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Physiological responses of chrysanthemum petals during senescence

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Abstract

Flower senescence was studied in three cultivars of *Chrysanthemum coronarium* L.: Snowball White, Yellow Chandrama (standard type flowers) and Spray Button (spray type flowers). Spray button flowers exhibited least respiration rate, less efflux of ions, minimum protease activity and less decline in fresh mass, relative water content and total soluble protein content with the progression of senescence. The Snowball White flowers showed highest respiration rate, great efflux of ions, maximum protease activity, high activity of hydrolytic as well as proteolytic enzymes, and high decline in fresh mass, relative water content, and total soluble protein content. Yellow Chandrama flowers showed responses similar to Snowball White flowers.

Additional key words: *Chrysanthemum coronarium*, ion efflux, petal senescence, protease activity, protein content, relative water content, respiration rate.

Introduction

Senescence is a deteriorative change that leads to death of whole plant, organ, tissue or cell. It is determined genetically and governed by environmental factors during development. The metabolic capacity and stress responses contribute to the life maintenance capacity of any organism and their interaction lead to senescence or longevity.

In general, flowers provide an excellent organ for studying senescence. The flower petals are generally short lived. It is usually the life span of petals, which determine the effective life of flower. The regulation of petal senescence is of great interest to horticulturist for improving the post harvest longevity of cut flowers.

Physiology of flower senescence has predominantly been studied in some plant families such as *Caryophyllaceae*, *Malvaceae* and *Orchidaceae*, where involvement of ethylene has been reported (Nichols 1966, Maxie *et al.* 1973, Halevy and Mayak 1979, Wulster *et al.* 1982, Van Altrovorst and Bovy 1995). Just prior to petal wilting, a rise in ethylene production occurs in ethylene sensitive flowers. In carnation the ethylene evolution peaked to a maximum of about 50 - 200 times from the basal level. The evolution of ethylene was not observed in *Chrysanthemum* and *Narcissus* (Nichols 1966). Flower senescence that is apparently not initiated by ethylene has been studied only in a few species such as *Freesia* (Spikeman 1989), *Gerbera* (Van Doorn and Stead 1994) and *Sandersdonia* (Eason and De Vre 1995). The mechanism of senescence in chrysanthemum is not fully understood. The present study is therefore aimed at the physiological basis for petal senescence in chrysanthemum.

Materials and methods

Chrysanthemum (*Chrysanthemum coronarium* L.) root suckers were planted in pots containing garden soil, leaf mould and farm yard manure in 1:1:1 ratio. Two suckers were planted initially and only one transplanted afterwards.

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Abbreviations: DAF - days after full bloom; PG - polygalacturonase; PME - pectin methylesterase; PVP - polyvinylpyrrolidone; R_D - respiration rate; RWC - relative water content.

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Proper stacking was done to prevent physical and wind damage. Fully opened flowers of chrysanthemum cultivars Snowball White, Yellow Chandrama and Spray Button were harvested late in the afternoon and the following physiological and biochemical parameters were recorded at an interval of 2 d. The flowers were kept in distilled water at 25 ± 1 °C immediately after the harvest and brought for analysis. Fresh mass of flowers was recorded. Since the flowers of the three cultivars differ in their respective mass, the rate of change in fresh mass with respect to fresh mass at full bloom were calculated. The rate of change was expressed in a percentage decrease during senescence.

Three flowers of equal size were selected, weighed and their respiration rate (R_D) was measured with the aid of portable photosynthetic system (*LI-COR 6250*, Lincoln, USA) with specially designed chamber. Five flowers of equal size were taken for measurement of respiration of Spray Button, as the flower size was small.

Relative water content (RWC) was determined gravimetrically following the method of Weatherley (1950). The flowers were enclosed in polythene bag just after harvesting from the plant. Petals were blotted dry and 10 discs of 2.47 cm^2 were punched and fresh mass was recorded. Petal discs were saturated in distilled water for 6 h and then dried to a constant mass at 80 °C overnight. RWC was calculated using the following formula: RWC = [(fresh mass - dry mass)/(saturated mass - dry mass)] × 100.

Leakage of ions from the petals was measured according to Bailly *et al.* (1996) with minor modifications. Petals were placed in 25 cm^3 distilled water at room temperature. The conductivity of the medium was measured with a conductivity bridge (*ELICO*, Hyderabad, India) after a three-hour incubation period. KCl (0.01M) was used as a standard that gives specific conductance of 1.41 mS cm^{-1}.

Total soluble protein content in the petals was estimated following enhanced alkaline copper assay (Lowry *et al.* 1951). Protease activity in the petals was measured indirectly according to the presence of free amino acids by ninhydrin method (Lee and Takahasi 1967, Arnon 1970). One g of petals was extracted in 1 cm^3 of 25 mM Tris (pH 7.5) and centrifuged at 10 000 *g* for 10 min. To 1 cm^3 of enzyme extract 1 cm^3 of Tris and 0.5 cm^3 of 4 % bacto-haemoglobin were added. The reaction mixture was incubated for 60 min at 40 °C and the reaction was stopped with 0.25 cm^3 of 40 % trichloroacetic acid. The mixture was further incubated for 30 min at 0 °C. The solution was centrifuged at 10 000 *g* for 1 min and 0.1 cm^3 sample was taken for free amino acid assay. The reaction mixture consisted of 0.5 cm^3 of ninhydrin (1 %), 0.2 cm^3 citrate buffer (0.5 M, pH 5.6), 1.2 cm^3 glycerol, and 0.1 cm^3 of sample extract. The resultant mixture was kept for 30 min in boiling water, cooled in running tap water and final volume was made up to 5 cm^3 with distilled water. The absorbances were read at 570 nm (spectrophotometer *M-36, Beckman*, USA).

The cell wall hydrolytic enzymes polygalacturonase (PG) and pectin methylesterase (PME) were assayed following the method of Hobson (1962) and Rouse and Atkins (1955), respectively with minor modifications. Petals (1 g) were homogenized in 4 cm^3 of sodium acetate buffer (0.3 M, pH 6) with a pinch of $Na_2S_2O_4$ and PVP. The homogenate was centrifuged at 15 000 *g* for 20 min at 4 °C. The supernatant was used for both PG and PME activity assay. For PG activity, to a 2 cm^3 of assay mixture (0.45 % m/v pectin and 0.1 % casein in 0.3 M sodium acetate buffer - pH 3.8) 0.2 cm^3 of enzyme extract was added and incubated at 37 °C for 2 h. From the supernatant 0.05 cm^3 were taken and 1 cm^3 of 5 % phenol, 5 cm^3 of 96 % H_2SO_4, 5 cm^3 of distilled water were added, mixed and cooled to room temperature. The absorbances were recorded at 490 nm. For PME activity the pH value of assay mixture (0.3 mM sodium acetate - pH 8 and 0.5 % pectin) was measured before adding 3 cm^3 of enzyme extract. After 5 min, 0.1 M NaOH was added to bring pH to its original value (pH value just before adding enzyme extract). The volume of 0.1 M NaOH consumed to restore the original pH was recorded.

The values of observations have been represented as means of seven replications and data analysis was done following Panse and Sukhatme (1995).

Results

There was a significant reduction in fresh mass of flowers after harvest in all the cultivars of chrysanthemum (Fig. 1). The decline in fresh mass was much more pronounced in cv. Snowball White compared to other two cultivars at 2 days after full bloom (DAF). The flowers of Spray Button showed the least decline in fresh mass. The respiration rate and relative water content also decreased in all the three cultivars after harvest (Fig. 1). However, the standard type flowers showed higher R_D than Spray Button during the course of senescence. There was an insignificant decrease in RWC at initial stages. However, at later stages, Snowball White showed the highest decline in RWC and Spray Button the least.

The loss of membrane integrity measured by the leakage of ions increased continuously in all the three cultivars (Fig. 1). The leakage was the highest in Snowball White during ageing in comparison to Yellow Chandrama and Spray Button. The latter two cultivars showed similar level of leakage.

It was observed that the total soluble protein content decreased continuously after harvest (Fig. 2). Spray type flowers showed higher protein content during ageing than standard type flowers with Snowball White showing the least protein content. The decrease in content of soluble

protein was greater than 50 % at later stages, *i.e.*, at 6 DAF. Proteolysis as expressed in terms of the content of free amino acids increased steadily with the progression of senescence (Fig. 2). Snowball White flowers expressed higher enzyme activity and Spray Button expressed the least activity.

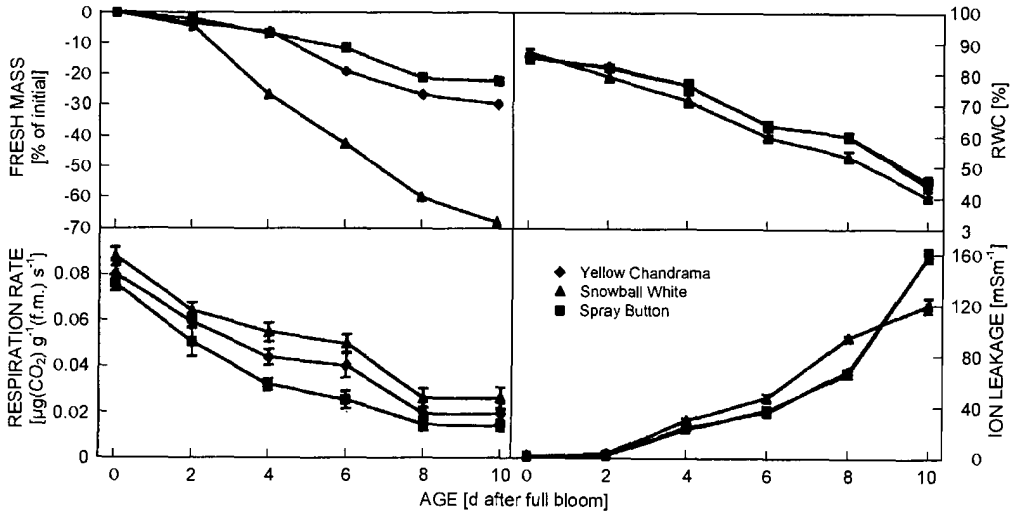

Fig. 1. Percent decline in fresh mass, respiration rate, relative water content and ion leakage from the petals of chrysanthemum cultivars during senescence.

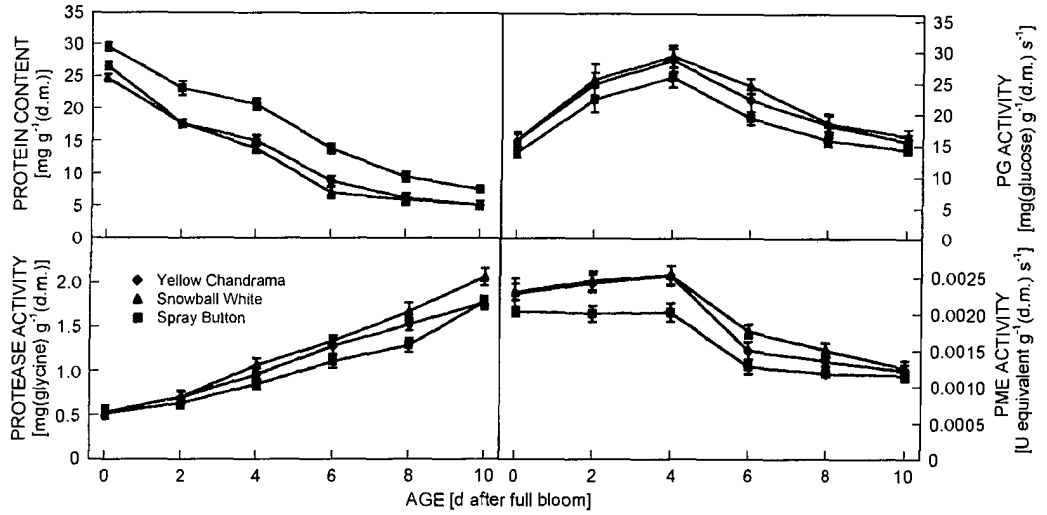

Fig. 2. Total soluble protein, protease activity, polygalacturonase activity (PG) and pectin methylesterase activity (PME) in the petals of chrysanthemum cultivars during senescence.

The polygalacturonase activity increased up to 4 DAF and thereafter decreased (Fig. 2) in all cultivars. Standard type flowers showed higher enzyme activity than spray type flowers. Within standard type flowers Snowball White exhibited the highest activity. The pectin methylesterase also increased up to 4 DAF and decreased by 50 % between 4 and 6 DAF. Similar to PG activity, the Spray Button flowers showed the least PME activity.

Discussion

The reduction in fresh mass is the result of decreased RWC, decreased dry mass due to respiration, and possibly increased ion leakage from the petals (Elanchezhian 1998). These results find support from the work of Bieleski and Reid (1992), Serek *et al.* (1995) and Borochov *et al.* (1995). The decrease in R_D after attaining the full bloom stage was also reported in other non-climacteric flowers like *Iris*, *Narcissus* (van Doorn and Stead 1994) and *Tulipa* (Jones

et al. 1995). It has been pointed out that metabolic energy is required to initiate senescence (Solomos 1988), which is supplied through respiration. In the present experiment, spray type flowers have lesser decline both in fresh mass and in R_D. These two factors are important for maintaining a longer life period after the full bloom stage. Decrease in water potential was reported in cut carnations (Eze *et al.* 1986) and roses (Orlandini *et al.* 1991). A higher R_D, low RWC and increased efflux of ions might have possibly contributed to the earlier senescence of Snowball White.

It is well established that there is a progressive loss of wide range of proteins, both soluble and membrane bound from plants during senescence. At least in leaves, senescence reflects a change in the equilibrium between rate of synthesis and degradation of particular proteins (Brady 1988). In the present investigation, we observed that the total soluble protein content decreased continuously after full bloom with cultivar variation in quantity of proteins. Similar observations have been reported by Borochov *et al.* (1986) in cut carnations. Apart from decrease in protein synthesis, protein degradation may also be enhanced during senescence of flowers. Genes encoding several different types of protease have been identified in senescing leaves from various plants. Buchanan-Wollaston (1997) reported increased cystein protease activity during senescence of rice and maize leaves. In addition the content of total free amino acids, indicator of proteolysis, increased after harvest in cut roses (Gao and Wu 1990). From these results, it may be suggested that decrease in protein may be due to the inhibition of protein synthesis and/or enhanced protein degradation by proteases, which lead to loss of functional capability of membrane resulting in higher efflux of ions and finally senescence and death.

The flower senescence, like fruit ripening, is characterized by the involvement of cell wall hydrolytic enzymes such as PG and PME (van Doorn and Stead 1997). In the present study PG and PME increased up to 4 DAF and thereafter decreased. It may be suggested that the hydrolytic enzymes degrade pectic substances thereby decreasing the ability to absorb water rapidly. The integrity of pectic substances, by virtue of lower enzyme activity, may contribute to higher absorption of water or maintenance of pressure potential in the cell. Snowball White recorded higher enzyme activity and lower RWC than Spray Button or Yellow Chandrama. Moreover the efflux of ions is definitely smaller when there is less degradation in cell wall or less activity of hydrolytic enzymes. Similar results were reported in cut *Gerbera* during its vase life (Jona *et al.* 1989).

Amongst the three cultivars Spray Button exhibited least respiration, less efflux of ions, minimum protease activity and less decline in fresh mass, RWC and total soluble protein with the progression of senescence. The standard type flowers (Snowball White) showed higher respiration rate, more efflux of ions, greater protease activity and more decline in fresh mass, RWC and total soluble protein. Snowball White also possessed higher activity of hydrolytic as well as proteolytic enzymes.

Thus it may be concluded that the respiration rate, RWC, ion leakage and activities of hydrolytic enzymes are important in the senescence of chrysanthemum flowers and the protein inactivation and degradation may explain the onset of senescence in chrysanthemum.

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