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Regulation of the expression of GA-insensitive gene homolog by light in *Oryza sativa* c.v. DongJin

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Abstract: *To understand the mechanism by which light regulates a gibberellin (GA)-insensitive gene in DongJinByeo (Oryza sativa cv. DongJin), both green and etiolated DongJinByeo seedlings were submerged in water and treated with GA. Total RNA from the seedlings was isolated and hybridized with cDNA of a GA-insensitive gene homolog. The amount of transcript for the GA-insensitive gene homolog was higher in green seedlings than in etiolated seedlings in the absence of GA. However, upon the addition of GA, greater accumulations of the gene transcript occurred in etiolated seedlings than in green seedlings. This result indicates the possibility that the expression of the GA-insensitive gene homolog transcript may be inhibited by light in the presence of GA. Light seems to regulate multilaterally the accumulation of the transcript of the GA-insensitive gene homolog in DongJinByeo (Oryza sativa cv. DongJin).*

Key words: *GAI gene homolog, Light, Regulation, DongJin*
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Introduction

Gibberellins (GAs) are plant growth substances involved in the promotion of stem elongation, the mobilization of seed reserves in seeds, fruit set and flower induction (Hooley, 1994; Swain and Olszewski, 1996; Carrera *et al*., 1999; Kim *et al.,* 2006). GAs are synthesized from isopentenyl pyrophosphate *via* geranylgeranyl pyrophosphate (Hedden and Kamiya, 1997; Lange, 1998). The formation of *ent*-kaurene from geranylgeranyl pyrophosphate, with copalyl pyrophosphate as an intermediary, is the first committed step of GA biosynthesis. This reaction is catalyzed by the enzymes *ent*-copalyl diphosphate synthase and *ent*-kaurene synthase, which have been cloned in many plant species (Carrera *et al*., 1999). The *ent*-kaurene is metabolized to GAs by monooxygenases and 2-oxoglutarate-dependent dioxygenases, which are enzymes responsible for the successive oxidation of C-20, with the loss of CO_2 and the formation of C-19 GAs.

GA-related mutants such as the GA-deficient mutant and GA biosynthesis inhibitors have been identified in several species, including rice and *Arabidopsis*. Previous genetic and physiological analyses of the GA-deficient mutations gai, D8 and Rht indicated that all are gain-of-function mutations conferring reduced GA responses and enhanced endogenous GA levels (Peng and Harberd, 1993; Carol *et al*., 1995; Wilson and Somerville, 1995). The endogenous GA levels found in the *gai*, D8 and Rht mutants were enhanced through the inhibition of the feedback control mechanism of negative regulation by GA. Genetic analysis showed that GAI is a repressor of GA responses, that GA could release this repression and that *gai* is a mutant repressor that is relatively resistant to the effects of GA (Peng and Harberd, 1993). Recently, the GA-insensitive rice DWARF1 was reported to encode a GA receptor, thus illuminating and raising questions about GA signaling (Ueguchi-Tanaka *et al.*, 2005; Hartweck and Olszewski, 2006; Ueguchi-Tanaka *et al.*, 2007). Moreover, a recessive GA-insensitive dwarf mutant of rice, *gibberellin-insensitive dwarf1* (*gid1*), has been identified using a proteomic approach (Hartweck and Olszewski, 2006; Tanaka *et al*., 2006). However, data on GA-insensitive gene regulation by light and GA in *Oryza sativa* cv. DongJin are not available. Thus, we examined the effect of light and GA on the transcript levels of genes encoding a GA-insensitive gene homolog in seedlings of DongJinByeo (*Oryza sativa* cv. DongJin).

Materials and Methods

Experimental plant: DongJinByeo (*Oryza sativa* cv. DongJin) seeds were soaked in 1% sodium hypochlorite for 15 min and then washed three times with sterilized water. The sterilized seeds were immersed in sterilized water and grown in a growth chamber at 28°C for 3 days until germination. The seedlings were cultured in sufficient water for up to 10 days under total darkness for etiolated seedlings and under a 12 hr light/12 hr dark cycle for green seedlings. To examine the down-regulation of the GA-insensitive gene homolog by light, seedlings were grown submerged in 500 ml of an aqueous solution containing10^{-6} M gibberellic acid (GA_3) for 2, 4, 6, 8 or 10 days. Seedlings were harvested and frozen in liquid N_2, and then stored at -80°C until used.

Polymerase chain reaction and plasmid: Polymerase chain reaction (PCR) primers were designed from the sequence of a maize d8 gene (AJ242530) that is homologous to the gibberellin-insensitive gene. A genomic fragment was generated by PCR using the genomic DNA isolated from T65/Tall rice as a template. The sense and antisense primers used for the PCR of GA-insensitive were 5'-GTG CAG CAG GAG AAC TTC TCC-3' and 5'-CTC GGT GAA GCG GTC GAG GAA-3', respectively. Using *Escherichia coli* XL-1 blue, the transformation was carried out basically as described by Sambrook and Russel (2001). The GA-insensitive cDNA fragment

was ligated with the pGEM-T vector, which had been cut with EcoR1, to form pGAI. The XL-1 blue-transformed pGAI was grown overnight in Luria-Bertani media containing 50 μg ml^{-1} ampicillin and then grown until optical density (OD) = 0.4 at 560 nm wavelength. Plasmid pGAI was isolated according to the alkali lysis method, as described by Sambrook and Russel (2001). The isolated plasmid was treated with EcoRI, and the GAI gene homolog was purified using an ultrafree-DA column (Millipore).

Total RNA extraction: Total RNA was isolated from the seedlings as reported earlier (Kim *et al*., 2006). 100 mg of seedling was homogenized in 1 ml of Trizol reagent Gibco BRL) and the homogenized samples were incubated for 5 min at 30°C to allow the complete dissociation of nucleoprotein complexes. After adding chloroform, the sample was centrifuged at 12,000 × *g* for 15 min at 4°C and the aqueous phase was then transferred to a fresh tube. Total RNA was precipitated from the aqueous phase by mixing with isopropyl alcohol. The RNA pellet was washed once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of Trizol reagent used for the initial homogenization. The RNA pellet was briefly air-dried and then dissolved in RNase-free water. After incubating for 10 min at 60°C, the RNA was quantified spectrophotometrically.

Northern hybridization: For Northern hybridization, 20 ug of total RNA was loaded per lane and run on agarose/formaldehyde gels as reported previously (Kim *et al*., 2006). The RNA was blotted onto a nitrocellulose filter and hybridized with a radioactively labeled (Rediprime kit, Amersham Life Sciences) GAI gene homolog. As a probe, a 1.4-kb GAI gene homolog was used. Unless stated otherwise, membranes were hybridized overnight at 65°C in a solution containing 0.5 M Na_2HPO_4 (pH 7.2), 7% SDS, 1 mM EDTA with 50% formamide, 5 × SSC, 50 mM Na_2HPO_4 (pH 6.3), 1 × Denhardt's, 0.1% SDS, 0.1 mg ml^{-1} denatured salmon sperm DNA, and with a random-primed ^{32}P-labeled cDNA fragment as a probe. Membranes were washed three times with 2 × SSC, 0.1% SDS, and then additionally with 0.1 × SSC, 0.1% SDS at 42°C, and 0.1 × SSC, 0.1% SDS at 65°C. The membranes were then autoradiographed with an intensifier screen for either 1 or 2 days Sambrook and Russel (2001).

Results and Discussion

The differential expression of many plant genes has been reported to be regulated via the interaction of hormones and light. However, it is not known exactly how light signals, mediated by phytochromes or hormones, are transduced to bring about changes in gene expression in DongJinByeo (*Oryza sativa* cv. DongJin). Moreover, detailed information concerning the direct relationship between light and gibberellin is limited. Light has also been reported to modulate the promoter activity, as well as the transcript level, of various genes through interactions with several hormonal signals in mungbean (Yi *et al*., 2003). In fact, the brassinosteroid effect on the promoter of the ACC synthase gene differed markedly between etiolated and green mungbean seedlings (Yi *et al*., 2003).

GAI is a repressor of GA responses, and GA can release this repression (Peng and Harberd, 1993). GA-deficient mutants and GA biosynthesis inhibitors were reported to be very useful in the examination of GA-related gene regulation (Ogawa *et al*., 2000; Hedden, 2003). The mutant repressor *gai* was resistant to the effect of GA. To investigate how light and GA affect the transcript level of the GA-insensitive gene homolog in DongJinByeo, we analyzed the level of GAI transcript expression with and without light using Northern hybridization (Fig. 1). The mRNA transcript for GAI accumulated notably in green seedlings without GA at 4 days, but did not accumulate in etiolated seedlings without GA over the entire period. However, upon the addition of GA to etiolated seedlings, the GAI transcript was induced significantly from 2 to 10 days (Fig. 2). The highest amount of transcript in etiolated seedlings was detected at 4 days. However, GAI transcript in green seedlings did not increase following GA treatment. These results indicate that the expression of GAI is up-regulated by the application of GA in the absence of light.

Time course of GAI transcript accumulation in green seedlings in the presence of GA was shown in Fig. 3. GAI transcript began to accumulate after 12 hr of GA treatment. The transcript level had increased by approximately two times at 24 hr. Moreover, similar transcript levels were maintained at 36 and 48 hr in green seedlings. In etiolated seedlings in the presence of GA, GAI transcript began to accumulate after 24 hr of GA treatment (Fig. 4). It decreased slightly at 36 hr and then increased at 48 hr of treatment with GA.

The expression of GA-oxidase was also regulated *via* negative-feedback control by the biosynthetic end-product GA in the light as reported previously (Kim *et al*., 2006). Greater transcript accumulation of the GA-20 oxidase gene homolog was observed in green seedlings than in etiolated seedlings. However, upon the addition of GA, higher accumulation of the gene transcript was found in etiolated seedlings than in green seedlings.

The RGA gene that encodes putative transcriptional regulators that repress GA signaling in *Arabidopsis* (Silverstone *et al*., 1997; Dill *et al.,* 2001) is homologous to the GAI gene. Immunoblot analyses indicated that the delta domain in RGA is a regulatory domain in the GA-induced degradation of RGA. The deletion of this region stabilized the rga-Delta 17 mutant protein and rga-Delta 17 was a constitutively active repressor of GA signaling, regardless of the endogenous GA status. It has been postulated that plants have both membrane-bound and soluble GA receptors; GID1 was found to be a soluble receptor mediating GA signaling in rice. Moreover, GID1 was reported to bind to a repressor of GA signaling in a GA-dependent manner in yeast cells (Ueguchi-Tanaka *et al*., 2007).

A recessive GA-insensitive dwarf mutant of rice, gibberellin-insensitive dwarf1 (gid1), showed a severe dwarf phenotype and contained high concentrations of endogenous GA (Tanaka *et al*., 2006). Also, gid1 exhibited the altered regulation of probenazole-inducible protein (PBZ1) in response to cold stress and pathogen attack (Tanaka *et al*., 2006).

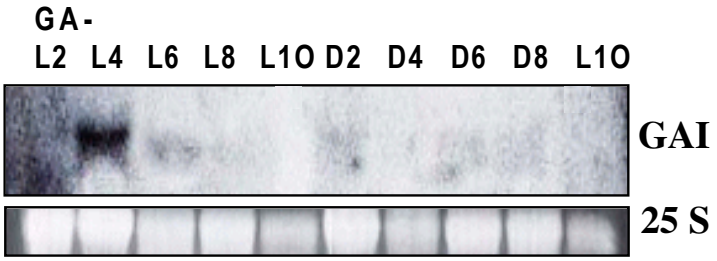

Fig. 1: The accumulation of GAI transcripts in green (L) and etiolated (D) DongJinByeo (*Oryza sativa* cv. DongJin) seedlings
Each lane contains 20 µg of total RNA extracted from seedlings grown for 2, 4, 6, 8, or 10 days
The transcript levels of the 25S rRNA gene were used as internal loading controls

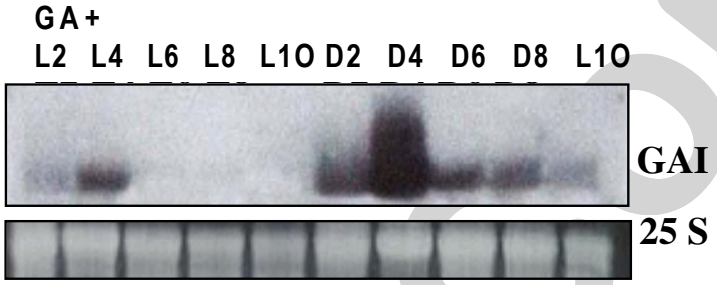

Fig. 2: Effects of gibberellin on the accumulation of GAI transcript in green (L) and etiolated (D) DongJinByeo seedlings
Each lane contains 20 µg of total RNA extract from seedlings grown for 2, 4, 6, 8, or 10 days in the presence of 10^{-6} M gibberellin. The transcript levels of the 25S rRNA gene were used as internal loading controls

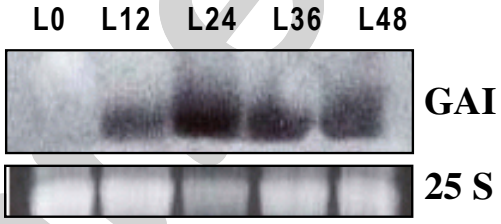

Fig. 3: Time course of GAI transcript accumulation in green seedlings of DongJinByeo in the presence of gibberellin
The seedlings were harvested after 0, 12, 24, 36, or 48 h of treatment with 10^{-6} M gibberellin under light

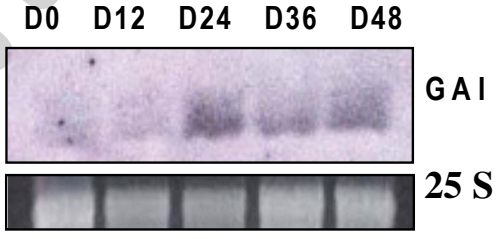

Fig. 4: Time course of GAI transcript accumulation in etiolated seedlings of DongJinByeo in the presence of gibberellin
The seedlings were harvested after 0, 12, 24, 36, or 48 h of treatment with 10^{-6} M gibberellin under light

The application of GA reduced the transcript levels of the GAI gene homolog in light-grown DongJinByeo seedlings, whereas GA increased the transcript level in dark-grown seedlings in this investigation. More detailed experiments are required to determine how light and GA interact at the molecular level and regulate the GAI gene multilaterally.

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References

Carol, P., J. Peng and N.P. Harberd: Isolation and preliminary characterization of gas1-1, a mutation causing partial suppression of the phenotype conferred by the gibberellin-insensitive (gai) mutation in *Arabidopsis thaliana* (L.) Heyhn. *Planta*, **197**, 414-417 (1995).

Carrera, E., S.D. Jackson and S. Prat: Feedback control and diurnal regulation of gibberellin 20-oxidase transcript levels in potato. *Plant Physiol*., **119**, 765-774 (1999).

Dill, A., H.S. Jung and T.P. Sun: The della motif is essential for gibberellin-induced degradation of RGA. *Proc. Natl. Acad. Sci. USA*, **98**, 14162-14167 (2001).

Hartweck, L.M. and N.E. Olszewski: Rice gibberellin insensitive dwarf1 is a gibberellin receptor that illuminates and raises questions about GA signaling. *The Plant Cell*, **18**, 278-282 (2006).

Hedden, P. and Y. Kamiya: Gibberellin biosynthesis: Enzymes, genes and their regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., **48**, 431-460 (1997).

Hedden, P.: The genes of the Green Revolution. *Trends Genet*, **19**, 5-9 (2003).

Hooley, R.: Gibberellins: Perception, transduction and responses. *Plant Mol. Biol*., **26**, 1529-1555 (1994).

Kim, S.K., T. K. Son, S. Y. Park, I. J. Lee, B. H. Lee, H. Y. Kim and S. C. Lee: Influences of gibberellin and auxin on endogenous plant hormone and starch mobilization during rice seed germination under salt stress. *J. Environ. Biol.,* **27**,181-186 (2006)

Kim, J.H., I.S. Yoon and M.Y. Lee: Effects of light on the feedback control of GA-20 oxidase gene homolog in DongJin Byeo seedlings. *J. Environ. Biol.*, **27**, 367-371 (2006).

Lange, T.: Molecular biology of gibberellin synthesis. *Planta*, **204**, 409-410 (1998).

Ogawa, M., T. Kusano, M. Katsumi and H. Sano: Rice gibberellin-insensitive gene homolog, OsGAI, encodes a nuclear-localized protein capable of gene activation at transcriptional level. *Gene*, **245**, 21-29 (2000).

Peng, J. and N.P. Harberd: Derivative alleles of the Arabidopsis gibberellin-insensitive (gai) mutation confer a wild-type phenotype. *Plant Cell*, **5**, 351-360 (1993).

Sambrook,J. and D.W. Russell: Molecular cloning a laboratory manual. 3rd Edn., Cold Spring Harbor Laboratory Press, New York, USA (2001).

Silverstone, A.L., P.Y. Mak, E.C. Martinez and T.P. Sun: The new RGA locus encodes a negative regulator of gibberellin response in *Arabidopsis thaliana*. *Genetics*, **146**, 1087-1099 (1997).

Swain, S.M. and N.E. Olszewski: Genetic analysis of gibberellin signal transduction. *Plant Physiol*., **112**, 11-17 (1996).

Tanaka, N., M. Matsuoka, H. Kitano, T. Asano, H. Kaku and S. Komatsu: Gid1, a gibberellin-insensitive dwarf mutant, shows altered regulation of probenazole-inducible protein (PBZ1) in response to cold stress and pathogen attack. *Plant Cell Environ.*, **29**, 619-631 (2006).

Ueguchi-Tanaka, M., M. Ashikari, M. Nakajima, H. Itoh, E. Katoh, M. Kobayashi, T.Y. Chow, Y.I. Hsing, H. Kitano, I. Yamaguchi and M. Matsuoka: Gibberellin insensitive dwarf1 encodes a soluble receptor for gibberellin. *Nature*, **437**, 693-698 (2005).

Ueguchi-Tanaka, M., M. Nakajima, A. Motoyuki and M. Matsuoka: Gibberellin receptor and its role in gibberellin signaling in plants. *Annu. Rev. Plant Biol.,* **58**, 183-198 (2007).

Wilson, R.N. and C.R. Somerville: Phenotypic suppression of the gibberellin-insensitive mutant (gai) of Arabidopsis. *Plant Physiol*., **108**, 495-502 (1995).

Yi, K.W., I.S. Yoon, D.H. Park, B.G. Kang and M.Y. Lee: Effects of light on the hormonal regulation of VR-ACS6 truncated promoter in transgenic tobacco. *Plant Physiol. Biochem*., **41**, 331-335 (2003).