Effect of Oxidative Damage Induced by γ Irradiation on Germination Potentials of Rice Seeds

Narasimhan Ramarathnam,* Toshihiko Osawa, Shunro Kawakishi, and Mitsuo Namiki

γ irradiation, for accelerated aging, of four Indica and four Japonica rice seeds, with and without intact hull, was carried out. At an irradiation dose of 20 kGy, all of the varieties with intact hull had lower electron spin resonance (ESR) signal intensity and thiobarbituric acid (TBA) values compared to those irradiated after dehulling. At a lower dose of 10 kGy, rice seeds irradiated with intact hull had higher germination potentials, while the ones irradiated after dehulling had lower germination abilities. The overall susceptibility to oxidative damage was less in the case of Indica rice seeds, indicating that the antioxidative defense system in Indica rice hull offered better protection to rice seeds in overcoming the oxidative stress than in Japonica rice hull.

Seeds in general deteriorate during prolonged periods of storage. They exhibit their maximum germination potentials soon after harvest, and as storage time increases, they lose vigor gradually and eventually die (Harman and Mattick, 1976). Aging or loss of vigor is evidenced by delayed germination and emergence or reduction in percentage germination and increased susceptibility to environmental stresses (Byrd and Delouche, 1971; Woodstock, 1973; Douglas, 1975; Mc Donald, 1976). This aging of dried seeds in storage is thought to be accompanied by changes in membranes (Heydecker, 1972; Villiers, 1973) and nucleic acids (Roberts and Osborne, 1973). Alterations in the membranes of aged seeds are considered to lead to a greatly enhanced leakage of solutes during seed imbibition (Ching and Schoolcraft, 1968; Parrish and Leopold, 1978). The rate of physiological aging generally increases with increased moisture content and storage temperature (Harrington, 1973).

Recently, seed aging has been the subject of extensive research, but still it is unclear what the fundamental processes of aging are. Several workers have argued that the alterations to seed membranes during aging may result from peroxidation (Koostra and Harrington, 1969; Villiers, 1973). Harrington (1973) and Pammenter et al. (1974) have suggested that oxidation of unsaturated lipids may lead to free-radical formation. Free radicals may then damage cellular membranes and react destructively with macromolecules. Similar suggestions have been presented to explain aging in several other systems (Marx, 1974; Harman and Mattick, 1976).

Seeds, on the other hand, have a broad spectrum of inherent antioxidants such as tocopherols, carotenoids, ascorbic acid, and many other phenolic compounds that are expected to inhibit lipid peroxidation and protect against damage to membrane functions. In a preliminary paper, Osawa et al. (1985) proposed the presence of an antioxidative defense system in rice hull. Moreover, we have also demonstrated the relationship between the antioxidative activity of rice hull and germination potentials of rice seeds (Ramarathnam et al., 1986). In this report, we demonstrate the differences in the extent of protection offered by the rice hulls of Japonica and Indica rice seeds against oxidative damage induced by γ irradiation.

It is well-known that γ irradiation of biological systems is believed to generate hydroxyl radicals, which can initiate lipid peroxidation (Nawar, 1977; Swallow, 1977). Moreover, the formation of free radicals in irradiated fats has been demonstrated with the aid of electron spin resonance spectroscopy (Nawar, 1977). The ESR signals produced by irradiation of triglycerides, fatty acid methyl esters, and a number of animal and vegetable fats have been studied (Lueck et al., 1963, 1964a, 1964b). However, the effect of γ irradiation as a rapid method to demonstrate aging of rice seeds has not been attempted so far. To supplement further evidence to our assumption that rice hull not only provides physical protection of the grain from attack by insects and fungi but also prevents rice lipid autoxidation during storage (Ramarathnam et al., 1986), further model experiments were carried out to induce oxidative damage, during accelerated aging using γ irradiation, in rice seeds irradiated with and without intact hull.

Department of Food Science and Technology, Faculty of Agriculture, Nagoya University, Chikusa-Ku, Nagoya 464, Japan.

MATERIALS AND METHODS

Rice Seeds. Eight rice cultivars (*Oryza sativa* L.), Katakutara, Century Patna, IR-8, K-184 (Indica), Koshihikari, Kusabue, Sachiwatari, and Himenomochi (Japonica), were cultivated under controlled conditions in the experimental farm of Nagoya University. Immediately after harvest, the seeds were sun-dried, for about 2 weeks, to a moisture level of around 10%. This also influenced the break in dormancy of newly harvested rice seeds.

γ Irradiation. Two types of irradiation experiments were carried out simultaneously. In the first type, rice seeds with intact hull were irradiated with doses of 5, 10, 15, and 20 kGy by a 4 kCi of ^{60}Co γ irradiator (676 Gy/h), while in the other rice seeds were dehulled first in a laboratory mill and the brown rice samples thus obtained were irradiated with different doses of 5, 10, 15, and 20 kGy. Both sets of experiments were carried out in duplicate.

Electron Spin Resonance (ESR) Spectral Measurements. Immediately after irradiation, rice seeds irradiated with intact hull were dehulled manually, and the respective brown rice grains of Indica and Japonica rice seeds irradiated with and without intact hull were analyzed, in duplicate, for increases in radical intensities on JES-ME type ESR spectrometer (Japan Electron Optics Laboratory Co. Ltd., Tokyo, Japan). Increases in ESR signal intensities were expressed in units/milligram of rice grain.

Lipid Peroxidation. The extent of lipid peroxidation in rice seeds, induced by γ irradiation of seeds with and without hull was monitored by the method of Ottolenghi (1959). Immediately after irradiation, 2 g of rice grains from seeds irradiated with and without intact hull was soaked in 0.1 M PO_4 buffer (pH 7.4) for 24 h. They were then homogenized, followed by centrifugation. Two-milliliter aliquots were assayed, in triplicate, for the increase

0021-8561/87/1435-0008$01.50/0

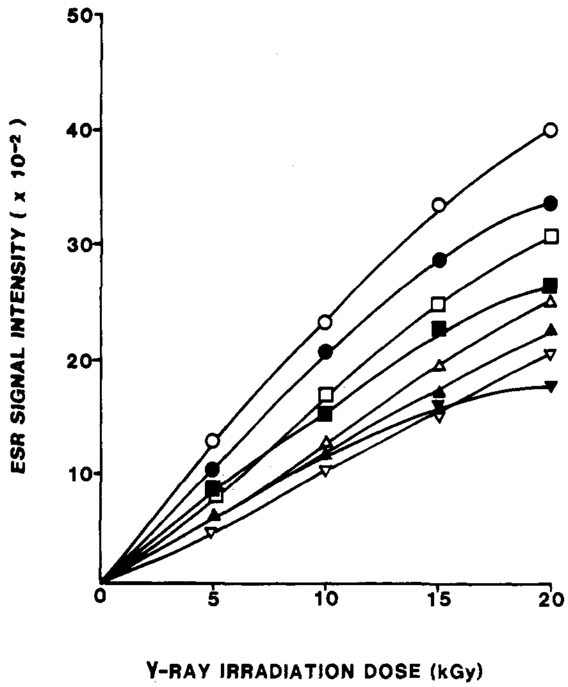

Figure 1. Effect of γ irradiation on ESR signal intensities of rice grains irradiated with intact hull. Indica: Century Patna (▽); IR-8 (△); Katakutara (▼); K-184 (▲). Japonica: Himenomochi (○); Koshihikari (■); Kusabue (□); Sachiwatari (●). Results are averages of duplicate experiments.

in TBA values by incubating with 1 mL of 20% trichloroacetic acid (Nakarai Chemicals Ltd.) and 2 mL of 0.675% thiobarbituric acid (Tokyo Kasei Kogyo Co., Ltd.) in a boiling-water bath for 10 min. The tubes were cooled and then centrifuged at 3000 rpm for 10 min. The increase in optical density (OD) was measured at 535 nm.

Germination Studies. The germination ability of the rice seeds irradiated with and without intact hull was carried out according to the method prescribed by the Association of Official Seed Analysts (1978). Germination tests, in duplicate, were carried out with 100 seeds of each variety on 9-cm Whatman No. 1 filter paper in a 9-cm covered Petri dish supplemented with 3 mL of water. The dishes were incubated at 30 ± 1 °C in the dark for 10 days. Germination ability was defined as the protrusion of coleoptile or radicle to the extent of at least 2 mm in length.

RESULTS AND DISCUSSION

Effect of γ Irradiation on the Increase in ESR Signal Intensities of Japonica and Indica Rice Seeds Irradiated with and without Intact Hull. Generation of free radicals during irradiation of rice seeds is illustrated in Figures 1 and 2. It was observed that the ESR signal intensities were far less intense in the case of seeds irradiated with intact hull (Figure 1) than in the case of seeds irradiated without hull (Figure 2). These observations indicated that better protection was offered by the enclosing hull to the edible grain portion constituting the germ, responsible for germination, against oxidative damage by γ irradiation in all of the eight varieties studied. However, the protection was distinctly more effective in the case of Indica rice seeds irradiated with intact hull than the corresponding Japonica varieties. This could be attributed to the presence of higher levels of phenolic constituents in the hull fraction of Indica rice seeds than in the case of Japonica rice varieties (Ramarathnam et al., 1986). Since the rice phenolic components are assumed to play the role as radical scavengers (Tajima et al., 1983), the quantitative difference in their levels in rice hull is

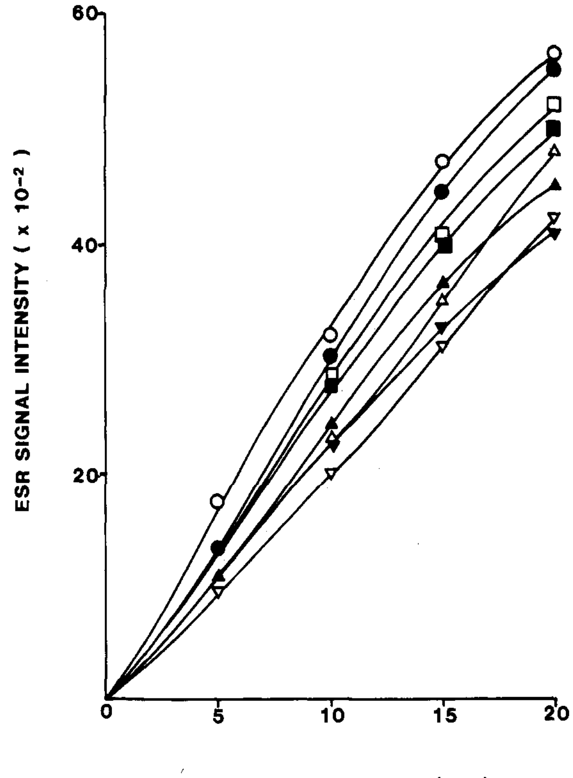

Figure 2. Effect of γ irradiation on ESR signal intensities of rice grains irradiated without intact hull. Indica: Century Patna (▽); IR-8 (■); Katakutara (▼); K-184 (△). Japonica: Himenomochi (○); Koshihikari (▲); Kusabue (□); Sachiwatari (●). Results are averages of duplicate experiments.

believed to reflect on the extent of protection offered to the inner edible grain protions in Indica and Japonica rice seeds, thereby showing differences in the ESR signal intensities among the varieities examined. In the case of rice seeds irradiated after dehulling, the radical intensities were almost closely identical with each other among the Indica and Japonica rice seeds (Figure 2). Among the Japonica rice seeds, Koshihikari proved to offer best protection while Himenomochi was found to be readily susceptible to oxidative damages by γ irradiation. Rice hull of Katakutara, belonging to Indica, exhibited the best shielding effect among all the varieties studied while that of Century Patna, K-184, and IR-8 showed decreasing abilities to protect the internal grain portion.

Effect of γ Irradiation on the Extent of Lipid Peroxidation in Japonica and Indica Rice Seeds Irradiated with and without Intact Hull. The extent of lipid peroxidation was determined by measuring the TBA values of buffer extracts of grain fractions from irradiated rice seeds. It was observed that all of the Indica rice seeds irradiated along with the hull had relatively lower values compared with the Japonica seeds (Figure 3). Among the Japonica varieties, Koshihikari was shown to have undergone less oxidative damage induced by γ irradiation. Kusabue, Sachiwatari, and Himenomochi seeds showed increasing extents of damages, reflected by higher TBA values at higher irradiation doses of 15 and 20 kGy. At all irradiation doses, Katakutara, Century Patna, IR-8, and K-184 showed little or no increase in TBA values in comparison with the Japonica rice seeds. All four Indica rice seeds showed higher TBA values on irradiation without hull (Figure 4). Koshihikari seeds (Japonica) seemed to have undergone oxidative damage to a greater extent on irradiation without hull. Among the Indica seeds irradiated without hull, Katakutara showed the least increase

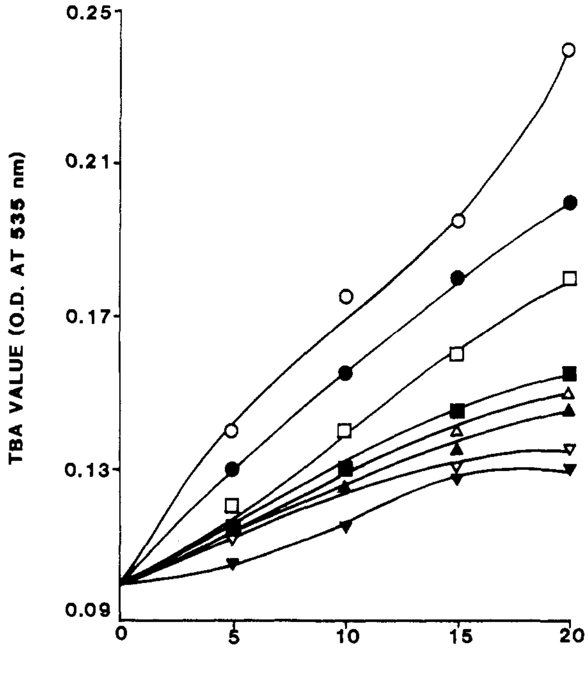

Figure 3. Effect of γ irradiation on TBA values of rice grains irradiated with intact hull. Indica: Century Patna (▽); IR-8 (▲); Katakutara (▼); K-184 (■). Japonica: Himenomochi (○); Koshihikari (△); Kusabue (□); Sachiwatari (●). Results are averages of three replicates.

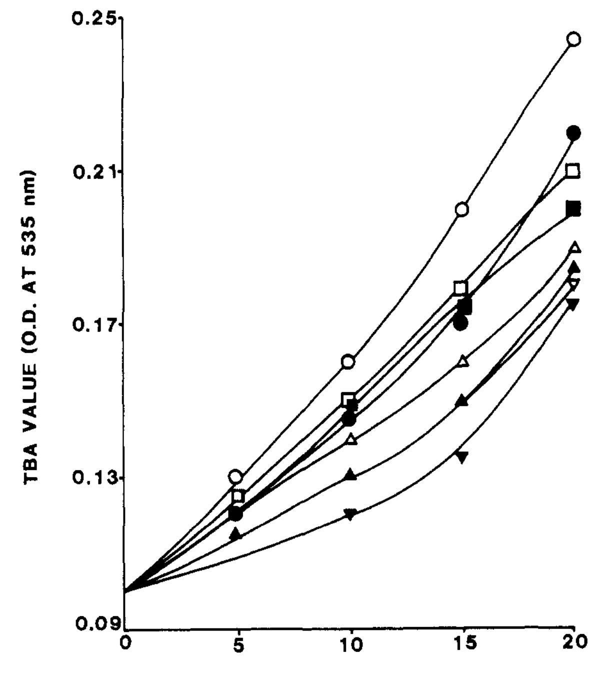

Figure 4. Effect of γ irradiation on TBA values of rice grains irradiated without intact hull. Indica: Century Patna (▽); IR-8 (△); Katakutara (▼); K-184 (▲). Japonica: Himenomochi (○); Koshihikari (□); Kusabue (■); Sachiwatari (●). Results are averages of three replicates.

in TBA value. All these observations clearly suggest the possibility of chemical protection offered by rice hull to the grain from undergoing oxidation damage induced by γ irradiation.

Effect of γ Irradiation on the Germination Potentials of Japonica and Indica Rice Seeds Irradiated with and without Intact Hull. Autoxidation of rice lipids has been reported to be one of the several major factors that cause the depletion of germination of rice seeds (Povarova et al., 1975). In the present investigation, attempts have been made to monitor the oxidative damage effects induced by γ irradiation on the germination potentials of rice seeds irradiated with intact hull (Table I) and without hull (Table II). It was observed that rice seeds irradiated with intact hull at irradiation doses of 5 and 10 kGy had higher germination potentials (Table I) compared to those irradiated without hull (Table II). Among the rice cultivars irradiated at 10-kGy dosage with hull, the Indica rice seeds, especially Katakutara, retained higher germination potentials ranging from 42.5 ± 0.5% to 54 ± 0.0%. Whereas, in the Japonica rice varieties, Koshihikari retained a higher ability to germinate (42.5 ± 0.5%), Kusabue, Sachiwatari, and Himenomochi had germination potential values of 35.5 ± 0.5%, 30.5 ± 0.5%, and 26 ± 1.0%, respectively. At an irradiation dose of 5 kGy, all the varieties had germination potentials in the range of 81.5 ± 0.5% to 88.5 ± 0.5%, except for Sachiwatari and Himenomochi, which had 74 ± 2.0% and 71 ± 1.0%, respectively (Table I). At irradiation doses exceeding 15 kGy, all of the eight varieties irradiated with or without intact hull showed their complete inability to germinate.

In the case of rice seeds irradiated without intact hull (Table II), the Japonica rice seeds had lower germination potential values in the range of 30.5 ± 2.5% to 41.5 ± 1.5% at an irradiation dose of 5 kGy, while the Indica varieties showed a higher value of 39.5 ± 1.5% to 50 ± 2.0%. Similar observations were found at an irradiation dose of 10 kGy when the Indica rice seeds retained their germination abilities in the range of 20.5 ± 1.5% to 28 ± 1.0% whereas the Japonica members showed relatively lower values of 18.0 ± 1.0% to 22 ± 2.0%.

The observations of germination experiments once again indicate that oxidative damage, induced by γ irradiation, was less pronounced when the seeds were irradiated with

Table I. Effect of γ Irradiation Dose on the Germination Abilities of Rice Seeds Irradiated with Intact Hull

	germination,[a] %				
cultivars	0 kGy	5 kGy	10 kGy	15 kGy	20 kGy
K-184	99.5 ± 0.5	85.0 ± 1.0	42.5 ± 0.5	0	0
Katakutara	100.0 ± 0	88.5 ± 0.5	54.0 ± 0	0	0
IR-8	98.0 ± 1.0	86.5 ± 1.5	48.0 ± 1.0	0	0
Century Patna	100.0 ± 0	85.5 ± 1.5	52.0 ± 0	0	0
Himenomochi	98.0 ± 1.0	71.0 ± 1.0	26.0 ± 1.0	0	0
Sachiwatari	98.0 ± 1.0	74.0 ± 2.0	30.5 ± 0.5	0	0
Koshihikari	100.0 ± 0	84.0 ± 1.0	42.5 ± 0.5	0	0
Kusabue	99.5 ± 0.5	81.5 ± 0.5	35.5 ± 0.5	0	0

[a] Germination of 100 seeds was observed after incubation for 10 days at 30 °C in the dark. Reported values are mean ± SD, $n = 2$.

Table II. Effect of γ Irradiation on the Germination Abilities of Rice Seeds Irradiated without Intact Hull

	germination,[a] %				
cultivars	0 kGy	5 kGy	10 kGy	15 kGy	20 kGy
K-184	99.5 ± 0.5	44.5 ± 2.5	24.5 ± 2.5	0	0
Katakutara	100.0 ± 0	50.0 ± 2.0	28.0 ± 1.0	0	0
IR-8	98.5 ± 0.5	39.5 ± 1.5	20.5 ± 1.5	0	0
Century Patna	99.5 ± 0.5	46.5 ± 2.5	25.5 ± 1.5	0	0
Himenomochi	98.0 ± 1.0	30.5 ± 2.5	18.0 ± 1.0	0	0
Sachiwatari	98.5 ± 0.5	38.5 ± 2.5	19.5 ± 1.5	0	0
Koshihikari	100.0 ± 0	41.5 ± 1.5	22.0 ± 2.0	0	0
Kusabue	98.5 ± 0.5	39.5 ± 1.5	19.5 ± 0.5	0	0

[a] Germination of 100 seeds was observed after incubation for 10 days at 30 °C in the dark. Reported values are mean ± SD, $n = 2$.

intact hull. It can also be concluded from ESR data (Figures 1 and 2) and TBA values (Figures 3 and 4) that the increases in free-radical intensities and TBA values were less prominent in the case of rice seeds irradiated before dehulling, thereby promoting a higher germination ability (Table I) and indicating a direct relationship between the oxidative damage induced by γ irradiation, deterioration of the rice lipid, mainly concentrated in bran and germ fractions, and fall in germination potentials of rice seeds. Rice seeds irradiated after dehulling, however, suffered higher damage due perhaps to the absence of protective defense systems such as phenolic components of the rice hull that could act either as free-radical scavengers or as natural antioxidants by inhibiting lipid peroxidation. As an overall conclusion, the defense system seems to be functioning relatively more effectively in the case of Indica rice seeds.

CONCLUSION

It has long been suggested that lipid peroxidation is a principal cause of seed deterioration, though the deteriorative changes, during long-term storage, capable of causing aging and death of seeds are probably manifold. The possibility that certain forms of degradation may be more severe during accelerated aging, induced by γ irradiation, than under natural storage conditions cannot be ruled out. Rice hull was always believed, in the past, to offer mere physical protection to the rice grain from attack of insects and microbes. The present investigation, however, focuses main attention on the protective role offered by the hull fraction of Indica and Japonica rice seeds in minimizing the detrimental effects of oxidative damage to cellular components, mainly in the membranal lipids. It can now be concluded from the present investigation that in addition to the presence of natural antioxidants such as α-tocopherol and oryzanol in rice bran certain inherent defense systems do exist in the rice hull. The main contribution of such defense systems toward long-term storability of rice seeds could be as free-radical scavengers or as natural antioxidants for inhibiting the autoxidation of rice lipids during storage. The defense systems in hulls of Indica rice seeds were relatively more effective than the Japonica ones.

ACKNOWLEDGMENT

We thank Dr. Tashiro Toru for providing his valuable assistance in the cultivation of rice samples. Funds to carry out this work were provided by the Foundation for Promotion of Food Sciences and Technology, Japan, for which we are deeply indebted.

LITERATURE CITED

Association of Official Seed Analysts *J. Seed Technol.* **1978**, *3*, 1.
Byrd, H. W.; Delouche, J. C. *Proc. Assoc. Off. Seed Anal.* **1971**, *61*, 41.
Ching, T. M.; Schoolcraft, I. *Crop Sci.* **1968**, *8*, 407.
Douglas, J. E. In *Cereal Seed Technology*; Food and Agriculture Organization of the United Nations: Rome, 1975; pp 87–107.
Harman, G. E.; Mattick, L. R. *Nature (London)* **1976**, *260*, 323.
Harrington, J. F. *Seed Sci. Technol.* **1973**, *1*, 453.
Heydecker, W. In *Viability of Seeds*; Roberts, E. H., Ed.; Chapman and Hall: London, 1972; pp 209–252.
Koostra, P. T.; Harrington, J. F. *Proc. Int. Seed Test. Assoc.* **1969**, *34*, 329.
Lueck, H.; Deffner, C. U.; Kohn, R. *Z. Lebensm.-Unters.-Forsch.* **1963**, *123*, 200.
Lueck, H.; Deffner, C. U.; Kohn, R. *Fette, Seifen, Anstrichm.* **1964a**, *66*, 249.
Leuck, H.; Deffner, C. U.; Kohn, R. *Fette, Seifen, Anstrichm.* **1964b**, *66*, 665.
Marx, J. L. *Science (Washington, D.C.)* **1974**, *186*, 1105.
McDonald, M. B. *Proc. Assoc. Off. Seed Anal.* **1976**, *65*, 109.
Nawar, W. W. In *Radiation Chemistry of Major Food Components*; Elias, P. S., Cohen, A. J., Eds.; Elsevier/North-Holland Biomedical: The Netherlands, 1977; pp 21–61.
Oswa, T.; Ramarathnam, N.; Kawakishi, S.; Namiki, M.; Tashiro, T. *Agric. Biol. Chem.* **1985**, *49*, 3085.
Ottolenghi, A. *Arch. Biochem. Biophys.* **1959**, *79*, 355.
Pammenter, N. W.; Adamson, J. H.; Berjack, P. *Science (Washington, D.C.)* **1974**, *186*, 1123.
Parrish, D. J.; Leopold, A. C. *Plant Physiol.* **1978**, *61*, 365.
Povarova, R. I.; Krasnook, N. P.; Vishnyakova, I. A.; Davidenko, E. K. *Izv. Vyssh. Uchebn. Zaved., Pisch. Tekhnol.* **1975**, *3*, 42.
Ramarathnam, N.; Osawa, T.; Namiki, M.; Tashiro, T. *J. Sci. Food Agric.* **1986**, *37*, 719.
Roberts, B. E.; Osborne, D. J. In *Seed Ecology*; Heydecker, W., Ed.; The Pennsylvania State University: University Park, 1973; pp 99–114.
Swallow, A. J. In *Radiation Chemistry of Major Food Components*; Elias, P. S., Cohen, A. J., Eds.; Elsevier/North-Holland Biomedical: The Netherlands, 1977; pp 5–20.
Tajima, K.; Sakamoto, M.; Okada, K.; Mukai, K.; Ishizu, K.; Sakurai, H.; Mori, H. *Biochem. Biophys. Res. Commun.* **1983**, *115*, 1002.
Villiers, T. A. In *Seed Ecology*; Heydecker, W., Ed.; The Pennsylvania State University: University Park, 1973; pp 265–288.
Woodstock, L. W. *Seed Sci. Technol.* **1973**, *1*, 127.

Received for Review April 7, 1986. Accepted August 6, 1986.