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Changes in The Activity of Enzyme Peroxidase (Ec 1.11.1.7) During Leaf Senescence in Sericultural Crop Morus Alba Linn

KEYWORDS: Enzyme peroxidase (EC 1.11.1.7), Morus alba. Linn

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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. The activity of enzyme peroxidase (EC 1.11.1.7) in young, mature and senescent leaves of the three mulberry cultivars VIZ. M5 (K2), V1 and S36. It is evident from the figure the mature leaves of three cultivars have relatively very high enzyme activity as compared to young and senescent leaves. The leaf senescence in mulberry is found to be accompanied by a general decline in the activity of this enzyme.*

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 cacoon 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, 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), 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.

Material and method-

To study the enzyme peroxidase (EC 1.11.1.7) activity the method of Maehly (1954) was followed. 500 mg fresh leaf material (of different categories VIZ. M5 (K2), V1 and S36) of each cultivar was homogenized in 15 ml ice-cold (0.1 M) phosphate buffer (pH 7) and filtered through 4 layers of muslin cloth. The filtrate was centrifuged at 10,000 rpm for 20 minutes and supernatant was used as source of enzyme. The reaction mixture contained 2 ml of 0.1 M phosphate buffer (pH 7), 1 ml of 20 mM guaiacol and 1 ml enzyme extract. The reaction was initiated by the addition of 0.05 ml H_2O_2 (1 mM) Changes in optical density due to oxidation of guaiacol was recorded after 30 min. at 470 nm. The soluble proteins in the enzyme extract were determined according to the method of Lowry *et al.,* (1951). The enzyme activity was expressed as unit h^{-1} mg^{-1} protein.

Result and discussion

The activity of enzyme peroxidase (EC 1.11.1.7) in young, mature and senescent leaves of the three mulberry cultivars VIZ. M5 (K2), V1 and S36 is shown in Fig.1. It is evident from the figure that the mature leaves of three cultivars have relatively very high enzyme activity as compared to young and senescent leaves. The leaf senescence in mulberry is found to be accompanied by a general decline in the activity of this enzyme.

Among the plant enzymes, peroxidase is perhaps the most extensively studied enzyme (Penel *et al.*, 1992). According to Crozier *et al.*, (2000), the enzyme peroxidase or IAA oxidase catalyzes decarboxylative catabolism of IAA by removing the 1'-carboxyl group and decarboxylated oxindoles (Oxindole-3-methanol, 3-methylene oxindole, and 3-methyloxindole) or indoles (indole-3-methanol, indole-3-aldehyde and indole-3-carboxylic acid). According to Gasper *et al.,* (1982), besides the possible involvement of peroxidases in many reactions, isoperoxidases play four major roles in growth and development through their control and participation in auxin catabolism and consequently the regulation of endogenous free auxin level, lignin formation and cell wall biogenesis, defense mechanism against pathogens and some respiratory processes. The leaf senescence represents a terminal developmental stage and there are many reports which indicate that there is an increase in peroxidase activity during leaf senescence (Lauriere, 1983). According to Birecka *et al.*, (1979), peroxidase has been implicated in plant senescence mainly due to its ability to oxidize IAA and to participate in lignin formation. An increase in the activity of this enzyme in particular of its distinctive isoforms, has been observed in many species in the course of physiological or ethylene induced senescence. In primary leaves of *Phaseolus vulgaris* peroxidase tend to increase in leaf homogenates with advancing senescence, but only low and essentially constant activity of peroxidase was detectable in chloroplasts during senescence (McRae and Thompson, 1983). According to Gasper *et al.,* (1982), besides the possible involvement of peroxidases in many reactions, isoperoxidases play four major roles in growth and development through their control and participation in auxin catabolism and consequently the regulation of endogenous free auxin level, lignin formation and cell wall biogenesis, defense mechanism against pathogens and some respiratory processes.Its association with electron transfer from $NADH_2$ to cytochrome during respiration was studied by Ivonova *et al.*, (1967). In the absence of H_2O_2, peroxidase can catalyze oxidation of NADH and NADPH with the help of atmospheric oxygen. Peroxidase shows oxidase activity besides the peroxidase activity i.e. it catalyzes the oxidation of different substances by atmospheric oxygen under aerobic conditions without exogenous peroxide i.e. $NADH_2$, $NADPH_2$, indol acetic acid and phenyl pyruvate (Fric, 1976). As early as 1968 Parish sug-

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gested that an increase in peroxidase activity is one of the most reliable indicators of maturity and senescence. Patra and Mishra (1979) studied the peroxidase activity during leaf development and senescence in 8 monocotyledonous (*Amomum aromaticum, Eleusine coracana, Hordeum vulgare, Oryza sativa, Pennisetum typhoideum, Sorghum vulgare, Triticum vulgare, Zea mays*) and 8 dicotyledonous spp. (*Arachis hypogaea, Boerhavia diffusa, Chenopodium album, Crotalaria striata, Hibiscus micranthus, Nicotiana plumbaginifolia, Raphanus sativus, Tabernaemontana coronaria*). They observed increase in peroxidase activity towards the basal leaves. Reddy and Srivastava (2003) reported loss in fresh weight and rise in respiratory activity in ripening mango cultivar, Baneshan. They noticed very high antioxidant enzyme peroxidase activity at mature green stage, which significantly declined as the ripening proceeded. In three varieties studied in the present investigation, peroxidase activity shows a similar trend during senescence. The level of enzyme peroxidase, in different developmental stages of the leaves showed marked variation in the MR-2 variety of mulberry leaves (*Morus alba* L.) (Sangeetha and Ramarethinam, 2000). The activity of peroxidase showed quite a significant decrease from the nodal leaves to the senescent mulberry leaves. At the same time, Ford and Simon (1972) suggested that the peroxidase increase can not be taken as an case of cucumber cotyledon senescence.. Singh *et al.,* (2004) examined the activity of peroxidase in bracts and in the upper most 1st and 3rd leaves of sunflower at flower opening stage and seed feeling stage. They noticed that upper most leaf had lower activity of peroxidase as compared to the 3rd leaf at flowering opening stage but activity increased significantly at seed filling stage. Thus, plant age may also influence the pattern of variations in the peroxidase activity in leaves of different ages. Ohe *et al.,* (2005) designed experiments to clarify the mechanism of the decline in oxidative stress tolerance of leaves during the course of senescence. They studied the difference in the levels of various antioxidants and antioxidant enzymes in leaves of different ages, in 8 week old tobacco plants. Even though the chlorophyll content had not changed, the protein content and the photosynthetic capacity were significantly decreased in the older leaves which had higher levels of H_2O_2. Analysis of the components of the active oxygen species – scavenging system revealed that older leaves had significantly lower level of the antioxidant enzymes, especially ascorbate peroxidase. The values presented in the part –'Results and Discussion' represent average of three independent determinations.

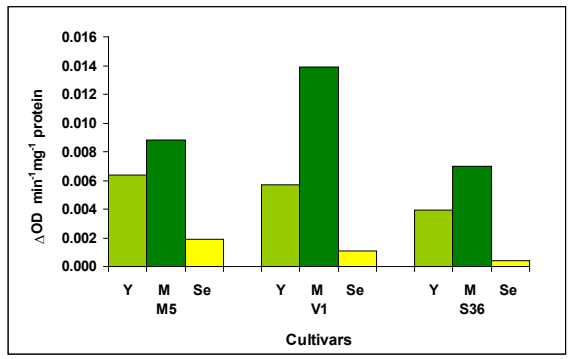

Fig.1: Changes in the activity of enzyme peroxidase during leaf senescence in sericultural crop *Morus alba* **Linn. (Y = Young, M = Mature and Se = Senescent)**

CONCLUSION-

The findings suggested that the decline in the levels of antioxidants and antioxidant enzymes associated with leaf senescence lead to lower photo oxidative stress tolerance, which might in turn accelerate the propagation of senescence. In the senescent leaves of the three mulberry cultivars probably similar situation may arise since, there is a decline in the overall peroxidase activity.

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