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Effects of osmotic stress on antioxidant enzymes activities in leaf discs of P_{SAG12}-*IPT* modified gerbera

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Abstract: Leaf senescence is often caused by water deficit and the chimeric gene P_{SAG12}-*IPT* is an auto-regulated gene delaying leaf senescence. Using in vitro leaf discs culture system, the changes of contents of chlorophylls, carotenoids, soluble protein and thiobarbituric acid reactive substance (TBARS) and antioxidant enzymes activities were investigated during leaf senescence of P_{SAGl2}-*IPT* modified gerbera induced by osmotic stress compared with the control plant (wild type). Leaf discs were incubated in 20%, 40% (w/v) polyethylene glycol (PEG) 6000 nutrient solution for 20 h under continuous light [130 μmol/(m^2·s)]. The results showed that the contents of chlorophylls, carotenoids and soluble protein were decreased by osmotic stress with the decrease being more pronounced at 40% PEG, but that, at the same PEG concentration the decrease in the transgenic plants was significantly lower than that in the control plant. The activities of superoxide dismutase (SOD), catalases (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and dehydroascorbate reductase (DHAR) were stimulated by PEG treatment. However, the increases were higher in P_{SAG12}-*IPT* transgenic plants than in the control plants, particularly at 40% PEG treatment. Lipid peroxidation (TBARS content) was increased by PEG treatment with the increase being much lower in transgenic plant than in the control plant. It could be concluded that the increases in the activities of antioxidant enzymes including SOD, CAT, APX, GPX and DHAR were responsible for the delay of leaf senescence induced by osmotic stress.

Key words: Antioxidant enzymes, Gerbera, Leaf disc, Leaf senescence, Osmotic stress, P_{SAG12}-*IPT*
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INTRODUCTION

Leaf senescence always adversely affects higher plant production in agriculture (Dong *et al*., 2006). Senescence devalues ornamental plants and foliar vegetables during transportation, storage and on shelves (Li *et al*., 2006; Toit *et al*., 2004). Therefore, manipulation of leaf senescence may result in agricultural benefits. Senescence is regulated by several factors including plant hormones such as abscisic acid (ABA), ethylene, and cytokinin. ABA and ethylene have been shown to promote senescence whereas cytokinin application inhibits leaf senescence (Gan and Amasino, 1997). The *IPT* gene encodes a bacterial isopentenyl transferase which catalyses the rate limiting step in cytokinin biosynthesis. The *IPT* gene under the control of a chalcone synthase promoter has been reported to increase chlorophyll levels and stem thickness, and delay flowering onset and flower development in tobacco plants (Wang *et al*., 1997). In order to control leaf senescence, an autoregulatory senescence inhibition system, in which a highly senescence specific promoter SAG12 from *Arabidopsis* is fused to *IPT*, has been developed (Gan and Amasino, 1995). This fusion directs the expression of *IPT* in leaves at the onset of senescence and subsequently increases the cytokinin level that prevents the leaves from senescing, and high cytokinin level resulting in

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down regulation of the senescence specific promoter that prevents cytokinin from accumulating too high, otherwise that will interfere with normal plant development. In modified tobacco, senescence was delayed by *IPT* expression under control of the senescence-specific SAG12 promoter in leaves under elevated atmospheric CO_2 (Ludewig and Sonnewald, 2000). The *IPT* gene under control of the senescence-specific SAG12 promoter from *Arabidopsis* also significantly delayed developmental and post-harvest leaf senescence in mature heads of transgenic lettuce (McCabe *et al*., 2001). Among ornamentals, introduction of the *IPT* gene into petunia, linked to senescence-associated SAG promoters from *Arabidopsis*, has been reported to inhibit senescence of the lower leaves, although transgenic lines show stronger symptoms of nutritional deficiency (Jandrew and Clark, 2001).

Environmental factors, such as drought, induce plant leaf senescence (Guo and Gan, 2005). Reactive oxygen species (ROS) have been linked to stress-induced or normal cell death (Pastori and Rio, 1997). ROS are a group of very reactive, short-lived chemicals produced during normal metabolism or after an oxidative reaction. ROS include superoxide anion, hydrogen peroxide, hydroxyl radical (Sun, 1990). ROS, at low concentrations, have been implicated in the regulation of several physiological processes such as proliferation (Shibanuma *et al*., 1990), differentiation (Allen and Balin, 1989), senescence (de Haan *et al*., 1996), and apoptosis (Mignotte and Vayssiere, 1998). At high concentrations, ROS are highly cytotoxic by inducing DNA damage, lipid peroxidation, and protein degradation (Sun, 1990). Antioxidant defense systems have been developed in aerobic cells to counteract the damaging effects of ROS. Plants possess antioxidant defense systems comprised of enzymatic and non-enzymatic components, which normally maintain ROS balance within the cell. For instance, they use a diverse array of enzymes like superoxide dismutase (SOD), catalases (CAT) and peroxidases as well as low molecular mass antioxidants (glutathione and ascorbate) to scavenge different types of ROS (Foyer *et al*., 1994). SOD catalyses the dismutation of superoxide to hydrogen peroxide and oxygen. However, hydrogen peroxide is also toxic to cells and has to be further detoxified by CAT and/or peroxidases to water and oxygen.

The antioxidant enzymes' activities play an important role in scavenging ROS and therefore their improvement could increase the ability of stress tolerance and delay the senescence (Alscher *et al*., 2002). P_{SAG12}-*IPT* is a chimeric gene associated with cytokinin synthesis and therefore inhibits leaf senescence. However, whether the changes of antioxidant enzyme activities are involved in the regulation of leaf senescence in P_{SAGl2}-*IPT* modified gerbera is unknown. Using in vitro leaf discs culture system, the changes of antioxidant enzymes activities were investigated during leaf senescence of P_{SAGl2}-*IPT* modified gerbera induced by osmotic stress. The objectives are to know whether antioxidant enzymes activities are involved in the regulation of leaf senescence in P_{SAGl2}-*IPT* modified gerbera and clarify the physiological mechanisms of senescence delay induced by a chimeric gene P_{SAGl2}-*IPT*.

MATERIALS AND METHODS

Plant materials and growth conditions

Gerbera plants, *Gerbera jamesonii* 'Yuyan', which are P_{SAG12}-*IPT* transgenic plants obtained through particle bombardment transformation, and control plants, were cultured in mixed media with peat, perlite and vermiculite in the proportion of 3, 1 and 1, respectively. The plastic pot was 4 L in volume, 18 cm in diameter and 18 cm in height. The concentration of nutrient elements were as follows: 5.5 mmol/L K^+, 3.0 mmol/L Ca^{2+}, 1.0 mmol/L Mg^{2+}, 11.25 mmol/L NO_3^-, 1.5 mmol/L NH_4^+, 1.25 mmol/L $H_2PO_4^-$, 1.25 mmol/L SO_4^{2-}, 35 μmol/L Fe, 5.0 μmol/L Mn, 4.0 μmol/L Zn, 0.75 μmol/L Cu, 30 μmol/L B and 0.5 μmol/L Mo. Fe was added as Fe-EDTA. The plants were grown in a greenhouse. The air temperature was set at 20 °C (night) and 30 °C (day), relative humidity ranged from 60% to 80% and light intensity was 500 μmol/(m^2·s) at noon. The photoperiod was day 14 h and night 10 h. The plant materials were cultivated under the growth conditions until flowering. Leaf discs (11 mm diameter) were prepared from fully expanded leaves using a cork borer. Leaf surface was cleaned with water and immediately transferred into a medium (5 mmol/L HEPES, 10 mmol/L KCl, 1.5 mmol/L $CaCl_2$, pH 6.0) (Huguet-Robert *et al*., 2003).

Osmotic treatments

Osmotic treatments were applied by adding polyethylene glycol (PEG) 6000 at specified concentrations, 0, 20%, 40% (w/v) to the medium (Huguet-Robert *et al*., 2003) and pH was readjusted to 6.0. Leaf discs were placed in Petri dishes with covers, with each containing 15 discs in 20 ml of stress medium, placed under continuous light [130 μmol/(m^2·s) at the leaf discs level], shaken continuously at 20 °C and incubated for 20 h. All treatments were replicated three times concurrently with control assays performed with leaf discs incubated in flasks containing 20 ml of reference medium.

Enzyme extraction

For enzyme assays, leaf discs were ground with 4 ml ice-cold 50 mmol/L HEPES buffer (pH 7.8) containing 0.2 mmol/L EDTA, 2% PVPP and 2 mmol/L ascorbate. The homogenates were centrifuged at 4 °C for 20 min at 12000×g and the resulting supernatants were used for determination of enzymatic activities (Zhu *et al*., 2000). All spectrophotometric analyses were conducted on a SHIMADZU UV-2410PC spectrophotometer.

Determination of enzymatic activities

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Stewart and Bewley, 1980). CAT activity was measured using the reduction in absorbance at 240 nm due to the decrease of extinction of H_2O_2 (Patra *et al*., 1978). Guaiacol peroxidase (GPX) activity was measured by the increase in absorbance at 470 nm due to guaiacol oxidation (Nickel and Cunningham, 1969). Ascorbate peroxidase (APX) activity was measured by the decrease in absorbance at 290 nm as ascorbate was oxidized (Nakano and Asada, 1981). The assay of dehydroascorbate reductase (DHAR) activity was carried out by measuring the increase in absorbance at 265 nm due to reduced ascorbate formation (Nakano and Asada, 1981).

Determination of lipid peroxidation

Lipid peroxidation was estimated by measuring the concentration of thiobarbituric acid reactive substances (TBARS) (Shalata and Tal, 1998), the reaction mixture in a total volume of 4 ml containing 1 ml of extracts, 3 ml of 2% (w/v) TBA (2-thiobarbituric acid) made in 20% trichloroacetic acid (TCA). The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. After centrifugation at 10000×g for 10 min, the absorbance of the supernatant was measured at 532 nm. A correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The lipid peroxides were expressed as (nmol TBARS)/(g DW) by using an extinction coefficient of 155 $(mmol/L)^{-1}\cdot cm^{-1}$ (Shalata and Tal, 1998).

Determination of soluble protein content

Soluble protein concentration was measured using bovine serum albumin as standard at 595 nm according to the method of Bradford (1976).

Determination of chlorophylls and carotenoids content

Contents of chlorophyll (a and b) and carotenoids were determined in alkaline acetone extracts (Lichtenthaler, 1987).

RESULTS

Chlorophylls and carotenoids

Fig.1 indicates that the contents of chlorophyll a, chlorophyll b and total chlorophylls in leaf discs were significantly decreased by osmotic stress (incubation in PEG solution) with the decrease being more pronounced in 40% PEG than in 20% PEG. However, at the same PEG concentration the decrease in transgenic plants was significantly lower than that in the control plants, especially at 40% PEG. The contents of carotenoids in leaf discs were reduced by PEG treatment, especially in the control plants at 40% PEG treatment.

Soluble protein content

The soluble protein content in leaf discs was decreased by PEG treatment with the decrease at 40% PEG being higher than that at 20% PEG. However, the decrease induced by PEG in modified plants was significantly lower than that in the control plants, especially at 40% PEG (Fig.2).

Antioxidant enzyme activities

After osmotic stress, the changes of activities of SOD, CAT, APX, GPX and DHAR had similar

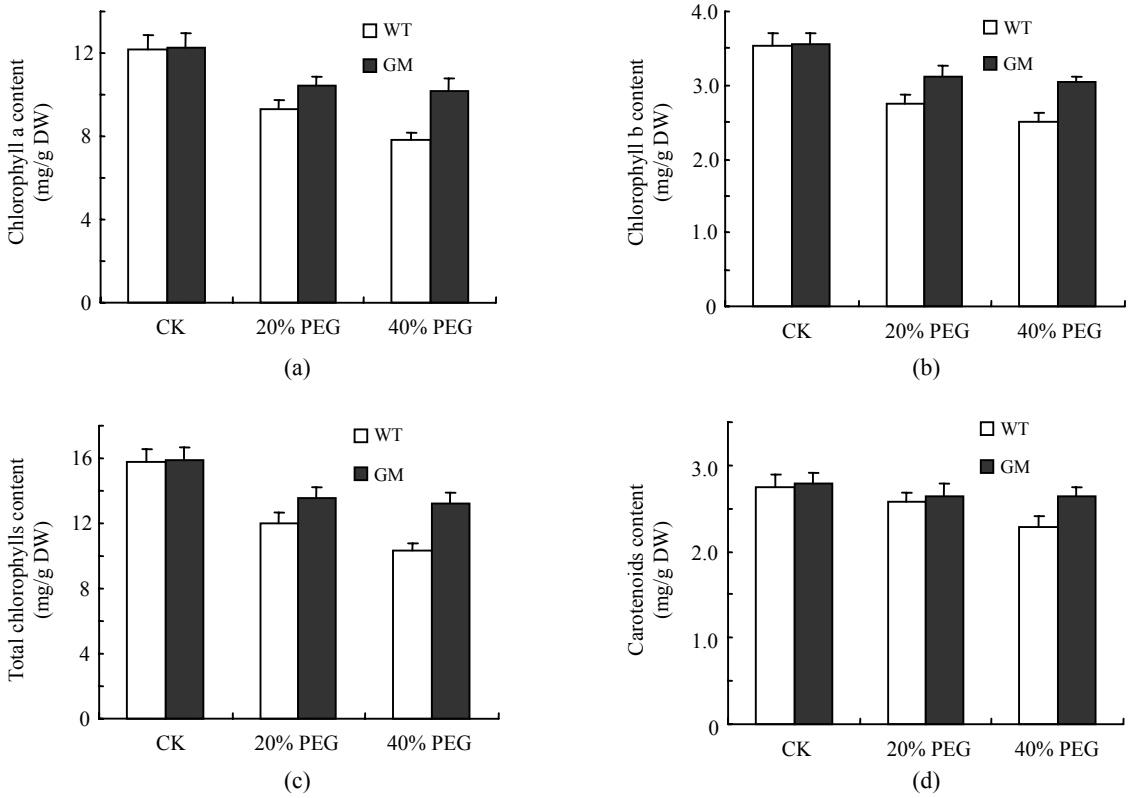


Fig.1 Effects of osmotic stress on the contents of chlorophyll a (a), chlorophyll b (b), total chlorophylls (c) and carotenoids (d) in leaf discs of P_{SAG12}-*IPT* transgenic (gerbera modified, GM) and control (wild type, WT) gerbera plants. Data are the mean±*SD* of three replicates

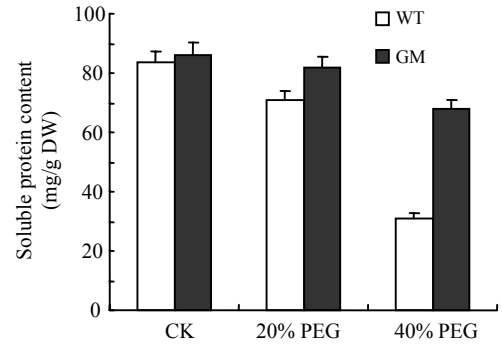


Fig.2 Effects of osmotic stress on soluble protein content in leaf discs of P_{SAG12}-*IPT* transgenic (GM) and control (WT) gerbera plants. Data are the mean±*SD* of three replicates

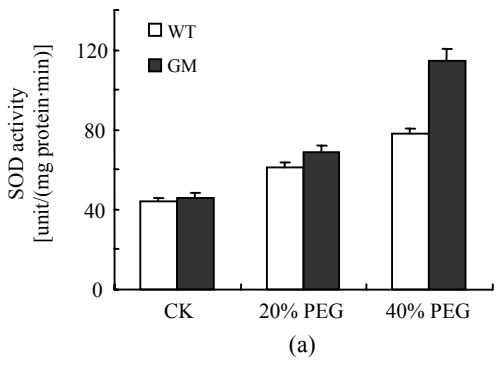
tendency. The activities of SOD, CAT, APX, GPX and DHAR were stimulated by PEG treatment. However, the increases were higher in P_{SAG12}-*IPT* transgenic plants than in the control plants, particularly at 40% PEG treatment (Fig.3).

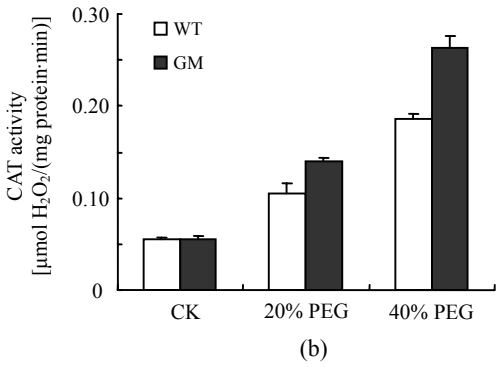
Lipid peroxidation

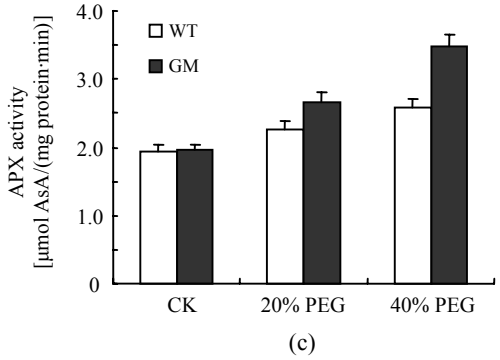
As an indicator of lipid peroxidation, TBARS concentration was measured. The TBARS concentration in the leaf discs was increased by osmotic stress with the increase being higher at 40% PEG than at 20% PEG. However, this increase was significantly higher in the control plants than in transgenic plants (Fig.4).

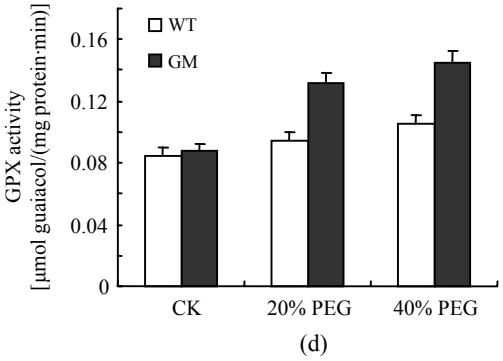
DISCUSSION

Genotypic differences in drought tolerance could be attributed to the ability of plants to growth (Turtola *et al*., 2006). Leaf senescence can be affected by a complex array of factors and manipulating any one of these factors will result in either promotion or prevention of leaf senescence, for example, water deficit promotes leaf senescence (Thomas *et al*., 1995). In many studies, PEG 6000 is often used as an inducer of









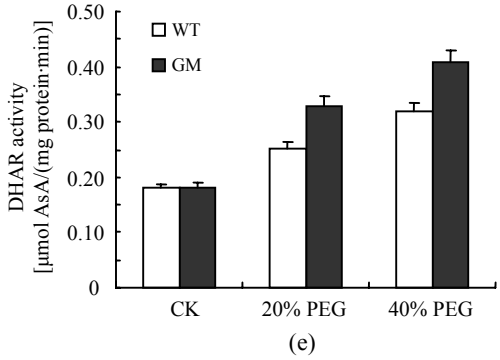


Fig.3 Effects of osmotic stress on SOD (a), CAT (b), APX (c), GPX (d) and DHAR (e) activities in leaf discs of P_{SAG12}-*IPT* transgenic (GM) and control (WT) gerbera plants. Data are the mean±*SD* of three replicates

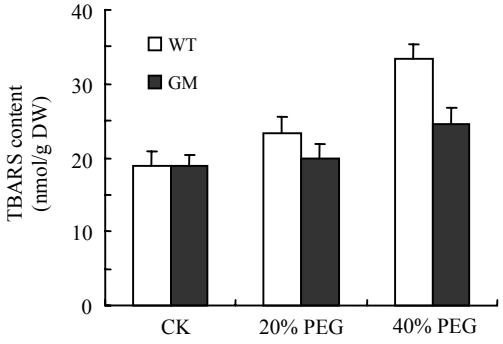


Fig.4 Effects of osmotic stress on TBARS content in leaf discs of P_{SAG12}-*IPT* transgenic (GM) and control (WT) gerbera plants. Data are the mean±*SD* of three replicates

water deficit (osmotic stress) and promotes plant senescence (Behera *et al*., 2003). In the present experiment, adding PEG in the incubation medium of leaf discs induced leaf senescence, indicated by the considerable decrease of chlorophylls and soluble protein content (Figs.1 and 2). However, the decreases of chlorophylls and soluble protein content in P_{SAG12}-*IPT* modified gerbera plants were significantly reduced compared to the control plants, indicating that leaf senescence was significantly delayed by the particle bombardment transformation of the chimeric gene P_{SAG12}-*IPT*.

Even under optimal conditions, many metabolic processes produce ROS. The production of toxic oxygen derivatives is increased as a result of all types of abiotic or biotic stresses. Plants possess efficient systems for scavenging active oxygen species that

protect them from destructive oxidative reactions (Foyer *et al*., 1994). As part of this system, antioxidant enzymes are key elements in the defense mechanisms. Many changes have been observed in the activities of antioxidant enzymes in plants under osmotic stress. Superoxide dismutase and ascorbate peroxidase activities were found to be enhanced in osmotically stressed leaf discs of rape plants compared with the control (Aziz and Larher, 1998). An increase in ascorbate peroxidase, superoxide dismutase and glutathione reductase activities was also observed in the drought tolerant wheat cultivar when the leaves were subjected to osmotic stress in vitro (Lascano *et al*., 2001). The results of the present experiment showed that the activities of SOD, CAT, APX, GPX and DHAR in leaf discs were stimulated by PEG treatment (osmotic stress) with the increases in leaf discs of P_{SAG12}-*IPT* transgenic plants being higher than those of control plants, particularly at 40% PEG treatment. Higher activities of SOD, CAT, APX, GPX and DHAR in leaf discs of P_{SAG12}-*IPT* transgenic plants with PEG treatment coincided with decrease in the TBARS concentration, suggesting that oxidative damage induced by osmotic stress could be alleviated by transformation of the chimeric gene P_{SAG12}-*IPT*. Such protection system against oxidative stress may play a crucial role in preventing leaves from senescence induced by osmotic stress. This result was consistent with the observation of Dertinger *et al*.(2003). They found that the activities of antioxidatve enzymes (ascorbate peroxidase and glutathione reductase) in old leaves of P_{SAG12}-*IPT* modified tobacco plants were higher than in wild-type leaves.

In summary, leaf senescence under osmotic stress was delayed by the transformation of P_{SAG12}-*IPT* gene in gerbera. The increases in the activities of antioxidant enzymes including SOD, CAT, APX, GPX and DHAR may be responsible for the delay of leaf senescence, indicated by the reduction of the decreases in chlorophylls and soluble protein content and the increases in TBARS content in leaf discs under osmotic stress.

References

Allen, R.G., Balin, A.K., 1989. Oxidative influence on development and differentiation: an overview of a free radical theory of development. *Free Radic. Biol. Med.*, **6**(6):631-661. [doi:10.1016/0891-5849(89)90071-3]

Alscher, R.G., Erturk, N., Heath, L.S., 2002. Role of superoxide dismutases in controlling oxidative stress in plants. *J. Exp. Bot.*, **53**(372):1331-1341. [doi:10.1093/jexbot/53.372.1331]

Aziz, A., Larher, F., 1998. Osmotic stress induced changes in lipid composition and peroxidation in leaf discs of *Brassica napus* L. *J. Plant Physiol.*, **153**(5):754-762.

Behera, S.K., Nayak, L., Biswal, B., 2003. Senescing leaves possess potential for stress adaptation: the developing leaves acclimated to high light exhibit increased tolerance to osmotic stress during senescence. *J. Plant Physiol.*, **160**(2):125-131. [doi:10.1078/0176-1617-00791]

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of micogram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**(1-2):248-254. [doi:10.1016/0003-2697(76)90527-3]

de Haan, J.B., Cristiano, F., Iannello, R., Bladier, C., Kelner, M.J., Kola, I., 1996. Elevation in the ratio of Cu/Zn-superoxide dismutase to glutathione peroxidase activity induces features of cellular senescence and this effect is mediated by hydrogen peroxide. *Hum. Mol. Genet.*, **5**(2):283-292. [doi:10.1093/hmg/5.2.283]

Dertinger, U., Schaz, U., Schulz, E.D., 2003. Age-dependence of the antioxidative system in tobacco with enhanced glutathione reductase activity or senescence-induced production of cytokinins. *Physiol. Plant.*, **119**(1):19-29. [doi:10.1034/j.1399-3054.2003.00095.x]

Dong, H.Z., Li, W.J., Tang, W., Li, Z.H., Zhang, D.M., Niu, Y.H., 2006. Yield, quality and leaf senescence of cotton grown at varying planting dates and plant densities in the Yellow River Valley of China. *Field Crops Res.*, **98**(2-3):106-115. [doi:10.1016/j.fcr.2005.12.008]

Foyer, C.H., Lelandais, M., Kunert, K.J., 1994. Photooxidative stress in plants. *Physiol. Plant.*, **92**(4):696-717. [doi:10.1034/j.1399-3054.1994.920422.x]

Gan, S., Amasino, R.M., 1995. Inhibition of leaf senescence by autoregulated production of cytokinin. *Science*, **270**(5244):1986-1988. [doi:10.1126/science.270.5244.1986]

Gan, S., Amasino, R.M., 1997. Making sense of senescence. *Plant Physiol.*, **113**(2):313-319.

Guo, Y.F., Gan, S.S., 2005. Leaf senescence: signals, execution, and regulation. *Current Topics in Developmental Biology*, **71**:83-112.

Huguet-Robert, V., Sulpice, R., Lefort, C., Maerskalck, V., Emery, N., Larher, F.R., 2003. The suppression of osmoinduced proline response of *Brassica napus* L. var oleifera leaf discs by polyunsaturated fatty acids and methyljasmonate. *Plant Sci.*, **164**(1):119-127. [doi:10.1016/S0168-9452(02)00343-6]

Jandrew, J., Clark, D.G., 2001. Selectively induced nutrient deficiency in transgenic P_{SAG12}-*IPT*, P_{SAG13}-*IPT* and P_{SAG12}-*Kn1* petunias. *HortSci.*, **36**:518-519.

Lascano, H.R., Antonicelli, G.E., Luna, C.M., Melchiorre, M.N., Gómez, L.D., Racca, R.W., Trippi, V.S., Casano, L.M., 2001. Antioxidant system response of different wheat cultivars under drought: field and in vitro studies. *Aust. J. Plant. Physiol.*, **28**(11):1095-1102.

Li, W.X., Zhang, M., Yu, H.Q., 2006. Study on hypobaric storage of green asparagus. *J. Food Eng.*, **73**(3):225-230. [doi:10.1016/j.jfoodeng.2005.01.024]

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Meth. Enzymol.*, **148**:350-382.

Ludewig, F., Sonnewald, U., 2000. High CO_2-mediated down-regulation of photosynthetic gene transcripts is caused by accelerated leaf senescence rather than sugar accumulation. *FEBS Letters*, **479**(1-2):19-24. [doi:10.1016/S0014-5793(00)01873-1]

McCabe, M.S., Garratt, L.C., Schepers, F., Jordi, W.J.R.M., Stoopen, G.M., Davelaar, E., van Rhijn, J.H.A., Power, J.B., Davey, M.R., 2001. Effects of P_{SAG12}-*IPT* gene expression on development and senescence in transgenic lettuce. *Plant Physiol.*, **127**(2):505-516. [doi:10.1104/pp.127.2.505]

Mignotte, B., Vayssiere, J.L., 1998. Mitochondria and apoptosis. *Eur. J. Biochem.*, **252**(1):1-15. [doi:10.1046/j.1432-1327.1998.2520001.x]

Nakano, Y., Asada, K., 1981. Hydrogen peroxide scanvenged by ascorbated specific peroxidase in spinach chloroplast. *Plant Cell Physiol.*, **22**(5):867-880.

Nickel, R.S., Cunningham, B.A., 1969. Improved peroxidase assay method using Ieuco 2,3,6-trichlcroindophenol and application to comparative measurements of peroxidase catalysis. *Anal. Biochem.*, **27**(2):292-299. [doi:10.1016/0003-2697(69)90035-9]

Pastori, G.M., Rio, L.A., 1997. Natural senescence of pea leaves, an activated oxygen-mediated function for peroxisomes. *Plant Physiol.*, **113**(2):411-418.

Patra, H.L., Kar, M., Mishre, D., 1978. Catalase activity in leaves and cotyledons during plant development and senescence. *Biochem. Physiol.*, **172**(4):385-390.

Shalata, A., Tal, M., 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. *Physiol. Plant.*, **104**(2):169-174. [doi:10.1034/j.1399-3054.1998.1040204.x]

Shibanuma, M., Kuroki, T., Nose, K., 1990. Stimulation by hydrogen peroxide of DNA synthesis, competence family gene expression and phosphorylation of a specific protein in quiescent Balb/3T3 cells. *Oncogene*, **5**(7):1025-1032.

Stewart, R.C., Bewley, J.D., 1980. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.*, **65**(2):245-248.

Sun, Y., 1990. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radic. Biol. Med.*, **8**(6):583-599. [doi:10.1016/0891-5849(90)90156-D]

Thomas, J.C., Smigoli, A.C., Bohnert, H.J., 1995. Light-inducible expression of *ipt* from *Agrobacterium tumefaciens* results in cytokinin accumulation and osmotic stress symptoms in transgenic tobacco. *Plant Mol. Biol.*, **27**(2):225-235. [doi:10.1007/BF00020179]

Toit, E.S., Robbertse, P.J., Niederwieser, J.G., 2004. Temperature regime during bulb production affects foliage and flower quality of Lachenalia cv. Ronina pot plants. *Scientia Horticulturae*, **102**(4):441-448.

Turtola, S., Rousi, M., Pusenius, J., Yamaji, K., Heiska, S., Tirkkonen, V., Meier, B., Riitta, J.T., 2006. Genotypic variation in drought response of willows grown under ambient and enhanced UV-B radiation. *Environmental and Experimental Botany*, **56**(1):80-86. [doi:10.1016/j.envexpbot.2005.01.007]

Wang, J., Letham, D.S., Cornish, E., Stevenson, K.R., 1997. Studies of cytokinin action and metabolism using tobacco plants expressing either the *ipt* or the *GUS* gene controlled by a chalcone synthase promoter I developmental features of the transgenic plants. *Plant Physiol.*, **24**(5): 661-672.

Zhu, Z.J., Gerendas, J., Bendixen, R., Schinner, K., Tabrizi, H., Sattelmacher, B., Hansen, U.P., 2000. Different tolerance to light stress in NO_3^- and NH_4^+-grown *Phaseolus vulgaris* L. *Plant Biol.*, **2**(5):558-570. [doi:10.1055/s-2000-7498]