there were no clear differences detected. The reducing sugars, glucose and fructose, showed different patterns of changes in response to radiation (Fig. 2 and 3). Glucose markedly decreased to the minimum values on the 10th day. In contrast to glucose, the highest levels of fructose were detected on the 10th day. Considerable differences were observed between the rate and the time course of fructose accumulation in the stem (Fig. 3B). For low doses (0, 20 and 30 Gy), fructose content decreased until the 5th day and increased later for another 5 days, reaching the maximum values on the 10th day after cutting. After this maximum peak, the fructose content decreased. On the other hand, for the higher doses (50, and 70 Gy), fructose content increased considerably until the 5th day and reaching its maximum values on the 5th and 10th day, and then increased slightly (Fig. 3B). The starch content in the leaf increased from the 0th day until the 10th day and showed the maximum

peak on the 10th day, and then decreased until the 20th day (Fig. 4A). But the starch content in the stem increased slightly until the 20th day and reaching its maximum value on the 20th day. Differences of starch content in the leaf and stem between rooting durations and doses were evident. It has long been thought that carbohydrate content of cuttings is important to rooting, and that carbohydrates accumulate in the base of cuttings during rooting [20]. The final product, sucrose is synthesized from activated glucose and fructose-6-phosphate [14]. It is supposed that declined fructose content is converted into sucrose. Sucrose is the major transported form of carbohydrates in plants. Sucrose synthesis is regulated and closely coordinated with starch synthesis [21]. Decreased starch contents in the leaf indicated its move to the stem, convert into sucrose, and being used as energy for rooting. Effects of gamma radiation on rooting may have been due to stimulation and/or inhibition of enzymatic activities [22]. As seen in Fig. 1-3, soluble sugars (sucrose, glucose, and fructose) contents in the 20 Gy irradiated group were higher than those of the non-irradiated control. In contrast, 30, 50, and 70 Gy resulted in an inhibitory effect. However, further studies are needed to investigate the possible reasons behind such response of gamma radiation on rooting.

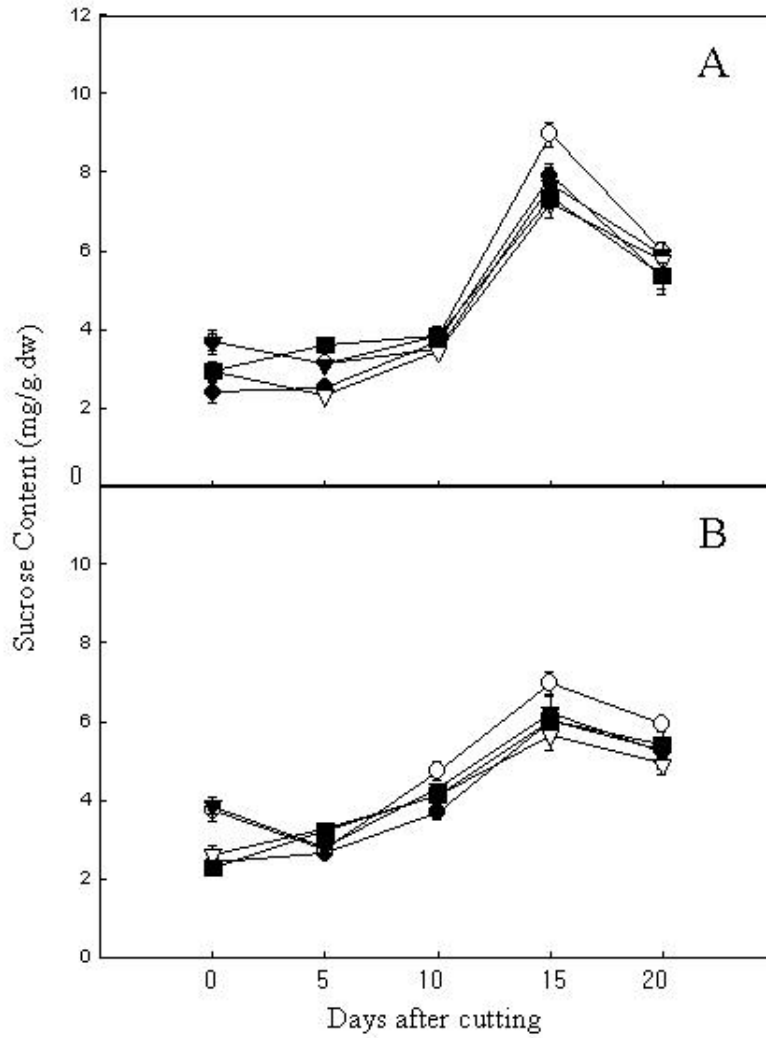


Figure 1. Effect of irradiation on the changes of sucrose content in the leaves (A) and stems (B) of chrysanthemum 'Emly' during propagation. (Filled Circle: 0 Gy, Empty Circle: 20 Gy, Filled Invert Triangle: 30 Gy, Empty Invert Triangle: 50 Gy, Filled Square: 70 Gy).

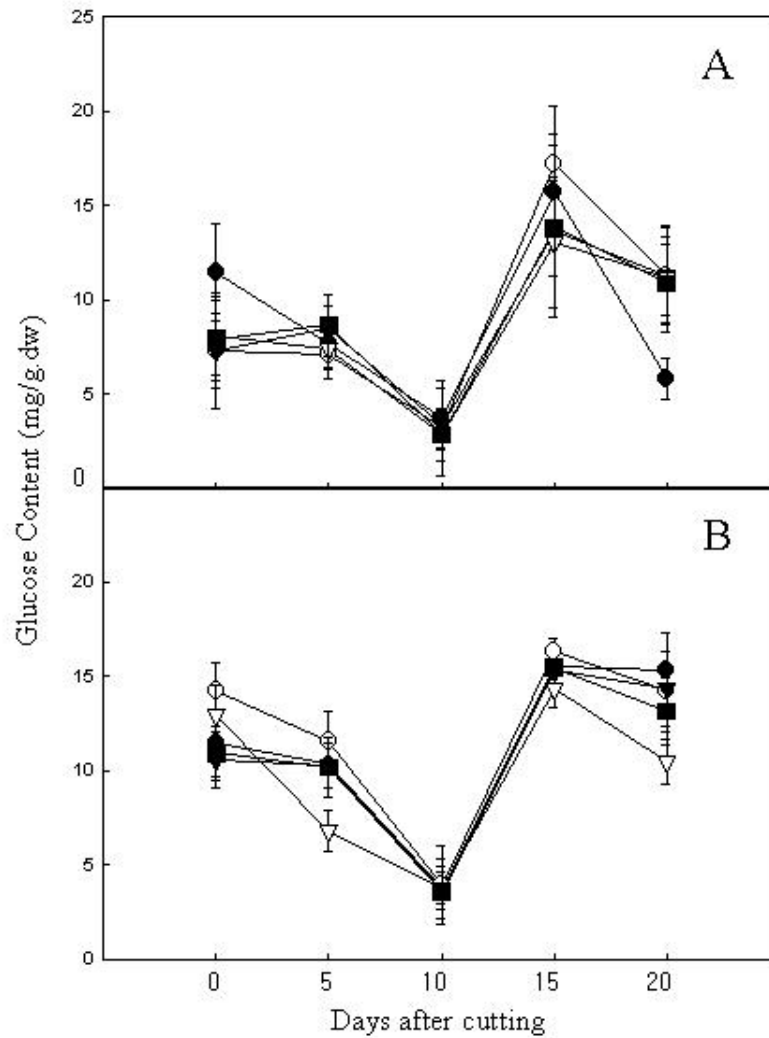


Figure 2. Effect of irradiation on the changes of glucose content in the leaves (A) and stems (B) of chrysanthemum 'Emly' during propagation. (Filled Circle: 0 Gy, Empty Circle: 20 Gy, Filled Invert Triangle: 30 Gy, Empty Invert Triangle: 50 Gy, Filled Square: 70 Gy).

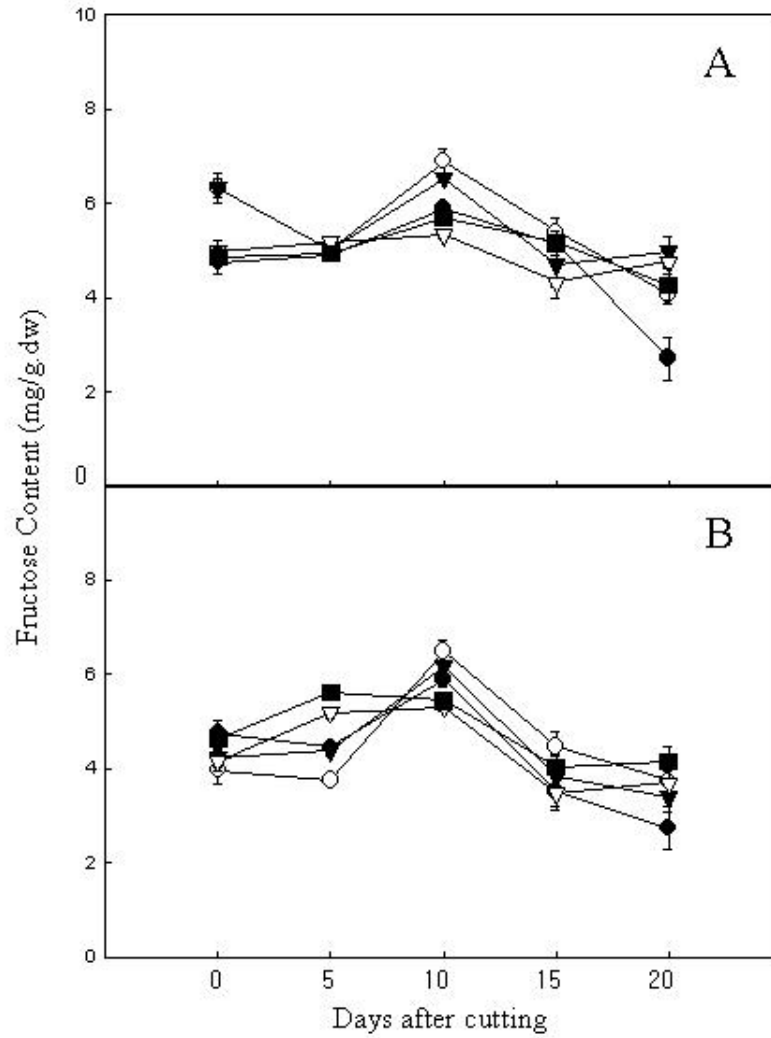


Figure 3. Effect of irradiation on the changes of fructose content in the leaves (A) and stems (B) of chrysanthemum 'Emly' during propagation. (Filled Circle: 0 Gy, Empty Circle: 20 Gy, Filled Invert Triangle: 30 Gy, Empty Invert Triangle: 50 Gy, Filled Square: 70 Gy).

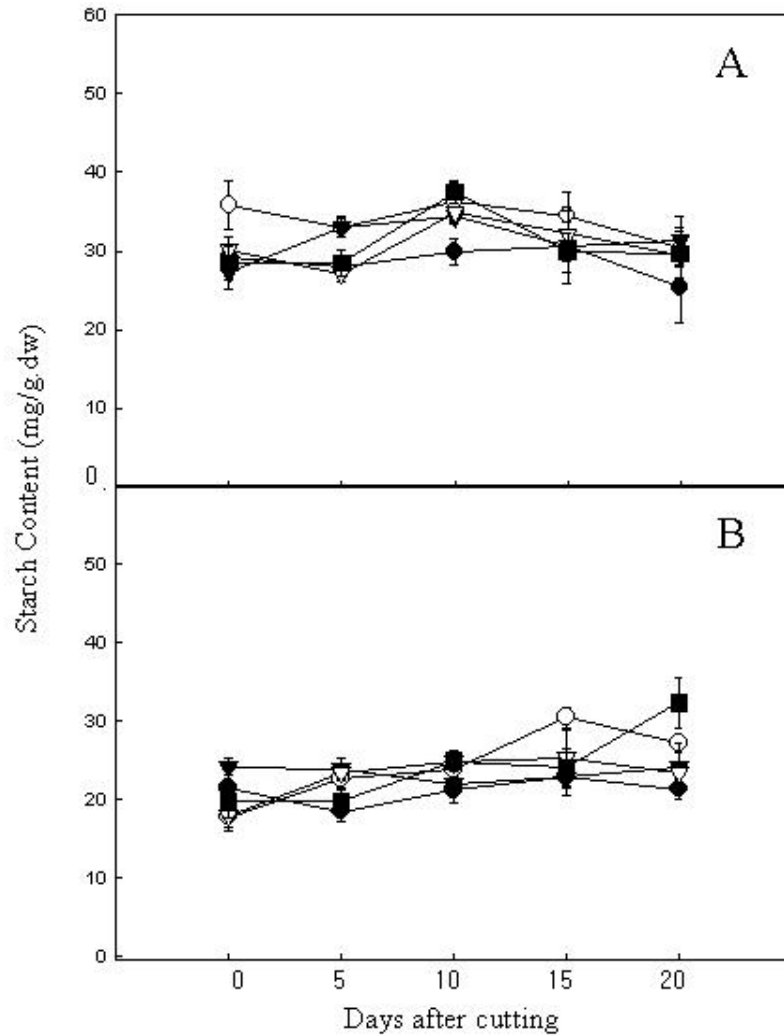


Figure 4. Effect of irradiation on the changes of starch content in the leaves (A) and stems (B) of chrysanthemum 'Emly' during propagation. (Filled Circle: 0 Gy, Empty Circle: 20 Gy, Filled Invert Triangle: 30 Gy, Empty Invert Triangle: 50 Gy, Filled Square: 70 Gy).

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