Isolation and Characterization of a Gibberellin Responsive Gene (*HvGR*) from initiated shoots from calli derived from mature embryo of barley

Rak Chun Seong[1], Jae Yoon Kim[2], Jae Woong Haam[3] and Yong Weon Seo[2]

[1]Division of Bioscience and Technology, Korea University, Seoul, Korea
[2]Division of Biotechnology and Genetic Engineering, Korea University, Seoul, Korea seoag@korea.ac.kr

[3]Woongjin Food Research and Development Center

Abstract

Immature barley embryos in culture show a high ability of plant regeneration, but the period during which immature embryos can be used is limited. To overcome this difficulty, mature embryos of barley (*Hordeum vulgare* L.) have been used to establish regenerable tissue cultures with potential use for transformation. In order to provide information on molecular events during plant regeneration, a cDNA library was constructed using tissues of the 6 day-old initiated shoots. A differential hybridization method was used to isolate genes expressed differentially during plant regeneration. Of the forty cDNA clones that were highly expressed during shoot initiation, gibberellin responsive gene (*HvGR*) was isolated and characterized. Northern analysis was conducted on total RNAs extracted from 3 week-old calli, 1, 3, and 6 day-old initiated shoots, and from shoots of plantlets. The *HvGR* gene expressed specifically during shoot development. Tissue localization of *HvGR* gene transcripts during different stages of shoot induction and callus tissue by *in situ* hybridization showed similar results. A high level of *HvGR* expression was observed in day 6 initiated shoots, where the signal was localized primarily in the developing shoot primordial. Effects of plant growth hormones, ABA, cytokinin (BAP), and gibberellic acid, and wounding on the expression of *HvGR* were analysed.

Media summary

A gibberellin responsive gene (*HvGR*) from initiated shoots from callus derived from mature embryo of barley was isolated and characterized

Key Words

Tissue culture, Mature barley embryo, Differentially expressed gene, Gibberellin responsive gene (*HvGR*).

Introduction

The success of plant regeneration in cereal crops is influenced by plant growth regulators, genotypic effects, explant, morphogenetic pathway, and cell type (Bhaskaran and Smith, 1990). Various explants have been used to initiate a regenerable culture. Immature embryos are the most frequently used as explants for the tissue culture. However, immature embryo-derived tissue culture has certain disadvantages. For example, the growth stage of immature embryo appropriate for isolation is strictly limited. Suitable embryo size for tissue culture varies with varieties and environmental conditions. Growth of donor plant and immature embryo isolation are all time-consuming, expensive and laborious. The use of mature embryo is easy to handle and available at any time. Plant hormones are known to be among the key factors involved in the control of organogenesis. Gibberellin (GA) plays a crucial role in the control of various physiological processes in the plant life cycle, including seed germination, enhancement of shoot growth, stimulation of cell division and the formation of flower buds. Identification of genes whose expression is controlled by GA is important for GA physiological studies. Genes whose transcript levels change following treatment with GA may encode proteins that act downstream of the GA signal transduction pathway and that may play a crucial role in regulating growth. The molecular mechanism of GA-induced growth, however, is currently not well understood, due to a lack of knowledge about the genes. From growing shoots, several GA-responsive genes have also been isolated and characterized

(Cho and Kende, 1997). However, the relationship between GA action and these genes, particularly their transcriptional relation has not been well clarified. In addition, molecular studies on GA-induced shoot growth in monocotyledonous species are relatively few in number, despite their agricultural importance. The objective of this research was to isolate and characterize differentially expressed genes that were presumably related to plant tissue initiation from callus derived from mature embryos of barley.

Methods

Plant materials and Tissue culture conditions

Mature seeds were sliced obliquely between embryo and endosperm with a scalpel. The cut surfaces of the seeds with intact embryos were placed on the callus induction medium, which consisted of MS basal salts, 30 g/l maltose, 1.0 mg/l thiamine-HCl, 250 mg/l myo-Inositol, 1.0 g/l casein hydrolysate, 690 mg/l L-proline, 7 g/l agar, and three levels of 2,4-D 2.5 mg/l,and BAP 0.1mg/l (Cho et al. 1998). The regeneration medium contained MS medium, 40 g/l maltose, 1.0 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 100 mg/l myo-Inositol, 730 mg/l L-glutamine, 230 mg/l L-proline, 0.5 mg/l BAP, 1.5 mg/l indole-3-acetic acid, and 7 g/l agar. Callus cultures were incubated at 24?1?C in the dark for three weeks. For plant regeneration, culture room was kept at 25?C with a 16 h photo-period (1800 lux).

Isolation of HvGR gene

For total RNA extraction, mature embryo cultures were harvested on the 6th day after shoot formation cultures. The shoot regeneration tissues of the 6-day-old, initiated shoots (1g) were ground in liquid nitrogen. cDNA library construction and gene screening was carried out as described by Jang et al. (2002)

Northern blot analysis and In situ hybridization

The method of Jang et al. (2003) was modified in northern blotting and *in situ* hybridization.

Results

All the cultivars were cultured on media containing nine different concentrations of 2,4-D, and BAP. DH (Differential Hybridization) method was used to isolate differentially expressed genes during plant regeneration. Total RNA was extracted from 3 week-old calli, 1, 3, and 6 day-old initiated shoots, and plantlets (Fig 1). *HvGR* gene expression during barley shoot development was followed by northern blot analysis (Fig 2). *HvGR* showed lower transcript levels in callus culture and expression increased strongly three days after initiation of the shoot. Tissue localization of *HvGR* mRNAs during different stages of shoot induction and callus tissue by *in situ* hybridization are shown in Figure 3. All observations are similar to the RNA gel blot results. A relatively low level of *HvGR* expression was observed in the early stage of initiated shoot. The expression was randomly distributed throughout the dividing cells of the callus (Fig 3). In 3 day-old initiated shoots, expression was localized to pockets of subepidermal cells from where shoot primordial originate (Fig 3G). High levels of *HvGR* expression were observed in 6 day-old initiated shoot, where the signal was localized primarily in the developing shoot primordial (Fig 3H). Effects of plant growth hormones (100 uM of abscisic acid (ABA), cytokinin (BAP), and gibberellic acid (GA_3), containing 0.05% Tween 20) and wounding on the expression of *HvGR* were analysed (Fig. 4).

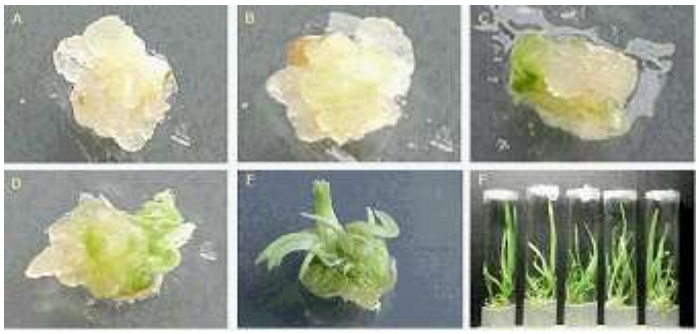

Fig. 1A-F Shape of different culture stages. (A):3 week-old calli, (B):1 day-old initiated shoots, (C): 3 day-old initiated shoots, (D): 6 day-old initiated shoots, (E): 10 day-old initiated shoots, and (F): plantlet

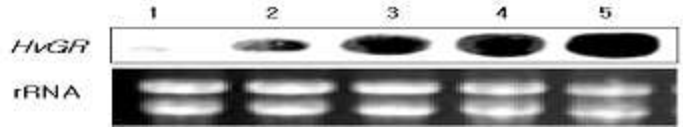

Fig. 2 Northern blot hybridization of *HvGR* gene in different culture stages. Total RNA (20 ?g per sample) of five tissues from the barley (cv.Sangroc) was fractionated on a 1% denaturing agarose gel. 1 :3 week-old callus, 2 :1 day old initiated shoots, 3 : 3 day-old initiated shoots, 4: 6 day-old initiated shoots, and 5: plantlets.

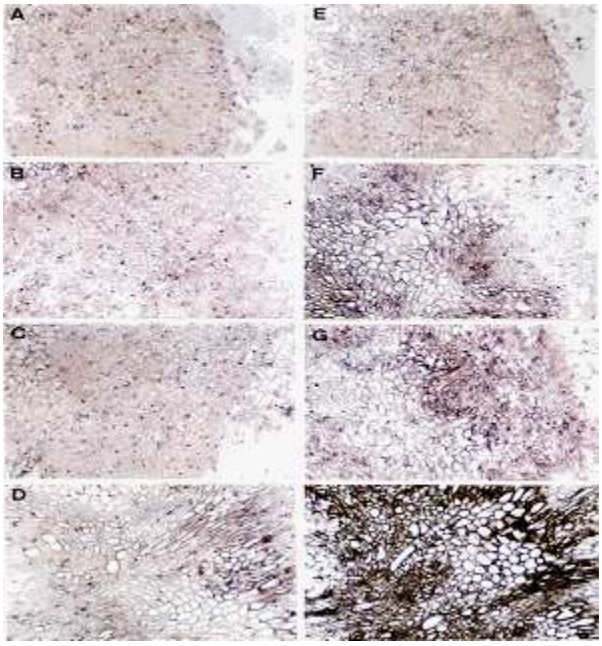

Fig. 3A-H *In situ* localization of *HvGR* transcripts in barley mature embryo cultures. Tissue sections were probed with a DIG-labelled gene specific probe of *HvGR* (A, B, C, D: control ; E, F, G, H: antisense probe). (A & E):3 week-old calli, (B & F):1 day old initiated shoot, (C & G): 3 day-old initiated shoot, (D & H): 6 day-old initiated shoot.No signal was detectable in the control.

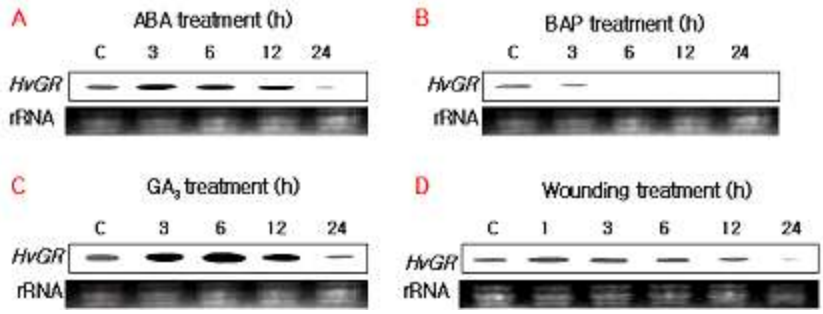

Fig. 4A-D Time course accumulation of *HvGR* in response to various treatments. Northern blot hybridization of a *HvGR* gene in young leaves of barley treated with ABA, BAP, GA3 and wounding. The leaf material was harvested from the plant sprayed with 100?M solution of each with ABA, BAP, and GA3 containing 0.05%(v/v) Tween 20. C: control, h: hours

Conclusion

The isolation and characterization differentially expressed gene in initiated shoot were described in this research. Since the cDNA library and the mRNAs that were used for the differential screen were isolated from different stage cell masses, the study was targeted to recover transcripts involved in the stage of regeneration. The results from the northern blot and *in situ* hybridization experiments showed the selection strategy was successful since *HvGR* showed the highest level of expression in the regeneration stage of the initiated shoot. These experimental results indicate that the expression of *HvGR* is regulated at the transcription level and is associated with development of shoots.

References

Bhaskarm, S and R.H. Smith (1990). Regeneration in cereal tissue culture: a review. Crop Sci. 30: 1328-1336.

Cho, H. T.,and H. Kende. (1997). Expression of expansion genes is correlated with growth in deepwater rice. Plant Cell 9: 1661-1671.

Cho, M. J., W. Jiang, and P. G. Lemaux. (1998). Transformation of recalcitrant barley cultivars through improvement of regenerability and decreased albinism. Plant Sci. 138: 229-244.

Jang, CS, DS Kim, SY Bu, JB Kim, SS Lee, JY Kim, JW Johnson, YW Seo. (2002). Isolation and characterization of lipid transfer protein (LTP) genes from a wheat-rye translocation line. Plant Cell Rep. 20: 961-966

Jang, CS, MS Lee, JY Kim, DS Kim, YW Seo. (2003). Molecular characterization of a cDNA encoding putative calcium binding protein, *HvCaBP1*, induced during kernel development in barley (*Hordeum vulgare* L.). Plant Cell Rep. 22: 64-70