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Quantification of total endogenous ascorbic acid from chrysanthemum (*Chrysanthemum* × *morifolium*) cultivars and its association with postharvest life

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ABSTRACT

In the present study, an effort was made to quantify the total endogenous ascorbic acid to reveal if it has any association with vase life of chrysanthemum (*Chrysanthemum* × *morifolium* Ramat) cultivars. Among 30 cultivars, maximum total ascorbic acid content (441.29 mg/100 g fresh weight) was recorded in cv. Red Gold followed by cv. Jaya (336.34 mg/100 g fresh weight) and minimum ascorbic acid content (93.55 mg/100 g fresh weight) was observed in cv. Arka Ravi followed by cv. Yellow Reflex (101.76 mg/100 g fresh weight). Chrysanthemum cultivars were found to contain maximum total ascorbic acid content in petals (235.00 mg/100 g fresh weight) and leaves (285.12 mg/100 g fresh weight) in stage II (fully opened flower). During all the stages of flower development total ascorbic acid content in leaves was recorded higher as compared to petals. It is concluded that there is a significant association between postharvest life and ascorbic acid content in leaves (r = 0.669) and petals (r = 0.547) at fully opened flower stage in chrysanthemum.

Key words: Antioxidants, Ascorbic acid, Leaf longevity, Senescence, Vase life

Chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.), a prime member of asteraceae family is the second largest cut flower in global market, also used as loose flower and pot plant purpose. Chrysanthemums belong to non-climacteric flower group having considerable keeping quality. However, premature leaf yellowing, wilting and failure of the flowers to fully open and petal senescence are major post-production problems in chrysanthemum (Jain *et al.* 2014). Flower senescence is a progressive oxidative deterioration of cells leading to the death of flowers (Cavaiuolo *et al.* 2013). Petal wilting usually determines the vase life of any cut flower (Halevy and Mayak 1979). Leaf yellowing occurs prior to the onset of flower senescence making the flowers unattractive and reduces its longevity (Doi *et al.* 2003). Various chemicals have been reported to have positive effect in delaying leaf and petal senescence in chrysanthemum (Ferrante *et al.* 2005, Kofranek and Halevy 1980, Petridou *et al.* 2001). However, research on ability of antioxidants in regulating senescence is limited. Some of the activated oxygen species such as hydrogen peroxide, superoxide radicals are highly reactive in plant cells and can cause damage to the cell structure and function in the absence of protective mechanisms. Plants show defense against oxidative stress like senescence by synthesizing antioxidants by eliminating hazardous oxygen molecules. Among antioxidants, ascorbic acid is known to play a central role in several physiological processes like photosynthesis, cell division, plant growth and stress responses. It is also reported that ascorbic acid is involved in regulation of some senescence related factors during oxidative stress-induced senescence in plants.

In recent times, researchers are considering utility of endogenous level of ascorbic acid due to its action against senescence and resistance to disease causing pathogens (Pastori *et al.* 2003, Pavet *et al.* 2005). Kotchoni *et al.* (2009) reported that higher inherent ascorbic acid content delayed flowering and senescence irrespective of environmental conditions like photoperiod and temperature. It has been reported that deficiency of ascorbic acid causes early senescence in plants due to rapid loss in leaf chlorophyll content (Conklin and Barth 2004). There is no inclusive information on ascorbic acid quantity and biosynthesis rates of different organs and tissues of an individual plant during its developmental phases. However, photosynthetic organs are likely the centers of ascorbic acid synthesis in plants. So far, no quantitative estimates of endogenous ascorbic acid levels and its antioxidant role in delaying leaf and petal senescence are available in chrysanthemum cultivars. Therefore, an attempt has been made to quantify endogenous ascorbic acid content in petal and leaf tissues

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from different cultivars and studied their association with postharvest life as well as leaf longevity.

MATERIALS AND METHODS

Chrysanthemum cultivars were grown at Research farm of Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi during 2016. The plant material utilized for conducting the experiments consisted of 30 chrysanthemum cultivars including both standard and spray type. Standard cultural practices were followed to obtain healthy plants having uniform, straight cut stems. Standard varieties were harvested when their outer petals were fully elongated and spray varieties were cut when they were fully open but before anthesis and containing 6-8 flower buds. Harvested stems were immediately placed in a bucket containing pure water to keep them turgid and were brought to the laboratory. These stems were cut back to uniform length of 60 cm and the leaves from the lower 1/3rd portion of the stem were removed. 2-3 cm basal portion of the stems was recut under water and placed in distilled water to record vase life. The ascorbic acid estimation from leaves and petals was made at three different stages of flower development (Stage I-visible flower bud color; Stage II-Fully opened flower; Stage III-Flower at incipient senescence). In case of leaves, fully expanded top leaves were selected for the estimation of ascorbic acid. Leaf and flower samples were collected during morning or evening hours in ice box, brought to the laboratory and stored in -80^{0}C for ascorbic acid analysis. Data was subjected to analysis of variance using completely randomized design with three replications and three samples (both flowers and leaves) per replication. Following observations were recorded for the study

Vase life and leaf longevity (days): The number of days was calculated after harvest till termination of vase life. Wilting of 50% flowers and outer three rows of ray florets in spray and standard varieties respectively was considered as the criteria for termination of vase life. Leaf longevity is the time period for which an individual leaf is alive and is expressed in days, months or years. Three top most fully expanded physiologically active leaves from each plant per replication were tagged to count leaf longevity. Longevity was recorded by counting the days from visible coloured flower bud stage till leaf turns to visible yellow colour.

Ascorbic acid content (mg/100 g fw): Ascorbic acid estimation was done by dipyridyl-ferric chloride reagent method given by Law *et al* (1983) with slight modifications. Its estimation is based on the formation of pink coloured complex due to the reduction of dinitrophenylhydrazine by ascorbic acid to phenyl hydrazone in acidic medium (Mukherjee and Choudhari 1983). Half gram of fresh petals or petals preserved in liquid nitrogen were extracted with 10 ml of 6 % trichloroacetic acid and homogenate was centrifuged at 5000 × g ('g' is relative centrifugal force) at 4^{0}C temperature in a refrigerated centrifuge. Supernatant was used for estimation of ascorbic acid. Reaction mixture consisted of 0.3 ml petal extract, 0.75 ml phosphate buffer and 0.3 ml distilled water for determination. Colour development of reaction mixture take place by addition of 0.6 ml of 10% trichloroacetic acid, 0.6 ml of 44% orthophosphoric acid, 0.6 ml of 4% dipyridyl and 0.3 ml of 0.3% ferric chloride. After vertex mixing, the mixture was incubated in water bath at 40^{0}C for 40 min. The absorbance was recorded at 525 nm. The concentration of ascorbic acid was quantified by referring from a standard curve plotted with known concentration of ascorbic acid in the range of 0-100 μg.

RESULTS AND DISCUSSION

Inherent ascorbic acid content estimated from leaves and petals of thirty chrysanthemum cultivars at three different stages of flower bud development is presented in Table 1. Among 30 cultivars, maximum total ascorbic acid content (441.29 mg/100g fresh weight) was recorded in cv. Red Gold followed by cv. Jaya (336.34 mg/100g fresh weight) and minimum ascorbic acid content (93.55 mg/100 g fresh weight) was observed in cv. Arka Ravi followed by cv. Yellow Reflex (101.76 mg/100 g fresh weight). Chrysanthemum cultivars were found to contain maximum total ascorbic acid content in petals (235.00 mg/100 g fresh weight) and leaves (285.12 mg/100g fresh weight) in fully open flowering stage (Fig 1). Leaves were found to possess higher ascorbic acid compared to petals during all stages of flower development. These results are supported with the findings of Cheruth *et al.* (2015) where the level of ascorbic acid in date palm increased from pre-flowering to full bloom stage. Moreover, antioxidant potential of ascorbic acid was triggered with winter stress during crop period which enhanced the biosynthesis of endogenous ascorbic acid. Higher ascorbic acid content in leaves may be attributed to the fact that the chloroplasts are the main hub of synthesis and is probably related to its central role in photosynthesis (Foyer *et al.* 1991). Photosynthetic organs and certain storage organs and meristems are reported to have high concentrations of ascorbic acid (Loewus *et al.* 1987). In *Arabidopsis,* ascorbic acid content notably increased in

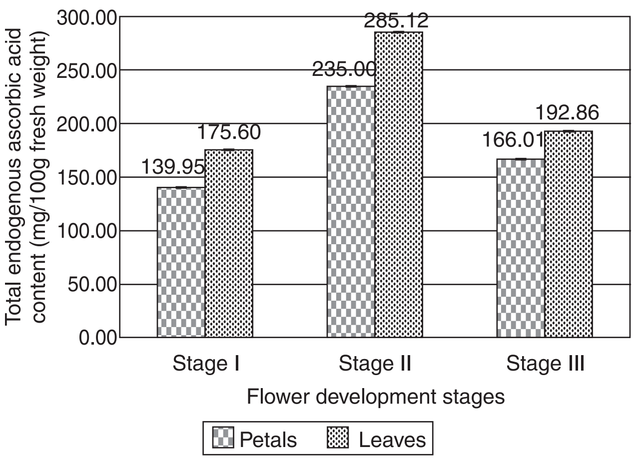

Fig 1 Variation in total endogenous ascorbic acid content of chrysanthemum cultivars at different stages of flower development (Stage I-visible flower bud color; Stage II-Fully opened flower; Stage III-Flower at incipient senescence).

Table 1 Total endogenous ascorbic acid content (mg/100g fresh weight) in leaves and petals of 30 chrysanthemum cultivars at three different flower development stages

Cultivar	Ascorbic acid content in petals			Ascorbic acid content in leaves			Mean
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III	
Pusa Centenary	74.86	175.57	135.57	177.71	243.43	197.00	167.36
Pusa Kesari	91.29	170.57	120.57	278.19	117.00	116.29	148.99
Tata Century	89.86	160.57	20.57	144.86	148.43	142.00	117.72
White Star	43.43	147.00	71.29	122.00	228.43	95.57	117.95
Yellow Star	209.14	232.24	66.29	138.43	67.00	49.21	127.05
Thai Chen Queen	229.14	247.00	224.14	302.00	352.71	282.00	272.83
Kundan	38.43	101.29	67.00	237.71	409.14	314.14	194.62
Arka Ravi	90.57	52.00	48.43	110.57	103.43	156.29	93.55
Shyamal	206.29	264.86	773.43	112.71	339.86	71.29	294.74
Jubilee	188.43	197.00	198.43	152.00	207.71	167.00	185.10
Maghi White	72.00	114.86	108.43	75.57	229.86	151.29	125.34
Karnal Pink	121.29	379.14	164.14	252.00	479.86	324.14	286.76
Kajal	174.14	484.14	131.29	252.95	254.14	159.86	242.75
Discovery	215.57	263.43	206.29	222.00	345.57	214.86	244.62
Mother Teresa	82.71	178.43	56.29	117.71	269.86	201.29	151.05
Shanti	72.00	124.86	154.14	109.14	239.14	196.29	149.26
Himanshu	152.00	183.43	24.86	174.86	253.43	138.43	154.50
Neelima	169.14	322.00	84.14	217.00	319.14	212.71	220.69
Yellow Reflex	59.14	106.29	54.14	93.43	191.29	106.29	101.76
Mahatma Gandhi	181.29	170.57	150.60	99.14	184.14	141.29	154.51
Birbal Sahni	194.86	262.71	149.86	217.00	374.14	153.43	225.33
Yellow Gold	299.14	364.86	184.86	263.19	477.00	293.43	313.75
Red Gold	303.43	496.29	533.43	219.86	567.71	527.00	441.29
Jaya	286.29	393.43	613.00	227.71	346.29	151.29	336.34
Haldighati	105.57	150.57	83.43	140.57	230.57	123.43	139.02
Basanti	86.29	362.00	122.00	157.71	464.86	332.71	254.26
Lalit	30.57	152.71	54.14	141.29	207.71	212.00	133.07
Jayanti	31.05	291.05	71.29	60.33	307.00	225.57	164.38
Ravi Kiran	223.43	369.86	279.14	289.14	398.43	223.43	297.24
John Baber	77.00	131.29	29.14	161.29	196.29	106.29	116.88
Mean	139.94	235.00	166.01	175.60	285.12	192.86	199.09
CD (P=0.05)	31.83	39.51	38.42	25.8	41.63	42.29	

Stage I-Visible flower bud color; Stage II-Fully opened flower; Stage III-Flower at incipient senescence

young leaves of plants approaching flowering (Attolico and De Tullio 2006). From the data it is clear that the total endogenous ascorbic acid content has been decreased towards incipient senescence stage due to diminished activity of antioxidants against ageing of plants. Similar findings to present study were indicated by Cavaiuolo *et al.* (2013). It was reported that there is rapid decline in ascorbic acid content during chrysanthemum petal senescence (Bartoli *et al.* 1997). It was suggested that endogenous level of ascorbic acid highly influences induction of flowering and regulation of senescence (Barth *et al.* 2006). Harman (1956) proposed that low levels of ascorbic acid would affect the photosynthetic mechanism resulting in accelerated senescence in plants.

The correlation data between total endogenous ascorbic acid content of different chrysanthemum cultivars (Table 2) and their leaf longevity and vase life, shows that there is a significant association between mentioned stages of flower development (Stage I-Visible flower bud color; Stage II-Fully open flower; Stage III-Flower at incipient senescence) with respect to their total endogenous ascorbic acid content in leaves and petals of all cultivars. Vase life of chrysanthemum cultivars were positively associated with inherent ascorbic acid content in leaves ($r = 0.669$) and petals ($r = 0.547$) at fully opened flower stage. Thus higher the endogenous ascorbic acid content in leaves and petals at fully open flower stage in chrysanthemum, longer will be the vase life. Sohal and Weindruch (1996) reported that there is an association between antioxidant capacity and potential life span of plants. There is positive link between decline in activities of antioxidants and plant senescence with simultaneous increase in destructive oxidants (Zimmermann

Table 2 Correlation matrix for total endogenous ascorbic acid of chrysanthemum cultivars and their vase life and leaf longevity

Observation	Ascorbic acid content in leaves			Ascorbic acid content in petals			Leaf longevity (days)
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III	
Stage II	0.483**						
Stage III	0.407*	0.765**					
Stage I	0.532**	0.519**	0.253NS				
Stage II	0.542**	0.729**	0.522**	0.666**			
Stage III	0.206NS	0.456*	0.189NS	0.640**	0.510**		
Leaf longevity (days)	0.179NS	0.002NS	-0.124NS	0.089NS	0.280NS	0.207NS	
Vase life (days)	0.479**	0.669**	0.241NS	0.329NS	0.547**	0.256NS	0.297NS

*Significant at P=0.05; ** Significant at P=0.01; NS - Non Significant. Stage I-Visible flower bud color; Stage II-Fully opened flower; Stage III-Flower at incipient senescence.

and Zentgraf 2005). Therefore, antioxidants concentration in tissues determines survivability of plant parts after detached from mother plant. Presence of higher ascorbic acid stabilizes chlorophyll content in leaves. Carbohydrate content of leaves was positively correlated with ascorbic acid levels in barley (Smirnoff and Pallanca 1996). Ascorbic acid synthesized in leaves and finally translocate to phloem tissues in plants (Franceschi and Tarlyn 2002). Stability of ascorbic acid is well maintained in phloem tissues of stems eventually cut stems of flowers live longer. There is pronounced association between vase life and ascorbic acid estimated from leaves (r = 0.479**) at visible flower bud color stage while non-significant association was found with ascorbic acid from petals from same stage. Leaf longevity (Fig 2) of 30 different cultivars was recorded. Life span of leaf decides survivability of plant and also acts as carbon sink for postharvest life of flowers. In present study, ascorbic acid content in leaves extracted from full flowering stage is poorly correlated with leaf longevity of all cultivars. This might be due to remobilization of nutrients from senescing tissues into young tissues and reproductive organs (flowers, seeds) maintaining vascular tissues until the very late stages of senescence as a part of recycling (Gan and Amasino 1997). In case of annual plants, there is a spontaneous decline in photosynthetic rates of leaves after full expansion (Hensel *et al.* 1993).There is a weak association between vase life and leaf longevity in chrysanthemum cultivars. Decline in photosynthetic rates of leaves acts as senescing signal in plants (Smart 1994). Life span of leaves on plant might have meager role in directing longevity of flower. The number of senescing leaves may increase during flowering and seed development.

Endogenous ascorbic acid in leaves and petals serves as important factor in deciding postharvest life of flowers. This study shows that there is a link between ascorbic acid level in plant parts (leaves and petals) at full flower open stage and vase life of chrysanthemum cultivars. Understanding the mechanism of a small antioxidant molecule in counteracting

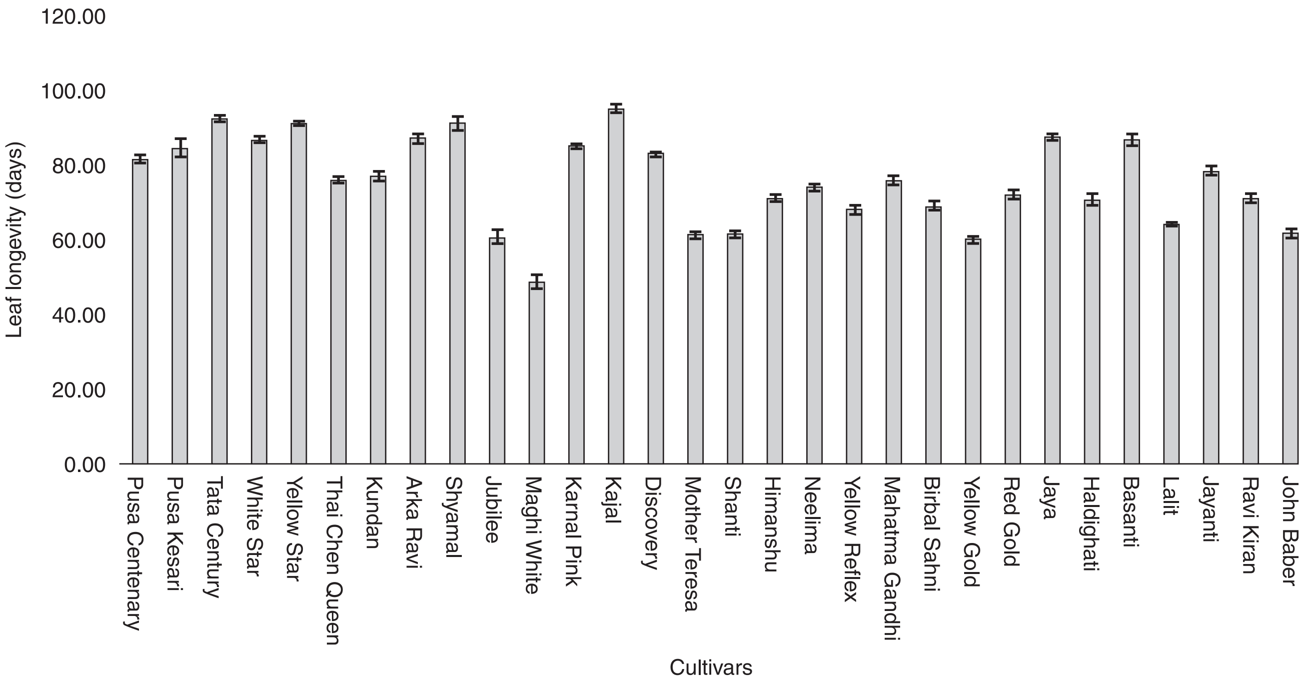

Fig 2 Leaf longevity of different chrysanthemum cultivars.

flower senescence is of crucial importance in flower crops. Controlling flowering time in *Arabidopsis* by artificial application of ascorbic acid provided an insight that the molecule is most significant in scheduling of flowering in cut flowers. Quantity of endogenous ascorbic acid present in chrysanthemum cultivars would help us in understanding and conducting effective postharvest studies towards senescence regulation. This study provides elite contrasting genotypes as breeding material for senescence triats. Cultivars with higher ascorbic acid content can be utilized to breed varieties with high longevity.The present study would be helpful for the farmers as well as florists to grow varieties with high longevity to meet consumer needs. However, postharvest studies on exogenous supply of ascorbic acid to chrysanthemum flowers might help to improve endogenous ascorbic acid level and understand precise role in deciding vase life of flowers.

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