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SHORT COMMUNICATION

Regulatory role of phenols in flower development and senescence in the genus *Iris*

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Abstract The mechanism of flower development and senescence involves a lot of biochemical and molecular changes. These changes are governed by various external (temperature, light and humidity) and internal factors, viz., protein turnover, protease activity, antioxidant activity, phenols and plant growth regulators. The role of proteins, growth regulators, changes in various antioxidant enzymes and protease activity has been studied to a great extent; however the contribution of phenols in flower development and senescence is still elusive. Generally, flower senescence is thought to be associated with a decrease in the total phenolic content, but the present study on various species of the genus *Iris* revealed that total phenolic content showed diverging trends, varying from species to species within the same genus. The total phenolic content has been shown to decrease during senescence in *Iris versicolor* and *Iris japonica*, but an increase in total phenolic content was registered in *Iris germanica*, *Iris kashmiriana* and *Iris ensata*. Fresh mass, dry mass and water content was shown to increase towards flower anthesis (stages I–IV) and a significant decrease was observed at V and VI stages of flower senescence in all the species of *Iris* under study. The ascorbate peroxidase activity during the various stages of flower development and senescence indicated that phenols have a more contributory role than just being free radical scavengers in regulating flower senescence of various species of the genus *Iris*.

Keywords Development · Flowers · Phenols · Pollination · Senescence

Flower senescence is the last developmental phase of the flower's life cycle. Although, previously thought as a deteriorative non-essential process, the importance of flower senescence can be visualized through its ecological benefits to the plants (Ahmad et al. 2013; Dar et al. 2014a). Flowers attract the pollinators by their showy appearance and once pollination is accomplished, the plants get rid of the flowers as it is a metabolically costly tissue. During the process of flower senescence the loss of membrane permeability leading to loss of turgor, increase in lipoxygenase activity, changes in the levels of various growth regulators, such as ethylene, abscisic acid, cytokinins, auxins, gibberellins, polyamines, brassinosteroids and jasmonates, protein degradation and decreased activity of various antioxidant enzymes leads to programmed cell death (PCD) of flowers and eventually flower senescence (Schmitzer et al. 2010; Dar et al. 2014b; Ahmad and Tahir 2015). Besides these biomolecules, phenols have been reported to play an important role in the regulation of flower development. Although enough information is available about the growth inhibiting/promoting activities of various phenols, not much is known about their role in flowers development and senescence (Cvikrova et al. 1994; Prakash et al. 2001).

Phenols possess one or more aromatic rings with one or more hydroxyl groups and are the most abundant and ubiquitous secondary metabolites of plants. Phenols have generally been reported to be involved in defense against pathogens, parasites and predators, besides contributing color to the plant parts. Phenols have also been reported to assist in mitigating the oxidative stress by scavenging the

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free radicals directly or by their function as electron donors to hydrogen peroxide in the reactions catalyzed by ascorbate peroxidase (APX) (Morina et al. 2008). The precise role of phenols in regulating flower development and senescence has not been studied in detail. Previous works on various flowers such as rose, *Hemerocallis, Dianthus, Nerine, Ranunculus* and *Helleborus* have shown that phenolic content showed a significant turnover at different stages of flower development and senescence (Mittler et al. 2004; Morina et al. 2008; Gul et al. 2011; Shahri et al. 2011). From the antioxidant behavior of phenols, it might seem that phenols should decrease towards senescence, but phenols have also been shown to increase towards flower senescence in some flowers like rose and *Nicotiana* (Mwangi et al. 2003; Nisar et al. 2015).

Keeping this in view, a study was conducted to investigate the turnover in the total phenolic content at different stages of flower development in various species of genus *Iris*. Ascorbate peroxidase (APX) activity of the tepal tissue at different stages of flower development and senescence was also recorded to gain an insight into the relation between APX activity and phenolic turnover during flower development and senescence.

Flowers of five species of the genus *Iris,* viz., *Iris versicolor, I. germanica, I. kashmiriana, I. ensata* and *I. japonica* growing in Kashmir University Botanic Garden (KUBG) were used for the present study (Fig. 1). Flower development and senescence was divided into six stages (stage I–VI): tight bud stage (I), mature bud stage (II), pencil stage (III), fully open stage (IV), partially senescent stage (V) and senescent stage (VI) (Fig. 2). Visible changes were recorded throughout flower development and senescence.

Fresh flowers (n = 10) were used to determine the fresh mass at each stage. These flowers were kept in paper bags and oven dried at 70 °C for 48 h to determine dry mass. Water content was calculated as the difference between fresh and dry mass.

For the estimation of total phenols, the plant material was fixed in hot 70% ethanol, macerated and centrifuged at $8000\times g$ and the phenolic content was estimated from a suitable volume of aliquot from the supernatant by the method of Swain and Hillis (1959).

For the determination of ascorbate peroxidase (APX) activity, flower petals were macerated in 100 mM sodium phosphate buffer containing 5 mM ascorbate, 10% glycerol and 1 mM EDTA. The APX activity was determined in 1 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM ascorbate and 0.3 mM H_2O_2. The decrease in the absorbance was recorded for 3 min at 290 nm (Chen and Asada 1989).

The values depicted in the figures and tables represent the mean of 10 independent replicates. The data was statistically analyzed and standard deviation (SD) was computed by:

$$S = \sqrt{\frac{(x - \bar{x})^2}{n - 1}}$$

The greenish buds of the various species of genus *Iris* opened into various hues, with dark blue colored flowers in *I. germanica*, light blue in *I. ensata*, pinkish blue in *I. versicolor*, white in *I. kashmiriana* and creamy white with yellow and blue spots in *I. japonica* (Fig. 1). The flowers remained open for two days in all the species of *Iris* under study, except *I. japonica*, where the flowers lasted for only

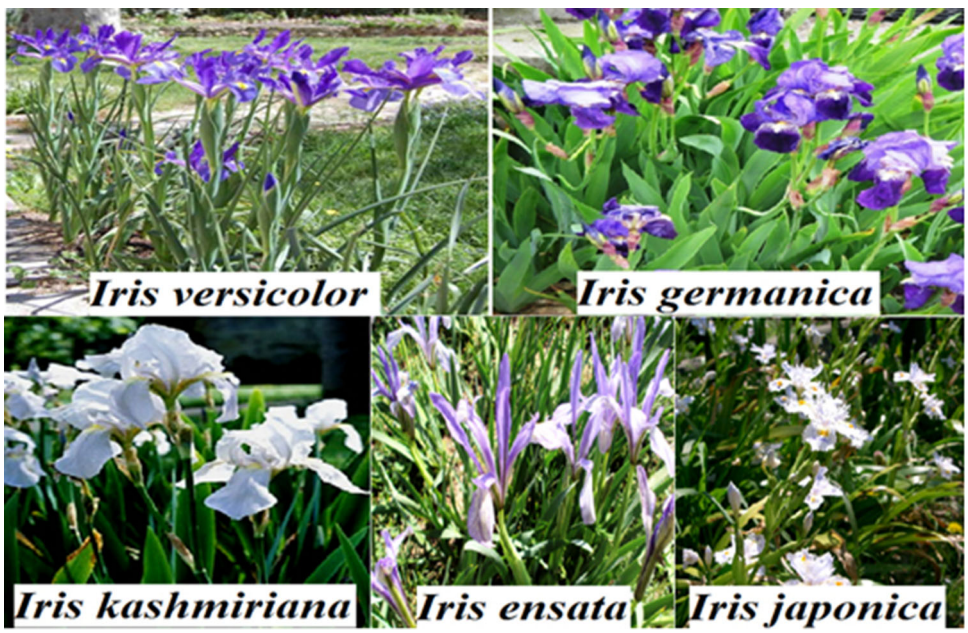

Fig. 1 Different species of the genus *Iris* in full bloom at Kashmir University Botanical Garden (KUBG)

Fig. 2 Stages of flower development and senescence (stages I to VI) in *Iris versicolor* (A), *Iris germanica* (B), *Iris kashmiriana* (C), *Iris ensata* (D) and *Iris japonica* (E)

1.5 days under field and laboratory conditions. Flower senescence is initiated by the loss of turgor at tepal tips followed by in rolling of the tepal margins. During the peak of senescence, the flowers turned pale and dried out completely. The fresh mass, dry mass and water content of the tepal tissues increased as the flowers opened in all the species and a sharp decrease was registered in these attributes with senescence (Figs. 3A–C). Loss of turgor and decrease in fresh mass of the flowers has been suggested as an early sign of senescence in various flowers like *Dianthus* and *Nicotiana* (Dar et al. 2014a; Nisar et al. 2015). Increase in the water content towards flower opening resulted in the increase in cell turgor, which is the prerequisite for flowers to open (Gul and Tahir 2009; Ahmad et al. 2013; Nisar et al. 2015).The decrease in the fresh and dry mass with senescence is because the flowers act as source during senescence for the allocation of resources to the developing plant parts as a part of resource allocation mechanism employed by the plants for ecological benefits (Zhou et al. 2005; Ahmad and Tahir, 2015; Dar et al. 2014a, b).

The changes in total phenolic content with flower development and senescence showed varying trends in the five species of the genus *Iris* under study. The total phenolic content in *I. versicolor* and *I. japonica* showed a steady increase from the bud stage up to flower anthesis (stage I to IV), and a sharp decline towards senescence (stages V and VI) (Fig. 4A). As such flower senescence in these two species was marked by a decrease in the total phenolics in the tepal tissues. Earlier studies on the members of the family Amaryllidaceae (*Nerine sarniensis*), Rosaceae (*Rosa hybrida*) and Astraceae (*Tithonia rotundifolia*) had shown that senescence in these flowers was associated with lower content of phenols in the tepal tissue (Mwangi et al. 2003; Schmitzer et al. 2010; Gul et al. 2011). Phenols are secondary metabolites and have long been implicated as having role in antioxidant defense mechanism (Mittler et al. 2004). Phenols play an important role in antioxidant defense by scavenging the free radicals and preventing the flower from oxidative stress, but a decline in the total phenolics makes the flower vulnerable to oxidative stress, which leads to programmed cell death and ultimately flower senescence (Mwangi et al. 2003; Schmitzer et al. 2010; Ahmad and Tahir 2015). Thus, a decrease in phenols towards senescence may be a driving factor for senescence to occur by exposing the tepal tissue to oxidative damage because of the accumulation of

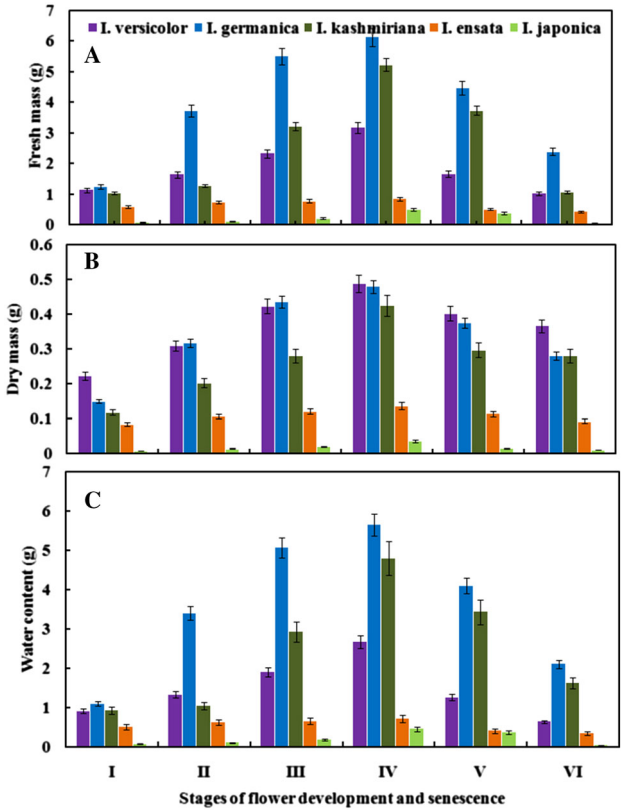

Fig. 3 Changes in the fresh mass (A), dry mass (B) and water content (C) with flower development and senescence in the five species of genus *Iris,* viz., *Iris versicolor, I. germanica, I. kashmiriana, I. ensata* and *I. japonica*

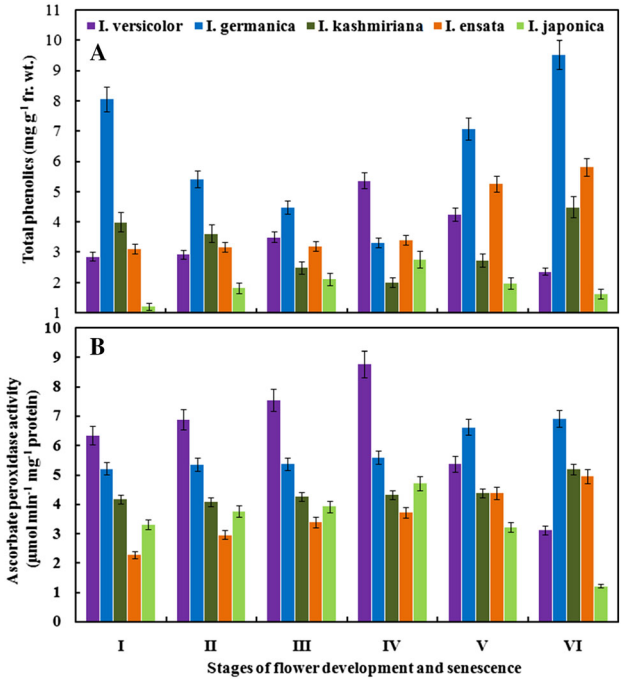

Fig. 4 Changes in the phenolic content (A) and ascorbate peroxidase activity (B) with flower development and senescence in the five species of genus *Iris,* viz., *Iris versicolor, I. germanica, I. kashmiriana, I. ensata* and *I. japonica*

reactive oxygen species and highly reactive free radicals. However, in case of *I. germanica* and *I. kashmiriana*, the tissue content of total phenols showed a decrease from bud to bloom (stage I to IV), but thereafter a sharp increase was registered towards senescence with a peak at completely senescent stage (Fig. 4A). In case of *I. ensata*, the total phenolic content in the tepal tissues showed a continuous and steady increase from the bud to senescent stage (Fig. 4A). Studies on some members of the family Caryophyllaceae (*Dianthus caryophyllus* and *D. chinensis*), Ranunculaceae (*Ranunculus asiaticus, Helleborus orientalis*), Liliaceae (*Hemerocallis fulva*) and Solanaceae (*Nicotiana plumbaginifolia*) have shown that senescence in these flowers is also associated with increase in phenolic content (Shahri et al. 2011; Dar et al. 2014a, b; Nisar et al. 2015).This increased phenolic content in the tepal tissues towards senescence can be due to failure of reallocation of the phenols towards the developing parts during senescence or by the increased synthesis of phenols towards senescence as part of the defense mechanism employed by flowers to delay senescence (Panavas and Rubinstein 1998; Mittler et al. 2004; Lattanzio et al. 2006; Schmitzer et al. 2010).

Moreover, the increase in the phenolic content towards senescence in *I. germanica, I. kashmiriana* and *I. ensata* was commensurate with an increase in the APX activity (Fig. 4B). Ascorbate peroxidase activity has been shown to increase with improved phenolic content as phenols act as electron donors to hydrogen peroxide in reactions catalyzed by peroxidase. Our studies on the activity of ascorbate peroxidase revealed that those species of *Iris*, which showed an increase in the phenolic content towards senescence also showed increase in APX activity towards flower senescence as a part of the defense mechanism to combat senescence (Fig. 4B). This increase in the APX activity is due to the availability of electrons for APX donated by phenols and as such the flowers are shielded from oxidative damage. Thus it seems that senescence in these flowers is not initiated by turnover in phenols and oxidative stress, but protein degradation or increase in protease activity may be involved in the initiation of senescence in these flower systems (Ahmad et al. 2013; Ahmad and Tahir 2015). Regarding *I. versicolor* and *I. japonica*, where senescence is associated with decreased phenolic content, APX activity showed an increase from bud to the open flower stages but a significant decrease was registered towards senescence (Fig. 4B). Down regulation of APX activity has also been reported to initiate senescence in various flowers such as daylily, gladiolus and carnation (Panavas and Rubinstein 1998). The primary

function of APX is to reduce H_2O_2 with the simultaneous oxidation of ascorbate to dehydroascarbate. APX is the key enzyme in detoxifying H_2O_2 in plants through ascorbate–glutathione cycle (Mittler et al. 2004). Promotion of senescence by decrease in APX activity is due to the endogenous accumulation of H_2O_2 that in turn inhibits superoxide dismutase activity (Mittler et al. 2004; Lattanzio et al. 2006). Thus decrease in the activity of antioxidant enzymes may lead to the initiation and execution of senescence in *I. versicolor* and *I. japonica*.

Earlier studies on the role of phenols in flower senescence have demonstrated that although phenols don't show a uniform trend during various phases of flower development and senescence, but within a particular family, the dynamics of phenols remains conserved, yet it may vary across families (Lattanzio et al. 2006; Schmitzer et al. 2010). During the present investigation, it was found that phenols follow a different trend during flower development and senescence even within species of the same genus, *Iris*. Although, the phenols registered an increase towards senescence in *I. germanica, I. kashmiriana* and *I. ensata*, but a significant decrease was registered in the phenolic content of *I. versicolor* and *I. japonica* towards senescence. The results, thus lead us to postulate that phenols may play an active role in the initiation of flower senescence in some flowers, while in other flower systems phenols may not be responsible for initiation or execution of senescence, but their turnover may be dependent on other primary changes taking place with senescence in these flowers.

The role of phenols in flower senescence is often confusing as the total phenolic content has shown opposing trends during flower development and senescence in various flowers like *Dianthus chinensis, Hemerocallis fulva, Helleborus orientalis* and *Nerine sarniensis*. Even though the previous studies have shown dynamism in the behavior of phenols, but phenols were shown to follow a particular trend within a family. But, our studies on the various species of genus *Iris* revealed that phenols may show diverging trends even among the various species of the same genus. An increase in phenolic content was registered towards senescence in *I. germanica, I. kashmiriana* and *I. ensata*, but a significant decrease was recorded in the phenolic content of *I. versicolor* and *I. japonica* towards senescence. Phenols may initiate flower senescence in some flowers and may not play a contributory role in others, but the precise role of phenols in flower senescence is yet to be elucidated. Detailed biochemical studies need to be undertaken to unfold the precise role of phenols in flower development and senescence.

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