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**SHORT COMMUNICATION**

# CHANGES IN SOME ASPECTS OF OXIDATIVE METABOLISM IN SENESCING MUNGBEAN COTYLEDONS AS AFFECTED BY SEEDLING DECAPITATION

**SURJENDU KUMAR DEY***

P.G. Department of Enviornmental Sciences, Fakir Mohan University, Vyasa Vihar, Balasore-756 019, Orissa

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**Decapitation effects on some aspects of oxidative metabolism in senescing mungbean (*Vigna radiata* (L.) Wilczek cv. PDM-54) cotyledons were studied. There was gradual decrease in fresh weight, dry weight and soluble protein contents. However, pigment contents recorded increase till 7$^{th}$ day and a subsequent decline. On shoot decapitation even though fresh weight, dry weight and protein contents showed lesser depletion, the photosynthetic pigment contents declined further. The superoxide dismutase activity increased where as catalase activity decreased in senescing cotyledons which favoured the chances of accumulation of $H_2O_2$ as a result increase in lipid peroxidation was noticed. However, on reversal of senescence by decapitation, there was decline in superoxide dismutase and increase in catalase activities that could limit the oxidative corrosion in the tissues, as observed in the form of lowering lipid peroxidation in rejuvenated cotyledons. Thus, catalase ($H_2O_2$ – scavenging) activity seems to be one of the key regulatory aspect of oxidative metabolism in senescing mungbean cotyledons.**

***Key words*: Catalase, cotyledon rejuvenation, lipid peroxidation, reactive oxygen species, superoxide dismutase**

Senescence is known to be a genetically programmed aging process that leads to the death of cells, organs and also of the whole organisms. This final phase of development in plants is accompanied by a sequence of events concerned with cellular disassembly, subsequent mobilization of materials and also transport of some of the degradation products to other parts (Trippi and Thimann 1983). Our knowledge on senescence process in plants has mainly come from various studies using the lateral organs. Cotyledons are such short lived lateral parts of plants which represent the specific reserve organs. However, in epigeal plants in addition to their reserve-mobilizing functions, the cotyledons also act as photosynthetic organs and their photosynthetic capabilities depend on the growth pattern of seedlings. Such cotyledons start greening and photosynthesize soon after their emergence and after achieving the oxygen evolution rates at par with those of expanded leaves, they senesce with decline in photosynthetic efficiency (La Rocca *et al*. 1996). Even though cotyledon senescence is not fundamentally different from leaf senescence, organ specific differences between cotyledons and primary leaves are there with respect to the photosynthetic activity regulation during natural senescence (La Rocca *et al*. 1996). Because of its dual nature as nutrient reservoir and photosynthetic organ during early stages of seedling growth, studies on the senescence pattern of epigeal cotyledons have always attracted the attentions of plant physiologists.

The oxidative metabolism in aerobic cells involves the steady state rate of formation of different reactive oxygen species (ROS) and their decomposition by the endogenous antioxidative systems. The alteration in such

*E-mail: surjendufmu@rediffmail.com

systems, as is known to occur under different developmental stages and also under biotic and abiotic stresses (Elstner *et al*. 1988, Cakmak *et al*. 1993, Dey *et al*. 2007), favours the formation of toxic ROS to a level beyond the capacity of the endogenous antioxidative protective systems to scavenge them off, creating a situation called oxidative stress. Thus oxidative stress situation is essentially a regulated process where the fate of plants is determined by the equilibrium between ROS generation and antioxidative efficiency of plants. Involvement of ROS in leaf senescence is a well established phenomenon. There are also some reports on the changes in antioxidative profile and other parameters in epigeal cotyledons during natural and dark induced senescence (Kanazawa *et al*. 2000, Ananieva *et al*. 2004). But reports on oxidative metabolic status of cotyledonary tissues undergoing senescence and the effect of shoot decapitation on it are scanty. Therefore, in this study some aspects of oxidative metabolism were analyzed during the early stages of senescing mungbean cotyledons. The effect of shoot decapitation on the changes in senescence pattern in terms of oxidative metabolism was also studied. As indicators of oxidative metabolism, the activities of antioxidative enzymes like superoxide dismutase (SOD) and catalase (CAT) along with lipid peroxidation level were determined.

Fresh and viable seeds of mungbean (*Vigna radiata* (L.) Wilczeck cv. PDM-54) were selected for uniformity of size and were sown in the soil (sandy loam and compost in the ratio of 3:1) in earthenware pots. The pots were kept open to sunlight and watered regularly with tap water. Since the cotyledons dried out within 8 to 10 days, the experiments were restricted within 9$^{th}$ day of sowing. Fresh cotyledons were collected from day 1 (24 hours after sowing) till 9$^{th}$ day, with one day intervals, for various biochemical analyses. For rejuvenation of senescing cotyledons, the epicotyl portions with differentiated leaves and apical buds in 7-day old seedlings were decapitated in one pot. The rejuvenated cotyledons of the same pot were collected on 9th day for various analytical purposes.

For fresh weight, cotyledons were weighed separately. The same cotyledons were kept in a hot air oven at 80 $^{o}$C for 48 hours and then weighed to record the dry weight. Photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) of the cotyledons were extracted with cold 80% acetone and were measured spectrophotometrically (Lichtenthaler and Wellburn 1983). Phosphate buffer extractable protein of the cotyledonary tissues was estimated following the phenol reagent method of Lowry *et al*. (1951). Malondialdehyde (MDA), a decomposition product of peroxidized polyunsaturated fatty acid components of membrane lipid, was extracted with 5% trichloroacetic acid (w/v) and was estimated by following the method of Heath and Packer (1968) as the thiobarbituric acid reactive material.

For extraction of enzymes, the cotyledons were homogenized under ice-cold conditions with a glass mortar and pestle in extraction buffers containing 10% (w/v) insoluble polyvinylpolypyrrolidone. The buffers used for extraction of enzymes were: 50 mM Tris-HCl buffer, pH 7.5 containing 0.1 mM ethylenediamine tetraacetic acid for SOD and 50 mM potassium phosphate buffer, pH 7.5 for CAT. The homogenates were centrifuged at 17, 000 *g* for 10 min at 0 $^{o}$C and the resulting supernatants were desalted by passing through gel filtration columns, packed with presoaked Sephadex G-25 (fine). The eluted fractions were tested for protein and the fractions responding to protein tests were collected and used for enzyme assays. For superoxide dismutase (SOD), the assay mixture was the same as formulated by Marshall and Worsfold (1978) and the detail schedules including the initiation and termination of reactions were performed as described by Giannopolities and Ries (1977). Catalase (CAT) activity was assayed following the method of Aebi (1983).

One unit of SOD was defined as the amount that inhibits the superoxide driven nitrobluetetrazolium reduction by 50% under the assay conditions. Activity of CAT was expressed as katals, i.e. mole of substrate ($H_2O_2$) used up per second. All the experiments were performed for five times, with three replicates in each time. The mean values are presented in figures. Standard deviations (SD) are also indicated as vertical bars.

Mungbean cotyledons are reserve organs whose basic functions are to nourish the developing seedlings till the photosynthetically efficient leaves emerge. Therefore, because of the reserve mobilization, decline in the fresh weight and dry weight of the cotyledons is

expected in a linear fashion with increase in the age. In this experiment also, from day 1 till 9th day, a steady decline in both fresh weight and dry weight of the cotyledons has been noted (Fig. 1). However, in 3-day old cotyledons, the fresh weight was slightly higher than that of the day 1 cotyledon. Since, there was no increase in dry weight of 3-day old cotyledons (Fig. 1), the increase in fresh weight of the same was simply because of the excessive hydration of the tissues. As expected, there was less decrease in both fresh and dry weight of the 9-day old cotyledons with shoot decapitation on 7th day, in comparison to its counterparts undergoing natural senescence (Fig. 1). This indicated that even within two days of shoot decapitation, there was less depletion of the reserves as a result increase in the fresh as well as dry weight of the cotyledons was observed. The cotyledons were, thus, rejuvenated. The life span of the rejuvenated cotyledons increased, about 4-5 days more, in comparison to their normal counter parts.

There was a rapid increase in the chlorophyll *a*, chlorophyll *b* and carotenoids contents in the cotyledons till 7th day and then there was decline (Fig. 1). Chlorophyll *a* content was always more than chlorophyll *b* and the ratio between chlorophyll *a* and *b* as well as between total chlorophyll and carotenoids increased till 5th day and then declined (Fig. 1). In 9-day old rejuvenated cotyledons with shoot decapitated on 7th day, no increase in pigment content was found in comparison to its normal counter parts, rather the pigment content further declined. However, the ratio between chlorophyll *a* and *b* and also between total chlorophyll and carotenoids increased in rejuvenated cotyledons which were due to further decrease in chlorophyll *b* and carotenoids contents in comparison to that of naturally senescing cotyledons. Recovery of total chlorophyll content has been reported in *Cucurbita pepo* cotyledons after 10 days of rejuvenation where there was almost two fold increase in the chlorophyll content in comparison to that of the normal cotyledons (Ananieva *et al*. 2004). Thus, the trend of the pigment contents in rejuvenated cotyledons could be understood by lengthening the rejuvenation period beyond two days. The reserve mobilization in the cotyledons was very fast during the study period, as has been observed in the form of sharp decline in soluble protein content (Fig. 1). As it was

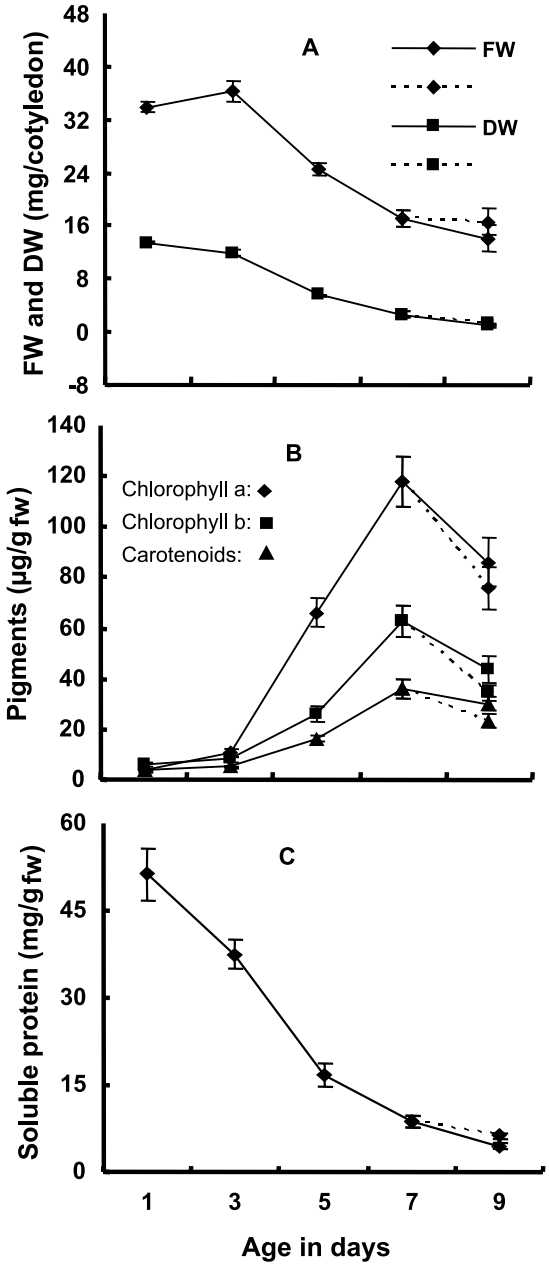


**Fig. 1. Changes in fresh weight (FW) and dry weight (DW) (A); photosynthetic pigment contents (B) and soluble protein content (C) of mungbean cotyledons undergoing natural senescence. Values for 9-day old cotyledons with shoot decapitation on 7th day are represented as broken lines (- - -). The values are mean of five independent experiments, each with three replicates. Vertical bars indicate SD**

expected, there was lesser depletion in soluble protein content of the rejuvenated cotyledons and due to this more soluble protein content was noticed in rejuvenated

cotyledons in comparison to that of the normal one of the same age. Thus, even though lesser depletion in the reserves was noticed in the rejuvenated cotyledons, no increase in the photosynthetic pigment contents was found in the same during the study period.

Superoxide dismutase and CAT are important antioxidative enzymes that prevent the accumulation of ROS like $O_2^{\cdot-}$ and $H_2O_2$ respectively in aerobic cells (Halliwell and Gutteridge 2007). The SOD activity dismutates the $O_2^{\cdot-}$ and generates $H_2O_2$ as the dismutation product where as CAT scavenges the $H_2O_2$ to $O_2$ and $H_2O$. Alterations in the activities of these two enzymes have been widely reported in different types of senescing organs, both under natural and artificial conditions (Pastori and del Rio 1994, 1997, Hodges and Forney 2000, Kanazawa *et al*. 2000). In this study, the SOD activity increased with the age of the cotyledons (Fig. 2). This indicated that the steady state levels of superoxide radicals in the tissues declined. Consequently, there was chances of accumulation of $H_2O_2$ (the dismutation product) in the tissues. At the same time, there was decline in CAT activity with increase in the age of cotyledons (Fig. 2). Thus, the protection against $H_2O_2$ was poor in the senescing cotyledons. With respect to SOD and CAT activities, the results of this study corroborate the studies of Pastori and del Rio (1994, 1997) in senescing leaves. But there are also reports of decreasing SOD activity in naturally senescing cucumber cotyledons (Kanazawa *et al*. 2000). However, as reported herein, the CAT activity also declined in the same study. In 9-day old rejuvenated cotyledons (with shoot decapitation on 7$^{th}$ day), there was decrease in SOD and increase in CAT activities in comparison to the same of the naturally senescing cotyledons (Fig. 2). Thus, in rejuvenated cotyledons although the protection against $H_2O_2$ was restored, $O_2^{\cdot-}$ scavenging efficiency declined. So far as SOD activity in rejuvenated organ is concerned, the results of this study contradict the findings of Dhindsa *et al*. (1981), where about 50% increase in SOD activity (on fresh weight basis) was reported in the reversal of senescence of tobacco leaves. But increase in CAT activity in rejuvenated cotyledons, as reported herein, is in agreement with the findings of several reports (Dhindsa *et al*. 1981, Venkatarayappa *et al*. 1984). Thus, perhaps during the reversal of senescence and rejuvenation process among the different antioxidative strategies of the cells, protection against $H_2O_{2-}$ plays important role.

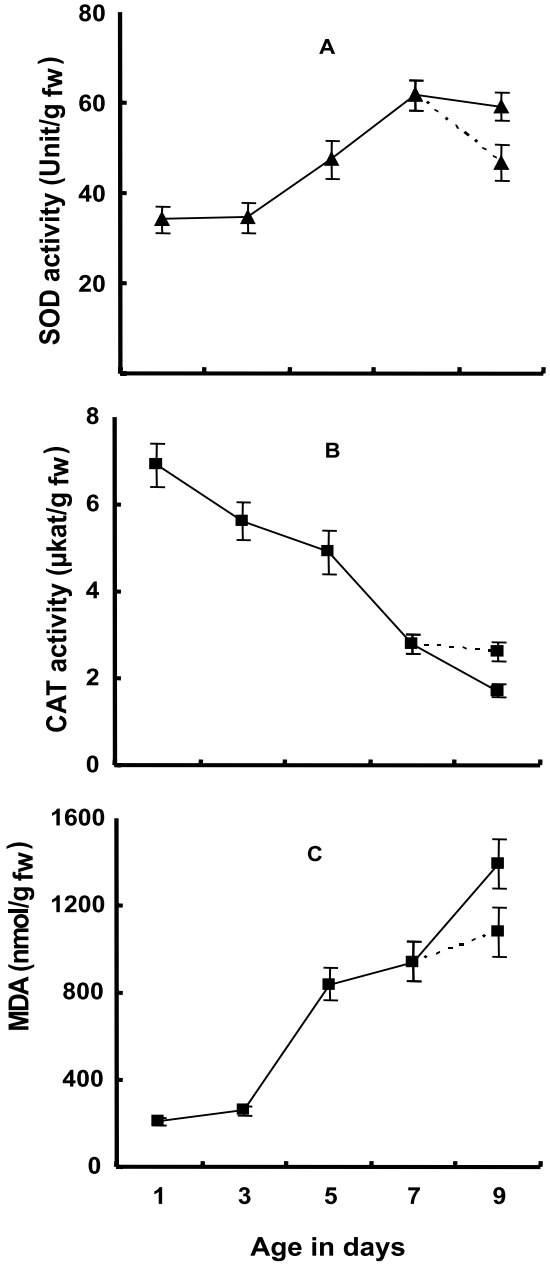


**Fig. 2. Changes in superoxide dismutase (SOD) (A) and catalase (CAT) (B) activities and lipid peroxidation (C) in mung bean cotyledons undergoing natural senescence. Values for 9-day old cotyledons with shoot decapitation on 7$^{th}$ day are represented as broken lines (- - -). The values are mean of five independent experiments, each with three replicates. Vertical bars indicate SD.**

In the present study, during the natural senescence of the cotyledons there was increase in the activity of SOD and decrease in the activity of CAT (Fig. 2) which favoured the accumulation of higher levels of $H_2O_2$ in the tissues. In aerobic cells, the hydroxyl radicals (the most toxic species of oxygen) are known to be formed from $H_2O_2$ in presence of transition metal ions (Halliwell and Gutteridge 2007). The unsaturated fatty acid components of membrane lipids are highly susceptible to hydroxyl radical attack and are peroxidized in presence of it. Therefore, lipid peroxidation is the consequence of free radical mediated oxidative reactions in the aerobic cells and acts as a good indicator of the prevalence of oxidative stress situation. Even though total peroxide level was not estimated in this study, increase in MDA content was found in the cotyledonary tissues during senescence (Fig. 2). Thus, there was increase in lipid peroxidation in the tissues. Dhindsa *et al.* (1981) have reported a good correlation between lipid peroxidation and increased membrane permeability in senescing leaves. Increase in lipid peroxidation during senescence has also been reported in many studies (Kar and Feierabend 1984, Hodges and Forney 2000). The increase in lipid peroxidation in the cotyledons indicated that there was prevalence of oxidative stress situation in the tissues during the natural senescence. But in the rejuvenated cotyledons, the lipid peroxidation level declined (Fig. 2) which indicated that there was also reversal in the oxidative metabolism during rejuvenation. This was mainly due to the increase in CAT activity (main decomposer of $H_2O_2$ in the aerobic cells) in the rejuvenated cotyledons (Fig. 2), because there was no elevation in SOD activity on reversal of senescence, as found in this study.

Thus, on reversal of senescence by shoot decapitation, there was lesser depletion in the reserves as a result the cotyledons were rejuvenated. However, no increase in photosynthetic pigment contents was noticed in the rejuvenated cotyledons during their short period of rejuvenation. There was imposition of oxidative stress in the cotyledons during senescence which was again reversed on reversal of senescence. As observed in this study, CAT activity ($H_2O_2$ – scavenging function) seems to be one of the key regulatory aspect of cotyledon senescence and subsequent cotyledon rejuvenation (reversal of senescence). However, the roles of peroxidase and other antioxidative enzymes involved in the ascorbate-glutathione pathway along with different low molecular weight antioxidants in the senescing cotyledons can not be over ruled. Therefore, the detail analysis of these aspects of oxidative metabolism would clarify the senescence mechanism of mungbean cotyledons, the lateral organs having dual functions of photosynthesis and nutrient reservoir.

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