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Expression Patterns of Diverse Genes in Response to Gamma Irradiation in *Nicotiana tabacum*

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We investigated the expression patterns of diverse genes at various time points after gamma irradiation of young tobacco plants. The first group of genes showed stimulation of transcript levels upon gamma irradiation, although their induction patterns varied. This group included glutathione-S-transferase, peroxidase, superoxide dismutase, and catalase. A second group, with post-irradiation reduction of transcripts, included genes encoding cytosolic ascorbate peroxidase, stromal ascorbate peroxidase, and a TMK1 receptor-like kinase. The third group of genes either showed no change in transcript levels or exhibited irregular patterns. These included genes encoding PR1a (pathogenesis-related protein), tobacco Ca^{++}-dependent protein kinase, the β-subunit of translational initiation factor 2B, and CHRK1, a chitinase-related receptor-like kinase. Thus, various genes displayed differential patterns of gene expression in response to gamma irradiation in tobacco plants, thereby suggesting a complex signaling mechanism is involved in the irradiation-induced defense by plants. In addition, many stress-responsive genes exhibited gene expression patterns upon gamma irradiation that differed from those resulting from other biotic and abiotic stresses. With the knowledge of distinctive expression patterns of diverse genes, irradiation-indicating marker plants could be developed by engineering and monitoring multiple radiation-responsive genes.

Keywords: active oxygen species (AOS), gamma irradiation, stress-inducible, tobacco

In both prokaryotic and eukaryotic cells, the level of active oxygen species (AOS) formed in normal cell metabolism increases under stress conditions. As with other organisms, plants have developed protective systems, either constitutive or inducible, to counteract oxidative stress (OS), and have also gained the ability to control and utilize the production of AOS (Bolwell et al., 1995; Inze and van Montagu, 1995). Changes in plant gene expression in response to various types of OS have been investigated. Photo-oxidative and other environmental stresses lead to the regulation of antioxidant enzymes, e.g., superoxide dismutase (Scandalios, 1997). The synthesis of pathogenesis-related proteins is also enhanced by OS and ozone attack (Kangasjarvi et al., 1994), as well as by UV irradiation (Green and Fluhr, 1995).

Ionizing radiation markedly affects cellular macromolecular components, such as plant cell walls, membranes, and DNA (Casarett, 1968; McLennan, 1988). These effects are considered a consequence of both the direct interactions between the ionizing radiation and the macromolecular structures, and the indirect action of AOS generated by water radiolysis. Those direct and indirect action of ionizing irradiation also induce other physiological changes in plants, such as enhanced respiration and increased ethylene production (Romani, 1984), induction of enzyme activities (particularly for phenolic metabolisms; Pendharkar and Nair, 1975), and the accumulation of sucrose (Hayashi and Aoki, 1985) and specific protein species, such as small heat-shock proteins (Ferullo et al., 1994). However, the effects of gamma irradiation on transcriptional regulation of various types of genes have not been fully investigated.

The objectives of this study were 1) to examine expression patterns of diverse genes in response to gamma irradiation in order to characterize the molecular effects of this stress on young tobacco plants, and 2) to eventually identify marker genes that may be useful in generating radiation-indicating marker plants.

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MATERIALS AND METHODS

Plant Materials and Treatments

Young tobacco plants (*Nicotiana tabacum* cv. Xanthi) at the 6- to 8-leaf stage were gamma-irradiated to 30 or 50 Gy with a ^{60}Co gamma-irradiator (KAERI, Korea), as described by Yun et al. (1999). At 2, 6, 18, and 42 h after irradiation, the stems and leaves of the plants were collected and frozen at –70°C until RNA preparation commenced.

RNA Gel Blot Analysis

Total RNA was prepared with a TRIzol™ Reagent (GIBCO/BRL), following the manufacturer's instructions. Approximately 50 μg of total RNA was electrophoresed on an agarose gel containing 5.1% (v/v) formaldehyde, then blotted onto a Hybond-N Nylon membrane (Amersham). As probes, the cDNA fragments of individual genes were labeled with [α-^{32}P]dCTP, using the Random Primed DNA labeling Kit (Boehringer Mannheim, Germany). Prehybridization and hybridization were carried out in 5x SSC, 5x Denhardt's solution, and 0.5% SDS, at 60°C overnight. The membranes were washed twice in 2x SSC and 1% SDS at room temperature, then washed in 0.1x SSC and 0.1% SDS at 60°C for 30 min.

RESULTS AND DISCUSSION

Young tobacco plants at 6-8 leaf stage were gamma-irradiated to 30 or 50 Gy using ^{60}Co gamma-irradiator, and at 2, 6, 18, and 42 h after irradiation the stems and leaves of plants were collected. Gamma irradiation at 30 to 50 Gy progressively inhibited growth of young tobacco plants, and the plant height growth and leaf emergence were completely stopped at 70 Gy (Yun et al., 1999). Total RNA was prepared from the collected tissues, and RNA gel blot analyses were carried out with the radiolabeled cDNA fragments of various genes from tobacco as probes.

Three general groups of genes were delineated according to their pattern of expression following gamma irradiation: 1) increased transcription levels, 2) decreased levels, or 3) an irregular pattern or no response. First, the expression patterns of some genes exhibited increased transcript levels upon gamma irradiation (Fig. 1). These included genes for GST, superoxide dismutase, peroxidase, and catalase. Although the level of glutathione-S-transferase (GST) transcripts was almost undetectable without the treatment, it was strongly induced at 2 h, followed by reduction at 6 h, and complete disappearance of the mRNA at 18 h after irradiation (Fig. 1A). The GST gene is induced by H_2O_2 generated by pathogen infection and elicitor (Levine et al., 1994). Thus the result indicates that H_2O_2 is also generated by gamma irradiation in tobacco leaves and is used as a signaling molecule for subsequent expression of defense genes.

Expression of the *sodCp* gene encoding tobacco CuZn-superoxide dismutase (SOD) was slightly

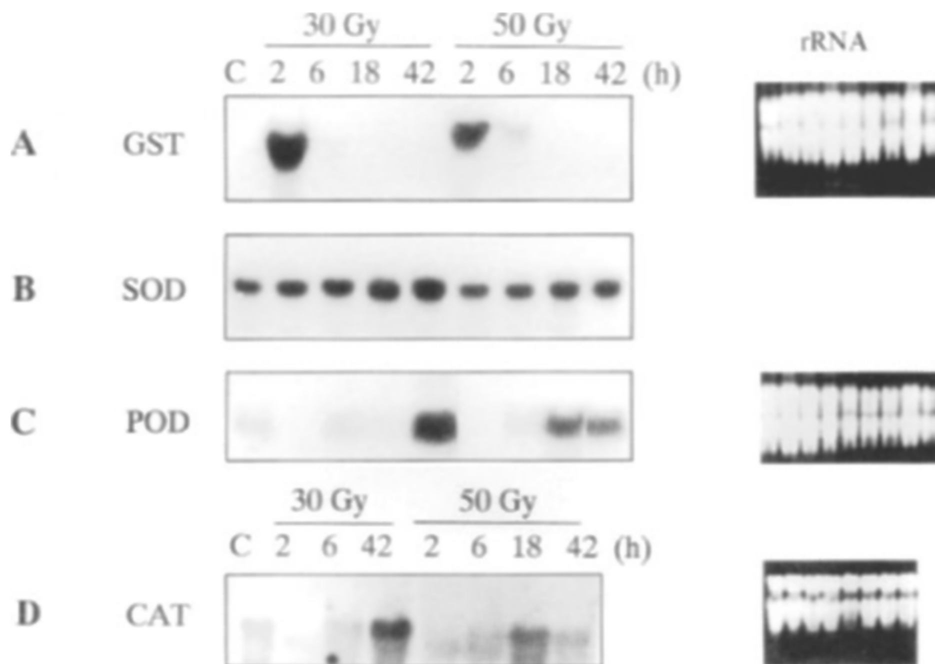

Figure 1. RNA gel blot analysis of genes that showed stimulated gene expression upon gamma irradiation. Each lane represents 50 μg of total RNA isolated from leaves and stems of young tobacco plants (6 to 8-leaf stage) collected at various time points after gamma irradiation at 30 and 50 Gy. The amount of EtBr-stained rRNA was shown to verify equal loading of RNA in each lane. The cDNAs encoding glutathione-S-transferase (GST), CuZn-superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were used as probes. The same RNA gel blot was used for GST and SOD.

enhanced by radiation at 42 h (Fig. 1B). The *sodCp* mRNA has been detected only in young plants or young leaves of mature plants (Kurepa et al., 1997). Expression of this gene was suppressed by the herbicide paraquat, but was greatly stimulated by Norflurazon-induced photooxidation. This resulted in a 10-fold increase in *sodCp* mRNA (Kurepa et al., 1997). In other plant systems, paraquat and a fungal toxin Cercosporin also stimulated the accumulation of maize SOD isozyme transcripts (Scandalios, 1997). Interestingly, maize scutella treated with H_2O_2 (0 to 0.1%) showed increases in *Sod4* and *Sod4A* transcripts (Kernodle and Scandalios, 1996).

After irradiation at 30 Gy, the *POD* transcripts encoding anionic peroxidase from tobacco disappeared at 2 h. However, the level increased to its normal level at 6 h then further increased to the higher level at 42 h (Fig. 1C). Upon irradiation at 50 Gy, the gene expression pattern was similar to that at 30 Gy, but the maximum level was achieved at 18 h and maintained until 42 h after irradiation (Fig. 1C). Previously, Kang et al. (1998) showed that the mRNA level of the same gene was sharply reduced by an acute hypersensitive response against TMV in tobacco, and by salicylic acid. Thus, *POD* gene expression in response to irradiation appears to be regulated in a different manner from its expression in response to pathogen infection.

Ngcat1 transcripts encoding catalase were detected in tobacco leaves prior to irradiation. However, after irradiation the transcript level decreased at the early time points butslightly increased later on (Fig. 1D). Yi et al. (1999) found that the expression of *Ngcat1* mRNA and its enzyme activity was repressed in tobacco plants undergoing hypersensitive reaction in response to TMV infection. They also observed this repression of the mRNA level following salicylic acid treatment. Thus, the regulation of *Ngcat1* gene expression upon gamma irradiation differs from that induced by pathogen or elicitor.

In the second group, the transcript levels of some genes were reduced in response to irradiation stress (Fig. 2). This group included *cAPX* (tobacco cytosolic ascorbate peroxidase), *NtTMK1* (tobacco TMK1 receptor-like kinase), and *stAPX* (tobacco stromal ascorbate peroxidase). The *cAPX* transcripts are abundantly expressed in young tobacco plants under normal greenhouse conditions. At 2 h after irradiation, the transcript level decreased to approximately one-fifth, and remained at the level until 48 h (Fig. 2A). Kang et al. (1998) had demonstrated that expression of the *cAPX* gene was suppressed during HR response by TMV infection, and mildly induced by salicylic acid. This is consistent with the report by Mittler et al., (1998). Suppression of *cAPX* gene expression may be post-transcriptional, possibly at the translation elongation stage (Mittler et al., 1998). Thus, gene expression of the tobacco *APX* gene is similar in response to either irradiation or pathogen infection. In other plant systems, expression of the *APX* gene was enhanced in response to various stresses, such as drought, salt, high light, chilling, UV radiation, ozone, wounding, and pathogen attack (Morita et al., 1999).

NtTMK1 transcripts were detected in young

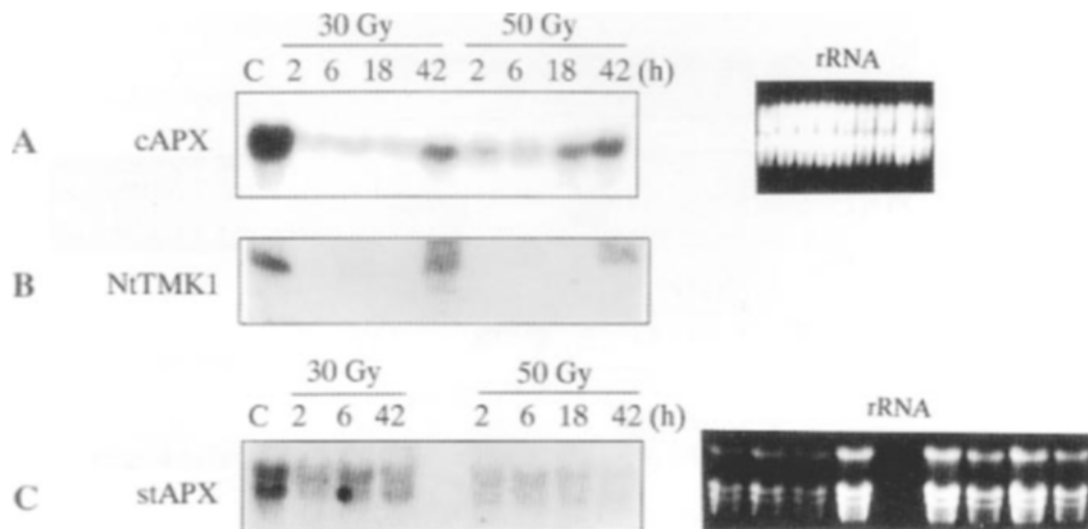

Figure 2. RNA gel blot analysis of genes that showed reduced gene expression upon gamma irradiation. The cDNAs encoding cytosolic ascorbate peroxidase (cAPX), NtTMK1 receptor-like kinase, and stromal ascorbate peroxidase (stAPX) were used as probes. The same RNA gel blot was used for cAPX and NtTMK1.

tobacco plants without treatment. They disappeared at 2 h after irradiation but increased to the basal level at 48 h (Fig. 2B). *NtTMK1* encodes a tobacco homologue of TMK1 receptor-like kinase of *Arabidopsis* (Chang et al., 1992; Cho and Pai, unpublished results). *NtTMK1* gene expression is developmentally regulated in various tissues and is sensitive to external stimuli, e.g., phytohormones, and abiotic and biotic stresses (Cho and Pai, unpublished results). Chang et al. (1992) have speculated that TMK1 is involved in cell proliferation. Cell-cycle arrest that is induced by radiation may be an underlying cause for suppression of *NtTMK1* gene expression.

The *stAPX* gene encoding stromal ascorbate peroxidase in tobacco was not as strongly affected by irradiation as was APX gene. After irradiation at 30 Gy, the *stAPX* transcript level decreased at 2 h, slightly increased at 6 h, then was maintained until 48 h. At 50 Gy, the transcript level was reduced at 2 h, and remained at that level until 48 h after irradiation (Fig. 2C). The response of the *stAPX* gene to other forms of stress has not been revealed yet.

The third group of genes either showed no change in their transcript levels or exhibited irregular patterns of transcript accumulation. This group included *NtCDPK1* (tobacco calcium-dependent protein kinase), *NeIF2Bβ* (β-subunit of translation initiation factor eIF2B), *PR1a* (pathogenesis-related protein), and *CHRK1* (chitinase-related receptor-like kinase). *NtCDPK1* gene expression in tobacco is stimulated by various stimuli, such as fungal elicitors, methyl jasmonate, wounding, calcium, and hormones (Yoon et al., 1999). Yet gamma irradiation did not significantly change the level of *NtCDPK1* transcripts, except for an initial decrease at 2 h (Fig. 3A). This indicates that the gamma irradiation-induced plant defense system does not completely overlap with that for other stresses.

PR1a transcripts were not detected either under normal conditions or upon gamma irradiation (Fig. 3B). However, Ryals et al. (1996) and Kang et al. (1998) have shown that, although *PR1a* transcripts were not detected under normal conditions, their levels were dramatically increased by pathogens and salicylic acid. In the current study, gene regulation induced by irradiation operated differently from that described for the pathogen-related scheme. Translation initiation factor 2B whose beta subunit is encoded by *NeIF2Bβ* in tobacco (Kim and Pai, unpublished results), is a key regulator of global protein synthesis rates in yeast and animals (Webb and Proud, 1997). The pattern of change in *NeIF2Bβ* transcript levels for our irradiated tobacco plants was irregular, depending on time point and dose (Fig. 3C). At 30 Gy, the transcript level generally increased after the initial decrease, whereas the level mostly decreased at 50 Gy.

Finally, the *CHRK1* receptor-like kinase gene from tobacco did not respond to gamma irradiation (results not shown). CHRK1 receptor-like kinase contains a chitinase-like sequence in its extracellular domain. *CHRK1* gene is specifically induced by both TMV and fungus infection; fungal elicitors, abiotic stresses, methyl jasmonate, BTH, and chitin fragments do not

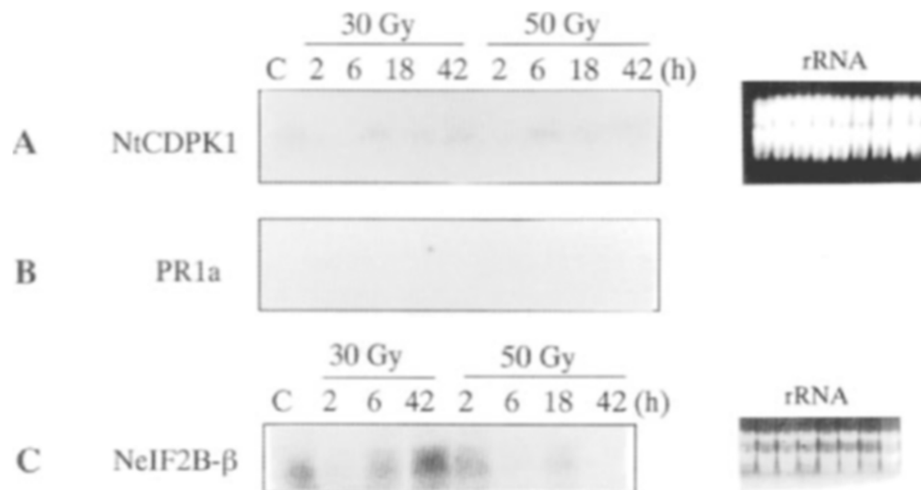

Figure 3. RNA gel blot analysis of genes that showed no change of transcript levels upon gamma irradiation. The cDNAs encoding tobacco calcium-dependent protein kinase (NtCDPK1), PR1a, and translation initiation factor 2B β-subunit (NeIF2B-β) were used as probes. The same RNA gel blot was used for NtCDPK1 and PR1a.

change mRNA levels (Kim and Pai, unpublished results).

In conclusion, gamma irradiation of young tobacco plants resulted in a wide range of gene expression patterns. This demonstrates that various genes respond differently to stress, thereby implicating a complex regulatory mechanism employed by plants to execute their irradiation-induced defense. Expression of many genes examined in this study has been previously shown to be modulated by other environmental and physiological stresses, such as pathogen infection, elicitors, and abiotic factors. This suggests that a common signaling pathway is shared for responding to different types of stresses. However, the patterns of gene expression in response to gamma irradiation may differ from those due to other stresses. This indicates that the signaling pathway governing irradiation-stress response also includes its unique components in addition to the common machinery. For example, several H_2O_2-inducible genes, such as GST and POD, became induced upon irradiation. Therefore, H_2O_2 produced during irradiation might play a role in the modulation of gene expression.

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