Indian J. Plant Physiol., Vol. 12, No. 2, (N.S.) pp. 138-145 (April-June, 2007)

EFFECT OF SIMULATED WATER STRESS ON THE PHYSIOLOGY OF LEAF SENESCENCE IN THREE GENOTYPES OF COWPEA (*VIGNA UNGUICULATA* (L.) WALP)

KAJAL G. PATEL AND T.V. RAMANA RAO*

Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar-388 120, Gujarat

Received on 8 May, 2006, Revised on 29 June, 2007

SUMMARY

The present study deals with the impact of simulated water stress on the physiology of leaf senescence in three genotypes of cowpea (*Vigna unguiculata*), viz. Akshay-102(A), Gomti vu-89(G) and Pusa Falguni(P). The induced water stress created by withholding water supply from flowering stage causes significant declines in the levels of chlorophylls with an increase in the amount of total carotenoids during senescence of the leaves of all the three presently studied genotypes of cowpea plants in comparison to that of control plants. A significant decrease in the amount of total protein is associated with the significant increase in the net pool of total free amino acids in Pusa Falguni and Gomti genotype during maturation and senescence stage of leaves. The increase in the levels of total phenols occur consistently, but significant increase occurs only at 65 and 75 days in Pusa Falguni and Akshay, while in Gomti it occurs from 55 to 75 days age of plants. The levels of nucleic acids (DNA and RNA) declined during maturation and senescent stage of leaves, but only Akshay exhibits non-significant decrease in the levels of its RNA. The specific activity of acid and alkaline proteases are more during the maturation and senescence of leaves in plants facing induced water stress condition than that of plants grown in the control plot, but this activity is found to be significant only in Gomti variety. While the specific activity of catalase decreases, that of peroxidase and polyphenoloxidase increases during development, maturation and senescence of leaves in all three genotypes. From the obtained data, it may be concluded that Akshay genotype displayed better drought tolerance than other two genotypes of cowpea.

***Key words*: Chlorophyll, leaf senescence, proteases, *Vigna unguiculata*, water stress**

INTRODUCTION

It is now evident from many earlier investigations that leaf senescence represents the final stage of its development and it is characterized by the transition from nutrient assimilation to nutrient remobilization (Masclaux *et al*. 2000, Hortensteiner and Feller 2002). Moreover, several researchers have shown that leaf senescence is a complex developmental programme involving highly co-ordinated changes in cell structure, metabolism, function and gene expression and, this programme, as with many other programmes, can be modulated by environmental conditions. Abiotic stresses are prevalent in nature and can substantially diminish plant yields (Elizabeth *et al*. 2000). Further, stresses involving water deficit may conceivably arise from drought conditions, saline soils or due to low temperature.

A review of literature shows that Kuhad *et al*. (1989) observed physiological responses like the rate of

*Corresponding author, E-mail: tadapanenirao@yahoo.com

photosynthesis, root and nodule respiration, and relative water content and water retension in the leaves of water stress imposed at preflowering genotypes of Pigeon pea (*Cajanus cajan* L.), while Pic *et al*. (2002) studied the leaf senescence induced by mild water deficit and observed that this kind of induced senescence follows the same sequence of macroscopic, biochemical, and molecular events as monocarpic senescence in Pigeon pea. Recently Garg *et al.* (2004) studied the effect of water stress on six genotypes of moth bean (*Vigna acontifolia)* and compared the metabolic alterations among them. However, it is evident from the published literature that so far no such attempt has been made to elucidate the impact of drought condition on the physiology of leaf senescence in the varieties of cowpea. Hence the present investigation is undertaken with a view to elucidate the biochemical changes associated with the water stress induced leaf senescence in the three selected genotypes of cowpea.

MATERIALS AND METHODS

The seeds of three varieties of cowpea (*Vigna unguiculata*) viz.: Akshay-102(A), Gomti vu-89(G) and Pusa Falguni (P) were procured from the local market and seeds of each variety were sown in two separate research plots of University Botanical Garden. Initially all the six plots were adequately watered up to flowering stage (i.e. till 45 days age), but thereafter only one of the plots for each variety was irrigated and considered it as a control plot, while no water was given to the second plot so as to have the impact of induced water stress and treated it as an experimental plot. The collection of leaf materials for their biochemical analysis was initiated when the leaves were of 5 days old from the plants of 25 days (Days after sowing) age and subsequently leaf samples were collected at regular interval of 10 days till the leaves attained 55 days age (i.e. plants attained the age of 75 days after sowing).

The methodology followed for the biochemical analysis of the collected leaf samples are as follows: The extraction and estimation of pigments (chlorophylls and total carotenoids) was carried out by following the methods cited by Thimmaiah (1999), while the quantitative analysis of total proteins, total free amino acids and total phenols has been done by following the method of Lowry *et al*. (1951) and Moore and Stein (1948) and Malick and Singh (1980) respectively. Extraction and estimation of DNA and RNA were carried out as per the procedures described by Pratibha Devi (2002). The method cited by Sadasivam and Manikam (1977) was followed for assaying the specific activity of catalase, while that of alkaline and acid proteases and polyphenoloxidase and peroxidase were carried out as per the methods cited by Thimmaiah (1999) and Pratibha Devi (2002) respectively. All the data were statistically analyzed by using student's t test.

RESULTS AND DISCUSSION

The quantitative analysis of pigments in the developing leaf (i.e. up to the 35 days age of the plant) shows that initially the amount of chlorophyll 'a' increases, but thereafter it decreases in the leaves of plants grown in both control and experimental plots. However, the decrease in the amount of chlorophyll 'a' is found to be relatively high in the leaves of plants grown under the simulated water stress condition (Gomti with 29%, Akshay with 22 % in Pusa Falguni with 19%) in comparison with that of the leaves of the 75 days old plants grown in the control plot (Fig. 1a). In contrast, the amount of chlorophyll 'b' decreases in the leaves of plants grown in the experimental plots by 48.6%, 55.6 % and 62.9% in Pusa Falguni, Akshay and Gomti genotypes respectively (Fig. 1b). The level of total chlorophylls also exhibits more or less similar trends as the amount of total chlorophylls increases initially in the leaves of control plants up to their 55 days age and up to 45 days age in the stress induced plants (Fig. 1c) but thereafter a sharp and significant decline occurs in the quantity of total chlorophylls during senescence of leaves in all the presently studied genotypes (Fig. 1c). Although water stress showed its impact on the quantity of total chlorophyll in all the three genotypes, its reduction is found to be more pronounced in Pusa Falguni with 35% decrease, while least reduction (30.1%) was noticed in Akshay genotype at their 75 days age over their counterparts in control plots. Thus the findings of the present investigation are in accordance with that of Rao and Mukherjee (1990) and Mukherjee (2003) who also noticed decline in the levels of chlorophylls in the leaves of C*ajanus cajan* after attaining maturity.

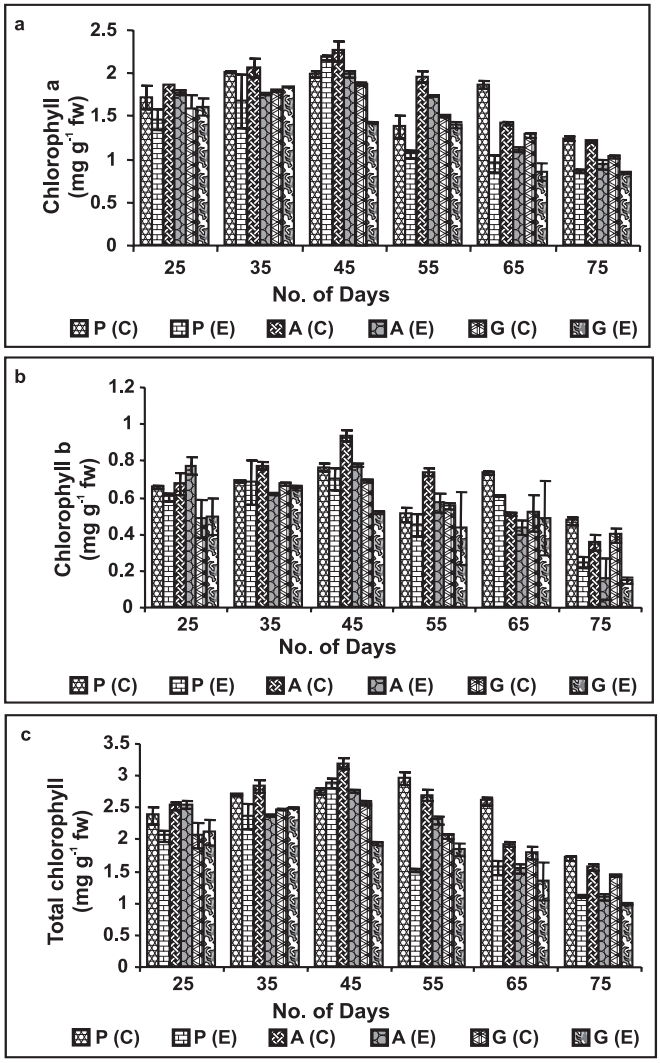

Fig.1. Changes in the chlorophyll a (a), chlorophyll b (b) and total chlorophyll (c) content (mg g^{-1} fw) in cowpea genotypes [Pusa Falguni (P), Akshay-102 (A) and Gomti vu-89 (G)] grown in control (C) and experimental (E) plots during development, maturation and senescence of leaves. Values are mean of triplicate ± S.D.

Like chlorophylls, carotenoids also exhibit a significant increase (17%) in their quantity in the 45 days old leaves of Gomti genotype of experimental plot (Fig. 2), whereas in the leaves of their counterpart Pusa Falguni and Akshay genotypes carotenoids increases to the levels of 27.2% and 13% respectively at their maturing stage. However, in the leaves of senescent stage the carotenoids increase to the level of 10%, 20.1% and 24.2% in the stress induced Gomti, Akshay and Pusa Falguni respectively as compared to that of the plants grown in control plots (Fig. 2). In contrast, from 25 days age onwards the mount of carotenoids continued to increase to the 1.5, 2.5, 1.6 fold in the stress induced plants than that of plants grown in control plot. However, the maximum amount of carotenoids occurs during senescence stage of leaves of both the categories of plants. Thus the amount of chlorophylls at this stage appears to be considerably less (Fig. 1, 2). According to Thimann (1980) the leaves turn yellow seems to show that carotenoids are more stable than chlorophylls. Dangl *et al*. (2000) also observed that in some cases where foliage of plants develops vivid colors before being shed, the loss of chlorophyll unmasks underlying carotenoids, which provide a yellow or orange background against which new pigment accumulates.

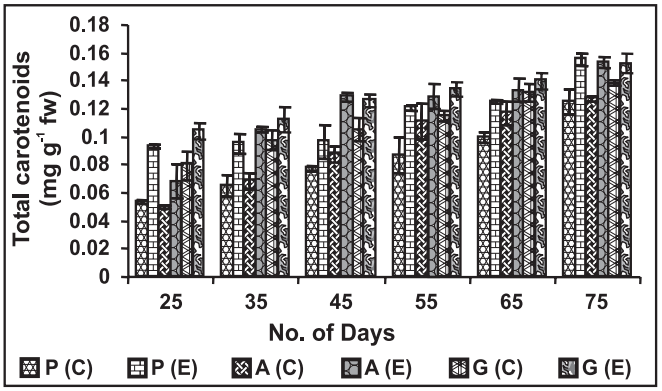

Fig.2. Changes in the total carotenoids content (mg g^{-1} fw) of cowpea genotypes [Pusa Falguni (P), Akshay-102 (A) and Gomti vu-89 (G)] grown in control (C) and experimental (E) plots during development, maturation and senescence of leaves. Values are mean of triplicate ± S.D.

Besides pigments, plants contain a large number of aromatic compounds with hydroxyl groups, which are collectively refered to as phenolics or phenols (Thimmaiah 1999). The quantum of total phenols increases gradually during the course of development, maturation and senescence of leaves of all the presently studied three varieties of cowpea, but significant increase (145%) of total phenols occurs in the leaves of stress induced plants of Gomti genotype during their 55 to 75 days age, while in Pusa Falguni it increases by 103% at maturing stage and only at senescent stage in Akshay genotype (Fig. 3). Thus there is 45.7%, 30.6% and 26% increase in the total phenols of the leaves of Pusa Falguni, Gomti and Akshay plants grown in the

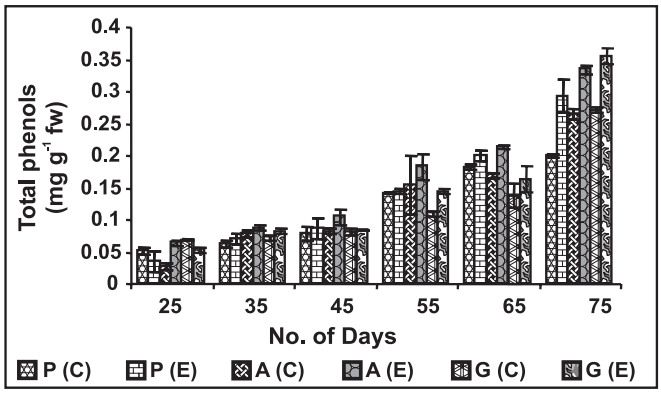

Fig.3. Changes in the total phenols content (mg g^{-1} fw) of cowpea genotypes [Pusa Falguni (P), Akshay-102 (A) and Gomti vu-89 (G)] grown in control (C) and experimental (E) plots during development, maturation and senescence of leaves. Values are mean of triplicate ± S.D.

experimental plants as compared to that of control plants. Thus results of the present findings are in accordance with the findings of Kar and Mishra (1976), who showed an increment in phenolic content with advancement of age (time). Buren (1970) also suggested that phenols were originally by-products of the metabolism of aromatic amino acids.

The quantitative levels of RNA increases initially (during early development) in the leaves of all the three genotypes, both control and stress simulated plants, studied under the present investigation, but thereafter they decrease, especially towards their maturation and senescent phases of leaves. A further analysis of RNA quantity patterns indicate that in Gomti it decreases significantly from 55 to 75 days, while in Pusa Falguni more decrease in RNA content occurs at its 75 days age, but at 45 and 55 days age significant decrease occurs in Akshay. At 75 days age 9%, 30.4% and 37.2% decrease was noticed in the leaves of water deficient plants over their counter control plants of Akshay, Gomti and Pusa Falguni genotype respectively. This finding is in line with the observations made by Makrides and Goldthwaite (1981) who reported that RNA rose to a peak during leaf growth, declined rapidly, and finally leveled off during maturity and senescence in bean leaves. The probable mechanism lying behind quantitative decline in RNA is mainly due to decrease in ribosomal RNA (rRNA), which is the most abundant cellular RNA in both the chloroplast and cytosol. This generalized loss of RNA is due to an increase in the RNase activities during senescence (Penarrubia and Moreno 1995).

Unlike the amount of RNA, the quantity of DNA decreases steadily and gradually in the leaves of control as well as stress induced plants of all the presently worked out cowpea genotypes as it is evident from 48% decrease in the DNA of Akshay, while 63% decrease in Pusa Falguni and 53.9% decrease from its initial amount in Gomti genotypes facing simulated water stress condition during their senescence stages. A statistical analysis of data reveals that the decrease of DNA is more significant only during maturation of leaves i.e. during 45 and 55 days age of all the genotypes. As compared to control plants, least reduction (only 8%) is noticed in Gomti, while Akshay genotype shows maximum reduction (13%) during the senescent phase of their leaves. These results are in accordance with the findings of Smillie and Krotkov (1961) who reported steady decrease in the DNA content during leaf senescence in pea from early stages onwards.

An increase in the quantity of total proteins occurs in the leaves of control plants from their 25 days age (i.e. during development phase of leaves) reaches to a peak when these plants become 55 days old, but thereafter decline in proteins occurs gradually during maturation and senescent phase of leaves of all the genotypes studied presently. This kind of decrease in the proteins leads to nearly a complete protein degradation with the least amount of it recorded at 75 days age of these plants (Fig. 4). However, plants growing under water stress condition show increase in their protein content and the maximum amount of it occurs at their 45 days age (i.e. when water was withhold), but again subsequently a steep decrease occurs (Fig. 4). Thus it may be attributed that water stress significantly decreased the content of proteins in Pusa Falguni and Gomti at 75 days age but it is found to be non significant in Akshay genotype (Fig. 4). Among all the three presently worked out varieties of cowpea reduction in the amount of protein is found to be maximum (46.2%) in Gomti genotype at its 75 days age over its control counterpart. These findings are in line with the findings of Rao and Mukherjee (1990) and Mukherjee (2003) who also reported decline in the

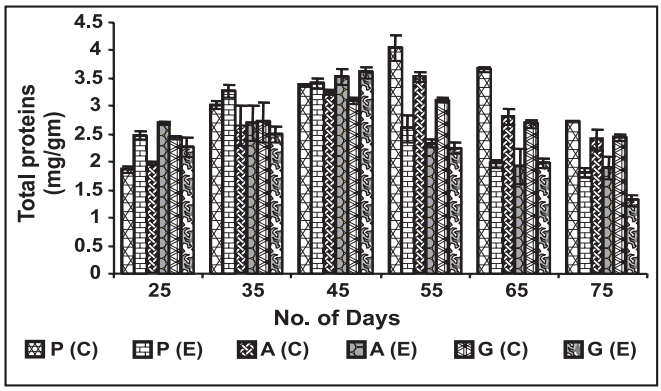

Fig. 4. Changes in the total proteins content (mg g^{-1} fw) of cowpea genotypes [Pusa Falguni (P), Akshay-102 (A) and Gomti vu-89 (G)] grown in control (C) and experimental (E) plots during development, maturation and senescence of leaves. Values are mean of triplicate ± S.D.

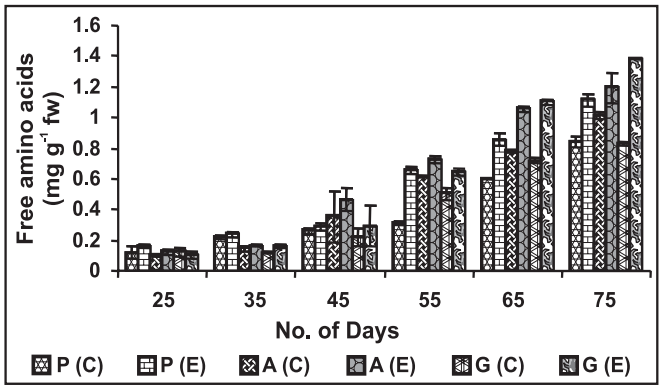

Fig. 5. Changes in the free amino acids content (mg g^{-1} fw) of cowpea genotypes [Pusa Falguni (P), Akshay-102 (A) and Gomti vu-89 (G)] grown in control (C) and experimental (E) plots during development, maturation and senescence of leaves. Values are mean of triplicate ± S.D.

protein content in *Cajanus cajan* leaves during their senescence. Moreover, loss of proteins during senescence is said to be one of the most common biochemical changes (Thimann 1980). In terms of total protein content, leaf senescence is characterized by the progressive loss of proteins (Brady 1988). Penarrubia and Moreno (1995) opined that increased protein breakdown may result from different mechanisms such as *de novo* synthesis of proteolytic enzymes, activation of pre-existing proteases, decompartmentalization of proteases and their substrates or susepetibilization of protein substrates to degradation.

In the present investigation, decline in the protein content is associated with an increase in the net pool of free amino acids level (Fig. 5). However, when the leaves reach the senescent phase a sharp and significant increase occurs in the quantity of free amino acids in the presently studied cowpea genotype, but with an exception of Akshay which (perhaps) exhibits its tolerance to drought condition (Fig. 5). At 75 days age of the plants, 65.9%, 32% and 17.4% increase is observed in the amino acid levels of Gomti, Pusa Falguni and Akshay varieties respectively as compared to that of control plants. Results of the present findings are in accordance with the findings of Garg *et al*. (2004) who noticed increased level of amino acids while studying the effect of water stress on moth bean genotype. According to Mukherjee (2003) increased level of the amino acids in senescing tissues is due to increased proteolysis. Proteases are the enzyme responsible for hydrolysis of proteins (Huffaker 1990). During the course of present study a significant increase in the specific activity of acid and alkaline protease is recorded in Pusa Falguni and Gomti genotype indicating their sensitivity to water stress condition (Fig. 6) whereas Akshay genotype exhibited non significant increase in activity of these enzymes during senescence of its leaves, which indicates its tolerance to drought condition (Fig. 6). Many workers including Mae *et al*. (1985) have correlated the decline in protein content with that of high protease activity during leaf senescence. According to Sawhney and Naylor (1982) decrease in soluble protein associated with an increase in free amino acids content, indicates stress induced increase in proteolytic activity. In the present investigation also a steady increase in the specific activity of acid protease is noticed in the plants grown in both the plots (Fig.6a) where as specific activity for alkaline protease decreased during development and maturation of leaves, but thereafter it increases during senescence of leaves in plants grown in both the plots (Fig.6b). More or less similar kind of increase in the activity of proteases at the time of senescence has been noticed by several researchers (Wittenbach 1979, Mae *et al*. 1985, Rao and Mukherjee 1990, Mukherjee 2003).

The data obtained from the present study indicates that the specific activity of alkaline protease is more

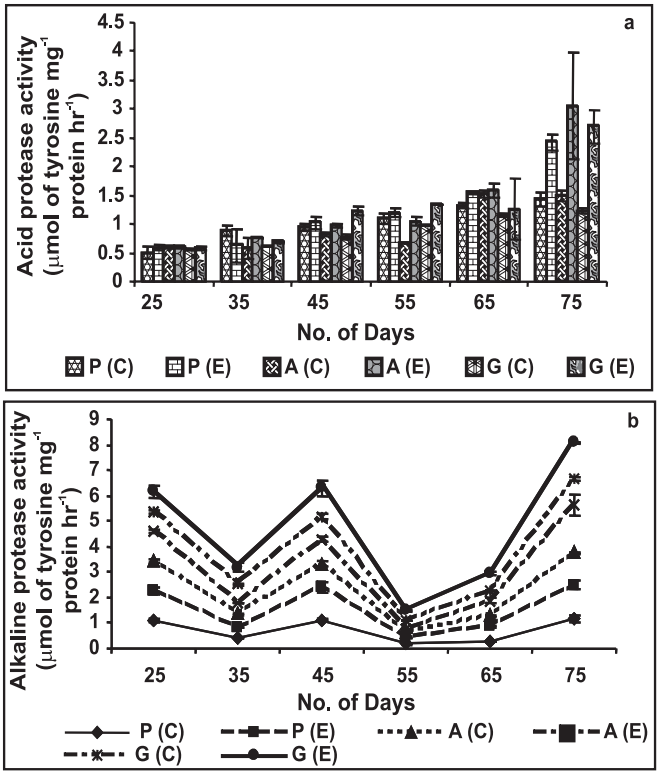

Fig. 6. Changes in the specific activity of acid protease (a) and alkaline protease (b) (μmol of tyrosine mg^{-1} protein hr^{-1}) of cowpea genotypes [Pusa Falguni (P), Akshay-102 (A) and Gomti vu-89 (G)] grown in control (C) and experimental (E) plots during development, maturation and senescence of leaves. Values are mean of triplicate ± S.D.

effective on proteolysis than that of acid protease because loss found in the protein content was accompanied with the concomitant rise in the specific activity of alkaline protease.

Catalase is an enzyme, which catalyzes the breakdown of H_2O_2 to water and molecular oxygen, present in nearly all plant cells, A steady decrease in the specific activity of catalase occurs in all three presently studied genotypes during development, maturation and senescence stage of leaves in plants grown in control as well as in the experimental plots (Table. 1), but only significant decrease is noticed in Pusa Falguni at its 65 days age, while in Akshay and Gomti the decrease in the activity of catalase is found to be non significant. The result reported here are in agreement with the findings of Dhindsa *et al.* (1981) who correlated the leaf senescence with the decreased levels of catalase.

Peroxidase has been implicated in plant senescence because of its ability to oxidize 3-indole acetic acid (IAA) (Parish 1968). As compared to normally irrigated plants, water stress significantly increased the specific activity of peroxidase in Gomti variety from 45 to 75 days age, while in Pusa Falguni and Akshay genotype only up to 65 days age (Table. 2). At 75 days age the specific activity of peroxidase increases by 21.1%, 41% and 59.7% over its respective control of Akshay, Pusa Falguni and Gomti genotypes respectively (Table. 2). Results obtained from the present study are in accordance with the findings of Parish (1968), Kar and Mishra (1976), Patra and Mishra (1979) and Mukherjee (2003) who also reported an increased level of peroxidase during senescence.

As in case of peroxidase, the specific activity of polyphenoloxidase also found to get increased

Table 1. Changes in the specific activity of catalase (units/mg protein) of cowpea genotypes during development, maturation and senescence of their leaves.

No. of days #	Age of leaves	Pusa Falguni		Akshay-102		Gomti vu-89	
		C	E	C	E	C	E
25	5	48.654±0.878	41.866±8.105	64.699±9.950	61.284±6.335	39.701±2.563	50.361±7.402
35	15	37.827±6.412	21.329±2.908	41.125±13.769	22.531±7.495	9.136±0.431	33.137±8.534
45	25	21.740±1.727	29.718±4.741 NS	36.207±1.667	47.887±7.596 NS	44.456±5.297	40.559±2.162 NS
55	35	18.051±3.091	21.639±1.661 NS	17.398±6.833	13.871±0.905 NS	10.710±1.042	19.588±3.140 NS
65	45	20.184±2.062	24.638±1.169*	31.941±6.074	32.441±6.324 NS	30.589±9.278	38.925±9.619 NS
75	55	12.313±0.647	13.793±1.387 NS	8.141±0.445	9.543±0.838*	10.503±0.309	12.640±2.288 NS

=Days after sowing of seeds, C = control, E = Experimental (induced water stress)
*= Significant at 5% level, NS=Non Significant, Values are mean of triplicate ± S.D.

Table 2. Changes in the specific activity of peroxidase (units mg^{-1} protein) of cowpea genotypes during development, maturation and senescence of their leaves.

No. of days #	Age of leaves	Pusa Falguni		Akshay-102		Gomti vu-89	
		C	E	C	E	C	E
25	5	0.989±0.209	0.605±0.160	1.369±0.263	1.455±0.425	0.441±0.058	0.681±0.040
35	15	1.421±0.237	3.058±0.511	1.524±0.120	3.746±0.177	1.140±0.219	1.331±0.068
45	25	3.411±0.104	4.942±0.187*	6.779±0.225	9.416±0.857*	4.013±0.837	7.747±0.097*
55	35	6.452±0.454	11.119±0.157*	14.670±1.632	17.514±1.920*	7.848±0.805	12.313±1.230*
65	45	14.093±0.618	25.019±1.797*	18.542±1.004	31.251±2.541*	14.704±1.174	24.585±2.121*
75	55	3.800±4.244	47.668±11.713 NS	46.762±6.478	56.634±11.269 NS	34.681±4.622	55.390±12.038*

=Days after sowing of seeds
*= Significant at 5% level, NS=Non Significant, Values are mean of triplicate ± S.D.

significantly during development, maturation and senescence of the leaves of Gomti variety plants grown in control as well as in the experimental plots (Fig. 7). In contrast to this, Akshay genotype exhibited non-significant increase for the same. But Pusa Falguni exhibited significant increase only at 55 and 65 days age (Fig. 2). In 75 days old plants of Akshay, Gomti and Pusa Falguni genotypes the specific activity of polyphenoloxidase increases to the tune of 11.9%, 15.6% and 22.1% respectively as compared to that of control plants. In this regard the findings of Patra and Mishra (1979) are noteworthy as they indicated high activities of Polyphenoloxidase during senescence of leaves and low activity in mature leaves as a specific feature of the rice plant.

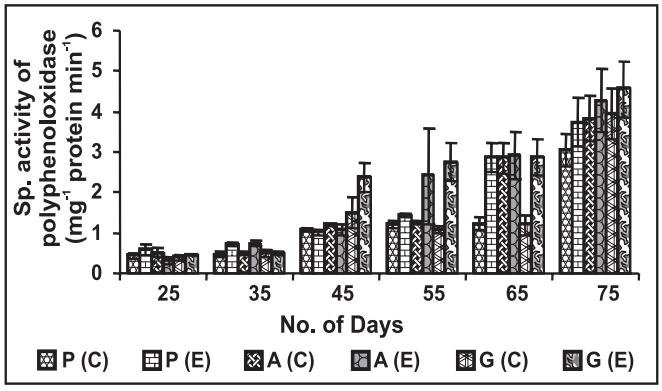

Fig.7. Changes in the specific activity of polyphenoloxidase (U mg^{-1} protein) of cowpea genotypes [Pusa Falguni (P), Akshay-102 (A) and Gomti vu-89 (G)] grown in control (C) and experimental (E) plots during development, maturation and senescence of leaves. Values are mean of triplicate ± S.D.

From the findings of the present study it may be concluded in general that Pusa Falguni and Gomti genotypes are more susceptible to drought condition as both these genotypes are invariably exhibited more metabolic alterations in terms of loss of chlorophyll, enhanced rate of synthesis of carotenoids and phenols, and disintegration of RNA, increase in the specific activity of peroxidase and polyphenoloxidase, proteolysis and associated accumulation of free amino acids. In contrast, Akshay genotype exhibits drought tolerance as it is found with less metabolic derangements of all the presently studied parameters, except for pigments and catalase activity. Furthermore, data obtained from the present investigation shows that simulated water stress condition affects the same metabolic events with enhancement of senescence leading to organ or plant death.

REFERENCES

Brady, C.J. (1988). Nucleic acid and protein synthesis. In: L.D. Nooden and A.C. Leopold (eds.), Senescence and Aging in Plants, pp. 147-181. Academic Press, London.

Buren, J.V. (1970). Fruit phenolics. In: A.C. Hulme (ed.), The Biochemistry of Fruits and their Products, Vol. 1, pp. 269-304. Academic Press, New York.

Dangl, J.L., Dietrich, R.A. and Thomas, H. (2000). Senescence and programmed cell death. In: B.B. Buchanan, W. Gruissem and R. Jones (eds.), Biochemistry and Molecular Biology of Plants. American Soc. Plant Physiologists, Rockville, Maryland.

Dhindsa, R.S., Plumb, P. and Thorpe, T.A. (1981). Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* **32:** 93-101.

Elizabeth, B.A., Bailey-Serres, J. and Weretilnyk, E. (2000). Plant responses to abiotic stresses. In: B.B. Buchanan, W. Gruissem and R. Jones (eds.), Biochemistry and Molecular Biology of Plants. American Soc. Plant Physiologists, Rockville, Maryland.

Garg, B.K., Burman, U. and Kathju, S. (2004). Effect of water stress on moth bean [*Vigna acontifolia* (Jacq.) Marechal] genotypes. *Indian J. Plant Physiol.* **9:** 29-35.

Hortensteiner, S. and Feller, U. (2002). Nitrogen metabolism and remobilization during senescence. *J. Exp. Bot.* **53:** 927-937.

Huffaker, R.C. (1990). Proteolytic activity during senescence of plants. *New Phytol.* **116:** 199-231.

Kar, M. and Mishra, D. (1976). Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. *Plant Physiol.* **57:** 315-319.

Kuhad, M.S., Nandwal, A.S. and Kundu, B.S. (1989). Physiological responses of pigeon pea (*Cajanus cajan* L.) genotypes to water stress. *Indian J. Plant Physiol.* **32:** 212-216.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193:** 265-271.

Mae, T., Hoshino, T. and Ohira, K. (1985). Proteinase activities and loss of nitrogen in the senescing leaves of field grown rice (*Oryza sativa*). *Soil Sci. Plant Nutr.* **31:** 589-600.

Makrides, S.C. and Goldthwaite, J. (1981). Biochemical changes during bean leaf growth, maturity and senescence. *J. Exp. Bot.* **32:** 725-737.

Malick, C.P. and Singh, M.B. (1980). Plant Enzymology and Histoenzymology. Kalyani publications, New Delhi.

Masclaux, C., Valadier, M-H., Brugiere, N., Morot-Gaudry, J-F., Hurel, B. (2000). Characterization of the sink source transition in tobacco (*Nictoana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. *Planta* **211:** 510-518.

Moore, S. and Stein, W.H. (1948). Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.*, **176:** 367-388

Mukherjee, D. (2003). Physiological changes in leaves, flowers and pods during development and senescence in *Cajanus cajan* L. *J. Indian Bot. Soc.* **82:** 78-84.

Parish, R.W. (1968). Studies on senescing tobacco leaf disks with special references to peroxidase. I. The effects of cutting and of inhibition of nucleic acid and protein synthesis. *Planta* **82:** 1-13.

Patra, H.K. and Mishra, D. (1979). Pyrophosphatase, peroxidase and polyphenoloxidase activities during leaf development and senescence. *Plant Physiol.* **63:** 318-323.

Penarrubia, L. and Moreno, J. (1995). Senescence in plants and crops. In: M. Pessarkli (ed.), Handbook of Plant and Crop Physiology, Marvel Dekker, Inc., USA.

Pic, E., Teyssendier, B., Tardieu, F. and Turc, O. (2002). Leaf senescence induced by mild water deficit follows the same sequence of macroscopic, biochemical and molecular events as monocarpic senescence in pea. *Plant Physiol.* **128:** 236-246.

Pratibha, Devi. (2002). Principles and Methods of Plant Molecular Biology, Biochemistry and Genetics. Agrobios, Jodhpur, India.

Rao, K.U.M. and Mukherjee, D. (1990). Some metabolic changes during development and senescence in the leaves of *Cajanus cajan*. *J. Indian Bot. Soc.* **69:** 311-314.

Sadasivam, S. and Manikam, A. (1991). Biochemical Methods. New Age International Publishers, New Delhi.

Sawhney, R. and Naylor, J.M. (1982). Dormancy studies in seeds of *Avena fatua*. 13. Influence of drought stress during seed development on duration of seed dormancy. *Can. J. Bot.* **60:** 1016-1020.

Smillie, R.M. and Krotkov, G. (1961). Changes in the dry weight, protein, nucleic acid and chlorophyll contents in growing pea leaves. *Can. J. Bot.* **39:** 891-900.

Thimmaiah, S.K. (1999). Standard Methods of Biochemical Analysis. Kalyani Publishers, New Delhi.

Thimann, K.V. (1980). The senescence of leaves. In: K.V. Thimann (ed.), Senescence in Plants. CRC Press, Boca Raton, Florida.

Wittenbach, V.A. (1979). Ribulose bisphosphate, carboxylase and proteolytic activity in wheat leaves from anthesis through senescence. *Plant Physiol.* **64:** 884-887.