Plant Biotechnol Rep (2007) 1:71–77
DOI 10.1007/s11816-007-0016-5

ORIGINAL ARTICLE

Particle bombardment-mediated transformation of barley with an *Arabidopsis* NDPK2 cDNA

Mi Ok Um · Tae Il Park · Young Jin Kim · Hong Yul Seo · Jung Gon Kim · Suk-Yun Kwon · Sang-Soo Kwak · Dae-Jin Yun · Song Joong Yun

Received: 30 August 2006 / Accepted: 3 January 2007 / Published online: 28 March 2007

Abstract As barley is recalcitrant to transformation with current methods, a new improved system is required to apply genetic transformation in breeding programs. In a previous study, we defined optimal conditions for plant regeneration (PR) using mature embryos. This study was conducted to establish an improved transformation system employing the previously adjusted regeneration conditions. Optimal DNA delivery condition for the embryogenic calli developed from mature embryos was bombardment pressure of 1,100 psi at the target distance of 6 cm. The feasibility of the regeneration and DNA delivery conditions was confirmed by developing transgenic barley plants transformed with the *Arabidopsis* nucleoside diphosphate kinase 2 (*AtNDPK2*) cDNA via particle bombardment of embryogenic calli from mature embryos. Stable integration of *AtNDPK2* cDNA into barley genome was confirmed by PCR and Southern blot analysis of *AtNDPK2* transgene. Transgenic plants showed about 10% reduction in membrane damage caused by methyl viologen, indicating the expression of *AtNDPK2* transgene. The results demonstrated that the transformation system developed in this study employing the PR from mature embryo-derived embryonic callus is applicable in transgenic barley production.

Keywords Barley · Nucleoside diphosphate kinase 2 · Transformation · Methyl viologen · Oxidative stress

M. O. Um · T. I. Park · Y. J. Kim · H. Y. Seo · J. G. Kim
Honam Agricultural Research Institute,
National Institute of Crop Science, RDA,
Iksan 570-080, South Korea

S.-Y. Kwon · S.-S. Kwak
Environmental Biotechnology Research Center,
Korea Research Institute of Bioscience and Biotechnology
(KRIBB), Yuesong, Daejeon 305-806, South Korea

D.-J. Yun
Division of Applied Life Science, Gyeongsang National
University, Jinju 660-701, South Korea

S. J. Yun (✉)
Division of Biological Resources Science, Institute of Rare Earth
for Biological Applications, Chonbuk National University,
Jeonju 561-756, South Korea
e-mail: sjyun@chonbuk.ac.kr

Introduction

Direct delivery of DNA by particle bombardment and *Agrobacterium*-mediated delivery are the two most widely used methods for genetic transformation. Barley (*Hordeum vulgare* L.) was found to be one of the most difficult crops to transform and it was by the development of particle bombardment method that barley was efficiently transformed (Wan and Lemaux 1994; Cho et al. 1998; Harwood et al. 2000). During the past decade, numerous reports of transient and stable expression of exogenous genes in barley cells and tissues have been available.

To develop barley showing high levels of tolerance to abiotic stresses, *Arabidopsis* nucleoside diphosphate kinase 2 (*AtNDPK2*) cDNA was transformed to barley via the particle bombardment method employing the plant regeneration (PR) procedure developed for mature embryo explants. NDPK is an ubiquitous enzyme in eukaryotes and prokaryotes. It catalyzes the transfer of phosphate from ATP to NDP, except ADP, through autophosphorylation (Parks and Agarwal 1973). In plants, NDPKs play a key role in the signaling of both stress and light. Transgenic *Arabidopsis* plants expressing NDPK2 had lower levels of reactive oxygen species (ROS) and showed tolerance

against several environmental stresses such as cold, salt, and hydrogen peroxide (H_2O_2) (Moon et al. 2003). Oxidative stress derived from ROS is one of the major damaging factors in plants exposed to environmental stresses. As demonstrated by numerous transgenic plants, the enhancement of antioxidant enzyme system could confer plants increased tolerance to various oxidative stresses (Kwon et al. 2002; Kim et al. 2003; Moon et al. 2003).

Objectives of this study were to establish an efficient transformation system in barley and to develop transgenic barley plants with abiotic stress tolerance, deriving from *Arabidopsis NDPK2* cDNA.

Materials and methods

Plant materials and culture conditions

Two barley varieties, Dooweonchapssalbori and Igri, were cultivated in the fields at the Honam Agricultural Research Institute, NICS, Korea, and harvested seeds were used for embryonic calli preparation with mature embryos essentially as described previously by Park et al. (2006). Briefly, calli was induced by incubating mature embryonic axis on CI medium containing 2.5 mg/l dicamba at 25°C under dark condition for 3 weeks. Embryogenic calli was obtained by incubating calli in CI3D medium [embryogenic callus (EC) medium supplemented with 3 mg/l 2,4-D, 50 g/l maltose and solidified with 4 g/l phytagel, pH 5.7] at 25°C for 2 weeks in the dark. Plants were regenerated from embryogenic calli on CIS medium [shoot induction (SI) + 1 mg/l IAA and 2 mg/l BAP, 30 g/l sucrose, solidified with 4 g/l phytagel, pH 5.7]. Roots were induced by incubating shoots/plantlets (3–4 cm in length) on PR medium containing 30 g/l sucrose, no plant growth regulators, and solidified with 4 g/l phytagel, pH 5.7 (Table 1).

Table 1 Composition of the media used for barley somatic embryogenesis

	CI	EC	SI	PR
MS salts	1×	–	–	1×
B5 salts	1×	–	–	1×
(NH_4) NO_3	–	165 mg/l	165 mg/l	–
KNO_3	–	1,900 mg/l	1,900 mg/l	–
KH_2PO_4	–	170 mg/l	170 mg/l	–
$MgSO_4$ $7H_2O$	–	370 mg/l	370 mg/l	–
$CaCl_2$ $2H_2O$	–	440 mg/l	440 mg/l	–
$MnSO_4$ $4H_2O$	–	16.9 mg/l	16.9 mg/l	–
$ZnSO_4$ $7H_2O$	–	8.6 mg/l	8.6 mg/l	–
H_3BO_3	–	6.2 mg/l	6.2 mg/l	–
KI	–	0.82 mg/l	0.82 mg/l	–
$CuSO_4$ $5H_2O$	–	0.025 mg/l	0.025 mg/l	–
Na_2MoO_4 $2H_2O$	–	0.025 mg/l	0.025 mg/l	–
Pyridoxine HCl	–	2 mg/l	2 mg/l	–
Nicotinic acid	–	2 mg/l	2 mg/l	–
Ca panthothenate	–	2 mg/l	2 mg/l	–
Biotin	–	0.02 mg/l	0.02 mg/l	–
Na pyruvate	–	10 mg/l	10 mg/l	–
$FeNa_2EDTA$ $2H_2O$	–	40 mg/l	40 mg/l	–
Thyamine HCl	1 mg/l	0.4 mg/l	0.4 mg/l	–
Citric acid	–	10 mg/l	10 mg/l	–
Casein hydrolysate	1 g/l	300 mg/l	300 mg/l	–
Myo inositol	250 mg/l	2,000 mg/l	2,000 mg/l	–
L-glutamine	–	256 mg/l	256 mg/l	–
L-proline	690 mg/l	250 mg/l	250 mg/l	–

CI callus induction, *EC* embryogenic callus, *SI* shoot induction, *PR* plant regeneration

Preparation of explants

Approximately ten EC clusters were pre-cultured by placing them on the callus formation medium (EC) for 1 day. Pre-cultured embryogenic tissue was placed in the designated 2.5-cm diameter target area on EC medium supplemented with 3 mg/l 2,4-D.

Preparation of gold particles and bombardment conditions

Microprojectiles (gold particle, 1.0 μm diameter) were suspended in the solution containing 5 μl of DNA solution (1 μg/μl). To the suspension, 50 μl of 2.5 M $CaCl_2$ and 20 μl of 0.1 M spermidine were added, mixed thoroughly and vortexed for 10 min. The solution was centrifuged for 1 min and the supernatant was discarded. The DNA-coated microprojectile pellet was resuspended and used by 1 μg DNA per shot for delivery.

All parts of the gun were thoroughly sterilized with 70% ethanol. Pre-cultured EC cluster was bombarded, one to two times using the Biolistic Particle Delivery System (PDS-1000/He, Bio-Rad). The dish containing explants was placed 60–90 mm below the rupture disk, with a gap distance of approximately 1.0 cm and a helium pressure of 900–1,100 psi.

Vector construction and histological GUS assay

The plasmid pACT1D containing rice actin promoter-GUS-NOS terminator (McElroy et al. 1990) was provided by Dr Wu R. (Cornell University, USA) through Dr Bennett J. (IRRI) and Dr Kim H.S. (NHAES) (Fig. 1). Bombarded embryogenic tissues were incubated in 5-bromo-4-chloro-3-indolyl-β-glucuronide (X-Gluc) solution over-

night at 37°C for histochemical GUS assay. Explants were decolorized with two to three rinses of absolute ethanol before microscopic evaluation.

Construction of recombinant *AtNDPK2* expression vector and selection of transgenic plants

The recombinant *AtNDPK2* expression vector was driven by the stress-inducible sweetpotato peroxidase (SWPA2) promoter (Kim et al. 2003), and contained bar gene as a selectable marker (Fig. 2). Two days after bombardment, callus was transferred to selection medium [CI3D medium supplemented with 5 mg/l phosphinothricin (PPT)] for 3 weeks in the dark. For SI, callus growing well on the selection medium was transferred to CIS medium supplemented with 5 mg/l PPT for SI, and was incubated for 7–8 weeks under the light intensity of 20 μmol/m^2/s, 16:8 h photoperiod at 26°C. Subculture was conducted every 2 weeks. Plantlets obtained from the calli were transferred for rooting to CIR medium without the selection agent, PPT.

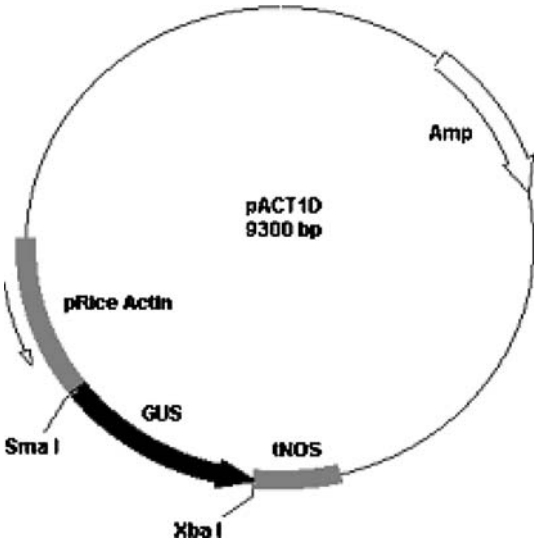
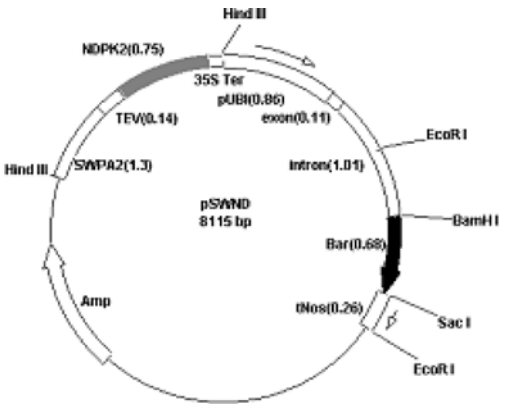
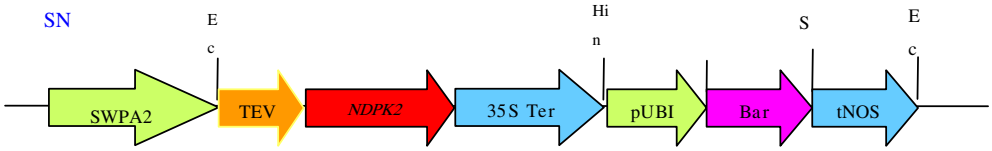


Fig. 1 Schematic diagram of pACT1D construct containing the GUS gene expression cassette driven by rice actin promoter (McElroy et al. 1990)

PCR and Southern blot analyses of *AtNDPK2* transgene

PCR amplification of *AtNDPK2* transgene fragment from the T_0 plants was carried out with the *AtNDPK2*-specific forward (5′- GGT GGG AGC GAC TGT AGT TAG T -3′) and reverse (5′- CCT CAT TCC CTT AGC CAT GAT GTA G -3′) primer pairs. PCR reaction was carried out using PreMix (Bioneer, Korea) containing 5 pM each primer and 10 ng genomic DNA. PCR was performed in a thermal cycler for 40 cycles and a cycle was consisted of heat denaturation (94°C, 30 s), annealing (65°C, 30 s), and extension (72°C, 1 min) steps. Initial denaturation (94°C, 5 min) or final extension (72°C, 10 min) step was additionally performed before the first and after the last cycle of the PCR reaction, respectively. PCR products were separated on a 1% agarose gel, transferred and fixed onto a nylon membrane. The membrane was hybridized with the *AtNDPK2* cDNA probe labeled using the AlkPhos kit (Amersham Pharmacia, UK), and the hybrid DNA molecules were detected with CDP-Star detection reagent (Amersham Pharmacia).





SWPA2 : sweet potato POD promoter, TEV : tobacco etch virus 5'-UTR

Fig. 2 Structure of the NDPK2 recombinant expression vector driven by oxidative stress-inducible SWPA2 promoter. Abbreviations used are: *SWPA2* sweetpotato peroxidase anionic 2 promoter, *pUBI* maize ubiquitin promoter, *TEV* tobacco etch virus 5′-UTR, *NDPK2*, nucleoside diphosphate kinase 2, *Bar* bialaphos resistance gene

Bioassay for stress tolerance

Tolerance to methyl viologen (MV)-induced oxidative stress was determined for the leaf disks (0.8 cm in diameter) from fully expanded leaves of plants grown for 40 days in the pots. Leaf disks were infiltrated with 0, 2, 5, 10, and 50 μM MV, respectively. After infiltration with MV, leaf discs were illuminated for 2 h followed by dark incubation for 12 h (Allen 1995). Ion leakage from the leaf discs was measured with an ion conductivity meter (Istek, model 455C) and means ± SE of the three replicates were obtained.

Results and discussion

Particle bombardment-mediated transformation via somatic embryogenesis

Compact EC clusters growing on CI3D medium were selected and used for bombardment. Optimal conditions for DNA delivery by particle bombardment were established based on histochemical transient GUS assay results (Jefferson 1987; Sanford et al. 1993). When subjected to GUS histochemical assay, the bombarded calli exhibited characteristic blue cells typical of transient expression of the GUS gene 24 h after DNA delivery. The transformed cells appeared randomly distributed on the surface of explants. In some cases, the entire embryonic explants stained totally blue, and this could be a result of the diffusion of GUS reaction products to adjacent tissues. Neither the control explants that were not bombarded nor those that were bombarded with microcarriers not coated with DNA exhibited GUS expression in any cells (Fig. 3). Similar GUS expression patterns were observed in the particle-bombarded barley immature embryos (Harwood et al. 2000).

The transient GUS expression indicated that the target distance and helium pressure significantly affected transient expression of the reporter gene. These findings are similar to those reported for cowpea and barley (Kononowicz et al. 1997; King and Kasha 1994; Harwood et al. 2000). The interaction between the bombardment pressure and target distance showed that the higher the pressure and the shorter the target distance, the higher the GUS expression. Highest GUS expression was obtained at the pressure of 1,100 psi and the target distance of 6 cm (Table 2).

To ascertain optimal selection conditions, PPT levels ranging from 3 to 6 mg/l were tested, and selection was performed after 4 weeks of culture essentially as described by Wan and Lemaux (1994) and Harwood et al. (2000). About 2% of the explants survived after 4 weeks of incubation on selection medium containing 5 mg/l PPT (Table 3).

Having established a PR system using mature embryos and set DNA delivery conditions by particle bombardment, transformation of barley was attempted with *AtNDPK2* cDNA by particle bombardment of embryonic tissues derived from mature embryos. The bombarded embryonic tissue explants were incubated on medium containing 5 mg/l PPT to select transformed tissues. During the selection, some calli grew vigorously, but some turned brown. To obtain callus lines devoid of non-transformed tissue, growing callus tissues were additionally subcultured once or twice onto fresh selection medium until uniform growth was obtained. Non-transgenic explants could not form root and shoot on the PPT selection medium. However, regenerated transgenic plants were obtained from PPT-resistant tissues through direct shooting followed by rooting from the shoots. The plants grew normally and set seeds. There was no significant difference in morphological and agronomic characteristics between the transformed and non-transformed plants (data not shown).

Table 2 Effect of microcarrier travel distance and helium pressure on GUS expression 24 h after bombardment[a]

Microcarrier travel distance (cm)	Helium pressure (psi)	Number of assayed calli	Percentage of GUS positive calli (%)
6	900	50	80 ± 3.4
6	1,100	50	82 ± 2.1
9	900	50	42 ± 1.2
9	1,100	50	68 ± 2.3

[a] GUS expression in callus from embryos of barley bombarded at different distances and helium pressure. Data are presented as mean values of at least five different experiments ± standard error

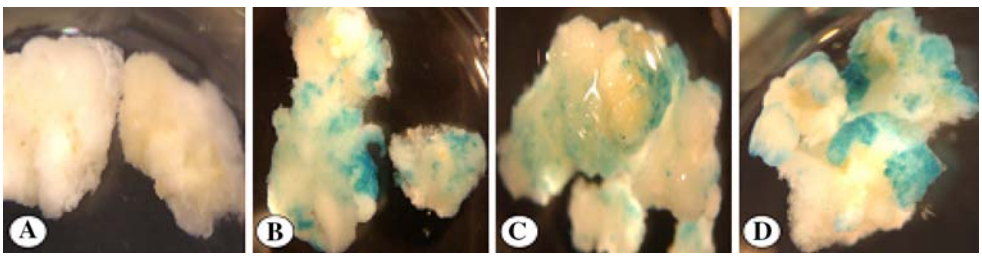


Fig. 3 Histochemical GUS expression in embryogenic tissues of barley. Explants were incubated in X-Gluc at 37°C. **a** Tissues bombarded by particles with no plasmid (control); **b–d** tissues bombarded by particles coated with the plasmid containing GUS gene

Confirmation of transformation by PCR and Southern blot analyses

A total of 20 regenerated plantlets, 9 and 11 from Dooweonchapssalbori and Igri, respectively, were obtained from 400 callus clusters cultured on the selection medium (Table 4). The transformed explants were successfully regenerated and were transferred to potting soil after acclimation in a glasshouse. PCR was performed to confirm whether the *AtNDPK2* sequence was integrated into genomic DNA of T_0 transgenic plants with the primer pairs specifically targeted at the *AtNDPK2* sequence to give a 756 bp amplification fragment. The fragment in the expected size was amplified from only 5 out 20 PPT-resistant plants. No DNA fragment was amplified from the control plants (Fig. 4). It was reconfirmed whether the amplified fragments were from the *AtNDPK2* transgene sequence by Southern blot analysis for the amplified fragment. A hybrid band in the same size as the amplified DNA fragment was detected from four, one from Dooweonchapssalbori and three from Igri, out of the five PCR-positive plants (Fig. 4). These results indicate the stable integration of *AtNDPK2* sequence into the barley genome. However, the transformation frequency was extremely low, at 0.05 and 0.15% for Dooweonchapssalbori and Igri, respectively (Table 4). This transformation frequency is estimated to be lower than that reported by Wan and Lemaux (1994) and Sharma et al. (2005) although no direct comparisons could be made among the experiments due to the nature of the data presented. Transformation frequency could be affected by many factors such as genotypes, explant types, medium compositions, and hormone combinations.

Table 3 Effects of phosphinothricin (PPT) in culture medium on the survival of regenerated plants after 8 weeks of incubation

PPT (mg/l)	No. of callus clusters inoculated	No. of shoots formated	No. of plants survived (%)[a]
0	50	21	20 (40)
3	50	18	12 (24)
4	50	7	3 (6)
5	50	2	1 (2)
6	50	0	0

[a] Plants with roots

Enhanced tolerance of transgenic plant against methyl viologen-induced oxidative stress

The stress tolerance of PPT-resistant transgenic plants to MV-induced oxidative stress was evaluated using a leaf disc incubation method. Ten leaf discs were incubated in 0, 2, 5, 10, and 50 μM MV solutions under the light (38 μmol m^2/s) for up to 48 h. After incubation, conductivity of the incubation medium was measured to estimate ion leakage from the leaf discs due to MV-induced membrane damage. Relative ion leakage was increased in a time- and MV concentration-dependent manner, and the change was most sensitive between 12 and 24 h of incubation in 5 μM MV (Fig. 5). Thus, 5 μM MV solution was used to estimate MV-induced oxidative damage in transgenic plants.

Leaf discs of one non-transgenic (B-018) and four transgenic plants (B-022, B-020, B-019, B-021) were subjected to incubation in 0 and 5 μM MV under the light for up to 48 h. Relative ion leakage of the two transgenic lines, B-019 and B-021, was significantly lower (about 10%) than that of the non-transgenic control plant in all incubation periods. However, the relative ion leakage of the transgenic line B-022 was about the same as that of non-transgenic plants (Fig. 6). This difference in the relative ion leakage between the transgenic lines could be associated, at least in part, with the copy number, position effect and silencing of the transgene. Further analysis of transgene constitution and expression in the advanced generations will result in direct evidence to explain the difference between the transgenic lines.

Transgenic *Arabidopsis* plants expressing *AtNDPK2* had lower levels of ROS and showed tolerance against several environmental stresses, such as cold, salt, and H_2O_2 (Moon et al. 2003). Thus, a significant reduction in ion leakage in the two transgenic lines could be the result of *AtNDPK2* transgene in the transgenic plants. This result suggests that *AtNDPK2* could interact with barley proteins that are phosphorylated by NDPK2, triggering H_2O_2-mediated MAPK signaling in barley (Moon et al. 2003). This is

Table 4 Transformation efficiency from embryogenic tissues of barley treated with particle bombardment

Varieties	Number of callus clusters bombarded (*A*)	Number of regenerated plantlets (%)	Number of positive explants		Transformation efficiency (*B*/*A*) (%)[a]
			PCR	Southern (*B*)	
Dooweonchapssalbori	200	9 (0.3)	2	1	0.05
Igri	200	11 (0.4)	3	3	0.15

[a] Transformation efficiency is defined as the percentage of callus cluster producing at least one PPT-resistant and NDPK2-positive shoot divided by the number of inoculated callus

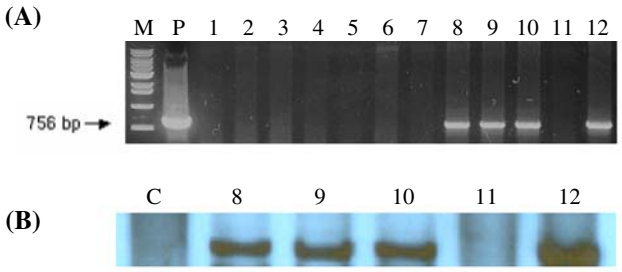


Fig. 4 **a** Amplification of a DNA fragment with the *AtNDPK2*-specific primer pair from the putative transgenic plants obtained via somatic embryogenesis. *M* Lamda DNA/*Hin*dIII ladder, *P* amplified product (756 bp in size) from the plasmid pSWND containing *AtNDPK2* DNA, *lane 1* non-transgenic plant (Igri), *lanes 2–10* transgenic plants (Igri), *lanes 11–12* transgenic plants (Dooweonchapssalbori). **b** Southern blot analysis for the transgenic T_0 plants with *AtNDPK2*-specific DNA probe. Total DNA was isolated from leaves and 10 μg of DNA were digested to completion with *Bam*HI. *Lane C* non-transgenic plant, *lanes 8–10* transgenic plants (Igri) transformed with plasmid pSWND containing *AtNDPK2* cDNA, *lanes 11–12* transgenic plants (Dooweonchapssalbori)

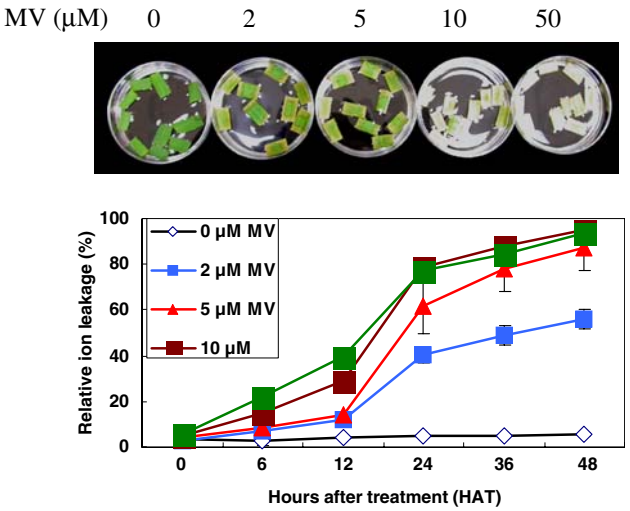


Fig. 5 Evaluation of methyl viologen (MV)-induced membrane damages in leaf discs. Leaf discs were floated on 2, 5, 10, and 50 μM MV solution, and then exposed to light for 24 h. Relative ion leakage was determined with respect to conductivity of the solution. Data are means ± SE of three replicates

consistent with the fact that the NDPK active sites are structurally identical, and almost all the residues involved in the active site are fully invariant from bacteria to human (Lascu et al. 2000). Consequently, this result indicates a practical feasibility of developing genetic lines with enhanced tolerance against multiple stresses by the modulation of NDPK2 gene expression in barley.

In summary, an efficient and reproducible PR system using mature embryos was established by setting the optimum medium composition and hormone combinations.

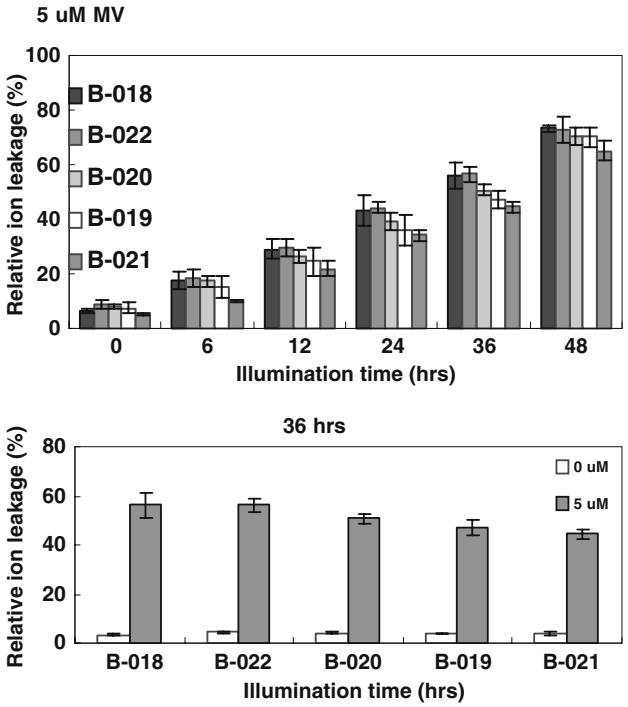


Fig. 6 Effect of 5 μM methyl viologen-induced membrane damges in leaf discs of transgenic plants. *B-018* non-transgenic plants; *B-022*, *B-020*, *B-019*, and *B-021* transgenic plants. Data are means ± SE of three replicates

Conditions to deliver DNA to the embryonic calli by particle bombardment were refined by adjusting optimum pressure and target distance for microcarrier discharge. The reliability of the regeneration and DNA delivery conditions were demonstrated by successfully generating transgenic barley plants expressing *AtNDPK2* gene employing the transformation system established in this study. These transgenic plants expressing *AtNDPK2* showed enhanced tolerance to MV-induced oxidative stress, suggesting their potential as valuable genetic lines for improving tolerance against multiple stresses.

Acknowledgments This work was supported by a grant from BioGreen 21 Program, Rural Development Administration, Republic of Korea. S.J. Yun was supported by Research Center for Industrial Development of BioFood Materials in Chonbuk National University, Jeonju, Korea. The center is designated as a Regional Research Center appointed by the Ministry of Commerce, Industry and Energy (MOCIE), Jeollabuk-do Provincial Government and Chonbuk National University.

References

Allen RD (1995) Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol 107:1049–1054

Cho MJ, Jiang W, Lemaux PG (1998) Transformation of recalcitrant barley cultivars through improvement of regenerability and decreased albinism. Plant Sci 138:229–244

Harwood WA, Ross SM, Cilento P, Snape JW (2000) The effect of DNA/gold particle preparation technique, and particle bombardment device, on the transformation of barley (*Hordeum vulgare* L.). Euphytica 111:67–76

Jefferson RA (1987) Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol Biol Rep 5:387–405

Kim KY, Kwon SY, Bang JWd, Kwak SS (2003) A novel oxidative stress-inducible peroxidase promoter from sweetpotato: molecular cloning and characterization in transgenic sweetpotato plants and cell cultured cells. Plant Mol Biol 51:831–838

King SP, Kasha KJ (1994) Optimizing somatic embryogenesis and particle bombardment of barley (*Hordeum Vulgare* L.) immature embryos. In Vitro Cell Dev Biol Plant 30:117–123

Kononowicz AK, Cheah KT, Narasimhan ML, Murdock LL, Shade RE, Chrispeels MJ, Filippone E, Monti LM, Bressan RA, Hasegawa PM (1997) Development of transformation system for cowpea (*Vigna unguiculata* (L.) Walp). In: Singh DR, Mohan R, Dashiell KE, Jackai LEN (eds) Advances in cowpea research. Copublication of International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS), IITA, Ibadan, pp 361–371

Kwon SY, Jeong YJ, Lee HS, Kim JS, Cho KY, Allen RD, Kwak SS (2002) Enhanced tolerance of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen-mediated oxidative stress. Plant Cell Environ 25:873–882

Lascu I, Giartosio A, Ransac S, Erent M (2000) Quaternary structure of nucleoside diphosphate kinases. J Bioenerg Biomembr 32:227–236

McElroy D, Zhang W, Cao J, Wu R (1990) Isolation of an efficient actin promoter for use in rice transformation. Plant Cell 2:163–171

Moon HJ, Lee BY, Choi G, Shin DJ, Theertha Prasad D, Lee OS, Kwak SS, Kim DH, Nam JS, Bahk JD, Hong JC, Lee SY, Cho MJ, Lim CO, Yun DJ (2003) NDP kinase 2 interacts with two oxidative stress activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. Proc Natl Acad Sci USA 100:358–363

Park TI, Um MO, Kim YJ, Hwang JJ, Choi KG, Yun SJ (2006) Somatic embryogenesis and plant regeneration in barley (*Hordeum vulfare* L). J Plant Biotechnol 33:237–242

Parks REJ, Agarwal RP (1973) Nucleoside disphosphokinases. In: Boyer PD (ed) The enzymes. Academic, New York, pp 307–334

Sanford JC, Smith FD, Russell JA (1993) Optimizing the biolistic process for different biological applications. Meth Enzymol 217:483–509

Sharma VK, Hansch R, Mendel RR, Schulze J (2005) Mature embryo axis-based high frequency somatic embryogenesis and plant regeneration from multiple cultivars of barley (*Hordeum vulgare* L.). J Exp Bot 56:1913–1922

Wan Y, Lemaux PG (1994) Generation of large numbers of independently transformed fertile barley plants. Plant Physiol 104:37–48