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Morphological changes induced by different doses of gamma irradiation in garlic sprouts

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Abstract

The objective of this work was to evaluate the effects of different doses of gamma rays applied in dormancy and post-dormancy on garlic bulbs in relation with some morphophysiological parameters. High (commercial) doses cause the complete inhibition of sprouting and mitosis (due to nuclear aberrations). Relatively low doses show no effects on bulbs but doses of 10 Gy applied in post-dormancy reduce sprouting and stop mitosis. This inhibition becomes noticeable from 150 days post-harvest onwards. Exogenous growth regulators can reverse these effects. Results may reinforce the good practice of radioinhibition processes in garlic.

Keywords: Gamma-irradiation; Garlic; Inner sprout; Commercial doses; Low doses; Dormancy; Reversion

1. Introduction

At the beginning of the post-harvest management, garlic bulbs can be stored for a long time. It is possible by the application of different methods that extend dormancy, in which, the bulbs, are kept out of the fast sprouting phase. During this last period, some catabolic processes are increased in the storage tissues, and nutrition substances are transported to the sprout. The consequence of this mobilization is the detriment of quality of the edible organ due to the loss of hydration and organoleptic properties.

The use of ionizing radiations has been proven to be very useful to inhibit sprouting and reduce weight loss during post-harvest storage (Curzio and Croci, 1988). The application of the ionizing radiation method in bulbs for conservation purposes is technical and economically convenient and has been accepted in many countries (US, France, Canada, Italy, Belgium, the Netherlands, Japan, etc.). It has been also adopted successfully in Argentina (Curzio and Croci, 1990) in view of MERCOSUR.

The dose and time of application of gamma rays become important due to the different effects that they produce on bulbs (Gunkel and Sparrow, 1961). The knowledge of this effects may be relevant in order to reinforce the good practice of radioinhibition processes in garlic.

The objective of this work was to evaluate the effects of different doses of gamma rays applied in dormancy and post-dormancy, in relation with some morphophysiological parameters.

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Table 1
Effects of low and high doses of gamma rays on sprouting of bulbs stored for 210 dph and bulbs sown at 180 dph and evaluated 30 days later. Each value represents the mean of 30 repetitions

	Sprouting (%)			
	Dormancy		Post-dormancy	
Doses of gamma rays	Stored	Sown	Stored	Sown
0 Gy (control)	11	100	11	100
Low: 2 Gy	9.3	100	10.3	100
5 Gy	19.4	100	12.5	100
10 Gy	1.6	100	1.4	43
High: 30 Gy	0	0	0	0
60 Gy	0	0	0	0
90 Gy	0	0	0	0
150 Gy	0	0	0	0

2. Materials and methods

Sound garlic bulbs var. Colorado, harvested in the southwest of Buenos Aires province, were used in this study. Bulbs were stored in commercial warehouse conditions, up to, and after treatments.

Bulbs were treated in dormancy (30 days post-harvest, dph) and post-dormancy (120 dph) with low (2, 5 and 10 Gy) and relatively high (30, 60, 90 and 150 Gy) doses of ^{60}Co gamma rays (Croci et al., 1994). The dose rate was 0.4 Gy/s and the dose uniformity ratio was 1.1.

The percentage of sprouting was determined for two experimental conditions: (a) cloves stored for 210 dph, and (b) cloves stored for 180 dph, then sown in vermiculite under greenhouse conditions, and emergence recorded 30 days later.

The number of leaf primordia from inner sprouts of control and treated cloves was determined from harvest to the end of post-harvest storage, at 30 days intervals.

Nuclear and cell morphology of apical meristematic cells from inner sprouts of control and treated cloves were observed at 150 dph. Garlic sprouts were fixed in 100° ETOH:glacial acetic acid (3:1) solution, for 24 h at room temperature and subsequently conserved in 70° ETOH at 4°C. The sprouts were hydrolysed in 2 N HCl solution at 60°C for 5 min. Apical meristems were removed, stained with lacto-propionic orcein and squashed (Dyer, 1963).

An in vitro culture experiment with exogenous growth regulators was carried out. Inner sprouts were removed from irradiated and non-irradiated cloves of post-dormancy treated bulbs. The explants were sterilized and transferred into culture within 6 h after irradiation (Ananthaswamy et al., 1972). AZ medium (Abo El-Nil, 1977) was supplemented with gibberellic acid (GA_3) (10^{-12} M), indole acetic acid (IAA) (10^{-8} M) and their combination. Twenty explants for each treatment were planted and maintained for 15 days in darkness in a controlled growth chamber at 18 ± 2°C. At 15 days the length of the explants was recorded.

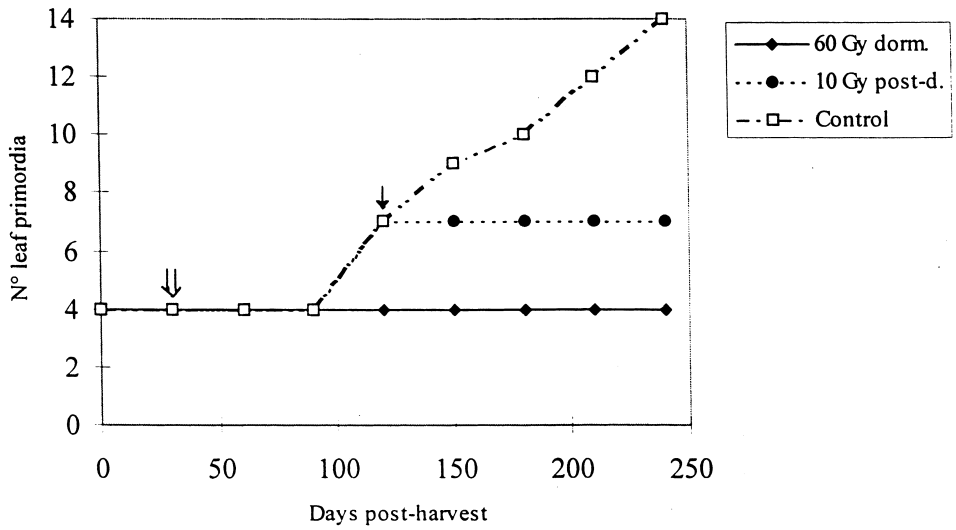

Fig. 1. Number of leaf primordia differentiated in the inner sprouts of control bulbs and treated with 60 Gy in dormancy, 10 Gy in post-dormancy recorded from harvest to the end of post-harvest storage. Irradiation treatments: ⇓ 60 Gy, 30 dph; ↓ 10 Gy; 120 dph.

Table 2
Effects of exogenous growth regulators on the growth of sprouts treated with 10 Gy in post-dormancy and without treatment. Each value represents the mean increment (in cm) of the length of 20 explants after 15 days of growth

Treatment	Control	10 Gy
Control	3.90	1.35[a]
AG_3	7.35	4.40
AIA	6.95	7.75
$AG_3 + AIA$	4.15	7.80

[a] Following values differ significantly (5%).

3. Results

Table 1 shows the results of the application of high doses of gamma rays in both dormancy and post-dormancy. This causes the total inhibition of sprouting in stored bulbs and sown bulbs. On the other hand, low doses of gamma rays applied in dormancy and post-dormancy had no effects on sprouting. Doses of 10 Gy in post-dormancy were the exception because they reduced sprouting more than in 50% on sown bulbs.

This biological material (sown bulbs treated with 10 Gy in post-dormancy) was useful for some microscopic and macroscopic observations comparable with the material irradiated with inhibitory doses (60 Gy in dormancy).

Fig. 1 shows the number of leaf primordia differentiated in treated and non-treated sprouts during all the post-harvest storage. It is important that, after each irradiation treatment, no new leaf primordia was differentiated.

At 150 dph mitosis was inhibited in all treated bulbs. However, microscopical observations showed that apical meristematic cells from bulbs irradiated with commercial doses of gamma rays in dormancy, were hypertrophic and presented nuclear aberrations such us fragmentations and micronuclei. In spite of the inhibition of mitosis in apical meristematic cells from bulbs treated with 10 Gy in post-dormancy, these cells did not show morphological abnormalities at this time, but they looked hypertrophic at 180 dph.

The exogenous application of growth regulators in the in vitro culture experience showed: (a) reversion and modulation of the irradiation effects (AIA > AG3) (Table 2); and (b) the differentiation of at least one leaf primordium (data not shown).

Seed cloves irradiated with 10 Gy in post-dormancy and sown in greenhouse conditions, developed into plants that showed deep morphological differences with the control ones (few and short roots, few and weak leaves, abnormal bulbils).

4. Discussion

Results show that high doses of gamma rays inhibit sprouting at any time of application. This can be explained on the basis that, at those treatment times, the plant material is highly radiosensible because of the high content of meristematic tissue (Gunkel and Sparrow, 1961). From the commercial point of view, it becomes important to have a wide period of time for the application of the irradiation treatment, in the sense that it allows some freedom in the post-harvest management of bulbs.

Low doses of gamma rays cannot inhibit sprouting in stored bulbs. Only doses of 10 Gy applied in post-dormancy reduced sprouting in sown bulbs and stopped mitosis in meristematic tissues. The development observed in plants coming from these bulbs may be due to apparent growth (cells elongate and become hypertrophic but no new cells are formed) (Fernandez and Aparicio, 1979).

The evidence that some metabolic activity happened immediately after the application of 10 Gy in post-dormancy, was the reason to try to reverse these effects using exogenous growth regulators. In these in vitro cultures it was recorded the growth in length and the differentiation of one leaf primordium in sown sprouts. In spite of these observations are not enough to achieve if radiation affects the synthesis mechanisms of these hormones, or changes their chemical structure, anyway they revealed the existence of repairing mechanisms. This was demonstrated when seed cloves irradiated with 10 Gy in post-dormancy were sown in greenhouse conditions and 25% of them developed into week plants with deep morphological differences with control ones.

From a practical point of view, it is important to have an efficient method to control the quality of irradiated garlic. The observations made in this work are simple, low cost and few samples are needed. They could be the basis for the development of methodologies able to detect if the commercial product has been treated with lower doses of gamma rays than those required by international codes of quality control procedures.

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