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CHANGES IN THE ACTIVITY OF ENZYME ACID PHOSPHATASE (E.C. 3.1.3.2) DURING LEAF SENESCENCE IN SERICULTURAL CROP MORUS ALBA LINN.

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ABSTRACT

An attempt has been made to study the changes in enzyme acid phosphatase during leaf senescence in mulberry (Morus alba Linn.). The changes in acid phosphatase activity during leaf senescence in the three mulberry cultivars namely M5 (K2), V1 and S36 are recorded. It is evident from the figure, that the activity of enzyme acid phosphatase is slightly increased with increase in leaf age of mulberry cultivars. Among the three leaf categories young leaves have the lowest enzyme activity in case of all the three cultivars .Thus, presence of the acid phosphatase in the leaves may affects silkworms, young and mature leaves of mulberry are fed to silkworm.

KEYWORDS : Enzyme Acid phosphatase, Morus alba Linn.

INTRODUCTION

Mulberry *(Morus alba* Linn.) leaves are used as food while rearing monophagous silkworm, *Bombyx mori* L. (Ullal and Narasimhanna,1981). Health and growth of the larvae, cacoon quality and raw silk quality are influenced by quality of leaf. Since, the physiological status of mulberry leaf is important in determining the nutritional quality; the age of leaf may influence silkworm feeding, Cacoon production depends mainly on nutrient composition of mulberry leaves (Krishnaswami *et al*., 1971; Bhuyian, 1981). Many aspects like health and growth of the larvae, cacoon 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) suggested that, over mature and yellow leaves with low protein content should be discarded to other nutritious feed to the silkworms. During present study nutritional constituents of young, mature and senescent leaves from three cultivars of mulberry (viz- M5, V1 and S36) has been studied and compared. 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), V1and S36) has been studied in the present investigation.

MATERIAL AND METHOD-

The enzyme acid phosphatase (E.C. 3.1.3.2) activity was assayed according to the method of McLachlan (1980). Five hundred milligrams of leaf material (of each category) was homogenized in 10ml ice cold 0.1M acetate buffer (pH 5.0). The extract was filtered through a four layered muslin cloth and centrifuged at 10,000 rpm for 20 minutes. The supernatant served as enzyme source. The assay mixture contained 3ml of p-nitrophenyl phosphate (0.1mg/ml of acetate buffer pH 5.0), 2 ml acetate buffer (pH 5.0) and 1ml enzyme. The reaction was allowed to proceed for 30 min. and then it was terminated by adding 1.5 ml of 1.68 N NaOH. Blank reaction mixture was prepared by adding all ingredients except the enzyme. The optical densities of developed pale yellow colour complex were read at 420 nm on Shimadzu double beam spectrophotometer. The soluble proteins in enzyme extract were estimated following the method of Lowry *et al.,* (1951) described earlier. The enzyme activity was expressed as Δ O.D. h^{-1} g^{-1} protein.

RESULT AND DISSCUSSION-

The changes in enzyme acid phosphatase (E.C. 3.1.3.2) activity during leaf senescence in the three mulberry cultivars namely M5 (K2), V1 and S36 are recorded in Fig.1. It is evident from the figure that the activity of enzyme acid phosphatase is slightly increased with increase in leaf age of mulberry cultivars. Among the three leaf categories young leaves have the lowest enzyme activity in case of all the three cultivars. The intracellular phosphatases, occurring in cytosol, plastids and vacuoles are causal factors for the release of Pi from organic compounds during seed germination, favoring internal Pi mobilization and translocation from senescent tissue (Lee, 1988 and Duff *et al*., 1994). Acid phosphatases comprise monomeric or dimeric glycoproteins which have subunit molecular masses of approximately 50-60 KD. These hydrolytic enzymes have a wide range of functions in plant metabolism. They have broad substrate specificity. According to Duff *et al.,* (1994), plant acid phosphatases (APases) do not normally exhibit an absolute specificity. Two distinct categories of plant APases have been distinguished on the basis of their relative substrate selectivity. The first type of plant APases are those truly 'non-specific' enzymes that show little or no substrate specificity. However, they are all possibly involved in the production, transport and recycling of Pi. The second category of plant APases are specialized enzymes such as the 3-P-glycerate (3 PGA) phosphatase from maize leaves (Randall and Tolbert, 1971) and the phosphoenol pyruvate (PEP) phosphatase of *Brassica nigra* (black mustard). Suspension cell cultures (Duff *et al*., 1989) which show clear but non absolute substrate specificity. These APases are important in the process of photorespiration and glycolysis (Plaxton, 1996). Phytases (E.C. 3.1.3.8 and 3.1.3.26) are well known class of acid phosphatase with a high affinity for inositol hexaphosphate (Phytate; IHP) and may therefore be particularly important for the hydrolysis of organic P sources in soils. These enzymes have been extensively studied from germinated seeds and grains (Nagai and Funahashi, 1962) and cotyledon tissue (Gibson and Ullah, 1988) of various higher plant species. According to Leigh and Walker (1980), the vacuole possesses acid phosphatase activity which could be distinguished by their very high susceptibility to low concentrations (100 µm) of ammonium molybdate. The acid phosphates was a soluble enzyme which hydrolyzed a large number of phosphate esters and had a pH optimum of 5.5. Acid phosphatase (APase) is believed to be involved in many physiological processes, especially regulation of phosphorus efficiency (Bieleski and Ferguson 1983). External and internal APase activities changes in response to phosphorus availability. Low phosphorus availability increases APase secretion to the rhizosphere in a number of plant species. Secretion of APase under phosphorus stress probably contribute to liberation of phosphorus from insoluble P sources in the soil (Tarafdar and Jungk,, 1987) and thus plays a role in P uptake. Cheour *et al*., (1992) noticed that in senescing leaf discs of cabbage, phospholipase-D, phosphatidic acid and phosphatase, lipolytic acyl hydrolase and lipozymes appeared to be involved in the breakdown of phospholipids. In cotton, imposing a water deficit on excised leaves by floating on PEG solution caused increased histochemical staining, for phosphatase and lipase in chloroplast. This increased activity coincided with symptoms of chloroplast senescence (Da Silva *et al*., 1974). Yeh *et al.,* (1995) noticed that abscisic acid and methyl jasmonate exhibited ABA-like effects by promoting senescence of detached rice leaves by inducing acid phosphatase activity. According Barrette-Lennard *et al.,* (1982), the possible roles of phosphatase and other hydrolytic enzymes in senescence might be more apparent if increased enzyme activities could be related to the senescence of specific organelles and irreversible metabolic changes. Besford and Spred (1979) suggested two possible functions for this increased activity; i.e. enhanced translocation of phosphate from mature to young leaves and transport of Pi across the plasmalemma.

Fruit ripening is regarded as a special type of senescence and an increase in acid phosphatase during fruit ripening has been noticed in number of studies (Domingos *et al.,* 1999).

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

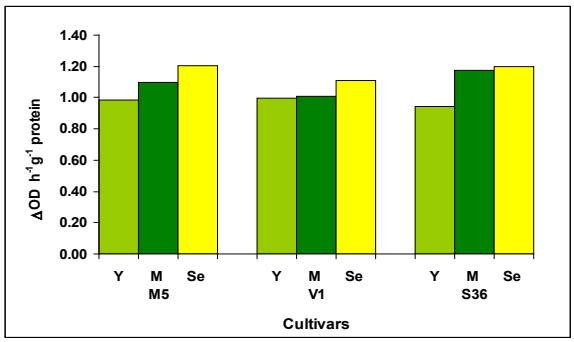

Fig. 1: Changes in the activity of enzyme acid phosphatase during leaf senescence in sericultural crop *Morus alba* Linn. (Y = Young, M = Mature and Se = Senescent)

CONCLUSION

In case of mulberry leaves, the mature leaves have greater phosphatase activity than the young leaves. Further, the activity of the enzyme in senescent leaves is slightly higher than both these categories inspite of the fact that the 'P' levels in the senescent leaves is reduced in comparison to young and mature leaves. As we have already observed in senescent leaves there is decrease in titratable acidity. Thus, the cellular environment also dose not appears to be congenial for the action of acid phosphatase activity. Hence, there is only marginal increase in this enzyme in the senescent leaves. The major hydrolysis of phosphates in such situation may be cared out by alkaline phosphatases.

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