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ORIGINAL ARTICLE

Impact of Seasonal Stress on Reactive Oxygen Species and Scavenging Enzymes of Two Crop Plants Growing Under Tropical Indian Conditions

Supatra Sen

[1] *Department of Botany, Asutosh College, Kolkata, Pin 700026. INDIA*

*E-Mail: ***supatra.sen@asutoshcollege.in***

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Nearly all metabolic changes and responses in the plant life cycle are influenced by seasonal environmental conditions which profoundly affect their growth, yield and metabolism. This work was carried out under tropical environmental conditions of Kolkata, West Bengal, India in three seasons –summer, rainy and winter in two stages – preflowering and postflowering to study the effect of seasonal variations (if any) on some select antioxidants and scavenging enzyme activities in Okra (*Abelmoschus esculentus* L. Moench) and Tomato (*Lycopersicon esculentum* Mill.) to determine the favourable/unfavourable seasons for growth and yield and thus correlate with the yield quality. Favourable seasons (summer for *Abelmoschus;* winter for *Lycopersicon*) recorded low reactive oxygen species production accompanied by elevated activities of scavenging enzymes while the unfavourable seasons (winter for *Abelmoschus;* rainy for *Lycopersicon*) showed the opposite trend. These periods were marked by abundant production of free radicals (meaured as MDA and total peroxide contents), accompanied by poor scavenging and reduced detoxification of these active oxygen species by the antioxidants (ascorbic acid) and scavenging enzymes (SOD, catalase, peroxidase, ascorbate peroxidase, glutathione reductase, ascorbic acid oxidase). These results could be well correlated with yield and yield quality of these two crop plants. The parameters under study served as useful bioassay indices of environmental stress, while the two plants acting as a measure of the prevailing environmental conditions, can serve as efficient bio indicator species. Thus, plant response to environment indicates the enormous impact of environmental stress on agricultural productivity.

Key words: Abiotic stress, Antioxidative defense, Bioindicator, Seasonal variations

Normal cell metabolism requires efficient scavenging and detoxification of active oxygen species. Antioxidant enzymes constitute an important defence system to clear up the detrimental reactive oxygen species *in vivo.* Plants possess superoxide dismutase (SOD) which catalyzes the disproportionation of $O^{\cdot-}_2$ to H_2O_2 and O_2. H_2O_2 is scavenged by two classes of related enzymes - the catalases and peroxidases. These enzymes catalyze the divalent reduction of H_2O_2 to $2H_2O$ using H_2O_2 as the electron donor in the case of catalases, or using a variety of reductants in the case of peroxidases. Catalase is localized in the peroxisomes where it scavenges H_2O_2 produced by glycolate oxidase, a very active enzyme responsible for the oxidation of glycolate to glyoxylate.

The ascorbate - glutathione - NADPH system is responsible for the scavenging of H_2O_2 in chloroplasts being catalyzed by three enzymes *viz.* ascorbate peroxidase, dehydroascorbate reductase and glutathione reductase. Ascorbate peroxidase reduces H_2O_2 to water using ascorbate as the reductant. H_2O_2 can also be reduced by the activity of glutathione - dependent dehydroascorbate reductase. Glutathione reductase converts oxidized form of glutathione generated by dehydroascorbate reductase back to the reduced form of glutathione. This enzyme serves to ensure that the ratio between reduced glutathione and oxidised glutathione of the cells is present mostly in the form of reduced glutathione with only a minute amount of oxidised glutathione (Esterbauer and Grill 1978). In addition to the antioxidant enzymes such as catalase, glutathione peroxidase and SOD, glutathione, a cysteine containing tripeptide provides an important cellular defence against oxidative stress (Sagara *et al.,* 1998). Glutathione is the most abundant sulfhydryl compound, comprising more than 95% of the total sulfhydryl content (Grill *et al.,* 1979).

In plants, the enzymes SOD, catalase, peroxidase, ascorbate peroxidase and glutathione reductase form the first line of defence. The second line of defence is provided by ascorbic acid or Vitamin C (scavenger of $O^{\cdot-}_2$, $^{\bullet}OH$ and $^1O_2$), α-tocopherol or Vitamin E (scavenger of $^1O_2$ and inhibitor of lipid peroxidation chain reaction), reduced glutathione (scavenger of $^{\bullet}OH$ and $^1O_2$) and carotenoids (scavenger of $^1O_2$ and inhibits the formation of $^1O_2$ from triplet chlorophyll by quenching excess excitation energy from chlorophyll). The enzyme ascorbic acid oxidase oxidises ascorbic acid to dehydroascorbic acid.

Nearly all metabolic changes and responses in the plant life cycle are influenced by seasonal environmental conditions which profoundly affect their growth, productivity, yield and yield quality (Sen and Mukherji 1998a,b,c,d,e, 1999a,b, 2000, 2002). This work was carried out to study the effect of seasonal variations (if any) on some select antioxidants and scavenging enzyme activities in Okra (*Abelmoschus esculentus* L. Moench) and Tomato (*Lycopersicon esculentum* Mill.) to determine the favourable/unfavourable seasons for growth and yield and thus correlate with the yield quality.

MATERIALS AND METHODS

The experiments were conducted in pot culture and studied in summer (March-June), rainy (July-September) and winter (November-February) seasons. Seed varieties which could germinate throughout the year in all the three seasons, were sown in earthen pots in the experimental garden of the University Campus at Kolkata (22.34° North, 88.24° East), West Bengal, India under tropical environmental conditions. Seeds of *Abelmoschus esculentus* (L.) Moench (Okra) variety Parbani Kranti and *Lycopersicon esculentum* Mill. (Tomato) variety Pusa Ruby were obtained from National Seed Corporation. For a particular season, the experiments were conducted in both a) Preflowering (vegetative) and b) Postflowering (reproductive) stages with fully expanded penultimate (second from top) leaves. In *Abelmoschus,* the leaves were collected 28 days after sowing (DAS) for experiments in the preflowering stage and 58 days after sowing (DAS) for experiments in the postflowering stage in all seasons. In *Lycopersicon,* the leaves were collected 35 days after sowing (DAS) for experiments in the preflowering stage and 75 days after sowing (DAS) for experiments in the postflowering stage in all the seasons.

Estimation of total peroxide was done by ferrithiocyanate method of Thurman *et al.* (1972) and Malondialdehyde was estimated by the method of Heath and Packer (1968). Glycolate oxidase enzyme activity was assayed according to the method of Zelitch (1953),

superoxide dismutase by the method of Marshall and Worsfold (1978), catalase enzyme activity was assayed by the method of Gasper and Lacoppe (1968), peroxidase enzyme activity was assayed spectrophotometrically according to Chance and Maehly (1955). Ascorbate peroxidase was assayed according to Nakano and Asada (1981) and Glutathione reductase was assayed according to the method of Gamble and Burke (1984). Sulfhydryl content was determined according to Ellman (1959), estimation of ascorbic acid by the method of Mitsui and Ohta (1961) and Ascorbic acid oxidase activity was assayed according to Oberbacher and Vines (1963).with slight modifications.

All the experiments were repeated thrice, and the data obtained from 3 replications were statistically analysed. Standard Error (S.E.) and Critical Difference (C.D.) values both at 5% and 1 % levels were calculated from the respective analysis of variance (ANOVA) tables.

RESULTS

Total peroxide content was markedly higher in the winter season of Abelmoschus. The content exhibited drastic reductions in the rainy and summer seasons (Fig. 1A). Total peroxides increased from the preflowering to the postflowering stages in all seasons. In *Lycopersicon*, total peroxide content was significantly higher in the rainy season. Summer, followed by winter recorded low peroxide contents (Fig. 1B). Postflowering leaf samples contained higher peroxide content as compared to the preflowering samples.

Malondialdehyde (MDA) content in *Abelmoschus* exhibited an identical trend as total peroxides. The content was the highest in winter with considerably lower amounts in the rainy and summer seasons (Fig. 2A). MDA content increased from the preflowering to the postflowering. *Lycopersicon* leaves possessed significantly higher MDA content in the rainy seasons with decreased amounts in the summer and winter seasons (Fig. 2B). Leaves contained higher MDA content in the postflowering stages.

Glycolate oxidase enzyme activity in *Abelmoschus* was maximum in winter but severely lowered in the rainy and finally in the summer season (Fig. 3A). As compared to the preflowering stages the enzyme activity became more pronounced in the postflowering stages. In *Lycopersicon,* enhanced activity of glycolate oxidase enzyme was observed in the rainy season which dropped significantly in the summer and winter seasons (Fig. 3B). The enzyme activity increased from the preflowering to the postflowering.

In *Abelmoschus,* superoxide dismutase enzyme activity peaked in winter and suffered severe reductions in the rainy and summer seasons (Fig. 4A). The enzyme activity dropped sharply from the preflowering to the postflowering stages in all seasons. Superoxide dismutase activity in *Lycopersicon* was elevated in rainy but considerably depressed in the summer and winter months (Fig. 4B). The postflowering leaf samples recorded a lower enzyme activity than the preflowering.

Catalase enzyme exhibited significantly higher activity in winter in *Abelmoschus*. The activity declined in the rainy and summer months successively (Fig. 5A). The enzyme activity decreased from the preflowering to the postflowering. In *Lycopersicon,* catalase enzyme activity enhanced in the rainy months and reduced successively in the summer and winter months (Fig. 5B). The enzyme activity diminished from the preflowering to the postflowering.

Peroxidase enzyme activity in *Abelmoschus* was maximum in winter which underwent drastic reductions in the rainy and summer seasons (Fig. 6A). The activity increased in the postflowering stages of all seasons. In *Lycopersicon,* the activity of peroxidase enzyme was the highest in the rainy season but declined significantly in the summer and winter months (Fig. 6B). The enzyme activity was more pronounced in the postflowering stages.

Activity of ascorbate peroxidase enzyme in *Abelmoschus* was significantly enhanced in the summer season but progressively declined in the rainy and winter months (Fig. 7A). The enzyme activity dropped from the preflowering to the postflowering. In *Lycopersicon,* ascorbate peroxidase exhibited significantly higher activity in the winter season. The enzyme activity was considerably inhibited in the summer followed by the rainy season (Fig. 7B). The enzyme activity diminished in the postflowering stages as compared to the preflowering.

Glutathione reductase enzyme activity in *Abelmoschus* was elevated in the summer season but lowered in the rainy and winter seasons (Fig. 8A). The enzyme activity remained higher in the preflowering stages as compared

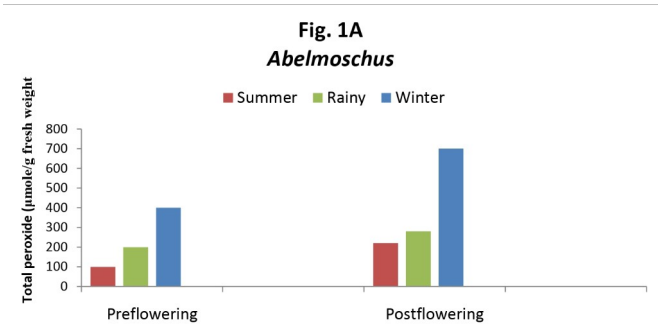
to the postflowering.

In *Lycopersicon,* glutathione reductase enzyme activity was maximum in the winter season but significantly reduced in the summer and rainy months (Fig. 8B). The enzyme activity decreased from the preflowering to the postflowering.

Sulfhydryl content in *Abelmoschus* peaked in summer with decreased amounts in the rainy and winter seasons (Fig.9A). Leaves contained higher sulfhydryl content in the preflowering stage. In *Lycopersicon,* higher sulfhydryl content was recorded in the winter season with decreasing amounts in the summer and rainy seasons (Fig. 9B). The content dropped from the preflowering to the postflowering.

In *Abelmoschus,* ascorbic acid content increased in the summer season but underwent considerable reductions in the rainy and winter seasons (Fig.10A). The preflowering leaf samples possessed higher level of ascorbic acid. In *Lycopersicon,* ascorbic acid content was elevated in the winter season but declined in the summer and rainy seasons (Fig.10B). Ascorbic acid content decreased from the preflowering to the postflowering.

Ascorbic acid oxidase in *Abelmoschus* was enhanced in the winter season with significant reductions in the rainy and finally summer seasons (Fig.11A). The enzyme activity was more pronounced in the postflowering stages in all the seasons. In *Lycopersicon,* ascorbic acid oxidase activity was higher in the rainy season. The enzyme activity dropped in the summer and winter seasons successively (Fig.11B). There was a marked rise in the activity of the enzyme from the preflowering to the postflowering stage.



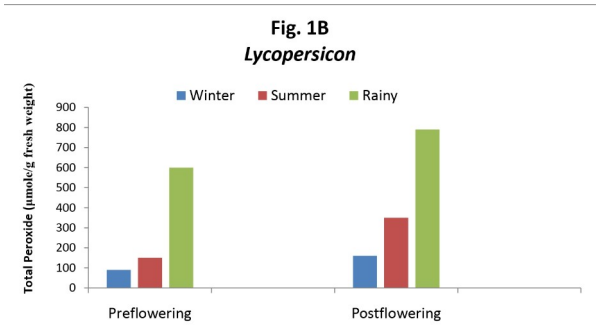


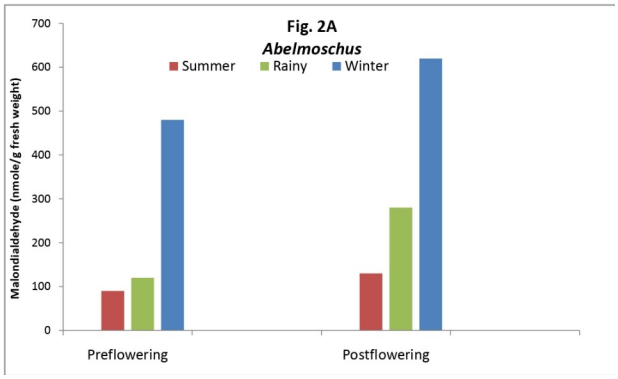
Figure. 1. Total Peroxide Content

A

S.E.= 10.26
C.D. of Season =10.55 (5%), 15.88 (1%)
C.D. of Stage = 8.62 (5%), 12.96 (1%)

B

S.E.= 8.39
C.D. of Season =8.63 (5%), 12.98 (1%)
C.D. of Stage = 7.04 (5%), 10.60 (1%)



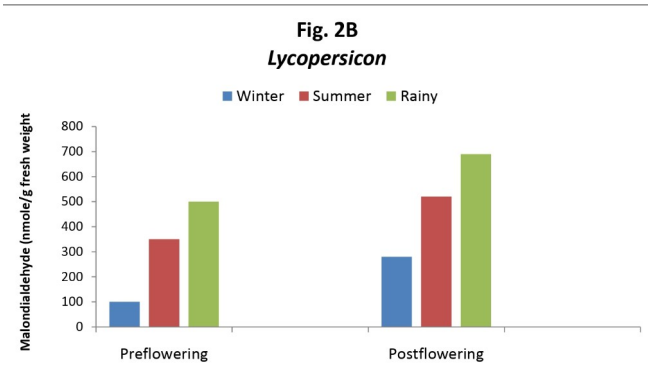


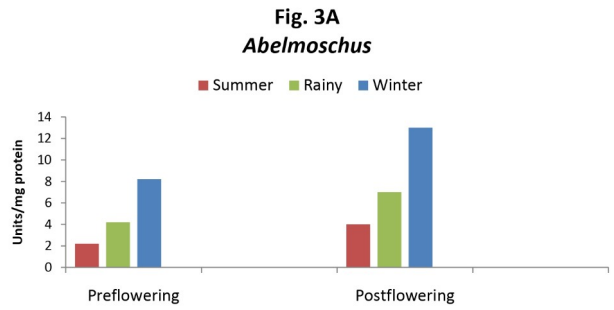
Figure 2. Malondialdehyde Content

A

S.E.= 3.54
C.D. of Season =3.64 (5%), 5.48 (1%)
C.D. of Stage = 2.97 (5%), 4.48 (1%)

B

S.E.= 10.73
C.D. of Season =11.04 (5%), 16.61 (1%)
C.D. of Stage = 9.01 (5%), 13.56 (1%)



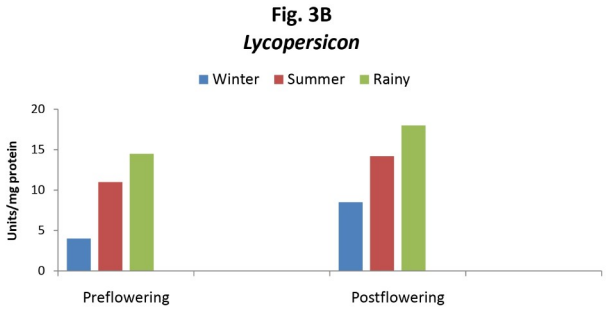


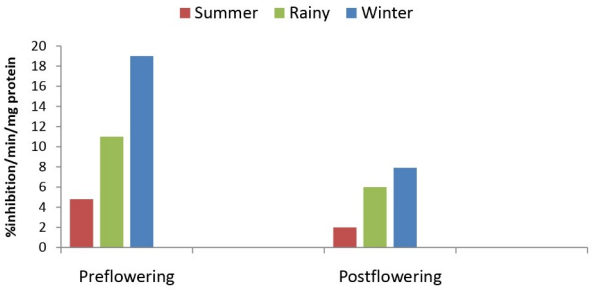
Figure 3. Glycolate oxidase enzyme activity

A

S.E.= 0.43
C.D. of Season =0.45 (5%), 0.68 (1%)
C.D. of Stage = 0.36 (5%), 0.55 (1%)

B

S.E.= 0.25
C.D. of Season =0.25 (5%), 0.38 (1%)
C.D. of Stage = 0.21 (5%), 0.31 (1%)



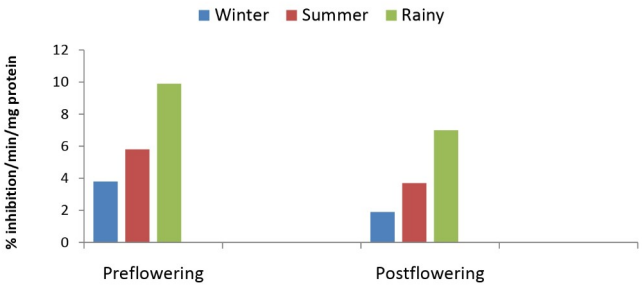


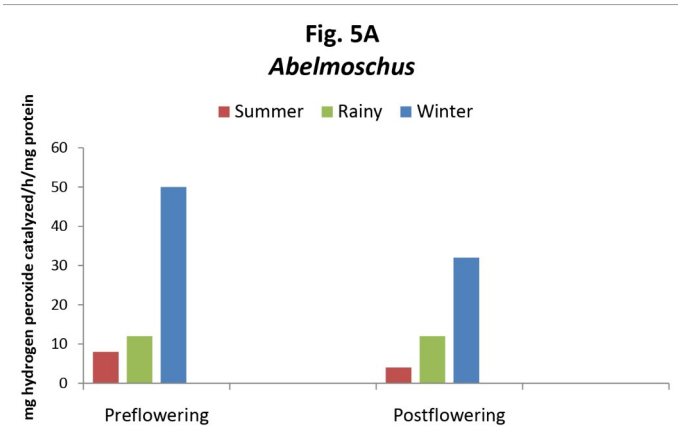
Figure 4. Superoxide dismutase enzyme activity

A

S.E.= 0.09
C.D. of Season =0.10 (5%), 0.15 (1%)
C.D. of Stage = 0.08 (5%), 0.12 (1%)

B

S.E.= 0.12
C.D. of Season =0.12 (5%), 0.18 (1%)
C.D. of Stage = 0.10 (5%), 0.15 (1%)



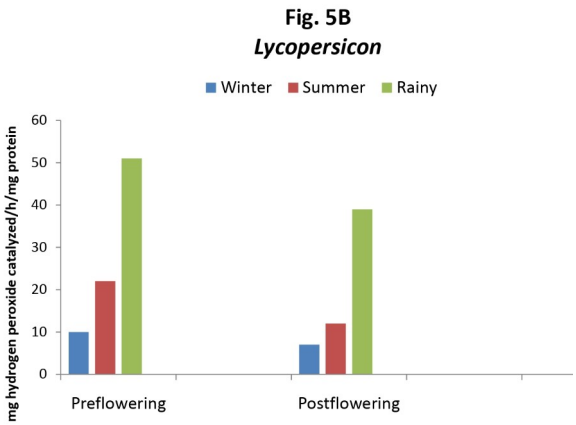


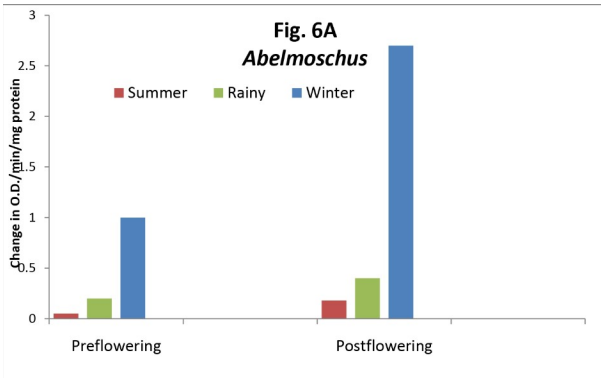
Figure 5. Catalase enzyme activity

A

S.E.= 0.26
C.D. of Season =0.27 (5%), 0.40 (1%)
C.D. of Stage = 0.22 (5%), 0.33 (1%)

B

S.E.= 0.73
C.D. of Season =0.75 (5%), 1.13 (1%)
C.D. of Stage = 0.61 (5%), 0.93 (1%)



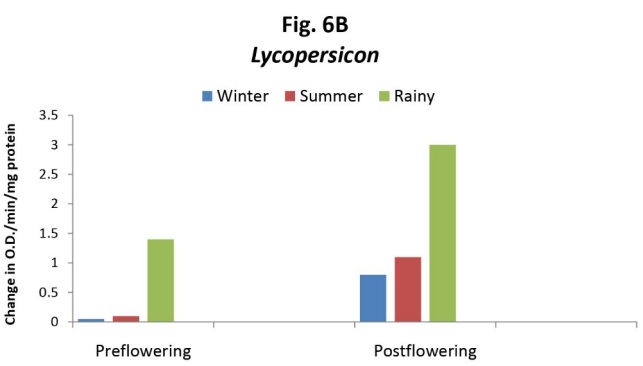


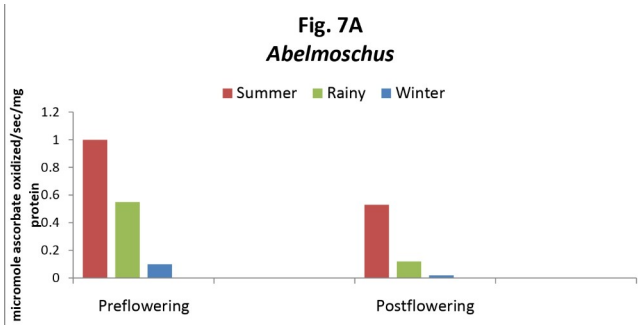
Figure 6. Peroxidase enzyme activity

A

S.E.= 0.07
C.D. of Season =0.07 (5%), 0.11 (1%)
C.D. of Stage = 0.06 (5%), 0.09 (1%)

B

S.E.= 0.11
C.D. of Season =0.11 (5%), 0.17 (1%)
C.D. of Stage = 0.09 (5%), 0.14 (1%)



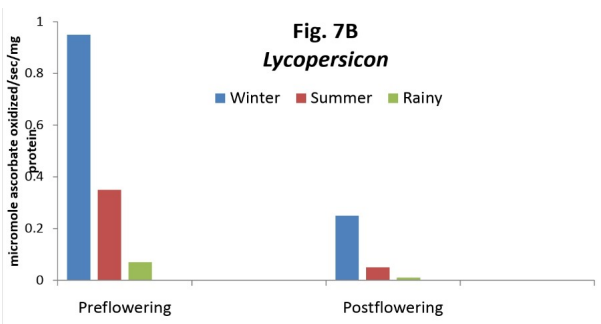


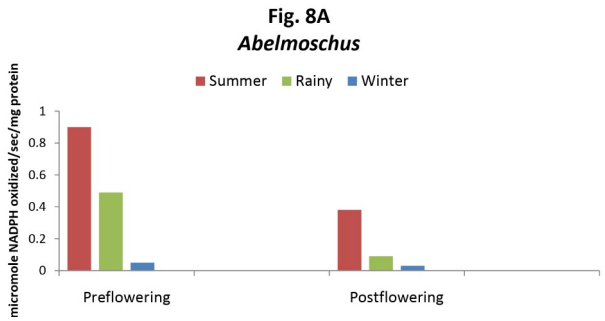
Figure 7. Ascorbate peroxidase enzyme activity

A

S.E.= 0.01
C.D. of Season =0.01 (5%), 0.02 (1%)
C.D. of Stage = 0.01 (5%), 0.02 (1%)

B

S.E.= 0.01
C.D. of Season =0.01 (5%), 0.02 (1%)
C.D. of Stage = 0.011 (5%), 0.017 (1%)



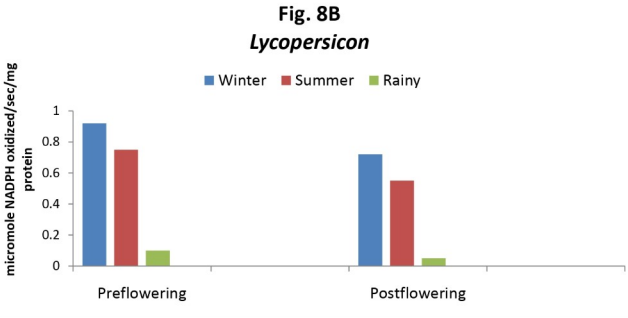


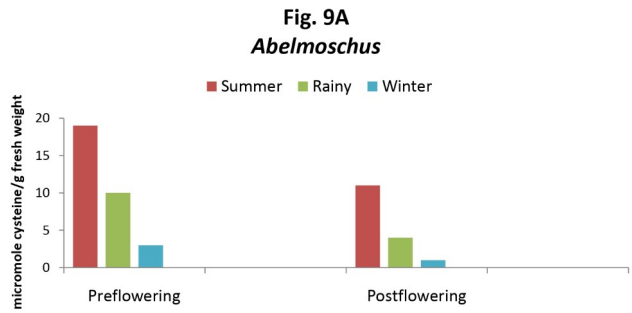
Figure 8. Glutathione reductase enzyme activity

A

S.E.= 0.01
C.D. of Season =0.011 (5%), 0.016 (1%)
C.D. of Stage = 0.009 (5%), 0.01 (1%)

B

S.E.= 0.01
C.D. of Season =0.01 (5%), 0.02 (1%)
C.D. of Stage = 0.01 (5%), 0.02 (1%)



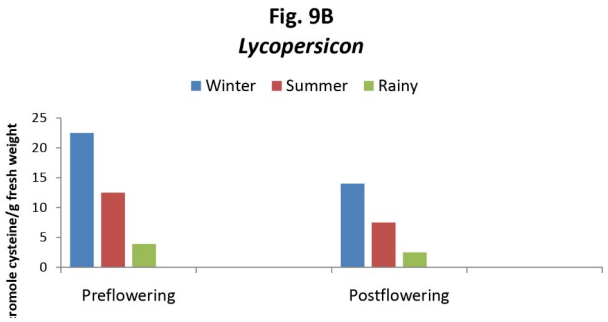


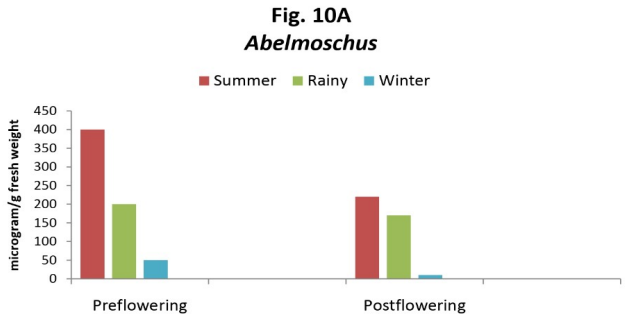
Figure 9. Sulfhydryl content

A

S.E.= 2.86
C.D. of Season =2.94 (5%), 4.43 (1%)
C.D. of Stage = 2.40 (5%), 3.62 (1%)

B

S.E.= 3.08
C.D. of Season =3.17 (5%), 4.77 (1%)
C.D. of Stage = 2.59 (5%), 3.89 (1%)



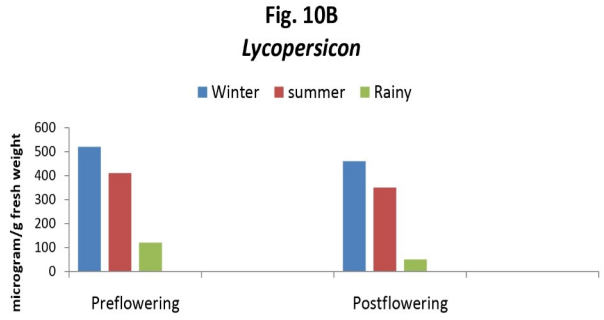


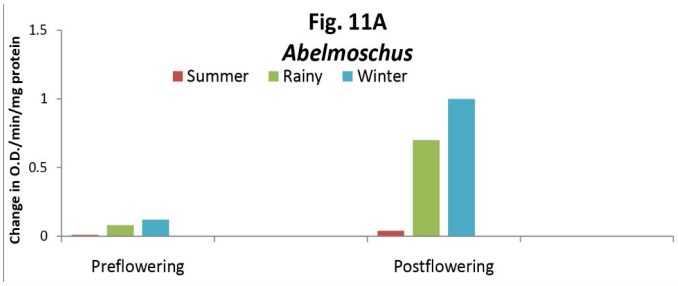
Figure 10. Ascorbic acid content

A

S.E.= 2.42
C.D. of Season =2.49 (5%), 3.75 (1%)
C.D. of Stage = 2.03 (5%), 3.06 (1%)

B

S.E.= 16.53
C.D. of Season =17.01 (5%), 25.59 (1%)
C.D. of Stage = 13.88 (5%), 20.89 (1%)



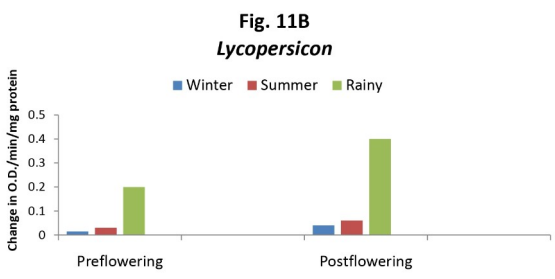


Figure. 11. Ascorbic acid oxidase enzyme activity

A

S.E.= 0.13
C.D. of Season =0.13 (5%), 0.20 (1%)
C.D. of Stage = 0.10 (5%), 0.16 (1%)

B

S.E.= 0.18
C.D. of Season =0.18 (5%), 0.28 (1%)
C.D. of Stage = 0.15 (5%), 0.22 (1%)

DISCUSSION

Utilization of oxygen represents an efficient mechanism for aerobic organisms to generate energy, but reactive oxygen species as the by-products during this process and other unfavourable events are also produced within the biological system (Tian *et al.* 1998). Under environmental stress conditions, which reduce the capacity to assimilate C, it has been suggested by Asada (1996) that photosynthetic electron flux to O_2 will increase, resulting in the increased production of superoxide anion radical ($O^{\cdot}_2$), hydrogen peroxide (H_2O_2) and hydroxyl ($^{\cdot}OH$) radicals. These active oxygen species are highly reactive and capable of damaging many biological macromolecules

such as DNA, RNA, protein and lipids (Tian *et al.* 1998). There is definite positive correlation between increased antioxidant activity and various abiotic stress tolerance. Scavenging enzymes play an important role against stress. After plant exposure to abiotic stresses such as salinity, drought, temperature extremes, herbicide treatment and mineral deficiency, the balance between the production of reactive oxygen species (ROS) and the scavenging activity of antioxidants is disturbed, often resulting in oxidative damage (Kusvuran *et al.* 2016).

Plants are protected against oxidative stress damages by antioxidative defense mechanisms. Plants possess very potent enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-S- transferase, GST) and non-enzymatic (ascorbic acid, ASH; glutathione, GSH; phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols) antioxidant defense systems which work in tandem to restrict uncontrolled oxidation and defend plant cells from oxidative damage by scavenging of ROS (Caverzan *et al.* 2016) .

In the present work, total peroxides estimated from the leaf samples increased in winter in *Abelmoschus* (Fig.1A) and rainy in *Lycopersicon* (Fig.1B). Lipid peroxidation was estimated by measuring malondialdehyde (MDA), a decomposition product of the oxidation of polyunsaturated fatty acids. MDA content was also found to be increased in winter in *Abelmoschus* (Fig.2A) and rainy in *Lycopersicon* (Fig.2B), thus indicating free-radical induced damage. MDA mediates photoperoxidative destruction of chlorophyll, which could be one of the reasons of low chlorophyll content in winter for *Abelmoschus* and rainy for *Lycopersicon* (Sen and Mukherji 1998b, 1999a). It also inhibits amino acid incorporation into proteins, reacts with and modifies the properties of proteins and amino acids possibly leading to their poor content in those same months (Sen and Mukherji 1998d). The increase of MDA produced by lipid peroxidation indicates the buildup of superoxide radical toxicity (Sen, 2016a,b). Lipid peroxidation has been found to be increased under a wide range of stresses including osmotic, drought, radiation and temperature extremes (Klebanov *et al.* 1996, Fryer *et al.* 1998). H_2O_2 has also been reported to increase under stress conditions (Jiang *et al.* 1994). Thus the seasonal conditions in winter for *Abelmoschus* and rainy for *Lycopersicon* are unfavourable, as they behave as periods of environmental stress for the two plants. The cumulative effects of temperature, light, humidity and rainfall in the winter and rainy seasons for *Abelmoschus* and *Lycopersicon* respectively, create a stressful environment, during which harmful free radicals are amply produced.

In the present work, both total peroxide and MDA contents were found to be increased in the matured leaf samples *i.e.* in the postflowering stage. Enhanced lipid peroxidation and increased peroxide accumulation were observed in aging tissues in other studies (Sen, 2016a,b). Lipid peroxidation increased during senescence due to significant decrease in the ability of scavenging free radicals (Sen, 2016a,b). Thus, activated oxygen radicals play an important role in leaf senescence and aging.

In this work, glycolate oxidase activity, which produces H_2O_2, increased in winter in *Abelmoschus* (Fig.3A) and rainy in *Lycopersicon* (Fig.3B). The increased activity of this enzyme in these two particular seasons, also coincided with the increase in total peroxide content (Fig.1A and 1B). The enzyme activity was more pronounced in the postflowering stages in the two plants and here too coincided with higher peroxide content.

Superoxide dismutase (SOD) activity was maximum in winter in *Abelmoschus* (Fig.4A) and rainy in *Lycopersicon* (Fig.4B). The postflowering leaf samples recorded a lower activity than the preflowering. Activity of SOD increased in those seasons in which lipid peroxidation and free radical formation were maximum, and this may well be assumed as an effort to scavenge and detoxify the active oxygen species. Activity of SOD has been reported to be elevated in the early phase of drought stress and also in temperature stress (Zhang and Kirkham 1994, Fryer *et al.* 1998). As per studies of Li *et al.* (1995), SOD activity has been reported to decrease in maturing or senescent leaves and the various reports are in conformity with my results. In fact, SOD enzyme activity might be employed as one of the biochemical indices in studying leaf senescence (Li *et al.* 1995), and along with catalase and peroxidase enzymes serve as effective indices of existing

environmental stress including heavy metal toxicity *viz.* arsenic toxicity (Sen 2016a,b). The plant SOD genes are controlled by development, tissue-dependent and environmental signals (Scandalios 2005).

In this work, catalase activity was significantly higher in winter in *Abelmoschus* (Fig.5A) and rainy in *Lycopersicon* (Fig.5B). The enzyme was more active in the preflowering stages of both plants. Like SOD, catalase activity showed an increase in the early phase of drought in wheat (Zhang and Kirkham 1994). Catalase is the potent H_2O_2 destroying enzyme whose activity increases manifold to scavenge the free radicals in the periods of environmental stress, as evidenced by the findings of the present work. Catalase activity in general, has always been found to decline during leaf senescence (Li and Mei 1989).

In my study, peroxidase activity was greatly enhanced in the winter of *Abelmoschus* (Fig.6 A) and rainy of *Lycopersicon* (Fig.6B). The enzyme was more active in the postflowering stages of both plants. Peroxidase has been found to be highly active under various conditions of stress. In fact, the activities of enzymes involved in the H_2O_2 - scavenging pathway were also observed to increase under various stress conditions (Mishra *et al.* 1995, Knorzer *et al.* 1996), and the present findings lend support to such observations. Increase in peroxidase activity during senescence has been well documented in different plant species (Mukherjee and Rao 1993, Sen 2014a, b, 2016a,b; Sen *et al*. 2014). Catalase and peroxidase enzyme activities may be taken as measures of leaf senescence (Sen *et al*. 2014).

In the present study, ascorbate peroxidase activity declined in the winter season of *Abelmoschus* (Fig.7A) and rainy in *Lycopersicon* (Fig.7B). The enzyme was more active in the preflowering stages of both crops. Decrease in ascorbate peroxidase activity during the periods of seasonal stress indicates the reduced efficiency of the chloroplasts to scavenge H_2O_2. Spruce seedlings under abiotic stress like temperature stress, showed decreasing ascorbate peroxidase activity, which corresponded to a sudden rise in lipid peroxidation, according to a study by Polle *et al.* 1996. Aging and senescence have been reported to suppress the activity of ascorbate peroxidase (Sung and Jeng 1994).

In the present work, glutathione reductase activity also declined in the winter season in *Abelmoschus* (Fig.8A) and rainy in *Lycopersicon* (Fig.8B). The enzyme activity was higher in the preflowering stages of both plants. Esterbauer and Grill (1978) reported that glutathione reductase activity showed an annual rhythm. It is known that reduced glutathione stabilizes protein-SH groups either by scavenging oxidising agents or by reducing formed S-S bonds in a nonenzyme reaction. These authors are of the opinion that since reduced glutathione is oxidised in both cases, it is evident that the protecting action of reduced glutathione is effective only as long as oxidised glutathione is reduced as soon as it is formed. Thus a decrease in glutathione reductase activity in the unfavourable seasons (winter of *Abelmoschus* and rainy of *Lycopersicon)* indicates a lessened ability to restore reduced glutathione leading to poor cleansing of the free radicals.

Results reported elsewhere suggest that changes in the physiological and environmental conditions affect the interaction of ascorbate - related enzymes *viz*. ascorbate peroxidase, mono-dehydroascorbate radical reductase, dehydroascorbate reductase and glutathione reductase in plants (Polle and Morawe 1995). Decreased activities of ascorbate peroxidase and glutathione reductase can be correlated with the general lowering of metabolism as indicated by stunted growth in wheat (Biemelt *et al.* 1998) which conform to my results exactly.

In the present study sulfhydryl content was the highest in summer in *Abelmoschus* (Fig.9A) and in winter in *Lycopersicon* (Fig.9B). Leaves contained higher sulfhydryl content in the preflowering stages. High sulfhydryl content, in general, is characteristic for young, physiologically active tissue (Pilet and Dubois 1968). It has been reported by Kok *et al.* (1981) that plants older than three months are almost incapable to accumulate sulfhydryl compounds. Glutathione is the most abundant sulfhydryl compound, comprising more than 95% of the total sulfhydryl content (Grill *et al.* 1979). Glutathione seems to have a dual role in plant metabolism: regulating S nutrition and defence against oxidative stress (Lappartient and Touraine 1997).

In the present work, ascorbic acid content was higher in summer in *Abelmoschus* (Fig.10A) and winter in *Lycopersicon* (Fig.10B). Higher ascorbic acid was present in the preflowering leaf samples. Ascorbic acid acts as a

potent and probably the most important hydrophilic antioxidant (Etsuo *et al.* 1995), and is an effective reducing agent and remover of $O^{\cdot-}_2$, $^{\cdot}OH$ and $^1O_2$ (Bodannes and Chan 1979). However, under conditions of stress, there is a decrease in the content of ascorbic acid (Sen *et al.* 2014b, 2016a,b). Decline in the ascorbic acid content during stress or in senescence as evidenced by my findings, imply decreased capacity of the leaves to scavenge the free radicals and maintain the general reducing environment.

In this present work, ascorbic acid oxidase increased in the winter of *Abelmoschus* (Fig.11A) and rainy of *Lycopersicon* (Fig.11B). This multi-copper enzyme catalyzes the oxidation of ascorbic acid to dehydroascorbate. From this work and a previous work on *Vigna radiata* (Sen *et al.* 2014) it is evident that ascorbic acid content and ascorbic acid oxidase activity are inversely related to one another (Fig. 35A, 36A and 35B, 36B). The enzyme was more active in the postflowering stages of both plants. The role of this enzyme has been thought to be important in regulating the levels of reduced and oxidised glutathione and NADPH (Malik and Singh 1980).

Unfavourable seasons (winter for *Abelmoschus;* rainy for *Lycopersicon*) were marked by abundant production of free radicals (meaured as MDA and total peroxide contents), accompanied by poor scavenging and reduced detoxification of these active oxygen species by the antioxidants (ascorbic acid, sulfhydryl) and scavenging enzymes (SOD, catalase, peroxidase, ascorbate peroxidase, glutathione reductase, ascorbic acid oxidase, glycolate oxidase). These results could be well correlated with yield, productivity and yield quality of these two crop plants. The parameters under study served as useful bioassay indices of the prevailing seasonal environmental stress, while the two plants acting as a measure of the existing environmental conditions, can serve as efficient bio indicator species. Thus, plant response to environment indicates the enormous impact of environmental stress on agricultural productivity.

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CONFLICT OF INTERESTS

The author declare that he has no potential conflicts of interest.

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