Studies in the Storage of Chestnuts Treated with Gamma Radiation. (II)

Takashi IWATA and Kuniyasu OGATA*

Laboratory of Processing and Physiology of Horticultural Products,
College of Agriculture, University of Osaka Prefecture, Sakai

(Received september 16, 1960)

Two varieties of chestnuts were irradiated with relatively high doses of gamma-rays, purporting the extension of storage life of the nuts through the inhibition of rooting and sprouting. The materials were treated with the doses of 1.5, 3 and 6×10^4 r on November 16, 6 weeks after the harvest, then stored in moist sawdust at 20°C. Almost complete inhibiting effect was obtained with all of the doses used regardless of varieties. The contents of ascorbic acid and of reducing sugar were not influenced directly by any dose of irradiation, but the content of non-reducing sugar was affected to some extent. Sharp increase of respiration was found both in the whole nuts and in the cotyledon part of the nuts immediately after irradiation. In the embryonic axis part of the irradiated nuts, the respiration did not show any change for a considerable period after irradiaiton; it became fairly lower than the control from the time when some control nuts initiated rooting, and the discoloration of this part took place about the same time. There was a rapid increase of rot incidence when the irradiated nuts had been stored until February at 20°C.

INTRODUCTION

In the previous investigation[1], it was observed that the sprouting and rooting of Imakita chestnuts (*Castanea crenata*) were considerably suppressed when they had been irradiated with gamma rays at the dose of 1.2×10^4 r one month atfer the harvest, but complete inhibition was not obtained. In the case of *C. mollissima* nuts (Chinese chestnuts), they were stored at the low temperature until May, and treated with the higher dose as much as 2×10^4 r, but the root inhibiting effect of irradiation was not so striking as it was expected from the results of Imakita chestnuts, showing the rooted materials of 34% three weeks after irradiation at room temperature. While, the roots of treated nuts did not elongate so extensively as those of the control nuts. From these results, chestnuts seemed to be less sensitive to gamma radiation as compared with onion bulbs and potato tubers which were completely inhibited the sprouting with the doses of 2 and 7×10^3 r, respectively[2]. In the present study, still higher doses of irradiation were employed purporting the extension of storage life of chestnuts without refrigeration through the inhibition of sprouting and rooting.

MATERIALS AND METHODS

Nuts of two varieties of *Castanea crenata*, Gin-yose and Imakita, were harvested

* 岩田　隆, 緒方邦安

from the orchard located at Todoromi, Osaka Prefecture, on October 3, and fumigated with carbon disulfide to destroy insects, then stored in moist sawdust at room temperature until irradiation. Irradiation was conducted with the doses of 1.5, 3 and 6×10^4 r on November 16, using the 2000C Co^{60} gamma-ray irradiation facility of the Institute for Chemical Research[3,4]. After the irradiation, both treated and control materials were stored in moist sawdust at the temperature of 20°C.

Reducing and total sugar contents were determined by Somogyi's method with some modification. The concentration of ascorbic acid was analyzed by the titration method of 2, 6-dichlorophenolindophenol.

Oxygen uptake in the slices of cotyledon and embryonic axis parts of the nuts was separately measured by Warburg manometric method, placing the tissues in 1/15 *N* phosphate buffer solution of pH 7.0 at the temperature of 30°C. At the same time, carbon dioxide evolution of the whole nuts was measured; the materials of 1.0 kg taken from each lot were placed in desiccators, and the carbon dioxide given off from the nuts was collected in 2 *N* KOH solution of 25 ml. placed at the bottom of desiccators, and was diluted to 250 ml. adding 10 ml of 25% $BaCl_2$, then titrated with 0.2 *N* HCl.

RESULTS

Rooting and sprouting. Figure 1 shows the definite effect of gamma radiation on the inhibition of rooting and sprouting of the chestnuts. When the vegetative organs of the nuts commence to grow, the roots come out first and elongate to a considerable length, then the appearance of sprouts follows. The developments of these organs are presented in Tables 1 and 2. At the room temperature, most of the chestnuts usually begin rooting about February. In this experiment, however, earlier rooting was observed on the untreated materials of both varieties because

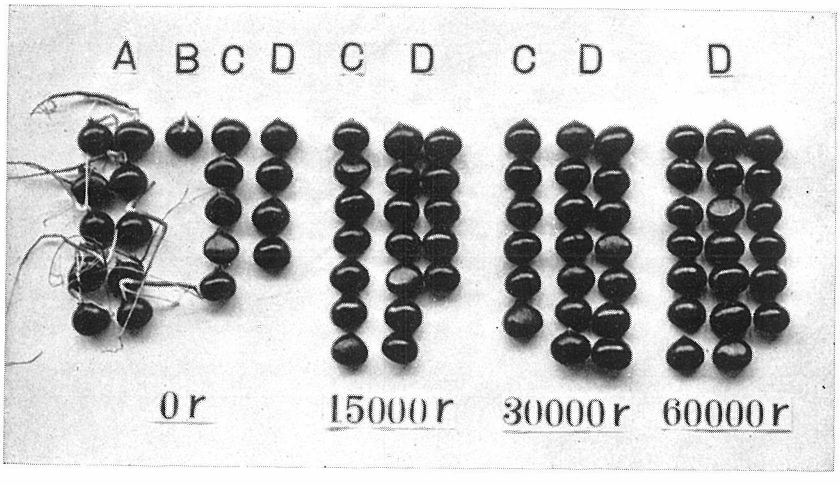

Fig. 1 The effect of gamma radiation on the rooting and sprouting of Imakita chestnuts. A: Rooted and sprouted materials. B: Rooted materials C: Rooting initiation. D: Not rooted sound materials.

all the materials were stored at the temperature of 20°C, which seems to be a favorable condition for rooting and sprouting. In the control lot of Gin-yose chestnuts, 11% of the materials elongated the roots by December 2. On December 24, there were 18% of nuts having both sprouts and long elongated roots, besides 62% of rooted nuts. On January 11, only 5% of the nuts remained sound. On the irradiated lots, 18% of the nuts in 1.5×10^4 r lot and 8% in 3×10^4 r lot initiated rooting, but they did not elongate after that. The chestnuts which received the dose of 6×10^4 r were completely suppressed the rooting throughout the experimental period.

Table 1. The inhibiting effect of gamma radiation on the rooting and sprouting of Gin-yose chestnuts stored at 20°C.

Date	Dose $\times10^4$ r	Sprouting	Rooting elongated	Rooting initiation	Rotting	Sound (not rooted)
Dec. 2	Control		11%	15%		74%
	1.5			3		97
	3			7		93
	6					100
Dec. 24	Control	18%	22	40	2	18
	1.5			18		82
	3			7	18	75
	6				7	93
Jan. 11	Control	51	11	25	8	5
	1.5			18		82
	3			8	22	70
	6				15	85

Harvest : Oct. 3, Irradiation : Nov. 16

Table 2. The inhibiting effect of gamma radiation on the rooting and sprouting of Imakita chestnuts stored at 20°C.

Date	Dose $\times10^4$ r	Sprouting	Rooting elongated	Rooting initiation	Rotting	Sound (not rooted)
Dec, 2	Control		2%	2%		96%
	1.5					100
	3					100
	6					100
Dec. 24	Control	9%	6	13		72
	1.5		2	8	2%	88
	3			6		94
	6					100
Jan. 11	Control	37	6	32	3	22
	1.5		3	33	2	62
	3			13		87
	6				8	92

Harvest : Oct. 3, Irradiation : Nov. 16

Similar effect was obtained with Imakita chestnuts, while the control materials commenced rooting later than Gin-yose, 22% of untreated nuts still remained sound (not rooted) on January 11, and some fruits of 1.5×10^4 r lot drew out short roots.

Rotting. Unfavorable secondary effect was found on the root-inhibited chestnuts. Gin-yose chestnuts treated with the doses of 3 and 6×10^4 r showed rotting of 22 and 15% by January 11, respectively, while 8% of materials decayed in the control lot (Table 1). The losses of Imakita nuts were much lower than Gin-yose in each lot (Table 2) and no rotted material was found in 3×10^4 r lot. Rapid increase of storage rotting, however, occurred in the irradiated lots when they were stored until the middle or the latter parts of February. The rotting amounted to 36-69% in Gin-yose nuts and 20-50% in Imakita on February 20, but no correlation was found between the rot per cent and the irradiation dose used. Figure 2 shows the section of rotted

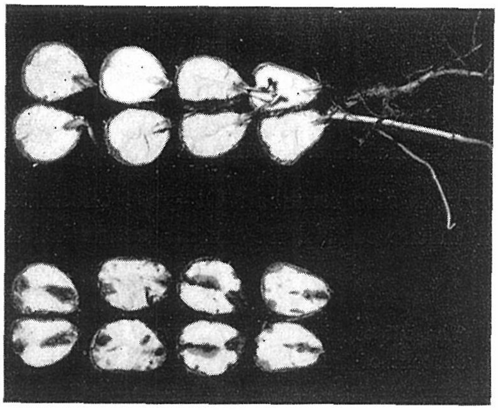

Fig. 2. Sections of nuts (Feb. 20). Upper: Control.
Lower : Rotted materials in irradiated lots.

nuts (lower), in which break down occurred in embryonic axis parts without exception. Almost all the control materials provided long elongated roots and sprouts at the time when the rotting increased sharply in the irradiated lots. Those materials or not rooted control nuts, which were exceptionally found, were cut to compare with the irradiated chestnuts (upper), and such features of rotting as those that was shown in the irradiated nuts were not observed.

Sugar and ascorbic acid. The reducing sugar content in Imakita chestnuts was not directly affected by any dose of irradiation used in this study. However, at the time when the content in the untreated nuts (not yet rooted) gradually increased toward the rooting period, less increase was observed in the irradiated nuts (Fig. 3).

The total sugar content of Imakita nuts treated with the dose of 3×10^4 r increased temporarily after the irradiation, which was followed by a decrease to reach to an extent of the control. In the 1.5×10^4 r lot, on the contrary, the immediate increase was not observed after irradiation, but, for a considerable period from early in December, the amount of total sugar was fairly higher than the other experimental lots. The nuts in 6×10^4 r lot did not show any difference from the control materials throughout the experimental period.

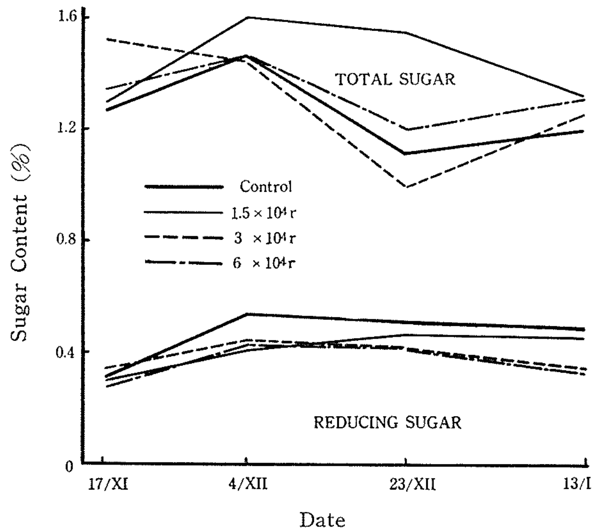

Fig. 3. The effect of gamma radiation on the sugar content of Imakita chestnuts. Irradiation : Nov. 16.

The same tendency was also found in Gin-yose chestnuts, that is higher content of reducing sugar in the control lot and an accumulation of non-reducing sugar in the 1.5×10^4 r lot.

Table 3. The effect of gamma radiation on the ascorbic acid content of Imakita chestnuts.

Date	Control	1.5×10^4r	3×10^4r	6×10^4r
Nov. 17	23.8mg%	22.0mg%	21.0mg%	24.1mg%
Dec. 13	20.6	21.5	19.2	23.6
Jan. 13	19.5	23.7	18.8	22.1

Irradiation : November 16

The concentrations of l-ascorbic acid were analyzed 1, 27, and 58 days after irradiation with Imakita chestnuts (Table 3), and no significant diffrence was found among irradiated lots and control lot.

Respiration. Fluctuations of respiratory activity were measured to investigate the effect of gamma radiation on the physiology of the chestnuts. Carbon dioxide evolution of the whole nuts immediately increased by the irradiation, twice as much as the untreated nuts on the day succeeding the treatment (Fig. 4). The stimulative effect was most striking with 1.5×10^4 r dose and the least with 6×10^4 r dose. The increased respiration in the treated nuts was followed by rapid decrease, and reached to a level near that of the control materials about two weeks after the irradiation.

Figures 5 and 6 show the oxygen uptake in cotyledon tissue, of which most part of flesh is composed, and in embryonic axis tissue. Measurements were conducted at the constant temperature of 30°C and the control values on the figures represent the oxygen consumption of not yet rooted nuts in the untreated lot. In

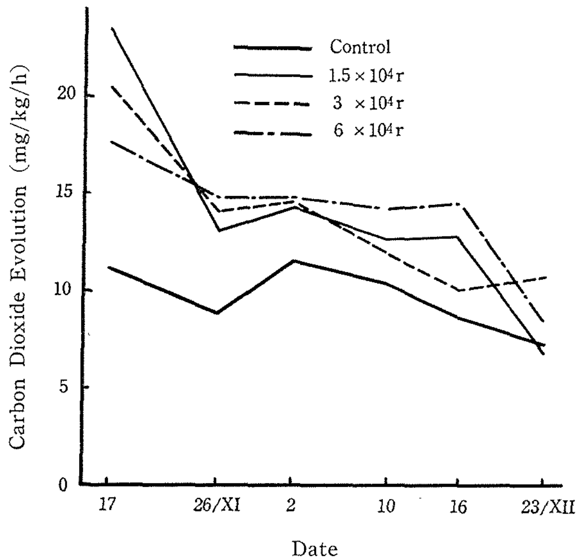

Fig. 4. The effect of gamma radiation on the carbon dioxide evolution of the whole nuts. Variety: Imakita Irradiation: Nov. 16

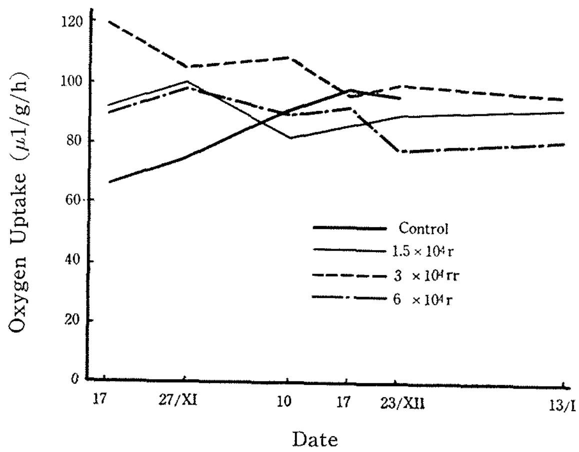

Fig. 5. The ffect of gamma radiation on the oxygen uptake in cotyledon tissue of the nuts. Variety: Imakita Irradiation: Nov. 16

the cotyledon parts, the respiration of control nuts increased toward the rooting period, while the amount of carbon dioxide given off from the whole nuts did not show such tendency because they were measured at the room temperature which was lowering with the advance of the storage period. In the irradiated nuts, the spurt of respiration was found as in the case of the whole nuts, but the most conspicuous increase was measured with the dose of 3×10^4 r.

In the embryonic axis parts, much higher respiration rate was measured. It amounted to twelve times as much as that of cotyledon parts at the middle of November on the base of fresh weight. It was expected that the embryonic axis tissue containing potentially growing cells would be more sensitive to gamma radiation than the cotyledon tissue which functions as a storage organ, but there was

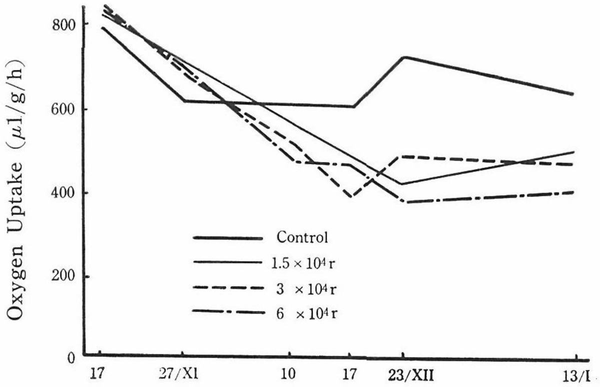

Fig. 6. The effect of gamma radiation on the oxygen uptake in embryonic axis tissue of the nuts. Variety : Imakita Irradiation : Nov. 16

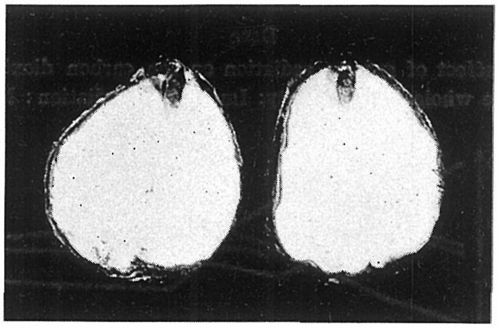

Fig. 7. Sections of irradiated chestnuts, showing the discoloration in the embryonic axis part.

not any change in oxygen uptake between control and irradiated lots immediately after irradiation through the end of November. However, from early December, when some of the control nuts began rooting, the oxygen uptake of the irradiated nuts became lower than the control. At the same time, the discoloration of the embryonic axis part was found in many fruits of every irradiated lot. Figure 7 shows the typical example; their embryonic axis part became light purple then turned brown.

DISCUSSION

In the former investigation on the sprout inhibition of onion bulbs[2], it was found that the inner bud of irradiated bulbs was browned and dead at the ordinary pre-sprouting period, which resulted in the complete inhibition of sprouting. Similar changes were observed in the irradiated chestnuts. The discoloration which was accompanied with the decrease of respiration occurred in the embryonic axis part of irradiated nuts just prior to the rooting period of untreated nuts. It was supposed, as in the case of the onion bulb, that the gamma radiation caused a certain change in the part which has relation to cell division, such as a change in chromosome, and it brought about the visible disorder in the embryonic axis at cell division stage preceeding the elongation of roots.

In the previous investigation[1], *C. mollissima* chestnuts were not suppressed the rooting completely even with the dose of 2×10^4 r. In the present study, however, profound inhibiting effect was obtained by the irradiation of 1.5×10^4 r dose with both varieties of *C. crenata*. This difference in effect might be due to the irradiation date rather than to the difference in species. The latter two varieties of chestnuts were irradiated at dormant period of the nut. *C. mollissima*, on the other hand, had been stored in refrigerator for a considerable period until irradiation was executed in May. It was accordingly assumed that the rooting was suppressed at low temperature, but the dormancy of nuts might have been broken during the storage and cell division probably took place in embryonic axis part by the time of irradiation. Consequently, when the nuts were shifted into the room of prevailing temperature, their roots would elongate to some extent owing to the enlargement of the previously divided cells in spite of the treatment with high dose gamma-rays.

Along with the beneficial effect of root inhibition, there came an undesirable effect of rotting of the nuts during the storage. It has been often reported[5,6] that rot incidence in the potato tubers increased when they received relatively high dose of gamma radiation. Gamma-radiatd chestnuts also seemed to become susceptible to storage rotting. Actual incidence of rotting, however, would greatly vary depending on the environmental condition in which the materials are stored; in the previous investigation, Imakita chestnuts treated with the dose of 1.2×10^4 r did not show any difference in rot incidence from the control nuts within the experimental period, *i.e.*, from November to the middle of February, at the same temperature (20°C) as used in the present study. At room temperature, the incidence of rot would be considerably lower than that occurring in this study.

ACKNOWLEDGMENT

The authors wish to express their sincere thanks to Prof. Tyôzaburô Tanaka, Head of the Laboratory, for his supervision and also to Prof. Rizaburô Nakai, Kyoto University, and Mr. Yasuyuki Nakayama, Institute for Chemical Research, Kyoto University, for the use of Co^{60} gamma-ray irradiation facility.

REFERENCES

(1) T. Iwata, and K. Ogata, *Bull. Univ. Osaka Pref., Ser. B*, 9, 59 (1959).
(2) K. Ogata, T. Iwata, and K. Chachin, This Bulletin 37, 425 (1959).
(3) S. Okamoto, Y. Nakayama, and K. Takahashi, This Bulletin, 37, 299 (1959).
(4) S. Shimizu, S. Tanaka, and Y. Nakayama, This Bulletin 37, 306 (1959).
(5) D. T., Duncan, W. J. Hooker, and F. Heiligman, *Food Research*, 23, 464 (1958).
(6) J. S. Schreiber, and M. E. Highlands, *Food Technol.*, 13, 159 (1959).