BIOLOGIA PLANTARUM 49 (4): 625-628, 2005

BRIEF COMMUNICATION

Effect of γ-radiation on development, yield and quality of microtubers *in vitro* in *Solanum tuberosum* L.

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Abstract

Explants obtained from *in vitro*-propagated plantlets of two potato cultivars, Shepody and Atlantic, were treated with five doses of γ-radiation (0, 2, 4, 6 and 8 Gy) to investigate the stimulating effects of low irradiation on the production and quality of microtubers *in vitro*. Microtubers of both cultivars treated with γ-radiation initiated 5 d earlier than in the non-irradiated control. The whole period of microtuberization was prolonged by 10 - 15 d with 4, 6 and 8 Gy irradiation treatment for cv. Atlantic. Irradiation of the plantlets (4 Gy) led to a significant increase not only in the microtuber number (116.7 and 34.5 % over the control) but also in the fresh mass (77.6 and 23.2 % in Shepody and Atlantic, respectively). Low dose irradiation (2 - 4 Gy) increased the starch content of microtubers. High doses (6 - 8 Gy) enhanced ascorbic acid and reducing sugar contents. 4 - 6 Gy doses also effectively increased the protein contents of microtubers.

Additional key words: ascorbic acid, microtuberization, plantlet, potato, protein, reducing sugar, starch.

Potato is propagated predominantly by asexual means (tubers and minitubers) and propagation by true seed is primarily used for breeding purposes. Micropropagation of virus-free potato plantlets grown *in vitro* (microplants) is an important method of potato propagation. With appropriate culture conditions, the axillary buds of such microplants can be induced to form aerial microtubers (Lopez-Delgado and Scott 1997). There are many factors that affect the microtuber induction and formation. Plant growth regulators play an important role in this progress and have been studied extensively (Lopez-Delgado and Scott 1997, Jackson 1999, Vreugdenhil and Sergeeva 1999, Zhang *et al*. 2002). Other factors influencing microtuber production *in vitro* include potato genotypes, explants and culture media such as sucrose, light and temperature (Ahloowalia 1994, Akita and Takayama 1994, Khuri and Moorby 1995, 1996, Jackson *et al*. 2000).

Low doses of γ-radiation have been reported to stimulate plant growth and development, and improve the yields and qualities of plants *in vivo* (Al-Safadi and Simon 1995, Wiendl *et al*. 1995, Paull 1996) and *in vitro* (Degani and Pickholz 1973, Al-Safadi and Simon 1990), apart from its induction of mutants in flower colour and shape (Misra *et al*. 2003).

To date, most of the reports about γ-irradiated potato were focused on the characteristics of the process and/or storage period (Ciesla and Eliasson 2002, Wang and Chao 2003). Limited information has been reported on the influence of γ-radiation on the potato production and

Received 2 March 2004, *accepted* 25 October 2004.
Abbreviations: AA - ascorbic acid; BAP - benzylaminopurine; CCC - chlorocholine chloride; MS medium - Murashige and Skoog medium.
Acknowledgements: The authors thank Shanxi Science and Technology Department (051039-1), Zhejiang Science and Technology Department (2005C22004) and Zhejiang Natural Science Foundation of China (Y304162) for financial supports and Zhejiang Irradiation Center, China for providing γ-radiation condition.
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quality development, especially on potato micropropagation material (microtubers). The purpose of this work is to investigate the stimulating effects of low doses of γ-radiation on the development, yield and quality of microtubers under *in vitro* condition.

In vitro plantlets of two leading commercial cultivars, Shepody and Atlantic, were propagated from single node sections in 100 cm^3 Erlenmeyer flasks with 30 cm^3 of Murashige and Skoog (1962; MS) propagation medium containing 30 g dm^{-3} sucrose and 8 g dm^{-3} agar (pH 5.8). Plantlets were cultured at temperature of 25 ± 1°C, 16-h photoperiod and irradiance of 100 μmol m^{-2} s^{-1}. After 4 weeks, 10 single-node explants obtained from propagated plantlets were transferred into each jar (250 cm^3 capacity) containing 40 cm^3 of the above medium and cultured under the same condition.

After 4 weeks culture, growing shoots were irradiated by ^{60}Co gamma radiation in the Zhejiang Irradiation Center, China. The irradiation doses were 0, 2, 4, 6 and 8 gray (Gy), at a dose rate of 1 kGy h^{-1}. After irradiation, each culture vessel was added 30 cm^3 of liquid MS induction medium (MS medium supplemented with 5 mg dm^{-3} benzylaminopurine (BAP), 500 mg dm^{-3} chlorocholine chloride (CCC) and 80 g dm^{-3} sucrose, pH 5.8). High sucrose content was used to promote the microtuber formation, since potato tuberization was triggered by high concentrations of sucrose (Perl *et al.* 1991, Khuri and Moorby 1995). The culture vessels were incubated in complete darkness at 20 ± 1°C. The microtuber number was recorded every 5 d during the growing period.

After 70 d culture, the fresh mass, diameter, number of harvested microtubers were investigated, and the vitamin C content of fresh microtubers and the starch, reducing sugar and protein contents of microtubers were determined after drying at 60 °C for 24 h.

The contents of starch, reducing sugars and proteins were estimated according to Men and Liu (1995). The method of starch determination involves dissolving starch in perchloric acid, diluting with distilled water, reacting with iodine solution and measuring the absorbance at 660 nm on a spectrophotometer (model *UV-2450*, *Shimadzu Co*., Tokyo, Japan). Ascorbic acid (AA) content was analyzed by the method of Nie *et al.* (1987) with some modifications. Oxalic acid (1 %) was used to extract the AA from the microtuber. After filtration, 2 cm^3 filtrate each was placed into 2 separate test tubes, added 1 drop of 10 % thiourea (thiocarbamide) in every tube. Then the sample tube was added 0.5 cm^3 of 2 % 2,4-dinitrobenzene, and the control tube leaving it blank. Both tubes were incubated at 37 °C for 3 h. After cooling, the sample tube was added another 0.5 cm^3 of 2 % 2,4-dinitrobenzene. After this, 2 cm^3 of 85 % H_2SO_4 were added to both tubes and stayed at room temperature for 30 min. The sample absorbance was determined at 540 nm using the blank one as the control.

The first microtuber initiated about 12 - 15 d after culture in the induction medium in darkness. Microtubers of both cultivars treated with various doses of γ-radiation initiated 5 d earlier than the non-irradiated control. In

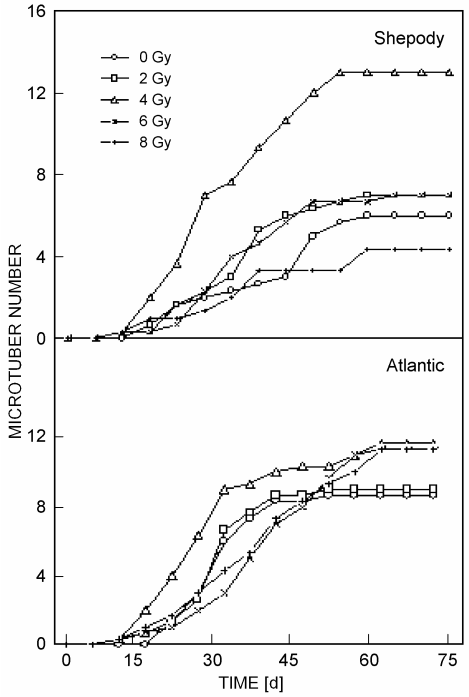

Fig. 1. Effect of γ-radiation on the formation of microtubers in two potato cultivars (Shepody and Atlantic) during 70 d *in vitro* culture.

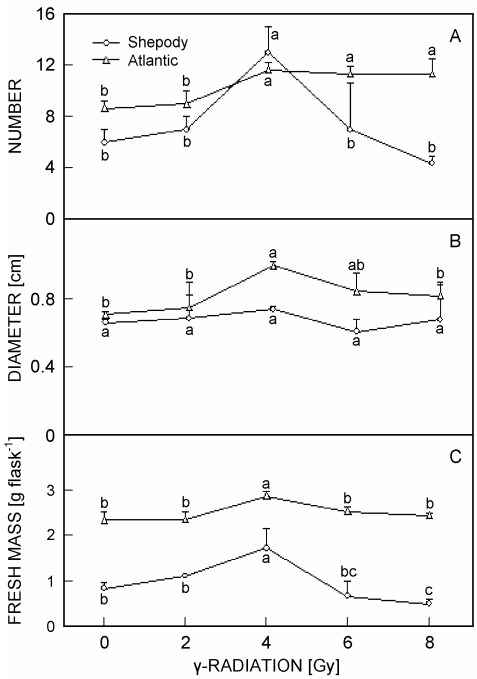

Fig. 2. Effect of γ-radiation on the mean number (*A*), diameter (*B*) and fresh mass (*C*) of microtubers of two potato cultivars (Shepody and Atlantic) after 70 d *in vitro* culture.

addition, the whole period of microtuberization was prolonged by 10 - 15 d with 4, 6 and 8 Gy irradiation treatment for cv. Atlantic, but this difference was less distinct for cv. Shepody (Fig. 1).

For cv. Shepody, the mean number of microtubers increased significantly at 4 Gy irradiation (116.7 % over the control). High doses of irradiation (6 and 8 Gy) did not increase the microtuber number (Fig. 2*A*). The diameter of microtubers exhibited no significant difference with the irradiation treatment. However, for cv. Atlantic, the mean number of microtubers increased significantly at the doses above 4 Gy (34.5 % over the control), the microtuber diameter increased at 4 Gy and then decreased at 8 Gy (Fig. 2*B*).

The fresh masses of microtubers for cvs. Shepody and Atlantic treated with 4 Gy radiation increased 77.6 and 23.2 % over the control, respectively (Fig. 2*C*). This increase of fresh mass of microtubers for cv. Shepody was mainly due to the increase of microtuber number, while for Altlantic, it attributed to not only the increase of the number but also the diameter of microtubers. High doses of irradiation (6 and 8 Gy) no longer increased the fresh mass of microtubers in Atlantic or even decreased fresh mass in Shepody microtubers.

With the increase of radiation doses, starch content increased at 2 Gy in Shepody and 4 Gy in Atlantic microtubers, and then decreased to the levels as of the control (Fig. 3*A*). All four treatments of γ-radiation significantly increased the AA content of microtubers in Shepody microtubers, while in those of Atlantic, the AA content increased only at 6 Gy irradiation and even decreased at low doses of radiation (2 and 4 Gy) (Fig. 3*B*).

Reducing sugar of microtubers increased progressively at the doses above 4 Gy of γ-radiation for Shepody, while that of Atlantic decreased at 2 Gy and then increased at 6 and 8 Gy irradiation (Fig. 3*C*). The protein content of microtubers increased at lower radiation dosage and then decreased at higher dosage. The highest protein contents were observed at 6 Gy for Shepody and 4 Gy for Atlantic, respectively (Fig. 3*D*).

Al-Safadi *et al.* (2000) reported that irradiation of the explants with 2.5 Gy of γ-radiation led to a significant increase in the number of microtubers, but the average mass of microtubers was not significantly influenced by low doses of γ-radiation. In our investigation, irradiation of the plantlets (dose 4 Gy) increased not only the microtuber number but also the fresh mass of the both cultivars. The difference from the result of Al-Safadi *et al.* (2000) may be due to the different induction system employed, apart from the given genotypes. In addition, microtubers of both cultivars treated with various doses of γ-radiation initiated 5 d earlier than the non-irradiated control. However, two potato cultivars exhibited different responses to the irradiation dosage in the respect of microtuberization period and microtuber development, indicating there was some genotypic-dependent difference.

Gamma irradiation of 20 kGy increased the potato starch gelatinization (Ciesla and Eliasson 2002). According to Wang and Chao (2003), the greater the dose (from 2 - 10 kGy) was, the lower the AA content and re-hydration ratio of dried potato. In our *in vitro* study where irradiation was treated on plantlets rather than directly on microtubers, low doses (2 - 4 Gy) of γ-radiation

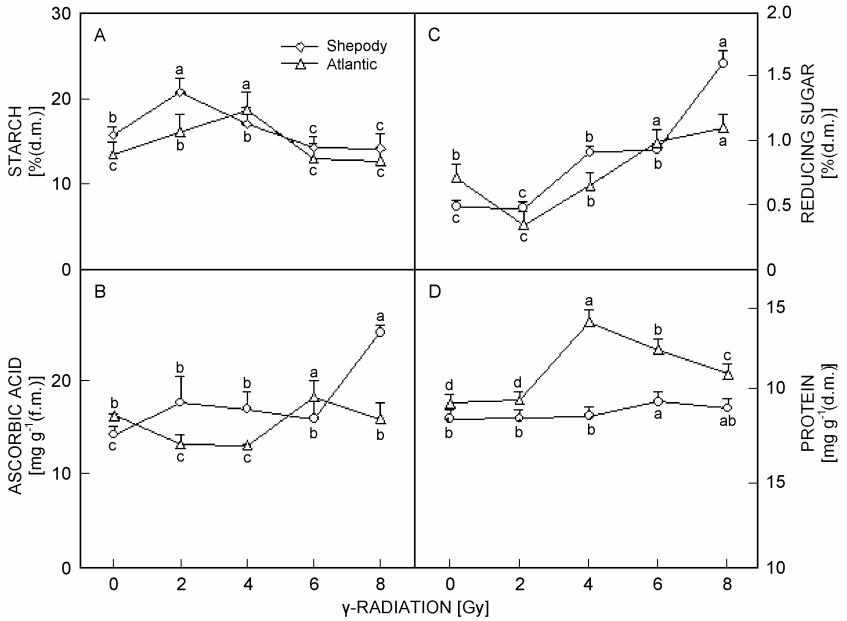

Fig. 3. Effect of γ-radiation on the starch (*A*), ascorbic acid (*B*), reducing sugar (*C*) and protein (*D*) contents of microtubers in two potato cultivars (Shepody and Atlantic) after 70 d *in vitro* culture.

increased the starch content of microtubers. High doses (6 - 8 Gy) increased AA and reducing sugar contents, although showing genotypic difference. In this work, protein content of two cultivars also exhibited increment at suitable radiation dosage (4 - 6 Gy). Higher dosage γ-radiation caused potato quality changes, mainly through the influence on the growth and development of plantlets. Comparison of the metabolite contents in microtubers with a range of published studies on soil-grown developing tubers showed that the two systems were similar both in the absolute contents and in the ratio between metabolites (Veramendi *et al*. 1999), so *in vitro*-grown microtubers are considered a suitable approach for the study of primary tuber metabolism.

In conclusion, irradiation of the explants with 4 Gy of γ-radiation led to a significant increase in the microtuber yield and also an improvement in most of the quality characters investigated. Since 4 - 6 Gy is a low radiation dose, it can be used to enhance tuberization *in vitro* without fear of genetic changes in the cultivars used. Therefore, the application of low doses of γ-radiation could be very useful for *in vitro* microtuber production in potato breeding programmes.

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