Effects of light on the feedback control of GA-20 oxidase gene homolog in DongJinByeo seedlings

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Abstract: The effects of gibberellin (GA) on the expression of GA-20 oxidase gene homolog were examined in light-grown seedlings and dark-grown seedlings of DongJinByeo. The growth rates of the stems of etiolated seedlings were faster than those of green seedlings. However, upon addition of GA to these seedlings, the stem growth rates of green seedlings were faster than those of etiolated seedlings. To understand the molecular mechanism of GA gene regulation in DongJinByeo, total RNA from DongJinByeo was hybridized with cDNA of GA-20 oxidase gene homolog. Greater accumulation of transcript of GA-20 oxidase gene homolog was observed in green seedlings than in etiolated seedlings. However, upon addition of GA, higher accumulation of the gene transcript was found in etiolated seedlings than in green seedlings, indicating that expression of the transcript of GA-20 oxidase gene homolog might be inhibited by light. These results suggest that light might regulate feedback control of the transcript of GA-20 oxidase gene homolog in DongJinByeo.

Key words: GA-20 oxidase gene, Light, Feedback control, DongJinByeo.

Introduction

Gibberellins (GA) are tetracyclic diterpenoid phytohormones with an essential role in plant growth and development. They are involved in the regulations of growth responses in higher plants, including stem elongation, seed germination, mobilization of seed reserves, fruit set and flower induction (Hooley, 1994; Swain and Olszewski, 1996; Carrera et al., 1999). 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). ent-Kaurene is metabolized to GAs by monooxygenases and 2-oxoglutarate-dependent dioxygenase enzymes responsible for successive oxidation of C-20, with loss as CO₂ and the formation of C-19 GAs. GA-20 oxidase is suggested to be one of the important points of regulation in the GA-biosynthesis. The final step of bioactive GA synthesis, from GAs₂⁵/GA₁₂ to GA₇/GA₆, is catalyzed through two parallel pathways (i.e. early-13-hydroxylation and non-13-hydroxylation pathways) by two soluble 2-oxoglutarate-dependent dioxygenases in the cytosol, GA-20 oxidase and GA-3 oxidase from rice (Sakamoto et al., 2004). GA-20 oxidase is a multifunctional enzyme and has been cloned and expressed from various plant species. Various cDNA clones were isolated from a number of plants, such as pumpkin, Arabidopsis, spinach, pea, rice, and French bean (Lange et al., 1994; Phillips et al., 1995; Xu et al., 1995; Martin et al., 1996; Wu et al., 1996; Garcia-Martinez et al., 1997; Toyomasu et al., 1997; Sakamoto et al., 2004). Comparison of these cDNAs has indicated highly conserved domains in all GA-20 oxidase proteins. Moreover, bioactive GAs have been reported to control their own synthesis through a negative-feedback mechanism of the GA-20 oxidase and 3β-hydroxylase genes (Chiang et al., 1995; Xu et al., 1995; Martin et al., 1996; Vidal et al., 2003). However, the information about GA-20 oxidase transcript regulation by GA as well as light in DongJinByeo is not available. The effects of light and GA on the transcript levels of genes encoding GA-20 oxidase homolog in DongJinByeo (Oryza sativa cv. Dong-Jin) seedlings were examined in this investigation.

Materials and Methods

Experimental plant: The seeds of DongJinByeo were soaked in a 1% sodium hypochlorite for 15 min, washed with sterilized water three times. The sterilized seeds were immersed in sterilized water and grown in a growth chamber at 28 °C for 3 days until germination. The sprouts were planted in flower pots containing vermiculite and then cultivated with sufficient watering for upto 10 days under total darkness for etiolated seedlings and under a 12hr light/12hr dark cycle for green seedlings. Seedlings were harvested at indicated days and frozen in liquid N₂, and then stored at –80 °C until used.

Application of gibberellin: To investigate the feed-back regulation of GA-20 oxidase gene in DongJinByeo, seedlings grown in pots were watered with 500 ml aqueous solution containing 10⁻⁶ M gibberellic acid (GA₃) for 6 days.

Polymerase chain reaction and plasmid: The complete open reading frame of a rice gibberellin-20 oxidase cDNA (U50333) was generated by PCR with the genomic DNA isolated from T65/Tall rice as a template. Sense primers used for PCR of
GA-20 oxidase was 5'-ATGAGCATGGTGGTGCAGCAG-3', and antisense primers for GA-20 oxidase was 5'-CTAGGAGTATATTGTTGGTTG-3'. E. coli XL-1 blue was used for transformation and the transformation was carried out basically as described by Sambrook and Russel (2001). GA-20 oxidase cDNA fragment was ligated with pGEM-T vector which had been cut with EcoR1 to the formation of pGOX20. The XL-1 blue transformed with pGOX20 was grown overnight in Luria-Bertani media containing 50 µg/ml ampicillin, and grown until OD=0.4 at 560 nm. Plasmid pGOX20 was isolated according to the alkali lysis method as described by Sambrook and Russel (2001). The isolated plasmid was treated with EcoR1, and the GA-20 oxidase gene homolog was purified by using ultrafree-DA column (Millipore).

**Total RNA extraction:** Total RNA was isolated from the seedlings by using Trizol reagent (Gibco BRL). 100 mg of the seedlings were homogenized in 1 ml of Trizol reagent, and the homogenized samples were incubated for 5 min at 30°C to permit complete dissociation of nucleoprotein complexes. After adding chloroform, the sample was centrifuged at 12,000 × g for 15 min at 4°C, and then the aqueous phase was transferred to a fresh tube. Total RNA was precipitated from the aqueous phase by mixing with isopropyl alcohol. 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. 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:** 20 µg of total RNA was loaded per lane and run out on agarose/formaldehyde gels. The RNA was blotted onto a nitrocellulose filter and hybridized with a radioactively labeled (Rediprime kit, Amersham Life Science) 1.2 Kb GA 20 oxidase gene homolog. Unless stated otherwise,

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**Fig. 1:** Effects of gibberellin on the heights of green seedlings and etiolated seedlings of DongJinByeo. The heights of these seedlings were measured at the indicated cultivation time without gibberellin (A) and with gibberellin (B).
membranes were hybridized overnight at 65 °C in a solution containing 0.5 M Na$_2$HPO$_4$ (pH 7.2), 7% SDS, 1 mM EDTA at 42 °C with 50% formamide, 5×SSC, 50 mM Na$_2$HPO$_4$ (pH 6.3), 1×Denhardt’s, 0.1% SDS, 0.1 mg ml$^{-1}$ denatured salmon sperm DNA, and with random primed $^{32}$P-labelled cDNA fragment as a probe. Membranes were washed three times with 2×SSC, 0.1% SDS, 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 one or two days.

Results and Discussion

It has been reported that expression of many plant genes is regulated via interaction of hormones and light. However, it is not known exactly how light signal mediated by plant phytochromes or hormones is transduced to bring about changes in gene expression. Moreover, detailed information concerning the direct relationship of light and gibberellin is limited. Light was also reported to modulate promoter activity as well as transcript levels of various genes through interacting with several hormonal signals (Yi et al., 2003). In the rice genome, four GA-20 oxidase-like genes were reported, three of which (OsGA2ox1, OxGA2ox2, and OsGAox3) were identical to the genes reported earlier (Sakamoto et al., 2001; Sakai et al., 2003), whereas seven copies have been previously reported in Arabidopsis (Schomburg et al., 2003). GA-deficient mutant and GA biosynthesis inhibitors were very useful when GA-20 oxidase gene regulation had to be examined (Hedden, 2003). Ancymidol was reported to block the conversion of ent-kaurene into ent-kaurenoic (Coolbaugh et al., 1978) and prohexadione was known to inhibit dioxygenases blocking GA biosynthesis at the 3β-hydroxylation step that converts GA$_{30}$ into GA$_1$.
Fig. 3: The effect of gibberellin on the accumulation of transcript level of GA-20 oxidase gene homolog under light and dark condition.

(Nakayama et al., 1990). In potato, inhibition of GA biosynthesis by application of ancymidol or prohexadione resulted in a much stronger accumulation of StGA20ox1 and StGA20ox3 transcripts than of transcript StGA20ox2 (Carrera et al., 1999). The feedback control of GA-20 oxidase gene by active GA was also reported in several plants through GA biosynthesis inhibitors similarly shown in potato (Carrera et al., 1999; Vidal et al., 2003).

Fig. 1 shows the effect of gibberellin on the height of light-grown DongJinByeo and dark-grown DongJinByeo. The heights of the etiolated seedlings were higher than those of green seedlings without GA. However, upon addition of GA to these seedlings, the growth rates of green seedlings were faster than those of dark seedlings. Moreover, the heights of GA-treated green seedlings were much higher than those of untreated green seedlings. Time course of gibberellin effect on the growth of the seedlings under light condition and dark condition was shown in Fig. 2. Stem elongation effect of GA began to occur at the green seedlings following GA treatment for 12 hours, while at etiolated seedlings following GA treatment for 24 hours. About 11% and 13% stimulations of the growths were shown in the green seedlings and etiolated seedlings, respectively, after 48 hour treatment of 10^{-6} M gibberellin. Longer treatments or higher concentrations of GAs were required to visualize notable effect of gibberellin as reported in potato (Carrera et al., 1999).

In order to investigate how light and GA affect the transcript level of GA-20 oxidase gene in DongJinByeo, we analyzed the level of the GA-20 oxidase transcript expression with and without light through northern hybridization. Fig. 3 indicates the differential accumulation of GA-20 oxidase transcripts with and without light. The expression levels of GA-20 oxidase transcripts in green seedlings were higher than those in etiolated seedlings in the absence of GA. However, the transcript levels in green seedlings were decreased greatly upon addition of GA. Thus the transcript levels in green seedlings were lower than in etiolated seedlings following gibberellin treatment. These results indicate that expression of GA-20 oxidase is regulated by negative-feedback control by the biosynthetic end-product GA in the light, while expression of GA-20 oxidase is regulated by positive-feedback control by GA in the dark. In many species, the expression of GA-20 oxidase is subject to feed-back regulation by the content of active GAs (Hedden and Kamiya 1997; Toyomasu et al., 1997). The evidence comes mainly from application experiments and work with GA biosynthetic mutants, where the contents of active GAs have been altered substantially. The levels of expression of all GA-20 oxidase mRNAs increased greatly in the ga1 dwarf mutant compared with wild-type plants. Treatment of wild-type plants with inhibitors of GA biosynthesis (ancymidol or prohexadione) increased the levels of expression of the StGA20ox1 and StGA20ox3 transcripts, but had little effect on the StGAox2 mRNA in potato (Carrera et al., 1999). In lettuce, two GA20x genes have been isolated and described as subject to feed-back regulation by applied GA1, but only one of them (LsGA20ox2) seems subject to feed-back regulation by the increase in endogenous GA1 following red-light irradiation (Toyomasu et al., 1998). The transcript levels of genes encoding GA-20 oxidase, as well as those encoding GA-3 oxidases are generally subject to feed-back regulation (Hedden and Kamiya 1997; Cowling et al., 1998), whereas the expression of the genes encoding GA-2 oxidase, a GA-inactivating enzyme, is under feed-forward regulation (Thomas et al., 1999). In agreement with this hypothesis, we also have
found that the application of GA reduced the transcript levels of GA-20 oxidase gene homolog in light-grown DongJinByeo seedlings, while GA increased the transcript levels in dark-grown seedlings. More detailed experiments will be needed to make sure how light and GA interact in the molecular levels and regulate GA-20 oxidase gene multilaterally in DongJinByeo seedlings.

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References


