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Physiological alteration in gladiolus flower during senescence as affected by abscisic acid

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ABSTRACT

Basic of senescence in ethylene insensitive flower has long stayed as a mystery and we proposed ABA as a key player in this process. The present study was carried out using gladiolus var. Snow Princess to understand the role of abscisic acid (ABA) for senescence in flower at biochemical level. Present study has provided the initial evidence that exogenously applied ABA (100 µM) accelerate the senescence process by enhancing the activity of several senescence processes such as lipid peroxidation and lipoxygenase activity. On the contrary, the antioxidant enzymatic activity was reduced, *viz.* superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione reductase, in petal tissue. Supplementation of GA_3 in the vase solution having ABA reversed various senescence related parameters, which were down or upregulated by ABA proving its role in senescence of gladiolus flowers.

Key words: Abscisic acid, GA_3, gladiolus, petal senescence.

INTRODUCTION

Senescence is natural deteriorative process that terminates functional life of plants or their parts. It is an active and highly ordered process that involves structural, biochemical and molecular changes (Buchanan *et al*., 4; Battelli *et al*., 3). Senescence of flower is genetically programmed and controlled by endogenous factors (Hoeberichts *et al*., 11). Gladiolus is one of the major commercial cut flowers, which belong to non-climacteric group, *i.e.* ethylene insensitive type. Activated oxygen species such as $O_2^{\cdot-}$ or H_2O_2 and their interaction product, hydroxyl radical ($OH^{\cdot}$) react with and degrade proteins, lipids and nucleic acids leading to senescence (Arora *et al*., 2). An increase in ROS level has been correlated with an increase in cell membrane permeability and senescence in daylily. Antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase, glutathione reductase, peroxidase and catalase are involved in scavenging of reactive oxygen radicals (Foyer *et al.*, 9). In the past decade molecular biological approaches have been utilized to identify genes that may be involved in the initiation and regulation of the senescence. Identification and characterization of these senescence genes have begun to provide us with an understanding of the process of senescence.

Normally cut spikes of gladiolus have a short vase-life of around 7 to 15 days depending upon the cultivar. Hence, prolonging vase-life with improved quality for an extended period would be highly beneficial. Earlier it has been reported that senescence in gladiolus flower is preceded by increase in ion leakage, reactive oxygen species, lipid peroxidation etc., but the signals that initiate the degradative changes during senescence are largely unknown for gladiolus. Therefore, in present study an attempt was made to determine ABA-mediated biochemical event alteration in gladiolus flower development.

MATERIALS AND METHODS

The experiments were conducted in the Division of Plant Physiology, IARI, New Delhi with gladiolus var. Snow Princess to understand the role of abscisic acid (ABA) in senescence of gladiolus flower. Healthy gladiolus bulbs were planted in the experimental field with recommended package of practices. The spikes were harvested just above ground level in the morning when the lower florets started to unfold the petals. The spikes were cut into a uniform length of 15 cm and all leaves were removed and placed in 25 mm dia. test tubes containing 30 ml of vase solution. Vase solution was added whenever needed and plugged with non-absorbent cotton to prevent evaporation loss. Test tubes were placed in laboratory conditions (22 ± 2°C; RH 70 ± 5% and under continuous illumination of light 20 µE $m^{-2}s^{-1}$) Various vase solutions used were ABA (100 µM; T_1), ABA (100 µM) + gibberellic acid (100 µM; T_2) along with control; T_0 (distilled water).

On fifth day of vase-life, five different developmental stages of flower were selected based on 3rd floret from the bottom of the spikes, *viz.* bud stage (colour visible), half-open stage, fully-open stage, incipient senescent

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stage and senescence stage. The treated petal tissues were used for measurement of all biochemical parameters of antioxidant enzyme activity, *viz.* superoxide dismutase (SOD) enzyme activity (Dhindsa *et al.*, 6), catalase enzyme activity (Teranishi *et al*., 20), total peroxidase enzyme activity (Castillo *et al*., 5), ascorbate peroxidase enzyme activity (Nakano *et al*., 14) and glutathione reductase enzyme activity (Smith *et al*., 19). Other senescence related biochemical parameters like; lipoxygenase (LOX) enzyme activity (Doderer *et al*., 7) and lipid peroxidation (Heath *et al*., 10) assay were also estimated in petal tissues. All the assays were conducted with three replicates and the data were analyzed statistically for critical difference in factorial completely randomized block design using AGRES software.

RESULTS AND DISCUSSION

Our results support the view that ABA is a natural regulator of flower senescence in gladiolus. TBARS (Thiobarbituric acid reactive substances) content and LOX (lipoxygenase) activity were gradually increased with the advancement of senescence. Lynch *et al.* (13) reported the processes that enhance the formation of oxy radicals initiate lipid peroxidation and upregulate enzymes such as LOX in senescing plant tissues. Lipoxygenase activity showed a gradual increasing trend from bud stage to partially open stage and abrupt hike after fully open stage (Fig. 1) in control as well as in ABA treated flowers. At any given stage, the activity of LOX was higher in ABA treated flowers as compared to control. Increasing trend of LOX activity was steadily maintained from stage III to V significantly. Increased activity of LOX (23.95%) was found in ABA treated flowers at senescence stage compared to control. It was observed that LOX activity was reduced to almost equal to control when flowers were treated with antagonist of ABA action, *i.e.* GA_3, which nullifies the senescence activity.

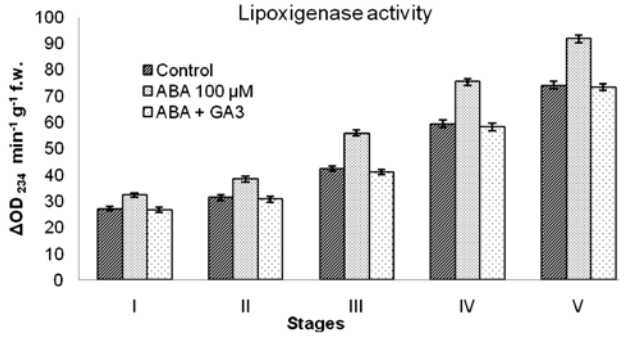

Fig. 1. Effect of ABA and GA_3 on LOX activity (ΔOD_{234} min^{-1} g^{-1} f.w.) in petals of gladiolus var. Snow Princess at different stages of flower development.

Thus, ABA (100 µM) treatment increases the activity of lipoxygenase enzyme, leading to hastening the senescence of gladiolus flowers. Similarly, Singh *et al.* (17) reported that the post-harvest application of GA_3 and sucrose lower LOX activity and lipid peroxidation in cut spikes of gladiolus. Lynch *et al.* (13) reported the processes that enhance the formation of oxy radicals initiating lipid peroxidation and upregulating enzymes such as LOX in senescing plant tissues.

The lipid peroxidation in terms of TBARS content (nmol.g^{-1} f.w.) increased gradually from stage I to senescence in both the treatments and control (Fig. 2). No significant difference was observed in TBARS content of bud stage in treatment over control, but at senescence stage. TBARS content was higher (30.86%) in ABA treated flowers. It was observed that TBARS content was reduced to almost equal to control when flowers were treated with GA_3, *i.e.* antagonist of ABA. Treatment ABA (100 µM) enhanced the process of lipid peroxidation, leading to hastening of senescence of gladiolus flowers. In contrast, the lower level of lipid peroxidation and lipoxygenase enzyme activities were reported in gladiolus spike treatments with chemicals like inositol (Vikas *et al*., 21) and calcium (Sairam *et al*., 16).

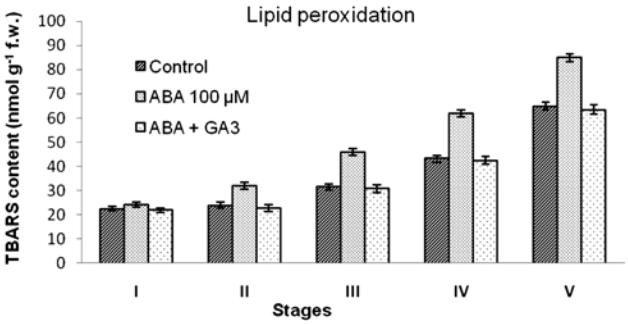

Fig. 2. Effect of ABA and GA_3 on lipid peroxidation in terms of TBARS content (nmol g^{-1} f.w.) in petals of gladiolus var. Snow Princess at different stages of flower development.

Various studies have demonstrated that vase life of flowers is modulated by antioxidants (Ezhilmathi *et al.*, 8; Sairam *et al.*, 16), suggesting the involvement of ROS in senescence. In the present investigation, the activities of antioxidant enzymes like SOD increased initially and then decreased sharply after stage II (Fig. 3). At any stage of flower development, SOD activity was less in ABA treated flowers as compared to control. These results were consistent with the pattern of SOD activity during senescence in gladiolus (Yamane *et al*., 22; Singh *et al.*, 18b; Ezhilmathi *et al*., 8)

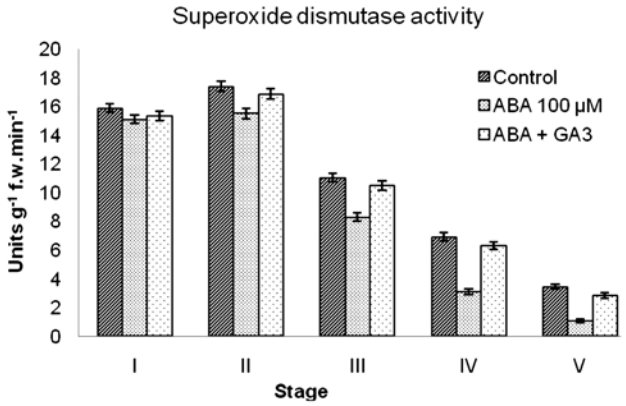

Fig. 3. Effect of ABA and GA_3 on superoxide dismutase activity (units g^{-1} f.w. min^{-1}) in petals of gladiolus var. Snow Princess at different stages of flower development.

and daylily (Panavas *et al.*, 15). Similarly, catalase (CAT) activity also continuously decreased from stage III to senescence (Fig. 4) in all the treatments. Thus, at senescence stage, ABA (100 µM) treatment decreases SOD activity (69%) and catalase activity (30%) over control, respectively. Sairam *et al.* (16) also observed similar trend, initial increase followed by decrease in activity of both SOD and CAT in gladiolus. In contrast, a steady decrease in catalase activity from flower opening to senescence stage was observed in daylily (Panavas *et al*., 15) and gladiolus (Singh *et al*., 18b; Ezhilmathi *et al*., 8). However, higher SOD and CAT activities were maintained in GA_3 + ABA treated flowers as compared to ABA alone. Thus, it is obvious to opine that petal wilting in gladiolus is associated with ROS induced lipid peroxidation, enhanced LOX activity and decrease in ROS scavenging system in form of SOD and CAT.

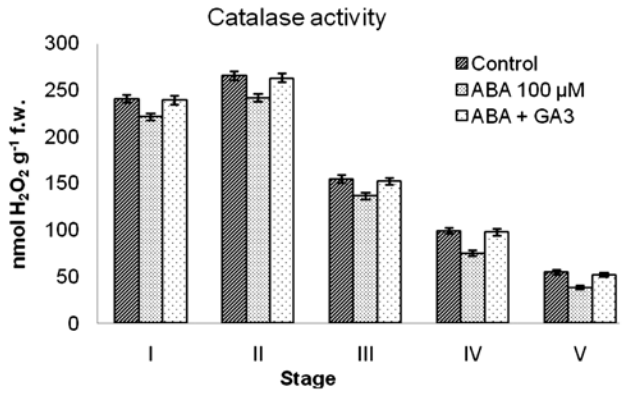

Fig. 4. Effect of ABA and GA_3 on catalase activity (nmol H_2O_2 g^{-1} f.w.) in petals of gladiolus var. Snow Princess at different stages of flower development.

Membrane bound ascorbate peroxidase (AP) is found to scavenge the H_2O_2, which was produced by the action of SOD on the superoxide radical (O_2^-) (Nakano *et al.*, 14). The ascorbate peroxidase activity is directly correlated with the reduction in free radicals induced membrane damage (Nakano *et al.*, 14). In our investigation, gladiolus spikes treated with ABA (100 µM) showed decreased levels of AP in last stage of senescence compared with the control (Fig. 5), whereas total peroxidase activity gradually decreased from bud stage to senescence stage (Fig. 6). Overall ABA (100 µM) treatment decreased AP activity by 30 and 31% reduction in POX activity over control. This result is in concurrence with the findings of (Sairam *et al*., 16; Hossain *et al*., 12; Ezhilmathi *et al*., 8) in gladiolus. Down regulation of AP activity seems to be the prerequisite for inducing senescence in

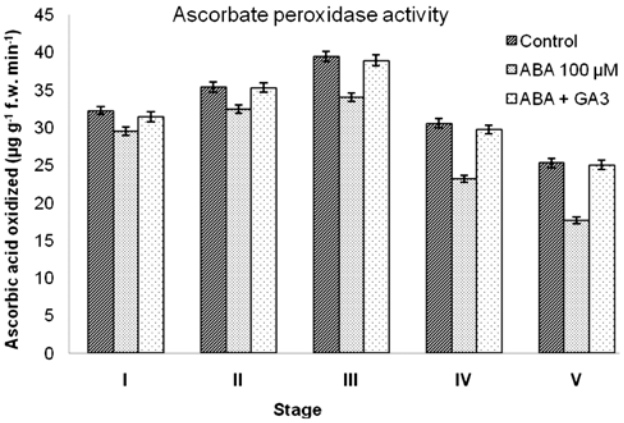

Fig. 5. Effect of ABA and GA_3 on ascorbate peroxidase activity in terms of ascorbic acid oxidized (µg g^{-1} f.w. min^{-1}) in petals of gladiolus var. Snow Princess at different stages of flower development.

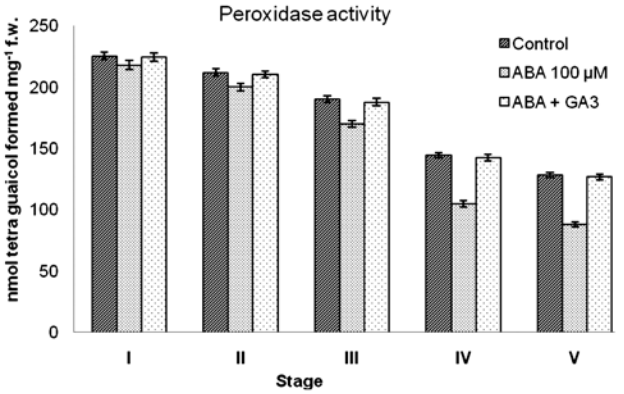

Fig. 6. Effect of ABA and GA_3 on total peroxidase (nmol tetra guaicol formed mg^{-1}f.w.) in petals of gladiolus var. Snow Princess at different stages of flower development.

gladiolus petals. Hence, it can be postulated that the decreased levels of AP in ABA treated gladiolus spikes in comparison to control may be one of the reasons to accelerate senescence process by producing more H_2O_2 and causes membrane damage.

Glutathione reductase (GR) is an enzyme which postulated to play an important role in plant protection against various forms of stress (Smith *et al*., 19). In the present investigation, the activity of GR initially increased upto fully open stage followed by decrease as the senescence proceeded in gladiolus petal (Fig. 7). At any given stage, the activity of GR was less in ABA treated flowers as compared to control. The same pattern of results was observed in gladiolus (Singh *et al*., 18; Hossain *et al*., 12; Ezhilmathi *et al*., 8) during senescence. Reduction in GR activity probably results in a decrease in the levels of reduced glutathione known to be an important factor in preventing oxidative injuries (Alscher, 1). In ABA treated gladiolus florets, GR activity considerably (43%) decreased in final stage of senescence over control.

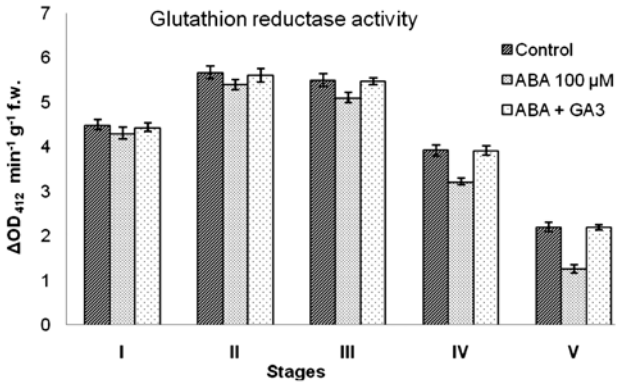

Fig. 7. Effect of ABA and GA_3 on glutathione reductase (GR) activity (Δ OD_{412} min^{-1} g^{-1} f.w.) in petals of gladiolus var. Snow Princess at different stages of flower development.

Thus, ABA preponed senescence of ethylene insensitive gladiolus flower not only by reducing antioxidant enzyme activity but also due to enhanced membrane deterioration in terms of increased lipid peroxidation and LOX activity. However, this trend was reversed when vase solution was supplemented with GA_3 along with ABA. Thus, the contemplated role of ABA in gladiolus, an ethylene insensitive flower is consolidated.

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