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PEROXIDASE ACTIVITY IN RELATION TO ANTHOCYANIN AND CHLOROPHYLL CONTENT IN JUVENILE AND ADULT LEAVES OF "MINI-STAR" *GAZANIA SPLENDENS*

GÜL CEVAHIR[*1], SEMAHAT YENTÜR[1], MEVZULE YAZGAN[1], MUAMMER ÜNAL[1] AND NADIM YILMAZER[2]

[1]Department of Botany, Faculty of Science, Istanbul University, 34460, Süleymaniye-İstanbul, Turkey,
[2]Department of Primary Education, Hasan Ali Yücel Faculty of Education, Istanbul University, Istanbul, Turkey

Abstract

Gazania splendens is an ornamental and decorative plant which is cultivated in parks and gardens and shows distinct juvenile and adult leaves in transition from the vegetative phase to the reproductive phase. There is no information in current literature that *Gazania* shows heterophylly. The objective of the present study was to assess the physiological parameters such as the changes of peroxidase activity (POD), anthocyanin and chlorophyll content in juvenile and adult leaves. It was found that POD activity and chlorophyll level increased in the juvenile leaves compared to the adult ones in which anthocyanin content decreased. These results indicate that there is an inverse correlation between the amount of anthocyanin and chlorophyll in the juvenile and adult leaf tissues, and increasing POD activity may limit anthocyanin content in the adult leaves.

Introduction

Polymorphism has concerned botanists over the years. One particular feature of polymorphism that has received recent attention is juvenile and adult phase changes of shoot systems in several plants, especially *Hedera helix* (Feldman & Cutter, 1970a, b; Frydman & Wareing, 1973a, b; 1974; Rogler & Hackett, 1975a, b; Young *et al*., 1995; Diggle, 1999; Huang *et al*., 2000; Orkwiszewski & Poethig, 2000; Srivastava, 2002; Yentür, 2003). Plants produce different types of leaves in their development. Variation in the size or shape of leaves produced along the axis of an individual plant is called heterophylly. Such individual variation in leaf size and shape on different parts of the same plant or at stages of development may arise by a number of different mechanisms. It is normally associated with a change in morphology from the juvenile vegetative phase to the adult reproductive phase (Schmidt & Millington, 1968; Franck, 1976; Mueller & Dengler, 1984; Goliber & Feldman, 1989; Lawson & Poetig, 1995; Bongard-Pierce *et al*., 1996; Lin & Young, 1999; Sylvester *et al*., 2001). In addition, it is generally thought that there is a switching on and off of gene activity from one phase to another, but data have yet to prove this (Simpson *et al*., 1999; Vega *et al*., 2002). At the same time, there are several investigations related to hormonal regulation of gene activity on both the physiological and molecular levels (Rogler & Dahmus, 1974; Young & Horton, 1985; Chandler & Robertson, 1994; Evans & Poetig, 1995; Heloir *et al*., 1998; Poetig, 2003). It is suggested that exogenous applications of gibberellic acid (GA_3) and abscisic acid (ABA) to the mature form may control morphological reversion to the juvenile form in

[*]Corresponding author
e-mail: gulcevahir@hotmail.com.
cevahirg@istanbul.edu.tr

Hedera helix and that growth retardants as well as ABA can stabilize the mature form of growth (Frydman & Wareing, 1974; Rogler & Hackett, 1975b). One of the most important subjects in plant physiology is the study of the changes in enzyme activity such as peroxidase (POD) which occurs in the several phases of development. The activity of peroxidase enzyme, also one of the stress parameters in plants, is directly or indirectly related to numerous physiological incidents. The increase in POD activity in plant tissues has played an important role in many biochemical reactions such as lignin biosynthesis (Jackson & Ricardo, 1994). Several biochemical markers can now be employed to distinguish the juvenile from the mature or adult developmental phases of certain plants e.g., differences in peroxidase and esterase isozymes (Huang *et al*., 2000; Huang *et al*., 2003). Anthocyanins, one class of flavonoid pigments, are derived from photosynthetically produced carbohydrates via enzyme catalyzed reactions of the shikimic acid, phenylpropanoids, and flavonoid biosynthetic pathways (Hahlbrock & Scheel, 1989; Murray & Hackett, 1991). It has been known that in the formation of this pigment, the genetic capacity is the primary factor. In addition, plants also accumulate in their structure secondary metabolites such as anthocyanin during the development processes or in order to resist the stress conditions (infections, low temperature, salinity, light intensity and wounding). Besides, Murray & Hackett (1991) have indicated dihydroflavanol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase of *Hedera helix* L. Plant pigments such as chlorophyll, xanthophyll and anthocyanin are indirect indicators of physiological function with age (Heloir *et al*., 1998; Kerstetter & Poetig, 1998; Poetig, 2003). Maturation (also called phase change or ontogenic aging) in plants is associated with chlorophyll content (Greenwood, 1995; Sestak & Siffel, 1997; Huang *et al*., 2003). The present report describes the physiological results such as POD activity, anthocyanin and chlorophyll content in the juvenile and adult leaves of reproductive phase in *Gazania splendens.*

Material and Methods

Leaves of *Gazania splendens* (Compositae) growing in pots under the greenhouse conditions were used as experimental material. A single individual can produce two types of leaves, one type which is entire and simple, and other type which is pinnatifid (Fig.1). The leaves were collected on the same date from a single individual.

Peroxidase activity measurement: The fresh juvenile and adult leaves were weighed and extracted in cold using 0.1 M phosphate buffer ($Na_2HPO_4.2H_2O$+ $NaH_2PO_4.2H_2O$, pH 7). Homogenates were then centrifuged at 13.000 rpm at 4°C for 20 minutes. The enzymatic activity was determined spectrophotometrically at 470 nm., using 15 mM guaiacol and 5 mM H_2O_2 as substrates. The kinetic measurements were carried out with a time-dependent increase of absorption (in 2 min within 10 sec of time intervals) and total POD activity was defined as ΔA/g.fw.min., quantitatively (Birecka *et al*., l973).

Determination of chlorophyll content: The fresh leaf materials were homogenized with some $CaCO_3$ powder and ice cold 80% acetone. Extracts were then centrifuged at 3000 g for 15 min and the volume of the supernatant was measured. Chlorophyll content was determined by reading absorbancy at 663 nm and 645 nm and calculated as mg total chlorophyll/g.fw. (Arnon, l949).

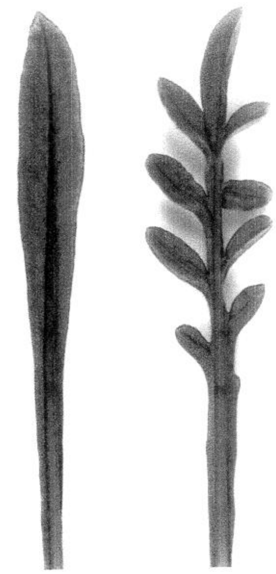

Fig. 1. Juvenile and adult leaf shapes of *Gazania splendens.*

Determination of anthocyanin content: Relative anthocyanin levels of *Gazania splendens* leaves were determined by incubating them overnight in 150 µL of methanol acidified with 1% HCl (v/v). After the addition of 100 µL of distilled water, anthocyanins were separated from chlorophylls with 250 µL of chloroform. Total anthocyanins were determined by measuring the A_{530} and A_{657} of the aqueous phase using a spectrophotometer. By subtracting the A_{657} from the A_{530}, the relative amount of anthocyanin was calculated as OD/g.fw. (Neff & Chory, 1998).

All spectrophotmetric assays were carried out with a SHIMADZU UV 160 spectrophotometer.

Statistical analysis: All experiments were performed 8 times with 2 replicates per samples. The results are given with standart deviations in Figure 2, and were evaluated with Student's *t*-test.

Results

Biochemical markers: The enzyme activity in the adult leaves of *Gazania splendens* was 8.9 ($P<0,05$) while it was 5.3 in the juvenile ones. Fig. 2*a* shows these data as histograms. Chlorophyll levels shown in Fig. 2*b* appeared to be higher in the adult leaves (2.069; $P>0,05$) than in juvenile leaves (1.471). Measurements of anthocyanin content were 0.443 for juvenile leaves and 0.199 ($P>0,05$) for adult leaves given in Fig. 2*c*.

Morphological Observations: *Gazania splendens*, a perennial rosette plant possesses leaves crowded in the basal part; juvenile leaves entire, adult leaves pinnatifid, green and brightly above, villous below. A gradual transition from juvenile phase to adult phase leaves photographed is given in Fig. 3.

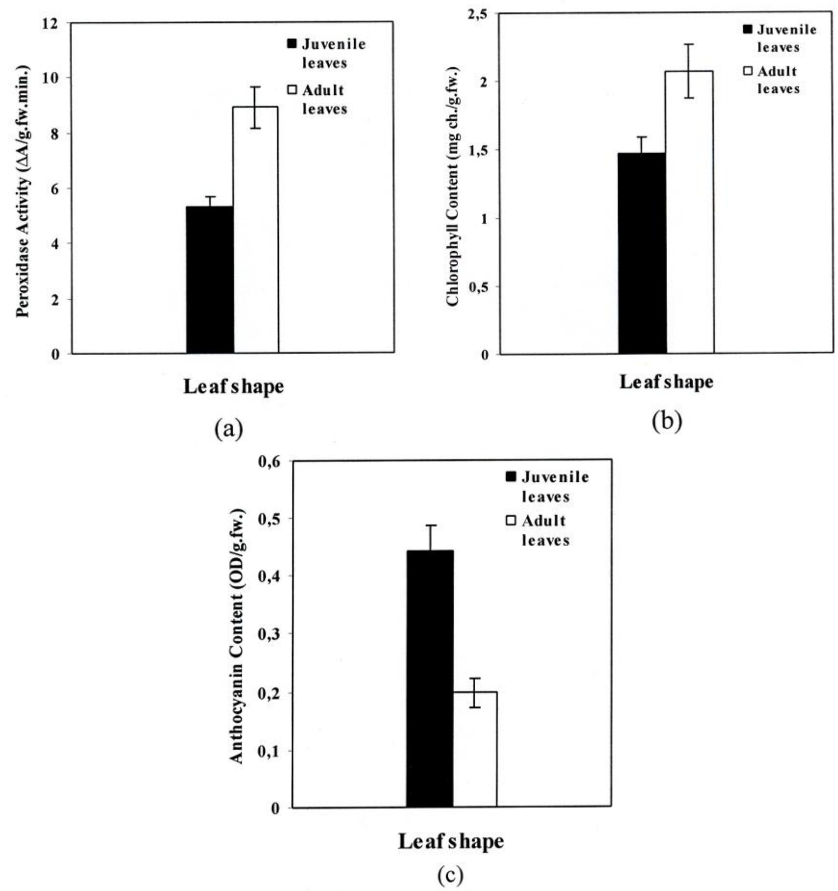

Fig. 2. The change of peroxidase activity ($P<0,05$), and chlorophyll and anthocyanin contents in juvenile and adult leaves of *Gazania splendens*, which are not of statistically significant ($P>0,05$, Student's *t*-test).

Fig. 3. Diagrammatic representation of variation in leaf shapes in *Gazania splendens.*

Discussion

Heterophylly is a term describing the occurrence of more than one type of leaf on the same plant, and is of considerable developmental interest (Rogler & Hackett, 1975a, b; Huang *et al*., 2000; Srivastava, 2002; Yentür, 2003). Plant peroxidases, which play an important role in the growth and differentiation of plants, are widely distributed in higher plants (Jackson & Ricardo, 1994; Huang *et al*., 2000; Huang *et al*., 2003). In addition, they are also enzymes responsible for the lignification of the cell wall and destructive process such as aging and senescence (Leopold & Kriedemann, 1985; Jackson & Ricardo, 1994). According to our measurements, peroxidase activity had especially higher values in the adult leaves than in the juvenile ones. We also observed an increase in peroxidase activity together with low anthocyanin content in the adult leaves. One can say that there is a correlation between lignification of cell walls in the adult leaves during the maturation (ontogenic aging or phase change) and POD activity ($P<0,05$). Murray & Hackett (1994) have reported that there was greater accumulation of phenylpropanoids in mature discs than in juvenile discs, but anthocyanin accumulated only in juvenile discs. In our research, it was found determined that in the juvenile leaves of this plant the amount of anthocyanin increases ($P>0,05$) while peroxidase activity and chlorophyll level decrease. The increasing anthocyanin content in the juvenile leaves might affect the activity of peroxidase enzyme negatively. Lewington *et al*., (1967) suggest that the aging of bean leaves is associated with an extended decline in chlorophyll content. In addition, leaf weight/leaf area and net photosynthesis increase with maturation, but chlorophyll content in *Hedera helix* L., does not change (Bauer & Bauer, 1980). In the present study chlorophyll level in adult leaves ($P>0,05$) was higher than in juvenile ones. This result is in concordance with the findings of Hutchison *et al*., (1990), who have proved that chlorophyll content in the leaves of eastern larch increases with maturation. We can therefore conclude that both of these metabolic activities (anthocyanin and chlorophyll biosynthesis) are performed in an opposite way. The fact of $p>0,05$ concerning anthocyanin and chlorophyll content implies that such environmental factors as light and temperature, and endogenous factors such as hormones, enzymes and certain proteins may play a role during phase change, thus being not considered an exact parameter for heterophylly in this plant. In addition, the fact that relative anthocyanin content of the adult leaves was lower than that of the juvenile form is most probably due to the inhibition of the anthocyanin biosynthesis or the lack of the anthocyanin accumulation. The increased POD activity in the adult leaves during reproductive phase,which is statistically significant, renders this enzyme to be regarded as an indicator of maturation. There is no doubt that molecular and physiological changes that underlie heterophylly are coordinated with special gene activation (Evans & Poetig, 1995; Heloir *et al*., 1998; Simpson *et al*., 1999; Vega *et al*., 2002; Poetig, 2003). Hence, heterophylly in our plant awaits to be cleared with molecular and genetical investigations.

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