



## Changes in The Ascorbic Acid Content During Leaf Senescence in Sericultural Crop *Morus Alba*. Linn

S. K. Khade

Department of Botany, Dattajirao Kadam Arts Science and Commerce College, Ichalkaranji, Dist: Kolhapur. (Maharashtra) – 416 115.

### ABSTRACT

The feeding of the silkworms on the leaves of mulberry is one of the significant components of sericulture. Thus, the leaf quality has an obvious impact on the performance of silkworms. Leaf senescence marks one of the critical phase in the life of leaves which is a genetically programmed and environmentally modulated event. Hence an attempt has been made to study the variations in Ascorbic Acid contents in young, mature and senescent leaves of the three mulberry cultivars namely M5 (K2), V1 and S36 are shown in the Figure. It is evident from the figure that, the young and mature leaves of three cultivars have the higher ascorbic acid content as compared to senescent leaves. Among the three cultivars, leaves of cultivar S36 contain the highest amount of ascorbic acid. In senescent leaves of all the three cultivars there is decline in the level of ascorbic acid content and this reduction is quite significant in the leaves of cultivar V1.

### KEYWORDS

Ascorbic acid, *Morus alba* Linn.

### INTRODUCTION-

The important agro industry sericulture involves rearing of silkworms for the commercial production of the silk. Mulberry leaves are used as food for rearing monophagous silkworm (*Bombyx mori* L) (Ullal and Narasimhanna, 1981). Mulberry leaves used as food for rearing of silkworms, larvae growth and development of silkworm and subsequent cocoon production depends mainly on the nutrient composition of mulberry leaves (Krishnaswami et al., 1971; Bhuyian, 1981). Many aspects like health and growth of the larvae, cocoon quality and raw silk quality are also influenced by quality of leaf. In addition to involving verities, different practices have been worked out to raise leaf production including irrigation, pruning and training types, application of fertilizers, etc. (Koul and Bhagat, 1991; Singh and Koul, 1997; Pandit et al., 1999). Ganga (2003), stated that, due to low protein level, declining (i.e. over mature, yellowing) leaves should be discarded. But at the same time there are several reports which indicate that leaf senescence in plants is promoted by several environmental constituents. Hence, In order to have further insight in to the above problem, a fate of various nutritional constituents during leaf senescence in the three cultivars of mulberry (viz- M5 (K2), V1 and S36) has been studied in the present investigation.

### MATERIAL AND METHOD-

A titrimetric method described by Sadasivam and Manickam, (1992) was followed to determine the leaf ascorbic acid content. The extract was prepared from fresh leaves (Young, Mature and Senescent) of the three cultivars of mulberry viz. M5 (K2), V1 and S36, in 4 % oxalic acid to reduce the pH and to stabilize its content by preventing catalytic oxidation. After centrifugation, clear supernatant was used to estimate the amount of Ascorbic Acid. Ascorbic Acid was oxidized to dehydroascorbic acid by reducing 2, 6 dichlorophenolindophenol (a blue dye) to a pink coloured solution. Oxalic acid was used as a titrant. The capacity of plant extract to reduce the dye is directly proportional to Ascorbic Acid content. Standard Ascorbic Acid (100 µg ml<sup>-1</sup>) was titrated against the dye till the appearance of persistent pink colour. The amount of the dye consumed was equivalent to the amount of Ascorbic Acid taken for titration and was calculated as follows,

Ascorbic acid (mg 100g <sup>-1</sup> )	=	0.5	X	Burette reading (extract)	X	Volume x 100
		Burette reading (Std)		5		Weight of sample(g)

### RESULT AND DISCUSSION-

It is evident from the figure.1. That, the young and mature leaves of three Mulberry cultivars VIZ. M5 (K2), V1 and S36 have the higher Ascorbic Acid content as compared to senescent leaves. Among the three cultivars, leaves of cultivar S36 contain the highest amount of ascorbic acid. In senescent leaves of all the three cultivars there is decline in the level of ascorbic acid content and this reduction is quite significant in the leaves of cultivar V1. Ascorbic acid in plants is important source of dietary vitamin. C. Its presence is noticed in all compartments of the cell. In chloroplast the concentration of Ascorbic Acid is about 20 mM. The site of biosynthesis of Ascorbic Acid is in mitochondria (Smirnov et al., 2001). L-galactone 1, 4-lactone is a main precursor for ascorbic acid biosynthesis (Smirnov et al., 2001). A very small amount of ascorbic acid is also synthesized through uronic acid pathway (Smirnov and Wheeler, 2000). Ascorbic acid (vitamin C) is utilized as a co-factor for violoxanthin deepoxide in chloroplast stroma, in quenching free oxygen radicals and reacting with hydroxy radicals, thus, protecting against O<sub>2</sub> mediated toxicity. According to Bendich et al., (1986), ascorbate is a good reducing agent, its free radical is very stable and Fairley non-reactive qualitative which are consistent with the relative non-toxicity of the latter and its antioxidant role in biological systems. In view of Loewus (1988) at least one functional role for L-ascorbic acid is now clearly identified that of participation in the mechanism for removal of active oxygen during photosynthesis. Ascorbic Acid peroxidase is involved in removing toxic hydrogen peroxidase (Arrigoni et al., 1992). It also acts as the precursor for biosynthesis of tartaric acid and oxalic acid. L-ascorbic acid serves as a co-factor for many enzymes (Arrigoni and De Tullio, 2000). Ascorbic acid is present in three forms in the plants viz. reduced ascorbic acid, non dehydroxy ascorbic acid (an unstable intermediate) and dehydro- ascorbic acid. Most of the ascorbic acid in plants is in the reduced form. Ascorbate affects many enzyme activities and physiological processes (Padh, 1990). According to Citterio et al., (1994), the main role of ascorbic acid may be related to its action in controlling the synthesis of hydroxyl proline containing proteins, which may be an essential requirement for cell progression through the G1 and G2 phases. Ascorbic acid metabolism in apoplast is described by Pingocchi et al., (2003). Apoplast mainly functions in control of stress, mainly the oxidative stress caused by environment, pathogen attack, growth and defense. Ascorbic acid is the major antioxidant buffer present in apoplast which is oxidized during these conditions. The oxidation as well as reduction of ascorbic acid is

controlled by enzyme ascorbate oxidase,  $\alpha$ -Tophopherol (vitamin E) is associated with cellular membranes, primarily the chloroplast membranes (Lichtenthaler et al., 1981), and at least one of its essential functions is to scavenging free radicals.  $\alpha$ - Topophenol acts as the primary antioxidant in leaf tissue and is regenerated by reactions with the water soluble antioxidants, ascorbic acid (vitamin C) (Flinch and Kunerst, 1985). Some workers have studied the effects of different concentrations of ascorbic acid on number of insects including silkworm (Etebari, 2002). It was reported that when 5th instar larvae of silkworm feed on leaves supplemented by 0.25-2% concentration of ascorbic acid significant increase was absorbed in larval and pupa weight, especially at 2% concentration (Etebari et al., 2004). In the present investigation also mature leaves of all the three mulberry cultivars are found to contain more ascorbic acid than the younger leaves. But in the senescent leaves a decline in ascorbic acid level is noticed and this is certainly not desirable in view of silkworm nutrition. The antioxidant activity of ascorbic acid is associated with resistance to oxidative stress and longevity both in animals and plants. Furthermore, the endogenous level of ascorbic acid has recently been suggested to be important in the regulation of various developmental processes including senescence and plant defense against pathogens (Pavet et al., 2005). Huang and Kao (2004) evaluated the protective effect of nitric oxide against senescence of rice leaves promoted by methyl jasmonate. Senescence of rice leaves was determined by evaluating decrease of protein content; methyl jasmonate treatment resulted in induction of leaf senescence and decrease in the level of reduced form ascorbic acid.

The values presented in the part –‘Results and Discussion’ represent average of three independent determinations.

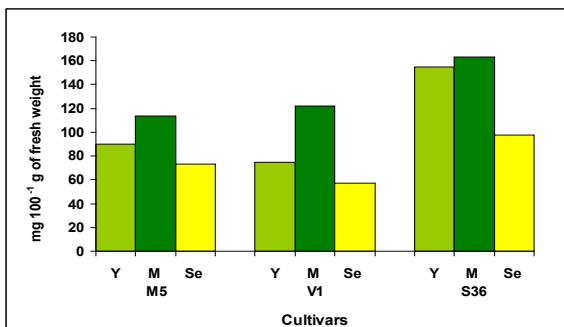


Fig. 1: Changes in the ascorbic acid content during leaf senescence in sericultural crop

*Morus alba* Linn. (Y = Young, M = Mature and Se = Senescent)

## CONCLUSION-

Ascorbic acid generally accepted all insects along with silkworms but in the senescent leaves ascorbic acid level is decreased so the senescent leaves are not used in silkworms feedings. The above findings indicate that, a decline in ascorbic acid in senescent mulberry leaves would lead to greater free radical damage in the senescent leaves and dismantling of the cellular machinery.

## REFERENCES-

1. Arrigoni, O. and De-Tullio, M. C. (2000). The role of ascorbic acid in cell metabolism: between gene-directed function and unpredictable chemical reaction. *Journal of plant physiology*, **157**: 481-488.
2. Arrigoni, O.; DeGara, L.; Tommasi, F. and Lisco, R. (1992). Changes in the ascorbate system during seed development of *Vicia faba* L. *Plant Physiol*, **99**: 235-238.
3. Bendich, A.; Machlin, L. J.; Scandura, O.; Burton, G. W. and Wyner, D. D. M. (1986). In: *advances in free radical Biology and Medicine* (Ed.) Pryor, W. A. (Pub.) Pergamon, New York Vol.2, pp.419-444.
4. Bhuyian, N. I. (1981). "Improvement of silkworm multiplication and silk production under Bangladesh condition", Bangladesh Agricultural University, pp. 60 – 61.

5. Citterio, S.; Sergio, S.; Stefania, S. and Elio, S. (1994). Ascorbic acid effect on the onset of cell proliferation in pea root. *Physiol. Plant.*, **92**: 601-607.
6. Etebari, K. (2002). Effect of enrichment of mulberry leaves (*Morus alba*) with some vitamins and nitrogenous compounds on some economic traits and physiological characters of silkworm *Bombyx mori* (Lep. bombycidae) M.Sc. Thesis, Isfahan University of Technology, Isfahan, Iran.
7. Etebari, K.; Kalival, B. B. and Matindoost, L. (2004). Different Aspect of Mulberry Leaves Supplementation with various Nutritional Compounds in Sericulture. *Int. J. Indust. Entomol.*, **9** (1): 15-28.
8. Flinch, B. F. and Kunerst, K. J. (1985). Vitamine C and E and antioxidative system agstem against in higher plants. *J.Agric Food Chem.*, **33**: 574-577.
9. Ganga, G. (2003). "Comprehensive Sericulture" Vol. I. Morigulture Oxford & IBH Publishing House Co. Pvt. Ltd. New Delhi, pp.181 – 183.
10. Huang, K. T. and Kao, C. H. (2004). Nitric oxide acts as an oxidant and delays methyl jasmonate-induced senescence of rice leaves. *J. Plant Physiol.*, **161**(1):43-52.
11. Koul, A. and Bhagat, R. L. (1991) : Effect of winter pruning on the spring leaf yield in mulberry. *Ind. J. Seric.* **30**: 131 – 134.
12. Krishanaswami, S., Kumararaj, S., Vijayraghavan, K. and Kasiviswanathan, K.(1971). Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety spacing and nitrogen fertilizer. *Ind. J. Seric.*, **9** (1): 79 – 89.
13. Lichtenthaler, H. K.; Prenzler, U.; Dource, R. and Joyard, J. (1981). Localization of prenylquinones in the envelope of spinach chloroplast. *Biochem Biophys, Acta*, **641**: 99-105.
14. Loewus, F. L. (1988). Ascorbic acid and its metabolic product. In: *The biochemistry of plants*. (Publ.) Academic Press, Inc. San Diego, California. pp. 85-107.
15. Padh, H. (1990). Cellular functions of ascorbic acid. *Cell Biol.* **68**: 1166-1173.
16. Pandit, R., Singh, D., Ram, K., and Koul, A. (1999): Effect of stump height on leaf yield in extensive plantation of mulberry. *Life Sci. Reporter* **1**: 15 – 17.
17. Pavet, V.; Olmos, E.; Kiddle, G.; Mowla, S.; Kumar, S.; Antoniw, J.; Alvarez, M.E. and Foyer, C.H. (2005). Ascorbic acid deficiency activates cell death and disease resistance response in Arabidopsis. *Plant Physiology*, **139**, 1291-1303.
18. Pingocchi, C.; Fletcher, J. E.; Barnes, S. and Foyer, C. H. (2003). The function of ascorbate oxidase (Ao) in tobacco (*Nicotiana tabacum* L.) *Plant Physiol.*, **132**: 1631-1641
19. Sadasivam, S. and Manickam, A. (1992). *Biochemical method for agricultural science*, Willey, Eastern Ltd. pp. 105.
20. Singh, D. and Koul, A. (1997): Effect of spacing on leaf yield in mulberry. *J. Seric.*, **5**: 17 – 19.
21. Smirnoff, N. and Wheeler G. L. (2000). Ascorbic acid in plants: Biosynthesis and function. *Critical Reviews in Biochemistry and Molecular Biology* **35**, 291-314.
22. Smirnoff, N.; Conclin, P. L. and Loewus, F. A. (2001). Biosynthesis of ascorbic acid in plants: a renaissance. *Annual Review of plant physiology and plant Molecular biology*, **52**:435-467.
23. Ullal, S. R. and Narasimhanna, M. N. (1981). *Handbook of practical sericulture*, **CSB**, Bangalore.