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LETTER TO THE EDITOR

Inactivation of tobacco mosaic virus using gamma irradiation and its potential modes of action

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Summary. – Gamma irradiation is a non-thermal processing technique used to disinfect harmful microorganisms in agriculture. This technology has been shown to be an effective method to control bacterial and fungal plant pathogens. However, its effect on viral plant pathogen is less understood. Gamma irradiation was evaluated for the inactivation of tobacco mosaic virus (TMV). TMV infectivity has gradually decreased following irradiation in a dose-dependent manner and virus was completely inactivated at a dose over 40 kGy. Transmission electron microscopy revealed that increased gamma irradiation disrupts the virion structure and degrades viral proteins, which results in TMV inactivation. The mechanisms, through which gamma irradiation inactivates TMV, can be directly associated with the damage to the virus constituents.

Keywords: coat protein; gamma irradiation; inactivation; tobacco mosaic virus; virion

Viral plant diseases can be found worldwide and cause severe economic losses in agriculture. The tobacco mosaic virus (TMV) is one of the most common viral pathogens and infects nine plant families and at least 125 individual species including tobacco, tomatoes, and peppers (*1*). TMV is the type member of the genus *Tobamovirus* and is a rod-shaped virus composed of single-stranded RNA encapsulated in a coat protein (CP) capsid. TMV has a hollow tube with genomic RNA attached to the internal face, and stable helical non-enveloped virions. Unlike chemicals used to control fungal and bacterial pathogens, to date there have been no efficient chemical treatments controlling TMV infection. Therefore, it is urgent to develop a novel, effective, and eco-friendly treatment to inactivate viral pathogens.

One potential technology for the inactivation of viral pathogens as an effective nonchemical treatment is irradiation technology. It has been suggested that ionizing radiation might be used to disinfect bacteria, insects, fungi, and pests, and incurs no significant risks to human health or the environment (*2*). The most commonly used types for disinfection are gamma irradiation and electron beam. Gamma irradiation has a much higher penetrability into materials than the electron beam. The exposure of microorganisms to ionizing radiation directly damages the cellular macromolecules (nucleotides, ribonucleotides, and to a lesser extent, proteins), or indirectly attacks the target macromolecules through the generation of a substantial flux of free oxygen radicals (especially hydroxyl radicals and hydrogen peroxide) (*3*). The unit of irradiation dose is the gray (Gy), which is the energy absorbed in J kg^{-1} of the material. Only UV treatment has been employed so far to inactivate certain plant viruses including tomato bushy stunt virus (BSV), tobacco necrosis virus (TNV), cowpea mosaic virus (CPMV), and barley stripe mosaic virus (BSMV) (*4, 5*). To date, no study has investigated the inactivation of plant viruses by gamma irradiation. Moreover, the mechanism of virus inactivation by gamma irradiation is poorly understood.

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Abbreviations: CP = coat protein; Gy = gray; TEM = transmission electron microscopy; TMV = tobacco mosaic virus

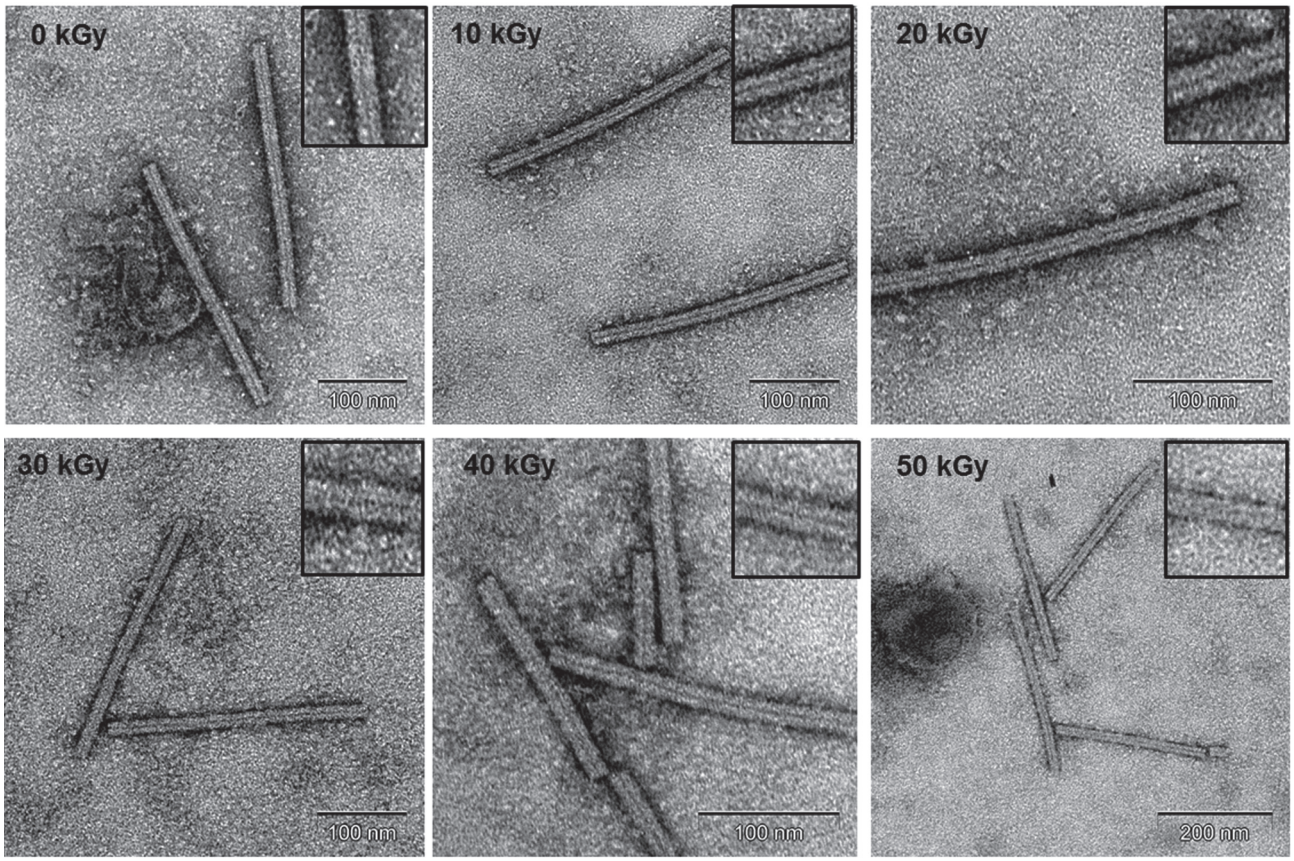

Fig. 1

Gamma irradiation damages TMV

Purified TMV was irradiated at doses of 10, 20, 30, 40, and 50 kGy. Treated and untreated virus particles were negatively stained with 1% ammonium molybdate and observed by transmission electron microscopy.

In this study, we evaluated the effectiveness of gamma irradiation on inactivation of TMV at different doses. We also determined the possible mechanism of virus inactivation by gamma irradiation through transmission electron microscopy (TEM). This study fills in the knowledge gap of how gamma irradiation affects the inactivation of TMV, and allows the direct idea for the control of plant viruses through gamma irradiation.

The TMV, which was obtained from the Plant Virus GenBank (PVGB) in Korea, was purified from wild type-infected leaves of *Nicotiana tabacum* vs. Samsun plants using a method previously described (*6*). Purified virus particles were resuspended in 2 ml of 0.1 mol/l phosphate buffer (pH 7.0) containing 5 mmol/l EDTA. A cobalt-60 gamma irradiator at the Korea Atomic Energy Research Institute, Jeongeup, Korea (150 TBq capacity; ACEL, MDS Nordion, Canada) was used for the irradiation. All of the absorbed doses were calibrated using alanine dosimeters with a diameter of 5 mm (Bruker Instruments, Rheinstetten, Germany) to determine the free-radical signals. Aliquots of purified TMV were irradiated at doses of 0, 10, 20, 30, 40, and 50 kGy of gamma radiation. Interestingly, leaves inoculated with virus treated with a dose over 40 kGy did not show any symptoms (light coloration between veins) in local and systemic leaves (data not shown). The TMV was reduced to non-detectable levels at a dose over 40 kGy. This allowed us to precisely determine what happens to the virus particles and viral components when no infectious virus particles remained. To determine whether gamma irradiation directly damages the virus particles, we analyzed the virus particles using TEM. Samples were fixed on a copper grid (Electron Microscopy Sciences, Hatfield, PA) and negatively stained with 1% ammonium molybdate (Sigma-Aldrich). Fixed samples were analyzed using a transmission electron microscope (JEOL Ltd., Tokyo, Japan) at 200 kV at the National Academy of Agriculture Science in Korea. For each grid, at least three fields of approximately 200 μm^2 were viewed. Images were taken and analyzed using a US1000 CCD camera and GATAN MICROGRAPH software (Gatan Inc., Warrendale, PA, USA). Considerable damage to the viral coat

protein was shown in the irradiated virus particles compared to the non-irradiated particles. For the untreated control, TMV particles were rod-like shaped virions of 300 nm in length and 18 nm in diameter, with no damage to the particles visible (Fig. 1). Through a treatment with doses over 10 kGy, the number of virus particles was significantly reduced. Moreover, the degree of microscopy stain around the virus particles was gradually decreased with increasing gamma irradiation compared to non-irradiated particles (Fig. 1). Clearly, this was due to the fact that the viral coat protein was degraded by the gamma irradiation. Subsequently, purified TMV was treated with increasing gamma radiation doses and subjected to SDS-PAGE analysis with TMV-CP antibody (Agdia, Elkhart, Indiana, USA) to determine if there was any effect on the viral proteins. For 40- and 50-kGy treatment, the TMV CP protein was undetectable, suggesting that the TMV CP protein was completely degraded (data not shown). Based on the results of the TEM and SDS-PAGE, it seems that gamma irradiation treatment degrades viral proteins. These results are similar to those obtained with other viruses. The food-born virus, tulane virus (TV) and the animal viruses, murine norovirus-1 (MNV-1) and vesicular stomatitis virus (VSV) were observed to lose their capsid protein after irradiation (*2*). This is the first evidence that the degradation kinetics of a plant virus capsid by gamma irradiation is similar to that of animal viruses. In response to irradiation of proteins, amino acids show high sensitivity to irradiation. Amino acids containing sulfur (cysteine, methionine) or aromatic compounds (tyrosine, phenylalanine) show high sensitivity because they react with hydroxyl radicals more easily than aliphatic (alanine, leucine, valine) amino acids.

In the present study, we demonstrated that it was possible to inactivate TMV by gamma irradiation through mechanistic approaches, a disruption of the virion structure and degradation of the viral proteins. Although gamma irradiation seems impractical to disinfect a plant virus in agricultural products because of high doses, irradiation combined with heat or eco-friendly agents will decrease the doses, thereby inactivating plant viruses in various products without their physical damage.

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