Effect of ethylene on antioxidant enzymes activity in ethylene-insensitive cut roses (*rosa hybrida* l.)

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Abstract

Cut roses (*Rosa hybrida* L.) have been classified as ethylene-sensitive, but the nature of the ethylene sensitivity changes in these flowers has not been well characterized. Therefore, in this work, ethylene-insensitive cut roses consisting *etr1-1* gene were evaluated. Cut roses prepared from an isolated greenhouse at commercial stage, after transferring to the laboratory, were compared regarding the content of antioxidant enzymes by ethylene treatment (0, 0.5 and 1 µl l^{-1}). Evaluations were carried out in wild type and transgenic line in bud and half-open stages. The research was performed in completely randomized factorial design by four replicates. After measuring and applying the statistical differences at the $P \leq 0.05$ level via SAS software was done. The results showed that ethylene significantly increased superoxide dismutase, catalase and guaiacol peroxidase activities in bud and half-open stages in wild type and transgenic line. Maximum antioxidant enzymes activity was observed in wild type roses in bud and half-open stages treated by 1 µl l^{-1} ethylene which had a significant difference in comparison with the same stage in transgenic line. According to the increasing trend of these enzymes activities in wild type and transgenic lines with higher concentrations of ethylene, it seems that, ethylene by inducing senescence oxidative damage could accelerate flower senescence. Therefore, genetic manipulation of ethylene receptor genes of ethylene-sensitive flowers as well as roses could decrease oxidatve stress during senescence and considerably improved longevity.

Keywords: *Antioxidant enzyme, Cut roses, Ethylene.*

Introduction

Roses are the most economically important ornamentals and belong to the top ten flowers in the worldwide. Cut roses account for about 21% and 31% of all cut flowers traded in China and in European auctions, respectively (Heinrichs, 2008; Meng *et al.*, 2013). Unfortunately, many consumers consider roses to have a very short vase life. Senescence is the end of the relatively short life of cut flowers that leads to the visible symptoms of various physiological characteristics (Rani and Singh, 2014). Ethylene, as a gaseous plant hormone, plays prominent role in acceleration of senescence phenomena of most plant concomitantly with increasing in endogenous ethylene production (Lei *et al*., 2009; Rani and Singh, 2014; Rogers, 2012). In general, during the senescence process, ROS[1] content increase and accelerate senescence process by increasing cell membrane permeability. In fact, low concentrations of ROS including singlet oxygen ($^1O_2$), the alkoxy radical ($RO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), hydroperoxyl radical ($HO_2^{\bullet-}$) and the peroxy radical ($ROO^{\bullet}$) are essential as secondary messengers for cell signaling to initiate biological processes, whereas oxidative stress during senescence denotes higher ROS levels which cause damage a range of macromolecules such as lipids, proteins and DNA (Lei *et al*., 2009; Rani and Singh, 2014; Rogers, 2012). When ROS overproduction is triggered with progressive senescence, antioxidant system comprising of enzymatic ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, catalase, superoxide dismutase and nonenzymatic components scavenge these compounds in plants (Chakrabarty *et al.*, 2009; Rani and Singh, 2014).

1 Reactive oxygen species

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Plant breeding by genetic engineering technique such as transformations by *ert1-1* mutated gene resulted in plants with considerably higher ethylene tolerance (Wilkinson *et al.*, 1997). In this regard, succeeding studies were reported in *Kalanchoe* (Sanikhani *et al*., 2008), *Campanula* (Sriskandarajah *et al*., 2007; Sriskandarajah *et al*., 2010), and *Dianthus* (Gubrium *et al*., 2000), which provided a better flower longevity. ETR1 is found to be both essential and sufficient for mutations among the ethylene receptors (Chen *et al.*, 2002; Dong *et al*., 2008; Grefen *et al*., 2008).

Senescence of rose flower is still not completely understood. In order to understand petal senescence, it is important to explore the mechanisms of oxidative stress management. In this study, the levels of endogenous antioxidant enzymes during senescence of *etr1-1* transgenic rose flowers pulsed with ethylene were investigated.

Materials and methods

Plant material and ethylene treatment

Cut roses, wild type (Wt) and transgenic line (Tr), at the bud commercial were obtained directly from Iranian Research Organization for Science and Technology (Tehran). Then cut flowers were selected for the uniformity in size, shape, and freedom from malformations or damage related with harvesting and transport handling. Lower leaves were removed and flower stems were trimmed to a uniform length of 35 cm and placed in dH_2O in a growth room equipped with a controlled environment maintained at 25 ± 2 °C, 60% ± 5% relative humidity and 15 µmol m^{-2} s^{-1} light intensity for 16 h day^{-1} by cool-white fluorescent lamps.

To investigate the effects of ethylene, the samples were subjected to 0, 0.5 and 1 µl l^{-1} exogenous ethylene for 24 h in the polyethylene plastic bag. In this regard, each flower was sealed in a 7 L polyethylene plastic bag with 1 ml KOH-type for respiration CO_2 absorbance. Aliquots of pure ethylene gas were injected by syringe into the polyethylene bag. Bag ethylene concentrations were checked by gas chromatography and flowers were held in dH_2O.

Petals were collected in bud (B) and half-opened (H) stages as shown in Fig. 1. In addition, when wild type was in half-open stage, transgenic line was in bud stage at the same time. Therefore, this stage in transgenic line was called TBH (transition bud to half-open stage). Transgenic petals were collected in this stage, too (Plate XIII, Fig. 1). In the following, immediately frozen in liquid nitrogen and then stored at -80 ºC.

Antioxidant enzyme assay

Superoxide dismutase activity

Total superoxide dismutase (SOD) activity was determined by measuring its ability to inhibit the photochemical reduction of MTT according to the method of Giannopolitis and Ries (1977) with some modifications (Chowdhury and Cboudburi, 1985; Zhang *et al*., 1995). The 3-ml reaction mixture contained 75 µM MTT, 4 µM riboflavin, 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.4). Riboflavin was added last. The test tubes containing the mixture were placed under two fluorescent lamps at 4000 lux. The reaction was started by switching on the light and was allowed to run for 15 min. The reaction was stopped by switching off the light, and the absorbance at 560 nm was recorded. A non-irradiated reaction mixture that did not develop color served as the control, and its absorbance was subtracted from A_{560}. The reaction mixture which lacked enzyme developed maximum color as a result of maximum reduction of MTT. The reduction of MTT was inversely proportional to the enzyme activity. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of MTT reduction at 560 nm.

Catalase activity

Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of H_2O_2 disappearance at 240 nm, taking at 240 nm as 40 mM^{-1} cm^{-1}(Patterson *et al*., 1984). The reaction mixture contained 50 mmol/L potassium phosphate (pH 6.8), 0.1 mmol/L H_2O_2. The reaction was run at 25°C for 2 min, after adding the enzyme extract containing 20 µg of protein, and the initial linear rate of decrease in absorbance at 240 nm was used to calculate the activity.

Guaiacol peroxidase activity

Guaiacol peroxidase (G-POD) activity was measured spectrophotometrically at 25°C by following the method of Tatiana *et al*. (1999). The reaction mixture (3 ml) consisted of 25 mmol/L potassium phosphate (pH 6.8), 40 mmol/L H_2O_2, and 20 mmol/L guaiacol. The reaction was started by the addition of an enzyme extract equivalent to 50 µg protein. The formation of tetraguaiacol was measured at 470 nm (ε = 26.6 mmol/ L^{-1} cm^{-1}).

Statistical Analysis

All the data were analyzed using SAS 9.1 for Windows. The data were initially compared by one way analysis of variance (ANOVA) and difference was detected using the Duncan test. The difference between treatments was

considered to be statistically significant when P values ≤ 0.05. Figures were performed using Excel and means ± standard error (S.E.) were shown. Experiments were repeated two times.

Results and discussion

SOD, CAT and G-POD activities increased during vase life and the highest activity was observed at the final stage after harvest in wild type (Plate XII, Fig. 2). Wild type had significant difference in all enzymes in comparison with transgenic line in the same stages (Plate XII, Fig. 2). It seems that ethylene exposure could be enhanced the activity of these antioxidant enzymes. Total SOD, CAT and G-POD activity enhanced in B and TBH stages in transgenic line by increasing ethylene to 0.5 $\mu l\ l^{-1}$. This up-trend continued in SOD activity by 1 $\mu l\ l^{-1}$ ethylene while the activity of CAT and G-POD showed down-trend (Plate XII, Fig. 2b, c). It is proved that ethylene existence is necessary during opening phase (Olsen et al., 2015; Rani and Singh, 2014). SOD activity in TBH stage significantly increased in compare to other stages (Plate XII, Fig. 2a). TBH was considered as a transition stage from bud to half-open in transgenic line. Increasing ROS and ethylene production in TBH stage confirmed these results (data not shown). Probably, higher SOD activity in this stage could be related to ethylene sensitivity and starting the opening phase.

It has been reported, an progressive increase in antioxidant enzymes activity and increase in endogenous H_2O_2 in some plant species such as carnation, daylily and rose during senescence and application of exogenous ethylene accelerated this process (Chakrabarty *et al.*, 2009; Rani and Singh, 2014). Moreover, enhanced antioxidant enzymes activity was associated with an increase in the level of peroxides and free radicals which react with cellular constituents (Rani and Singh, 2014). In addition, antioxidant enzymes activities was found to be much higher in senescent leaves than those of in young (Rani and Singh, 2014). In this research, the main emphasis is given on enzymatic components like CAT, SOD, and G-POD which showed antioxidant behaviour with progressive senescence. Catalase is considered as an important biological factor that its major function is in the process of superoxide metabolism and plays an important role in releasing oxygen and hydrogen peroxide free radicals and preventing creation of hydroxyl radicals (Spanou *et al.*, 2012). Superoxide dismutase plays a critical role in inhibition of superoxide and guaiacol peroxidase acts as detoxification of hydrogen peroxide (Miao and St Clair, 2009). It has been reported, CAT, SOD, and G-POD play role in protection of balance of oxygen in plant tissue (Miao and St Clair, 2009; Spanou *et al.*, 2012). Accordingly, the present research indicated antioxidant enzymes activities increased during vase life by increasing exogenous ethylene concentration in wild type and transgenic line (Plate XII, Fig. 2). The higher activity was observed in wild type compared with transgenic line in all developmental stages (Plate XII, Fig. 2). The induction of SOD, CAT and G-POD activities results in decreased sensitivity to free radical-induced cellular damage (Plate XII, Fig. 2). The increased SOD, CAT and G-POD activity that have observed in our study could reflect a similar process of oxidative stress with the implication of these enzyme activities as part of the antioxidant response against H_2O_2. In addition, these data are consistent to previous studies that the dominant ethylene-insensitive *etr1-1* mutant has reduced affinity for ethylene and delayed flower senescence (Müller and Stummann, 2003; Olsen *et al.*, 2015).

Conclusions

In conclusion, senescence could be slowed down by retarding peroxidation and high levels of antioxidant enzymes are correlated with delayed senescence. The compromise of oxidative stress in aging is a common feature to cut flowers, and this factor could be a key to designing adequate methods to prevent or delay deterioration and to improve

the conservation of cut roses. It seems that transformation by *etr1* in ethylene-sensitive flowers as well as roses could be increased longevity considerably based on the disruption in perceiving ethylene by increasing antioxidant enzymes activity.

References

Chakrabarty D, Verma AK, Datta SK 2009: Oxidative stress and antioxidant activity as the basis of senescence in Hemerocallis (day lily) flowers. Journal of Horticulture and Forestry **1**: 113-119

Chen YF, Randlett MD, Findell JL, Schaller GE 2002: Localization of the ethylene receptor ETR1 to the endoplasmic reticulum of Arabidopsis. Journal of Biological Chemistry **277**: 19861-19866

Chowdhury SR, Cboudburi MA 1985: Hydrogen peroxide metabolism as an index of water stress tolerance in jute. Physiologia Plantarum **65**: 476-480

Dong CH, Rivarola M, Resnick JS, Maggin BD, Chang C 2008: Subcellular co-localization of Arabidopsis RTE1 and ETR1 supports a regulatory role for RTE1 in ETR1 ethylene signaling. Plant Journal **53**: 275-286

Giannopolitis CN, Ries SK 1977: Superoxide dismutase. I. Occurrence in higher plants. Plant Physiology **59**: 309-314

Grefen C, Stadele K, Ruzicka K, Obrdlik P, Harter K, Horak J 2008: Subcellular localization and in vivo interactions of the Arabidopsis thaliana ethylene receptor family members. Molecular Plant **1**: 308-320

Gubrium EK, Clevenger DJ, Clark DG, Barrett JE, Nell TA 2000: Reproduction and horticultural performance of transgenic ethylene-insensitive petunias. Journal of the American Society for Horticultural Science **125**: 277-281

Heinrichs F 2008: International statistics flowers and plants. Institut für Gartenbauökonomie der Universität Hannover, Germany **56**: 16-90

Lei W, Geng-Guo T, Tong L 2009: Physiological, biochemical and ultrastructures of mesophyll cell changes in a senescing corolla of Lycoris longituba. Journal of Zhejiang Forestry College **26**: 498-502

Meng Y, Li N, Tian J, Gao J, Zhang C 2013: Identification and validation of reference genes for gene expression studies in postharvest rose flower (*Rosa hybrida*). Scientia Horticulturae **158**: 16-21

Miao L, St Clair DK 2009: Regulation of superoxide dismutase genes: implications in disease. Free Radical Biology and Medicine **47**: 344-356

Müller R, Stummann BM 2003: Genetic regulation of ethylene perception and signal transduction related to flower senescence. Food, Agriculture & Environment **1**: 87-94

Olsen A, Lutken H, Hegelund JN, Muller R. 2015: Ethylene resistance in flowering ornamental plants – improvements and future perspectives. Horticulture Research **2**: 1-9

Patterson BD, Pyane LN, Chen Y, Grahum D 1984: An inhibitor of catalase indused by cold chilling–sensitive plant. Plant Physiology **76**: 1014-1018

Rani P, Singh N 2014: Senescence and postharvest studies of cut flowers: A critical Review. Journal of Tropical Agricultural Science **37**: 159-201

Rogers HJ 2012: Is there an important role for reactive oxygen species and redox regulation during floral senescence? Plant, Cell and Environment **35**: 217-233

Sanikhani M, Mibus H, Stummann BM, Serek M 2008: *Kalanchoe blossfeldiana* plants expressing the Arabidopsis *etr1-1* allele show reduced ethylene sensitivity. Plant Cell Reports **27**: 729-737

Spanou CI, Veskoukis AS, Stagos D, Liadaki K, Aligiannis N, Angelis A, Skaltsounis AL, Anastasiadi M, Haroutounian SA, Kouretas D 2012: Effects of Greek legume plant of extracts on xanthine oxidase, catalase and superoxide dismutase activities. Journal of Physiology and Biochemistry **68**: 37-45

Sriskandarajah S, Mibus H, Serek M 2007: TransgenicCampanulacarpaticaplantswith reduced ethylene sensitivity. Plant Cell Reports **26**: 805-813

Sriskandarajah S, Mibus H, Serek M 2010: Introduction of ethylene insensitivity in campanula carpatica flowers by Genetic Manipulation with *Etr1-1*. In: Proc. 6th International Postharvest Symposium. (eds.): Erkan, M. and Aksoy, U. Acta Hort **877**: 1111-1114

Tatiana Z, Yamashita K, Matsumoto H 1999: Iron deficiency indused changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber root. Plant Cell Physiology **40**: 273-280

Wilkinson JQ, Lanahan MB, Clark DG, Bleecker AB, Chang C 1997: A dominant mutant receptor from Arabidopsis confers ethylene insensitivity in heterologous plants. Nature Biotechnology **15**: 444-447

Zhang J, Cui S, Li J, Wei J, Kirkham MB 1995: Protoplasmic factors, antioxidant responses, and chilling resistance in maize. Plant Physiology and Biochemistry **33**: 567-575

(1)

(2)

(3)

Fig. 1. Three studied stages of flower development in transgenic rose: (1) bud = B, (2) transition bud to half-open stage = (TBH) and (3) half-opened = (H)

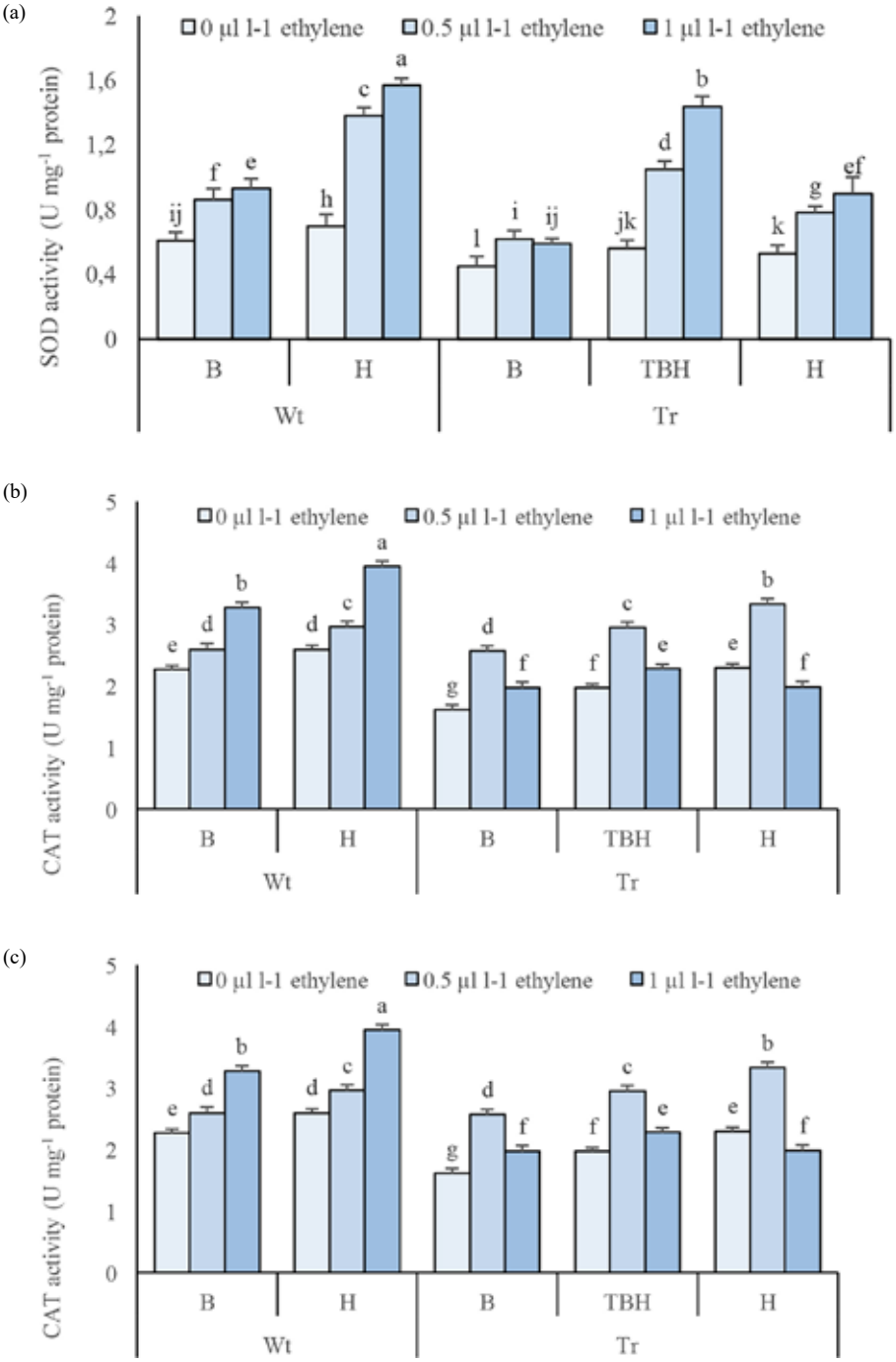

Fig. 2. Changes in the level of SOD, CAT and G-POD activity in the petals of wlid type and transgenic rose line during different stages of senescence. Different letters in each bar differ significantly according to Duncan test ($P \leq 0.05$)