J. Ecol. Field Biol. 29 (5): 479~484, 2006

Enhanced Resistance of Transgenic Sweetpotato (*Ipomoea batatas* Lam.) Plants to Multiple Environmental Stresses Treated with Combination of Water Stress, High Light and High Temperature Stresses

Song, Sun-Wha, Sang-Soo Kwak[1], Soon Lim[1], Suk-Yoon Kwon[1], Haeng-Soon Lee[1] and Yong Mok Park*

Department of Life Science, Cheongju University, Cheongju 360-764, Korea

[1]Laboratory of Environmental Biotechnology, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Korea

ABSTRACT: Ecophysiological parameters of non-transgenic sweetpotato (NT) and transgenic sweetpotato (SSA) plants were compared to evaluate their resistance to multiple environmental stresses. Stomatal conductance and transpiration rate in NT plants decreased markedly from Day 6 after water was withheld, whereas those values in SSA plants showed relatively higher level during this period. Osmotic potential in SSA plants was reduced more negatively as leaf water potential decreased from Day 8 after dehydration treatment, while such reduction was not shown in NT plants under water stressed condition. SSA plants showed less membrane damage than in NT plants. As water stress and high light stress, were synchronously applied to NT and SSA plants maximal photochemical efficiency of PS II (F_v/F_m) in NT plants markedly decreased, while that in SSA plants was maintained relatively higher level. This trend of changes in F_v/F_m between SSA plants and NT plants was more conspicuous as simultaneously treated with water stress, high light and high temperature stress. These results indicate that SSA plants are more resistive than NT plants to multiple environmental stresses and the enhanced resistive characteristics in SSA plants are based on osmotic adjustment under water stress condition and tolerance of membrane.

Key words: Electrolyte leakage, Multiple environmental stresses, Osmotic adjustment, Sweetpotato, Transgenic plant

INTRODUCTION

Global environmental change is one of the main topics in 21st century for human beings to solve because it may change natural ecosystem on the planet by causing environmental stress to plants, which in turn threaten survival of human beings (Osmond et al. 2004). Most of environmental stress in plants is associated with the increased production of toxic reactive-oxygen species such as super oxide (O_2^-), and hydrogen peroxide (H_2O_2) damaging plant cells (Allen 1995). Plants, however, have developed efficient defense mechanisms such as antioxidant enzymes detoxifying reactive oxygen species in the process of evolution (Asada 1999). Among environmental stress water is the most important factor determining plant distribution as well as crop productivity on the earth. Water stress causes various effects on plants through cessation of cells, stomatal closure, reduction of photosynthetic rate, wilting and even death (Hsiao 1973). As water stress often appeared together with heat stress researches of the effects of multiple environmental stresses on plant ecophysiology must be needed (Lu and Zhang 1999). Besides, development of resistive plants to multiple environmental stresses by biotechnology may be a way to cope with environmental stress resulting from global environmental change.

In fact, developed countries are trying to develop a strong and fruitful plants species for multipurpose such as securing foodstuffs and forage, and protecting natural disaster by using biotechnological techniques (Kwon et al. 2002). As a result, many-transgenic plants were made in a variety of plant species such as *Arabidopsis* spp. (Oh et al. 2005. Van Camp et al. 1996), maize (Caimi et al. 1996), pea (Donahue et al. 1997), alfalfa (McKersie et al. 1999, Samis et al. 2002), tobacco (Kwon et al. 2002, Sen Gupta et al. 1993), horseradish (Kawaoka et al. 2003) and rice (Suzuki et al. 2000). Most of transgenic plants showed enhanced tolerance to environmental stress though some of them failed to enhancing tolerance (Kwon et al. 2002, Torsethaugen et al. 1997).

However, to introduce transgenic plants to crop field or disturbed ecosystem, successfully, the most important thing must be an accurate evaluation on biohazard of transgenic plant in ecosystem and resistive characteristics to environmental stress. As a part of this kind of work we developed transgenic sweetpotato (SSA) plants expressing both CuZn superoxide dismutase (SOD) and ascorbate peroxidase (APX) in chloroplasts under the control of an oxidative

* Corresponding author; Phone: +82-43-229-8531, ecopark@cju.ac.kr

stress inducible SWPA2 promoter (Lim et al. 2004).

Here we evaluate ecophysiologically resistive characteristics of SSA plants to multiple environmental stress treated with combination of water stress, high light and/or high temperature stress.

MATERIALS AND METHODS

Two kinds of experiments were carried out, one evaluating the effect of water stress on plant water relations and membrane damage and the other assessing the effect of multiple environmental stress on potential photochemical efficiency.

The procedure of preparation for plant material was the same in both experiments except for experimental period as described below. Plant tissues for all measurements were sampled from fully developed.

Plant Material and Growth Condition

Plant materials were obtained by asexual propagation method from transgenic sweetpotato plants expressing both CuZn SOD and APX in chloroplasts (Lim et al. 2004). From SSA plants third-leaf stage shoots were cut and transplanted those into pots (7.5 cm in diameter and 14 cm deep) filled with mixed soil which was composed of sand and commercial soil (Supersangto, Soil and Fertilizer Technology Co., Korea.) in 1:1 volume ratio. All plants were well watered to run off every other day and given 50 mL of 1/500-strength Hyponex nutrient solution (Hyponex Plant Food 6.5-6-19, Hyponex Korea) four times. Plants were cultivated in a green house attached to Cheongju University, Korea. After cultivation for 4 weeks in the greenhouse plants were moved into a growth chamber with air temperature 25 ± 2℃, relative humidity 70 ± 10 % and photon flux density of 250 μmol m^{-2} s^{-1} with a photoperiod of 14/10h. Those conditions were maintained throughout the end of the experiment.

Stress Treatments

All treatments and measurements were carried out under the conditions of growth chamber mentioned above. Basically, all plants were subjected to water stress which was carried out by withholding water.

High light and high temperature stress were applied by exposing leaf surface to high light (1,800 μmol m^{-2} s^{-1}). Light was supplied through optical fiber from specially manufactured light source using halogen lamp (Lees OS Inc., Korea). The distance between light emitting point and leaf was maintained with 20 cm because it can keep high light intensity as well as high temperature of 33 ± 2℃.

High light stress was imposed by narrowing the distance between leaf and light emitting point, and by passing through a water body maintaining constant level of water in a 500 mL beaker filled with deionized water.

Measurements of Electrolyte Leakage

Electrolyte leakage from leaf tissues was determined according to Dioniso-Sese and Tobita (1998) with some modification. To quantify electrolyte leakage, leaf disk (25 mm^2 in diameter) was punched from fully developed leaves and put into a tube of 50 mL containing 20 mL of deionized water, and the tube was capped and then incubated on a rotary shaker (150 rpm) at room temperature for 30 min. The amount of electrolytes leaked from leaf sample was measured with conductivity meter (Checkmate II, Cornning, USA). Total amount of electrolytes in the leaf sample was determined after samples were autoclaved at 121℃ for 15 min and cooled to room temperature. Relative electrolyte leakage was calculated as the ratio of the amount of electrolytes before and after autoclaving.

Measurements of Leaf Water Relations and PSII Photochemical Efficiency

Leaf water potential, osmotic potential, stomatal conductance and transpiration rate were determined at an interval of 2 days. Leaf water and osmotic potential were measured by using dew point microvolt meter (Wescor, HR33T, USA). Leaf disk (7 mm in diameter) punched from fully developed leaves was placed in the sample chamber to reach equilibrium for 20 min, and then water potential of leaf disk was measured according to Premachandra et al. (1992) with some modification. After the measurement of leaf water potential leaf disk taken was put into an Eppendorf tube, and capped, and then stored in a deep freezer of −85℃ for 1 hr. The frozen leaf disk was allowed to thaw at room temperature condition and then osmotic potential was measured after sample thawing.

Stomatal conductance and transpiration rate were measured with steady state porometer (LI-1600, Licor, USA).

Chlorophyll a fluorescence was measured with potable chlorophyll monitoring system (PMS 2, Hansatech, UK). Before measurement of chlorophyll a fluorescence leaves were darkened for 20 min to open all PS Ⅱ reaction centers, and then minimal fluorescence (F_0) and maximal fluorescence yield (F_m) were measured and maximal photochemical efficiency (F_v/F_m) of PS II was calculated according to Schreiber et al (1994).

RESULTS AND DISCUSSION

Overall trend of leaf water potential was changed similarly in both NT plants and SSA plants (Fig. 1). Osmotic potential also showed almost identical changes in the two for 4 days from the start of dehydration treatment. However, osmotic potential of SSA plants decreased to −1.06 MPa on Day 6 thereafter, whereas that of NT plants showed −0.95 MPa. This difference in osmotic potential between NT plants and SSA plants continued the end of experi-

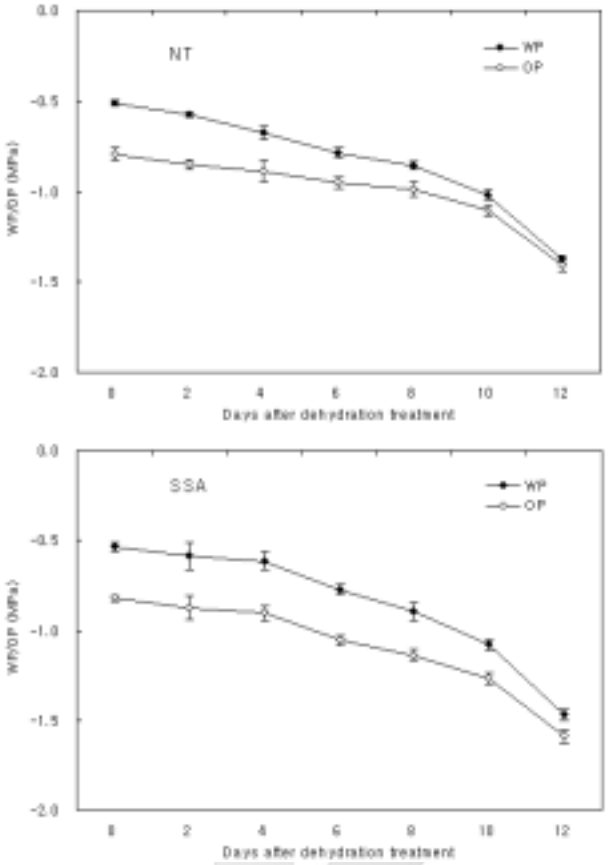

Fig. 1. Changes in leaf water potential (WP) and leaf osmotic potential (OP) of non-transgenic (NT) and transgenic (SSA) sweet potato (*Ipomoea batatas* Lam.) plants. Vertical bars represent standard errors of five measurements.

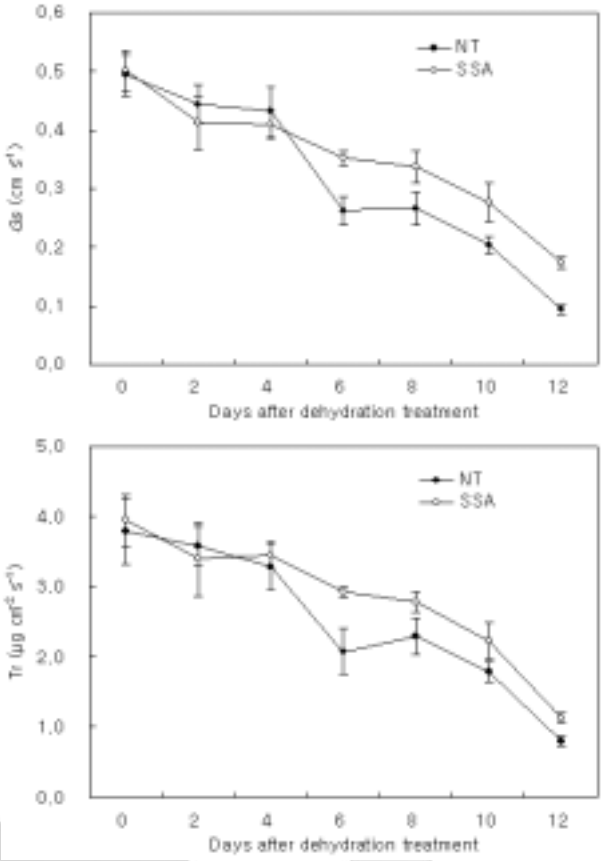

Fig. 2. Changes in stomatal conductance (*Gs*, upper) and transpiration (Tr, lower) of non-transgenic (NT) and transgenic (SSA) sweetpotato (*Ipomoea batatas* Lam.) plants. Vertical bars represent standard errors of five to seven measurements.

ment, and reached its peak on Day 8, showing the difference of 0.24 MPa. As a result, higher turgor pressure was maintained in SSA plants than in NT plants (Fig. 1), indicating osmotic adjustment in SSA plants. Turgor pressure at a particular water potential can be mediated either through the accumulation of solutes to low osmotic potential actively or by changes in wall elasticity (Jones 1979, Radin 1983, Evans et al. 1992). Though wall elasticity of NT plants and SSA plants were not determined in this experiment osmotic adjustment in SSA plants obviously contributed to the maintenance of turgor. Contribution of wall elasticity changes to turgor maintenance in water stressed plants is open to argument. Changes of wall elasticity in some crops and tree species with the absence of osmotic adjustment contributed to turgor maintenance (Nune et al. 1994, Fan et al. 1989). In some species, however, turgor was not maintained by change of wall elasticity in stressed plants (Martinez et al. 2006), and in some cases, both osmotic adjustment and changes of wall elasticity contribute together to turgor maintenance (Clifford et al. 1998). Thus, both factors may exert influence on turgor maintenance in SSA plants under dehydration treatment. SSA plants showed higher stomatal conductance and transpiration rate from Day 6 to the end of experiment (Fig. 2). Thus, higher turgor pressure in SSA plants than in NT plants must be reflected in stomatal conductance and transpiration rate, maintaining higher values than those in NT plants since Day 6 (Fig. 1). It is well known that photosynthetic rate in plants are highly affected by stomatal conductance (Farquhar et al. 1980). SSA plants also showed less membrane damage by dehydration treatment compared with NT plants (Fig. 3). Thus, SSA plants must have the advantage of other plants susceptible to water stress in the respect of matter production under drought conditions. Considering these facts, high stomatal conductance in SSA plants shown under water stressed condition is enough to arouse our interest (Fig. 2), expecting that SSA plants can contribute to stabilizing vegetation in the semiarid and arid regions, and enhancing agricultural productivity in those areas. This expectation is assured from the result of F_v/F_m, representing potential photosynthetic efficiency. In fact, SSA plants showed higher resistive

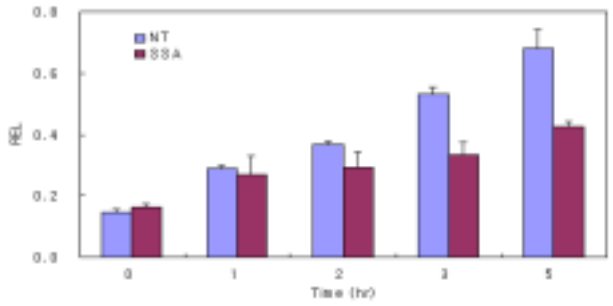

Fig. 3. Changes of relative electrolyte leakage (REL) in the leaves of non-transgenic (NT) and transgenic (SSA) sweet potato (*Ipomoea batatas* Lam.) plants after dehydration treatment. Each column indicates the mean of five measurements with standard error.

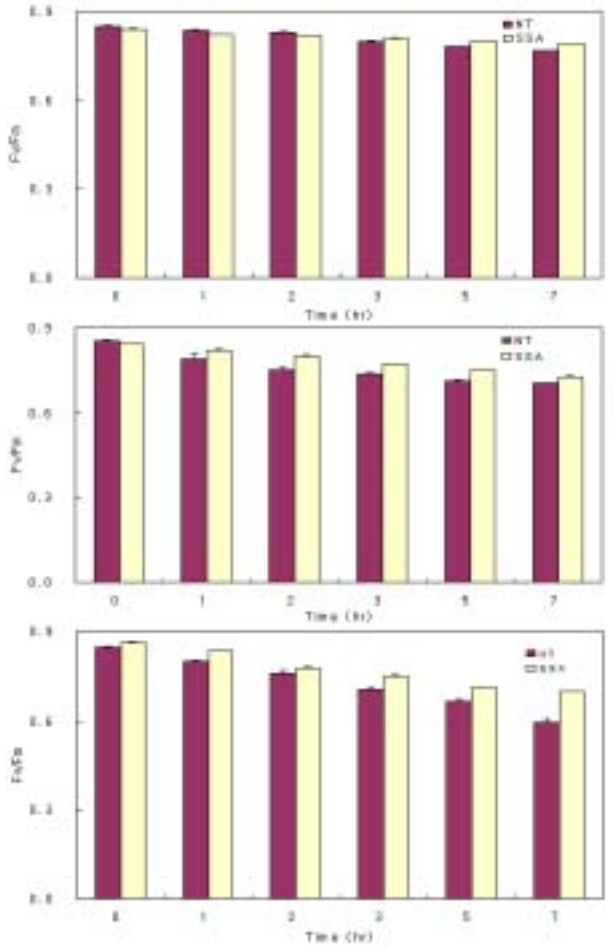

Fig. 4. Changes of maximum photosynthetic efficiency (F_v/F_m) of non-transgenic (NT) and transgenic (SSA) sweet potato (*Ipomoea batatas* Lam.) plants under high light stress imposed on Day 0 (upper), Day 4 (middle) and Day 8 (lower) after dehydration treatment. Each column indicates the mean of five measurements with standard error.

characteristics to multiple environmental stresses, combined water stress with high light stress (Fig. 4) and with high light and high temperature stress (Fig. 5). Under water stressed condition, high light stressreduced F_v/F_m in NT plants severely after 7 hr treatment on Day 8, compared with SSA plants. This reduction of NT plants was more obvious as multiple stress combined with high light and high temperature stress, was imposed simultaneously. F_v/F_m in NT plants decreased to 0.53 and 0.49 after 5 and 7 hr treatments on Day 8, respectively, while SSA plants maintained the values of 0.68 and 0.67, respectively.

Matter production by plants is the basis of biological diversity in an ecosystem as well as supporting ecosystems on the earth. Consequently, plants that have resistive characteristics to multiple environmental stress like SSA plants may play an important role in the extremely disturbed areas such as the source area of yellow dust, ongoing desertification area, and abandoned coal mine area. In

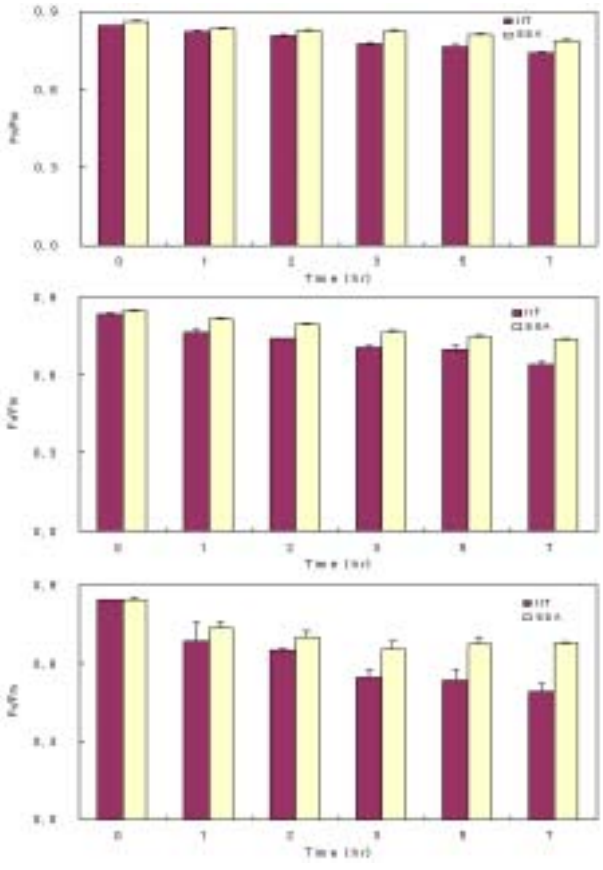

Fig. 5. Changes of maximum photosynthetic efficiency (F_v/F_m) of non-transgenic (NT) and transgenic (SSA) sweet potato *(Ipomoea batatas* Lam.) plants under high light and temperature conditions imposed on day 0 (upper), Day 4 (middle) and day 8 (lower) after dehydration treatment. Each column indicates the mean of five measurements with standard error.

recent, numerous trials to stabilize vegetation such as planting trees are carried out in the disturbed areas worldwide (Avery and Gordon 1983, Lewis 1985, Olofsson et al. 2005). In Korea, tree planting projects to protect yellow dust from the source area located at the border area between China and Mongolia are underway by various NGO groups. It is well known that the propagation of sweet potato plants depends on only asexual reproduction because sweet potato plants originating from tropical areas can not reproduce with sexual reproduction in cool temperate areas. Thus, the propagation of sweetpotato could be managed under human control. In this respect, SSA plants would play an important role in stabilizing vegetation in the source area of yellow dust and in restoring vegetation in disturbed area such as abandoned coal mine areas.

From these results, we conclude that SSA plants are more resistive than NT plants to multiple environmental stresses and the enhanced resistance to multiple stresses in SSA plants is resulted from osmotic adjustment and the resistive characteristic of membrane under water stressed condition. The use of transgenic plants as a restoring or revegetating plant species to extremely disturbed areas may be worth discussing.

ACKNOWLEDGEMENT

We are grateful to Mr Kwang-Sok Kim for his help in preparing plant materials. This research was supported by a grant from Bio-Green21 Program, Rural Development Administration, Korea.

LITERATURE CITED

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

Asada K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipitation of excess photons. Ann Rev Plant Physiol Plant Mol Biol 50: 601-639.

Avery M, Gordon J. 1983. New frontiers in agroforestry: consequences of pattern in tree and forage systems. In: Foothills for Food and Forests (Hannaway, DB, ed.). Timber Press, Beaverton, Oregon, USA, pp 261-270.

Caimi PG, McCole LM, Klein TM, Kerr PS. 1996. Fructan Accumulation and ucrose Metabolism in Transgenic Maize Endosperm Expressing a *Bacillus amyloliquefaciens* SacB Gene. Plant Physiol 110: 355-363

Clifford SC, Arndt SK, Corlett JE, Joshi S, Sankhla N, Popp M and Jones HG. 1998. The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk.). J Exp Bot 49: 967-977.

Dionisio-Sese ML, Tobita S. 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci 135: 1-9.

Donahue JL, Okpodu CM, Cramer CL, Grabau EA, Alscher RG. 1997. Responses of Antioxidants to Paraquat in Pea Leaves (Relationships to Resistance). Plant Physiol 113: 249-257.

Evans RD, Black RA, Loescher WH, Fellows RJ. 1992. Osmotic relations of the drought tolerant shrub *Artemisia tridentata*. Plant Cell Environ 15: 49-59.

Fan S, Blake TJ, Blumwald E. 1994. The relative contribution of elastic and osmotic adjustments to turgor maintenance in conditions. woody species. Physiol Plant 90, 408-13.

Farquhar GD, Shulze ED, Kupper K. 1980. Responses to humidity by stomata of *Nicotiana glauca* L. and *Colylus avellana* L. are consistent with the optimization of carbon dioxide uptake with respect to water loss. Aust J Plant Physiol 7: 315-327.

Hsiao TC. 1973. Plant responses to water stress. Ann Rev Plant Physiol 24: 519-570.

Jones, MM. 1979. Physiological responses of sorghum and sunflower to leaf water deficit (PhD Thesis). Australian National University, Canberra, Australia.

Kawaoka A, Matsunaga E, Endo S, Kondo S, Yoshida K, Shinmyo A, Ebinuma H. 2003. Ectopic expression of a horseradish peroxidase enhances growth rate and increases oxidative stress resistance in hybrid sspen. Plant Physiol 132: 1177-1185.

Kwon SY, Jeong YJ, Lee HS, Kim JS. Cho KY, Allen RD and Kwak SS. 2002. Enhanced tolerances 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.

Lewis CE, Tanner GW, Terry WS. 1985. Double vs. single-row pine plantations forwood and forage production. Southern J Appl Forestry 9: 55-61.

Lim S, Yang KS, Kwon SY, Paek KY, Kwak SS, Lee HS. 2004. Agrobacterium-mediated genetic transformation and plant regeneration of sweetpotato (*Ipomoea batatas)*. Kor J Plant Biotechnol 31: 267-271 (in Korean with English abstract).

Lu C, Zhang J. 1999. Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. J Exp Bot 50: 1199-1206.

Martinez JP, Silva H, Ledent JF, Pinto M. 2006. Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaeolus vulgaris* L.). Europ J Agro Doi :10.1016/j.eja. 2006. 08. 003.

McKersie BD, Bowl ey SR, Jones KS. 1999. Winter survival of transgenic alfalfa overexpressing superoxide dismutase. Plant Physiol 119: 839-848.

Nunes MA, Catarino F, Pinto E. 1989. Strategies for acclimation to seasonal drought in *Ceratonia siliqua* leaves. Physiol Plant 77: 150-156.

Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim YK, Nahm BH, Kim JK. 2005. Arabidopsis CBF3/DREB1A and ABF3 in

transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol 138: 341-351.

Olofsson J, Hulme PH, Oksanen L, Suominen O. 2005. Effects of mammalian herbivores on revegetation of disturbed areas in the forest-tundra ecotone in northern Fennoscandia. Landscape Ecol 20: 351-359.

Osmond B, Ananyev G, Berry J, Langdon C, Kolber Z, Lin G, Monson G, Nichol C, Rascher U, Schurr Um Smith S, Yakir D. 2004. Changing the way we think about global change research: scaling up in experimental ecosystem science. Global Change Biology 10: 393-407.

Premachandra GS, Saneoka H, Fujita K, Ogata S. 1992. Leaf water relations, osmotic adjustment, cell Membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. J Exp Bot 43: 1569-1576.

Radin JW. 1983. Physiological consequences of cellular water deficits: osmotic adjustment. In: Limitations to efficient water use in crop production (Taylor JM, Rains DW, Sinclair TR. eds). American Society for Agronomy (Publ.). pp. 267-76.

Samis K, Bowley S, McKersie B. 2002. Pyramiding Mn-superoxide dismutase transgenes to improve persistence and biomass production in alfalfa. J Exp Bot 53: 1343-1350.

Schreiber U, Bilger W, Neubauer C. 1994. Chlorophyll fluorescence as a noninstrutive indicator for rapid assessment of *in vivo* photosynthesis. In: Ecophysiology of Photosynthesis (Shulze ED, Caldwell MM. eds). Springer, Berlin, pp 49-70.

Sen Gupta A, Heinon J, Holaday A, Burke J, Allen R. 1993. Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc Natl Acad Sci USA 90: 1629-1633.

Suzuki S, Murai N, Burnell JN, Masao A. 2000. Changes in Photosynthetic Carbon Flow in Transgenic Rice Plants That Express C_4-Type Phosphoenolpyruvate Carboxykinase from *Urochloa panicoides*. Plant Physiol 124: 163-172.

Van Camp W, Capiau K, Van Montagu M, Inze D, Slooten L. 1996. Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts. Plant Physiol 112: 1703-1714.

Torsethaugen G, Pitcher LH, Zilinskas BA, Pell EJ. 1997. Overproduction of Ascorbate Peroxidase in the Tobacco Chloroplast Does Not Provide Protection against Ozone. Plant Physiol 114: 529-537.

(Received October 17, 2006; Accepted October 25, 2006)

KCI