Research Paper Botany

Change The Activity of Enzyme Dehydrogenase (Ec 1.1.1.4) 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 changes in levels of enzyme dehydrogenase in young, mature and senescent leaves of all the three mulberry cultivars Viz. M5 (K2), V1 and S36. It is evident from the figure, that the young leaves of three cultivars have the highest dehydrogenase activity as compared to mature and senescent leaves. The young leaves of cultivar V1 have shows the highest enzyme activity followed by significant decrease in the enzyme activity in mature and senescent leaves as compared to cultivars S36 and M5 (K2).

KEYWORDS : Enzyme Dehydrogenase, 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 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. 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:-

Healthy young, mature and senescent leaves of mulberry cultivars –M5 (K2), V1 and S36 were collected from identical positions and brought to the laboratory. The activity of enzyme dehydrogenase (EC 1.1.1.4) was studied the tetrazolium method of Kittoch and Law (1957). 100 mg fresh leaf material (belonging to three categories) was cut into small pieces with razor. The leaf slices were incubated in dark place for 1h in a vial containing 4 ml of 0.2% T.T.C. (2-3-5 triphenyl tetrazolium chloride). After this incubation period, plant tissue was washed 2-3 times with distilled water, then surfaces were blotted and treated with 5ml of methoxy ethanol for extraction of the red coloured formazon, which is formed due to the activity of dehydrogenase. The optical density of coloured formazon was measured at 470 nm. The enzyme activity is expressed as $\Delta OD\ h^{-1}g^{-1}$ fresh tissue.

Result and discussion

The changes in activity of enzyme dehydrogenase (EC 1.1.1.4) in young, mature and senescent leaves of all the three mulberry cultivars namely M5 (K2), V1 and S36 are shown in Fig.1. It is evident from the figure that, the young leaves of three cultivars have the highest dehydrogenase activity as compared to mature and senescent leaves.

According to Weimberg (1970), the enzyme dehydrogenase is one of the major energy yielding enzymes in cell metabolism and any fluctuations in its activity would result in the disruption and alterations of the growth. These are oxidizing enzyme catalyzing electron transfer from the donor to an acceptor other than molecular oxygen. Various dehydrogenases are involved in glycolysis, pentose phosphate pathway and TCA cycle in seeds (Chakravorti and Burma, 1959). Glyceraldehyde 3 phosphate dehydrogenase is an important glycolytic enzyme. The conversion of pyruvate an end product of glycolysis, to acetyl CoA is catalysed by multienzyme pyruvate dehydrogenase complex. The important function of pentose phosphate cycle is to provide adequate amount of NADPH for various synthetic processes and this is accomplished with two dehydrogenases. Since, the respiratory process is compartmentalized in cytoplasm and mitochondria. The cytosolic dehydrogenases generate reducing potentials NADH and NADPH which are utilized in various metabolic activities in growing tissues and also replenish the mitochondrial compartment with reducing powers in the event of metabolic limitations (Chen *et al.,* 1988). The reaction catalysed by enzyme glucose 6 phosphatase dehydrogenase (GDH) is regarded as regulatory step of pentose phosphate pathway. Similarly isocitrate dehydrogenase is important regulatory enzyme of TCA cycle. A relationship between dehydrogenase activity and respiratory rate has been established (Price and Thimann, 1954). It acts like oxidizing enzyme catalyzing electron transfer from the donor to an acceptor other than molecular oxygen. NADPH produced during light reaction of photosynthesis provides substrate for various dehydrogenases in the Calvin cycle as well as malate dehydrogenase of C_4 pathway. Sorbitol dehydrogenase (SDH), has been identified as the primary enzyme that metabolites sorbitol in apple fruit (Beruter, 1985) so it may play a critical role in defining sink activity in apple. The fate of different dehydrogenases during the course of leaf senescence has been studied by several workers. Calle *et al.,* (1986) studied the sub cellular localization of NAD^+ dependent glutamate dehydrogenase (GDH) in leaves of barley (*Hordeum vulgare* L.) during the leaf senescence induced by detachment and incubation in the dark. GDH strongly increased in the cytoplasmic fraction during senescence. It also showed a retarded and low increase in the mitochondrial fraction, no GDH was detected in the chloroplast fraction. Masclaux *et al.,* (2000) have demonstrated that glutamate dehydrogenase is one of the factor involved in sink to source transition in case of tobacco leaf development. Vera *et al.,* (1990) found that senescent chloroplast have high ferricyanide reducing activity, probably related to NADH dehydrogenase. Pistelli *et al.,* (1992) recorded increase in activities of malate dehydrogenase during the foliar senescence of leaf beet. In peroxisomes from senescent leaves the Km of Isocitrate dehydrogenase (ICDH) decreased almost 11 fold. This kinetic behavior resulted in the catalytic efficiency approximately 12 times higher for peroxisomal ICDH from senescent leaves (Corpas *et al*., 1999). However, the protein levels of ICDH in peroxisomes were not altered during senescence. The physiological significance of the change in the Km of peroxisomal ICDH during senescence is probably double; first, to compete with isocitrate lyases , an enzyme of the glyoxylate cycle which is present in the peroxisomes from senescent leaves , for the intracellular pool of isocitrate; and second , to provide a higher and constant supply of NADPH in order to eliminate, by the ascorbate glutathione cycle, the excess of H_2O_2 producing during senescence when catalase activity decreases dramatically (Pastori and del Rio,1997) . Jordi *et al.,* (1996) studied leaf senescence of *Alstroemeria* cut flowering stem in the dark. Two dimensional (2D) electrophoresis reveled that a polypeptide with an apparent molecular mass of 50 (+ - 2) KDa and isoelectric point of 5.0 (+ - 0.1) accumulated during the senescence process. Treatments which delayed leaf senescence (Light and

Gibberellic acid) diminished the accumulation of these polypeptides. This polypeptide was purified and one of the peptides resulting from digestion with trypsin was sequenced. The sequence shows a high degree of homology to that of 3 isopropylmalate dehydrogenase enzyme involved in leucine metabolism. According to these workers possibly, leucine plays role in carbon partitioning between sources and sink in the plant.

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

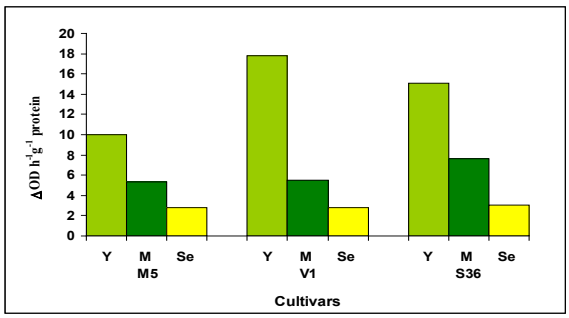

Fig. 1: Change the activity of enzyme dehydrogenase during leaf senescence in sericultural crop *Morus alba* **Linn. (Y = Young, M = Mature and Se = Senescent)**

CONCLUSION-

The above findings indicate that, in the leaf metabolism different kinds of dehydrogenase are involved and their behavior during leaf senescence is not uniform. TTC (Trizolium tetrachloride) reduction assay of dehydrogenase gives us a general idea of the metabolic status of the plant tissue. Hence, a decline in TTC reduction in senescent mulberry leaves indicates a general decline in vitality and metabolic turnover during the course of senescent in mulberry leaves.

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