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RESEARCH ARTICLE

Chloroplast pigments, proteins, lipid peroxidation and activities of antioxidative enzymes during maturation and senescence of leaves and reproductive organs of *Cajanus cajan* L.

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Abstract A comparative investigation was undertaken with pigeon pea leaves and attached flower buds/flowers/pods during their developmental stages including senescence in a natural system in experimental plots. Alterations in chloroplast pigments, total soluble proteins, lipid peroxidation, malondialdehyde (MDA) content and activities of guaiacol peroxidase (POD, EC 1.11.1.7) and superoxide dismutase (SOD, EC 1.15.1.1) were studied at 5-day interval from initial to 40-day stage. Chloroplast pigments and proteins of leaves increased upto 15 and 20-day stages respectively followed by a steady decline. Reproductive parts, however, exhibited rise in chloroplast pigments upto 25-day and protein till last stage as developing pods gain the amount from the senescing leaves which are nearest to them. Senescing leaves show very high POD activity than the developing and senescing pods and POD appears to be associated with chlorophyll degradation. Considerably higher activity and amount of LOX and MDA respectively have been noticed in senescing leaves than in flowers and pods. Increase in SOD activity during early stage of leaf growth and maturation indicates protective role that declined at senescent stages. Pods are unique in having very high SOD activity, only last stage of senescence does show a decline.

Keywords *Cajanus cajan* · Leaf · Lipoxygenase · MDA · POD · Reproductive organs · Senescence · SOD

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Introduction

Pigeon pea [*Cajanus cajan* (L.) Mill sp.], a member of the family Fabaceae is one of the major legume crops of the tropics and subtropics. It is used as a protein source for humans and livestock, a fuel source and a versatile intercropping and rotational plant (Nene and Sheila 1990). Legume plants, although flower profusely but the majority of flowers drop off without setting pods due to heavy flower shedding and senescence (Sinha 1974; Sheldrake and Narayanan 1979; Mukherjee and Ponmeni 2004). Flowering and fruit growth (in case of legume, pod setting and pod growth) can be considered as the signal for the mobilization of nutrients from the leaves followed by their death at later stages (Lindoo and Nooden 1976). Leaf senescence is characterized by complete cessation of photosynthesis, breakdown of chlorophyll pigments and proteins and also other macromolecules, recycling of nutrients to other growing parts of the plant, conversion of peroxisomes into glyoxysomes and a significant increase in the production of reactive oxygen species (ROS) (Pastori and del Rio 1997a; Prochazkova et al. 2001). It is a genetically controlled developmental process whose regulation depends on both endogenous and environmental factors (Gan and Amasino 1997; Nooden et al. 1997; Yoshida 2003). The onset of senescence in the vegetative and reproductive organs is characterized by a decrease of the antioxidant metabolism and overproduction of ROS might be one of the basis of the process (Pastori and del Rio 1997b; Prochazkova and Wilhelmova 2004). Extensive studies have been carried out with detached systems in relation to senescence. However, biochemical changes involved in intact system are far less documented.

Earlier studies on attached leaves of *Cajanus cajan* from our laboratory revealed how amino acids get distributed during various stages of senescence (Jakhar and Mukherjee

2006). Higher accumulation of amino acids has been associated with higher protease activity. Further, attempts have not been made earlier to make a comparative study of the vegetative organs (like leaves) and closely localized reproductive organs (floral buds/flowers/pods) with regard to metabolic changes, membrane degradation and activities of antioxidant enzymes during developmental stages including senescence.

It was, therefore, thought to carry out a comparative investigation of both leaves and reproductive organs of *C. cajan* during their developmental stages to record not only the alterations in chloroplast pigments and proteins but also the malondialdehyde (MDA) content, activities of liopxygenase (LOX), guaiacol peroxidase (POD), and superoxide dismutase (SOD).

Materials and methods

Experimental design

Plants of *Cajanus cajan* L.cv.UPAS-120 (commonly known as pigeon pea) were grown within the experimental cage of Botany Department, Kurukshetra University, Kurukshetra under natural condition of day and night. The length, breadth and height of the experimental cage had been 12×12×2.5 m respectively. Nine experimental plots had been prepared inside, each measuring 4×2 m^2. Each experimental plot was provided with 80 kg of dung manure, mixed properly to ensure uniformity of soil. The seeds were sown in the pulverized seed beds of the cage in rows. Pigeon pea plants produce flowers twice in a year; first and second appearance recorded in the months of September and January respectively when seeds were sown in the month of May. Here, experiments have been carried out with leaves and flowers during and after the month of January, which has been termed as 2nd flowering season, when plants were of 250–260 days old. Leaflets that appear on the 7th node (from the top) were tagged uniformly in the axil of which flowers appear on short branches. These leaves and closest flower buds which transformed into mature and senescent leaves as well as open flowers, developing and senescent pods respectively during 40 days were used in the present investigation (Plate 1, A–D). Leaflets continued to show expansion upto 20-day stage after tagging followed by gradual degradation of chloroplast pigments as witnessed from their appearance and colour. Corresponding to 0-day leaf stage, reproductive organs showed flower buds only whereas 5 and 10-day stages included open flowers. Young pods appeared at 15-day followed by developing and senescent pods. Pod senescence could be noticed at 35 and 40-day stages.

Estimation of chlorophylls and carotenoids

Leaf sample (200 mg) was grinded in chilled 80 % acetone (AR grade) with 20 mg of $CaCO_3$ and the absorbance was recorded at 645 and 663 nm in case of chlorophylls and at 480 and 510 nm for carotenoids in relation to peaks obtained in their absorption spectra using UV-Visible Specord-205 spectrophotometer (Analytik-Jena, Germany). The amount of chlorophylls was estimated using the formula of Arnon (1949), while that of carotenoids was calculated by the method of Holden (1965).

Estimation of total soluble protein

The total soluble proteins were estimated by the method of Bradford (1976) using Coomassie Brilliant Blue G-250 which can bind to protein rapidly and the complex remains dispersed in solution for a long time. For protein extraction, 50 mg of fresh leaf tissue (earlier stored in a freezer) was homogenized in 80 % ethanol after boiling in the same ethanol for 15 min. This was centrifuged at 10,000 g for 5 min. The residue was re-extracted with 5 % perchloric acid followed by centrifugation at 10,000 g for 5 min. The residue was then treated with 1 N NaOH (5 ml) and kept in warm water (40–50 °C) with regular shaking for 30 min. The clear supernatant was used for further analysis.

Measurement of peroxidase (POD) activity

The total peroxidase activity was measured following the method of Maehly (1954) using guaicol and H_2O_2. Breakdown of H_2O_2 by peroxidase with guaiacol as hydrogen donor is determined by measuring its activity (due to formation of tetraguaicol) on the basis of maximum colour development at 420 nm. Specific activity of peroxidase was expressed as mg^{-1} protein min^{-10}.

Measurement of lipoxygenase (LOX) activity

The activity has been estimated according to the method of Doderer et al. (1992) where the efficiency of enzyme depends upon the breakdown of fatty acid (linoleic acid). Plant material (0.2 g) was homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 μM EDTA with pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes, centrifuged at 4 °C in Remi refrigerated centrifuge (Compufuge, CPR-24) for 15 min at 15,000 g. The supernatant was transferred to 30 ml test tubes and referred to as enzyme extract. Five ml distilled water having 50 μl tween 20 was added to 35 μl linoleic acid (substrate) with a pH of 8.5–8.6. The final pH was adjusted to 9 by adding 0.2 M NaOH drop by drop so that linoleic acid dissolved completely. The pH was adjusted again to 6.5 by adding 0.2 M HCl. To

this solution, 0.1 M phosphate buffer of pH 6.5 was added and the final volume of substrate was raised to 100 ml with the same buffer. Substrate solution of 2.95 ml was taken in a cuvette and considered as blank set. In reaction set, 0.05 ml of the enzyme extract was added to the cuvette containing 2.95 ml of the substrate solution at zero time. Absorbance was noted at 234 nm for every minute upto 5 min. The activity was expressed as change in absorbance per minute per mg protein (The amount of protein from the enzyme extract was estimated by the method of Bradford 1976).

Measurement of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) has been assayed on the basis of its ability to inhibit the photochemical reduction of p-nitro blue tetrazolium chloride (NBT) into blue formazon which has a wavelength maximum at 560 nm (Giannopolitis and Ries 1977). Reactions involved are:

(i) Photoreduction-oxidation reaction between riboflavin and methionine with the reduction of flavin,
(ii) Release of superoxide ($O_2^{-\cdot}$)from reoxidation of reduced flavin,
(iii) Reduction of NBT by superoxide into blue formazon and
(iv) Inhibition of step (3) by SOD resulting in the formation of H_2O_2 and O_2.

For the extraction, 50 mg of fresh leaf tissue crushed in 2 ml of 0.1 M EDTA-phosphate buffer of pH 7.8 containing K_2HPO_4 and EDTA and the final volume was raised to 100 ml with double distilled water (DDW). This was centrifuged at 15,000 g and resultant supernatant was used as crude extract. The reaction mixture was prepared by adding 0.1 ml of crude extract followed by 0.9 ml of DDW, 0.5 ml of 300 mM Na_2CO_3 (pH-10.2), 0.5 ml of 378 μM p-nitrobluetetrazolium chloride (NBT), 0.5 ml of 78 mM L-methionine and 0.5 ml of 7.8 μM riboflavin. The final reaction mixture was 3 ml. The reaction was carried out in similar test tubes at 25 °C for 15 min. in 100 μmol photon $m^{-2}s^{-1}$ PFD from fluorescent lamp. The initial rate of reaction as measured by the difference in increase in absorbance at 560 nm in the presence and absence of extract was proportional to the amount of enzyme. The unit of SOD activity was obtained as that amount of enzyme which under the experimental conditions caused a 50 % inhibition of the reaction observed in the absence of enzyme (Giannopolitis and Ries 1977).

Measurement of lipid peroxidation

The level of lipid peroxidation in test samples was measured in terms of estimating the end product, i.e. MDA (malondialdehyde) (Heath and Packer 1968). Leaf sample (0.2 g) was homogenized in 3 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 15,000 g for 15 min. To 1.0 ml aliquot of the supernatant, 2.0 ml of 0.5 % thiobarbituric acid (TBA) in 20 % TCA was added. The mixture was heated at 95 °C for 30 min on the water bath and then cooled in an ice bath. After centrifugation at 10,000 g (Remi Compufuge, CPR-24) for 10 min and the absorbance of the supernatant was recorded at 532 nm. The value for non-specific absorption of each sample at 600 nm was also recorded and subtracted from the absorbance recorded at 532 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Samples in triplicates of both leaves and reproductive organs (flower buds, open flowers and developing pods with the progress of transformation) were collected at initial (0-day, i.e. day of tagging), 5, 10, 15, 20, 25, 35 and 40-day stages for various biochemical analyses. Atleast three aliquots were used for each sample during estimation.

Statistical analysis

All experimental data are based on average of nine values. The obtained data were statistically analyzed for the mean ± SD and the difference during days were analyzed by one way ANOVA taking $p<0.05$ and $p<0.01$ significant level.

Results

Morphological changes in leaves and reproductive organs

Selected branches of pigeon pea have been depicted in Plate 1-A showing the position of leaves and inflorescence. Flower bud and open flowers can be also seen (Plate 1 B). Various leaf stages which grow and mature can be witnessed in Plate 1 C. Stages of leaf maturation represent 0 to 20-day stage whereafter they entered the senescent phase. The progress of leaf senescence could be seen during 25–40 day stages. Initial stage of reproductive organ is represented by flower bud only while open flower stages include 5 and 10-day stages. Young pods are set at 15-day which grow and mature at later stages. Pods at 40-day is represented by senescent stage where they turned brown (Plate 1 D). Initial stage of leaves (0-day) corresponds to initial stage of reproductive organ (0-day), i.e. flower buds. At each stage, comparison has been made between leaf and corresponding reproductive organ in all biochemical analyses.

Biochemical analysis

The chlorophyll contents of leaves and reproductive organs as illustrated in Tables 1 and 2 showed an increasing trend with growth and development between 0 to 15-day and 0 to 25-day

Table 1 Changes in the amount of chlorophyll-a (Chl-a), Chl-b, total chlorophylls, carotenoids (mg g^{-1} dry weight) ± S.E., in tagged leaves of *C. cajan* L. cv. UPAS – 120 during growth and senescence

DAT	Leaves				
	Chl. a	Chl. b	Total Chl.	Chl a/b	Carotenoids
0	0.542±0.181	0.387±0.076	0.929±0.104	1.40	0.425±0.134
5	0.600±0.043	0.403±0.034	1.003±0.131	1.48	0.447±0.045
10	0.700**±0.111	0.400±0.082	1.100±0.075	1.75	0.504±0.031
15	0.764**±0.101	0.416±0.072	1.180*±0.097	1.84	0.431±0.022
20	0.523±0.041	0.398±0.045	0.921±0.056	1.31	0.401±0.022
25	0.409**±0.023	0.266**±0.053	0.675*±0.034	1.54	0.318**±0.034
35	0.376**±0.056	0.200**±0.023	0.576**±0.083	1.88	0.254 **±0.023
40	0.160**±0.012	0.130**±0.004	0.290**±0.072	1.23	0.162**±0.104

* and ** - 5 % and 1 % significant level for days interval; *DAT* days after tagging; 0 to 40 represent various stages of leaves

stages respectively (Fig. 1). Chlorophyll and carotenoid pigments were characterized by a steady decline from 20 to 15-day stages respectively in leaves. A decline of about 70, 66 and 69 % in chlorophyll-a (Chl. a), chlorophyll-b (Chl. b) and total Chl. respectively were observed in senescent leaves at 40-day in comparison to respective initial values. Chl. a/b ratio too increased from the initial stage (1.40) to 15-day (1.84) followed by a sharp decline at 20-day; again increased between 25 to 35-day stages and finally the value dropped to 1.23 at 40-day stage. Among chloroplast pigments, percent reduction of Chl. a was maximum followed by Chl.b and carotenoids during 40 days as compared to the initial values.

Amounts of chloroplast pigments as depicted in Table 2 indicate a slight drop in chl. a and chl. b at 5-day stage when open flowers appeared while total carotenoids exhibited a rise which continued till 10-day stage. Fifteen-day stage showed increment in both chl. a and chl. b and a decrease in carotenoids when younger pods were first analyzed for reproductive organs. Later stages of pod growth till 25-day stage was characterized by rise in chl. a and chl. b; whereafter marked decline could be noticed. Carotenoids, however, declined gradually in developing pods between 15 and 40-day stages. The percent decline in the total chlorophylls and carotenoids was 75 and 70 respectively in pods with respect to initial values.

Total soluble proteins in leaves showed a gradual increment of about 184 % at 20-day stage after tagging (Fig. 2). The amount reduced thereafter and a sharp decline was noticed at 35-day, when the value became less than one third in comparison to its maximum value at 20-day (8.886 mg/100 mg). The reduction in protein level from 0 to 40-day was about 61 % as compared to initial. The amount of protein in the flower buds at 0-day was lower than adjacent leaves but flowers exhibited higher amount than leaves at 10-day stage. Total soluble proteins accumulated during the development of pods where it had shown an increment of about 830 % at 40-day from its initial amount at bud stage. POD activity registered a gradual increase of about 9, 41, 169, 306, 356,

Table 2 Changes in the amount of chlorophyll-a (Chl-a), Chl-b, total chlorophylls, carotenoids (mg g^{-1} dry weight) ± S.E., in tagged buds, flowers and pods of *C. cajan* L. cv. UPAS – 120 during growth and senescence

DAT	Bud♠/Flower♣/Pods				
	Chl. a	Chl. b	Total Chl.	Chl a/b	Carotenoids
0	0.329♠±0.039	0.101♠±0.019	0.330♠±0.876	3.25♠	0.443♠±0.034
5	0.303♣±0.083	0.095♣±0.021	0.298♣±0.053	3.18♣	0.540♣±0.041
10	0.228♣**±0.089	0.075♣±0.012	0.303♣±0.042	3.04♣	0.640♣*±0.034
15	0.558**±0.173	0.150*±0.032	0.708**±0.023	3.72	0.572*±0.056
20	0.789**±0.165	0.200**±0.022	0.989**±0.021	3.94	0.526±0.067
25	0.889**±0.150	0.160**±0.023	1.049**±0.028	5.55	0.400±0.048
35	0.289**±0.031	0.121±0.032	0.410*±0.023	2.38	0.275*±0.063
40	0.056**±0.011	0.026**±0.010	0.082**±0.012	2.15	0.132**±0.034

* and ** - 5 % and 1 % significant level for days interval;; DAT- days after tagging; 0-day stage shows the presence of only flower buds; 5 and 10-day stages show open flowers and remaining stages show developing pods

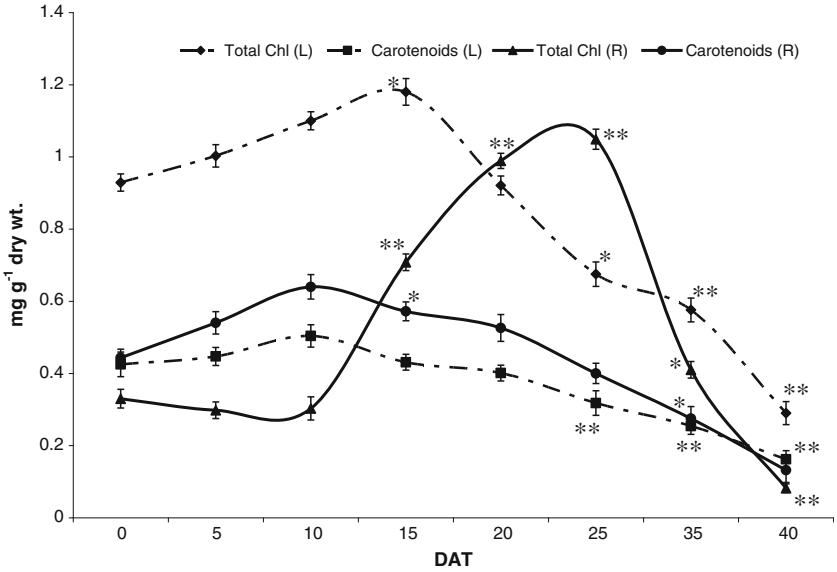

Fig. 1 Amount of total chlorophylls and carotenoids in leaves (L) and reproductive organs (R) during development and maturation in *Cajanus cajan* L. cv. UPAS – 120 (* and ** 5 % and 1 % significant level for days interval; *DAT* days after tagging). In reproductive organs, 0 day represents flower buds; 5 and 10 days represents open flowers and 15, 20, 25, 35 and 40-day stages represent various stages of pods

526 and 894 % during 5, 10, 15, 20, 25, 35 and 40-day respectively (Fig. 3). A sharp increment in POD activity has been witnessed between 0 and 20-day whereafter leaves attain full maturity. In reproductive organs which are represented by flower buds, open flowers and pods, POD activity increased slowly during 0 to 20-day stage and then it declined when pods fully matured and entered into senescent phase. It exhibited a reduction of about 24, 29 and 47 % during 25, 35 and 40-day respectively (Fig. 3).

Derived from the measurement of MDA content during development of leaves and reproductive organs during natural senescence process, the variations in MDA content were shown in Fig. 4. In both vegetative and reproductive organs, MDA content increased gradually with development and senescence. MDA levels tended to be higher in leaves than flower buds, open flowers and pods at all stages. Surprisingly, MDA content in open flowers and young pods increased slowly as compared to its increment in young leaves. However, a very high increment of about 535 and 919 % was recorded at 35 and 40-day respectively in pods at the onset of senescent phase.

Results obtained in our study showed that LOX activity increased gradually but significantly from 0 to 40-day in leaves. A very sharp increment has been recorded between 25 and 35 day stage when it exhibited an increment of about 208 %. At 40-day, LOX activity has been increased by about 534 % from 0-day. In comparison to young leaves at 0-day, flower buds had considerably higher LOX activity. In reproductive organs, LOX activity initially declined in flowers as compared with buds having least activity at 10-day when flowers were fully opened. Pod growth and development from 15 to 25-day was exhibiting a gradual rise in LOX activity but

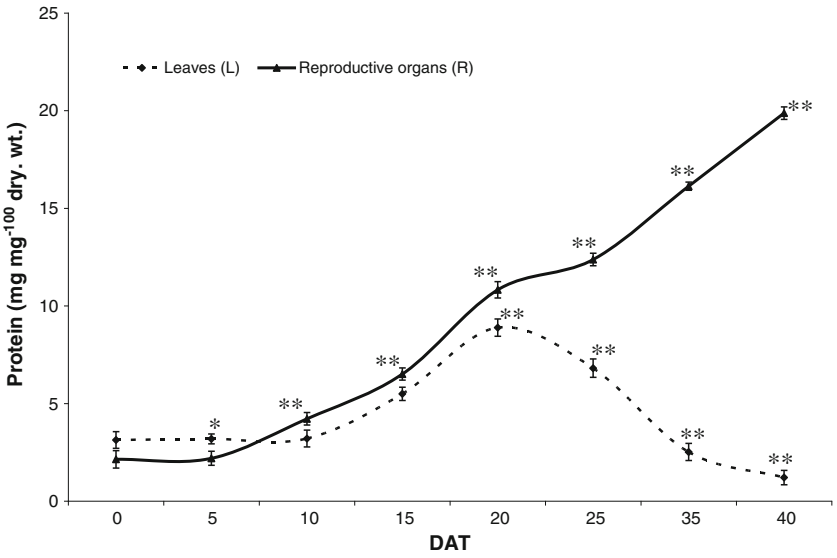

Fig. 2 Protein content in leaves (L) and reproductive organs (R) during development and maturation in *Cajanus cajan* L. cv. UPAS – 120 (* and ** 5 % and 1 % significant level for days interval; *DAT* days after tagging). In reproductive organs, 0 day represents flower buds; 5 and 10 days represent open flowers and 15, 20, 25, 35 and 40-day stages represent various stages of pods

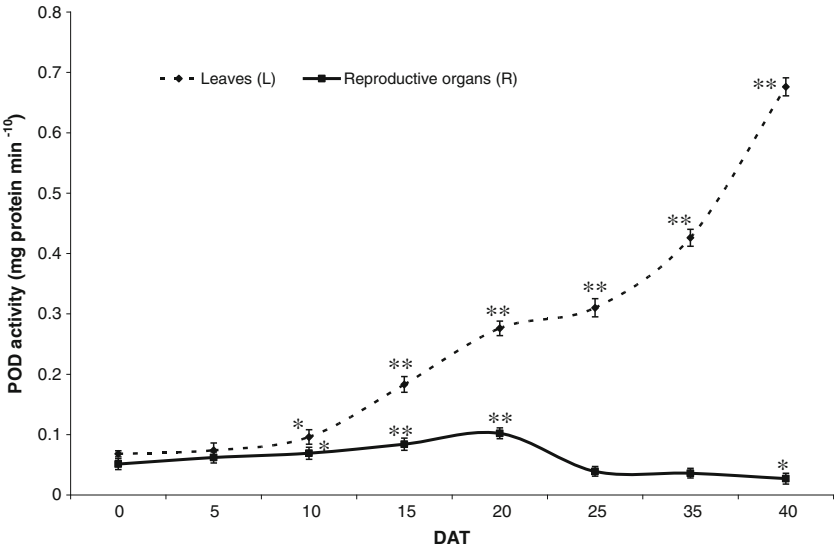

Fig. 3 Specific activity of peroxidase in leaves (L) and reproductive organs (R) during development and maturation in *Cajanus cajan* L. cv. UPAS – 120 (* and ** 5 % and 1 % significant level for days interval; *DAT* days after tagging). In reproductive organs, 0 day represent flower buds; 5 and 10 days represents open flowers and 15, 20, 25, 35 and 40-day stages represent various stages of pods

these values were lower than the initial value of flower buds (Fig. 5). The percent decline noted was about 20, 85, 79, 55 and 30 at 5, 10, 15, 20 and 25-day respectively, whereafter an increment of about 20 and 161 % was recorded at 35 and 40-day respectively when pod walls had turned blackish brown.

SOD activity in leaves registered an increment from 0 to 15-day (developing phase) when a maximum increase of about 191 % was observed as compared to the initial value. Although activity of SOD started to decline thereafter but still it has considerably higher value than the initial (0-day) stage. A significant decline in the activity of enzyme was recorded at 35 and 40-day (senescent phase) where it exhibited a reduction of about 14 and 74 % from initial value (Fig. 6). Regarding reproductive organs, SOD activity increased upto 20-day, where it showed its maximum activity; the values of percent increment recorded in flower bud, open flower and young pods were about 19, 73, 141 and 168 in 5, 10, 15 and 20-day stages respectively. Forty day old pods which represented senescent stage, there is a reduction of about 11 % when compared to initial value but the decline was about 157 % from its maximum activity at 20-day (3.307 Units min^{-1} mg^{-1} protein). Developing leaves, flowers and pods were characterized by a steady increment in SOD whereas onset of senescence exhibited its reduction.

Discussion

Present study has revealed an increment in Chl. a, and Chl. b upto 15 days; and carotenoids upto 10 days of development in leaves followed by a gradual decline in *C. cajan* whereas subtending reproductive organs recorded increment of both Chl. a and b upto 25-day and carotenoids upto 10-day (Tables 1 and 2; Fig. 1). Leaf proteins were unique in

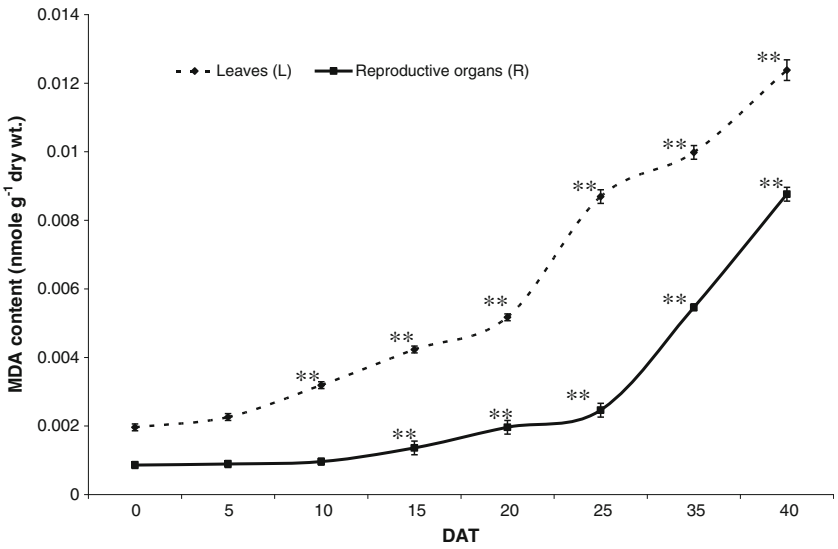

Fig. 4 MDA content in leaves (L) and reproductive organs (R) during development and maturation in *Cajanus cajan* L. cv. UPAS – 120 (* and ** 5 % and 1 % significant level for days interval; *DAT* days after tagging). In reproductive organs, 0 day represent flower buds; 5 and 10 days represents open flowers and 15, 20, 25, 35 and 40-day stages represent various stages of pods

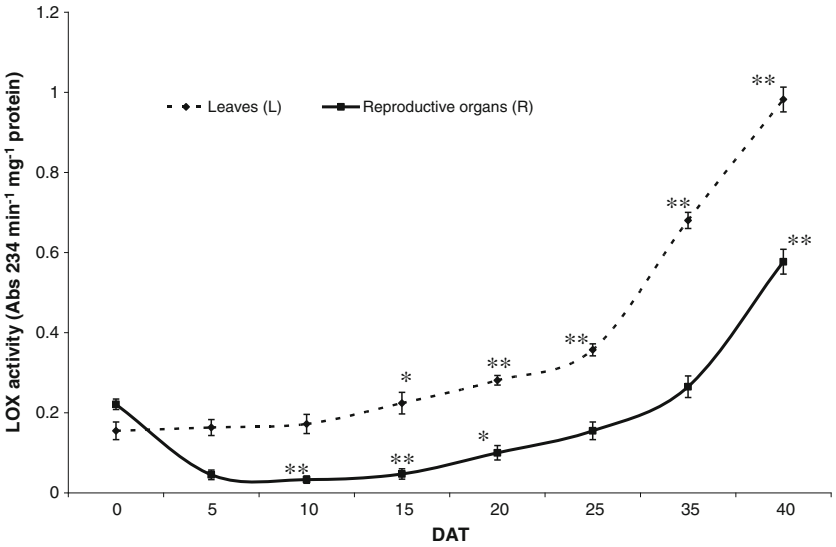

Fig. 5 LOX activity in leaves (L) and reproductive organs (R) during development and maturation in *Cajanus cajan* L. cv. UPAS – 120 (* and ** 5 % and 1 % significant level for days interval; *DAT* days after tagging). In reproductive organs, 0 day represent flower buds; 5 and 10 days represents open flowers and 15, 20, 25, 35 and 40-day stages represent various stages of pods

exhibiting a steady increase upto 20 days followed by a rapid fall between 20 and 40 days. Total soluble proteins of flower buds and flowers exhibited very little increase during first 10 days but thereafter with the pod setting and subsequent growth, the amount significantly increased upto 40 days. Optimal value of leaf protein at 20-day when reproductive organ (pods) exhibited almost half maximal rise may suggest the mobilization of metabolites from the adjacent leaves to the developing pods by the depletion of proteins (Fig. 2). Numerous investigators including Simpson et al. (1983), Grover et al. (1985) and Rao and Mikherjee (1990) have suggested earlier that requirements for nitrogen in grains and pods could be largely satisfied by vegetative structures like leaves which are closest to them. The distribution pattern of protein in leaves and reproductive organs gets support from earlier study in our laboratory where DNA, RNA and proteins have been analyzed in *C. cajan* (Mukherjee and Kumar 2007). Free amino acids released during protein breakdown in senescent leaves are mobilized to younger leaves for protein synthesis (Jakhar and Mukherjee 2006). Similar mobilization can also be noticed in developing flowers and pods which are situated very near to these leaves.

Smaller increments of POD in leaves have been noticed when reproductive parts were represented by flower buds or open flowers. But with the appearance of pods and their subsequent growth, larger increase could be seen in POD at all stages in leaves. The values of POD in each stage was much lower in pods than that of leaves. Developing leaves after 15-day stage is characterized by not only a rapid decline in the amount of chlorophylls but also a significant rise of POD activity attaining about half maximal activity at 25-day (Fig. 3). This increment in POD appears to be associated with chlorophyll degradation. POD is known to bleach Chl. in presence of H_2O_2 (Martinoia et al. 1982; Ponmeni and

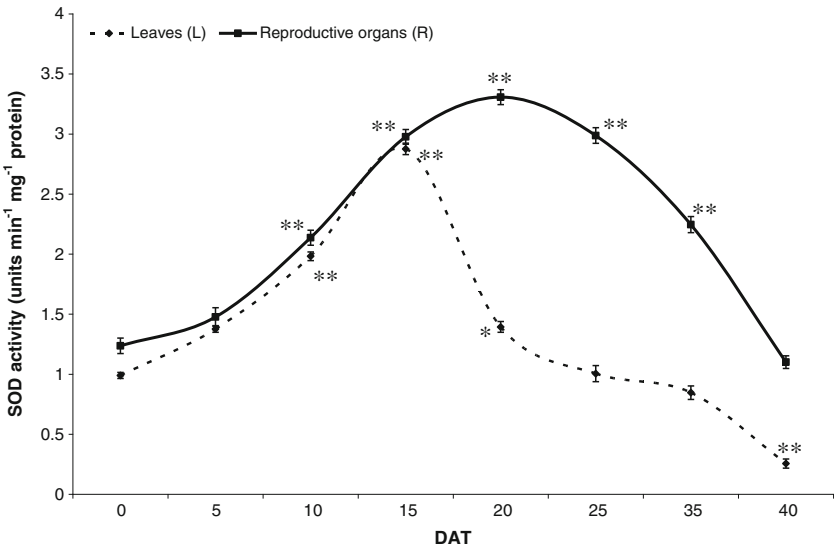

Fig. 6 SOD activity in leaves (L) and reproductive organs (R) during development and maturation in *Cajanus cajan* L. cv. UPAS – 120 (* and ** 5 % and 1 % significant level for days interval; *DAT* days after tagging). In reproductive organs, 0 day represent flower buds; 5 and 10 days represents open flowers and 15, 20, 25, 35 and 40-day stages represent various stages of pods

Mukherjee 1997). The increase of POD activity has been reported by Kanazawa et al. (2000) in the senescing cotyledons of cucumber. Several other investigators have assigned the role of Chl. catabolism to POD in their respective studies (Matile 1980; Huff 1981; Kato and Shimizu 1985; Yamauchi and Minamide 1986). However, Abeles and Dunn (1989) while reporting an increment in the level of POD during senescence commented that the increment was not involved in Chl. degradation. Shioi et al. (1991) have also shown that the breakdown of Chl. in *Chenopodium album* does not involve POD.

Stages of leaf expansion and maturation were characterized by a smaller increment in MDA content and LOX activity whereas a steady and significant rise was recorded in SOD (Figs. 4, 5, and 6). Progress of leaf senescence was, however, associated with a marked increase in lipid peroxidation and LOX activity after 20-day while SOD activity registered a steady decline after 15-day stage. Flowers and early stages of pods, on the contrary had a very slow increment in MDA content upto 20 to 25 day whereafter significant increase was noticed; that was associated with pod senescence. Among reproductive organs, flower buds had fairly higher LOX activity followed by lower activity in flowers and early stages of pods (5 to 20-day stages); but later stages of pods showed considerable increment in LOX activity. The degradation products of lipids are metabolized and converted to phloem-mobile sucrose for translocation out of the senescing leaf (Thompson et al. 1998; Kaup et al. 2002).

Flowers and pods had much higher activity of SOD than that in leaves; values increased upto 20-day, maintained fairly higher values at later stages also and only last stage showed a lower value. Increment of SOD in earlier stages of leaf development followed by a marked decline in senescent stages have also been reported in tobacco (Dhindsa et al. 1981), *Arabidopsis thaliana* (Ye et al. 2000), maize (Sairam et al. 2001), flag leaf of wheat (Srivalli and Khanna-Chopra 2001) and pea (Palma et al. 2006). Maintaining higher SOD values till the maturation of leaves and pods indicate protective mechanisms in *C. cajan* to get rid of deleterious superoxide radicals. Loss of this defense is clearly noticed especially in the advanced stage of senescence.

Lipid peroxidation, an important mechanism of leaf senescence being measured in terms of MDA rises with the increase in oxidative stress. Developing leaves and flowers do exhibit lower amount of MDA but it increased significantly with the progress of senescence in both leaves and pods as revealed in this investigation. Rise in the values of lipid peroxidation has been mentioned by many workers in their studies in relation to senescence (Dhindsa et al. 1981; Hurng and Kao 1994; Marie 1995). LOX causes peroxidative damage in membrane lipids resulting in a decrease in lipid unsaturation and membrane fluidity (Lesham 1988; Lee et al. 2005). LOX mediated polyunsaturated fatty acid (PUFA) oxidation product which may react with Chl. and oxidize them (Klein et al. 1984). The loss of Chl. in reproductive organs may be due to this enzyme (Eriksson and Svensson 1970; Galliard and Phillips 1971). Several studies have shown rise in LOX activity during senescence such as in leaves (Grossman and Leshem 1983), flower petals (Hossain et al. 2006) and tomato fruits (Thompson et al. 1990).

In conclusion it can be said that several mechanisms are operating at a given time for sustaining a plant structure in its active form and maximum efficiency viz. leaves or pods. Developing leaves (L) and reproductive organs (R) behave in different manner in exhibiting half optimal values of pigments, metabolites and antioxidant enzymes. Optimal values of chlorophylls has been recorded in the former at 15-day whereas at 20 to 25-day in the latter. Protein depletion initiated after 20-day in leaves whereas pods registered constant increase due to its specific biological requirement that is provided by subtending leaves. Amount of MDA exhibits two exponential like increases, a fast one for (R) with about 25 days and a slower one for (L) of about 20 days for half increase within a period of 40 days. Two exponential like increase has also been noticed for LOX but here the fast one

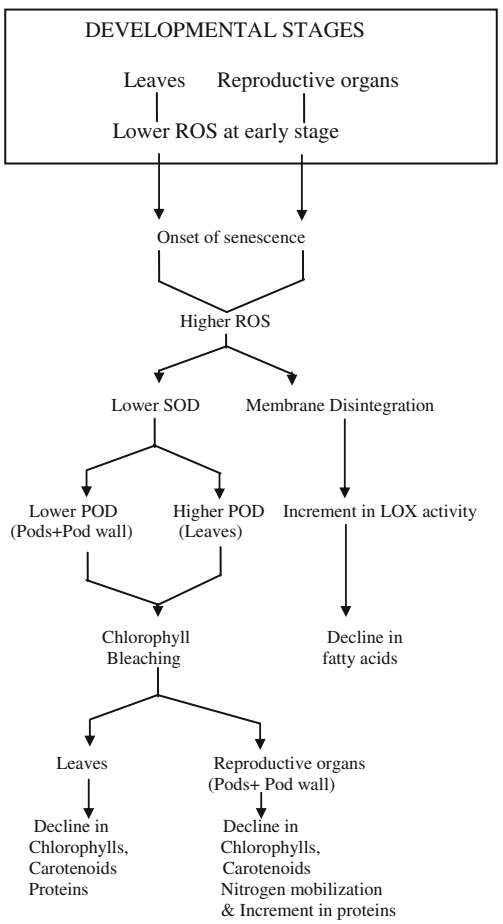

Fig. 7 A model showing occurrence of physiological and biochemical events during senescence of leaves and reproductive organs of *C. cajan*

is with (L) while slower one with (R). An optimal value of SOD has been witnessed at 15-day and 20-day in (L) and (R) respectively.

Much higher POD activity in senescing leaves appears to be associated with Chl. bleaching. Even younger stages of leaves, flowers and pods show the presence of MDA and LOX activity which becomes much greater when they entered senescent phase. SOD activity increased with leaf and pod maturation but the reduction starts with the onset of senescence. Various developmental processes that constitute senescence was finally responsible for cell disorganization and remobilization of metabolites and nutrients as shown in the proposed model. Figure 7 and which can be used in other plant organs such as from senescent leaves to young leaves as well as flower buds/developing flowers/developing pods. The destructive phenomenon leading to senescence and the protective role played by SOD, etc. are quite evident in leaves and reproductive organs during their development.

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Author contribution *Somveer Jakhar has carried out experiments, obtained results, analyzed data and took photographs. D. Mukherjee was responsible* for designing experiments, standardizing methods and verifying data. Both the authors contributed in preparing the manuscript.

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