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Season-controlled changes in biochemical constituents and oxidase enzyme activities in tomato (*Lycopersicon esculentum* Mill.)

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Abstract: *Season-controlled changes in biochemical constituents viz. carotenoids (carotene and xanthophyll) and pectic substances along with IAA-oxidase and polyphenol oxidase (PPO) enzyme activities were estimated/assayed in leaves of Lycopersicon esculentum Mill. (tomato) in two developmental stages – pre-flowering (35 days after sowing) and post- flowering (75 days after sowing) in three different seasons – summer, rainy and winter.Carotenoid content along with pectic substances were highest in winter and declined significantly in summer followed by rainy i.e. winter>summer>rainy. Carotenoid content was significantly higher in the pre- flowering as compared to post-flowering in all three seasons while pectic substances increased in the post- flowering as compared to pre- flowering throughout the annual cycle. IAA oxidase and PPO enzyme activities were enhanced in rainy and decreased sharply in summer and winter i.e. rainy>summer>winter. Both the enzymes exhibited higher activity in the post- flowering stage as compared to pre- flowering in all three seasons. These results indicate winter to be the most favourable season for tomato plants while rainy season environmental conditions prove to be unfavourable (stressful) with diminished content of carotenoid and pectic substances and low activities of IAA oxidase and PPO, ultimately leading to poor growth and productivity.*

Key words: *Seasonal variations, Lycopersicon esculentum, Carotene, Xanthophyll, Pectic substances, IAA oxidase, Polyphenol oxidase, Environmental stress*
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Introduction

The seasonal environment being variable in both time and space makes it difficult to predict responses of plants to the changing conditions of the environment. Physiological plasticity enables plants to withstand such seasonal fluctuations within the limits of tolerance. Biochemical constituents and enzyme activities serve as important indices of plant response and behaviour to seasonal variations, as evidenced by our earlier work (Sen and Mukherji, 1998a,b,c,d,e, 1999, 2000, 2002, 2006, 2007).

Our work aims to record and study certain biochemical constituents *viz*. carotenoids (carotene and xanthophyll) and pectic substances (both are nutritional quality parameters as well) along with the activities of two metabolically important oxidase enzymes – IAA oxidase and polyphenol oxidase (PPO) in leaves of tomato (*Lycopersicon esculentum* Mill.), an important crop plant with high nutritional attributes and antioxidant content, in three different seasons – summer, rainy and winter to trace the seasonal shifts in plant metabolism. While IAA- oxidase controls IAA levels in plants and is hence responsible for regulating growth, PPO is a stress marker enzyme, produced as a protective measure against disease and insect attack. Hence the above biochemical constituents and enzymes are selected for study.

Seasonal variations in carotenoids were earlier noted by Lamare and Hoffman (2004), in PPO by Golbeck and Cammarata (1981) and in IAA metabolism by Dangar and Basu (1985).

The results of our experimental work would be indicative of the seasonal patterns in the concentration/activity of the estimated biochemical constituents and enzymes, which would in turn explain their functional importance in plant growth and metabolism and finally account for the difference in yield and yield quality during different times of the year.

Materials and Methods

The work was carried out in pot culture in the experimental garden of the University Campus at Ballygunge, Kolkata (22.34^0 North and 88.24^0 East) in West Bengal, India under natural environmental conditions. The seasons under study were summer (March-June), rainy (July-September) and winter (November-February). Seeds of *Lycopersicon esculentum* var. Pusa ruby were obtained (National Seed Corporation, Kolkata) and sown in sandy loam soil and farmyard manure in the ratio of 3:1. Initially 12-15 seeds were sown in earthen pots of 12 inches diameter. When the seedlings were 5-6 inches tall, only the healthy ones of more or less uniform height were maintained, while the others were removed. This process was repeated thrice at different seasons, already mentioned, before the onset of the experimental observation, to ensure uniform and healthy plant material. Finally from the remaining 6-8 healthy plants, fully expanded, penultimate (second from top) leaves were collected at two stages of development – 35 days after sowing (pre-flowering) and 75 days (post-flowering) in all the three above-mentioned seasons. For all three seasons, identical water

management was maintained along with a constant nutrient status of the soil.

Carotene and xanthophyll were estimated according to the method of Davies (1965), pectic substances determined according to Ranganna (1977), IAA oxidase activity assayed according to Malik and Singh (1980) and polyphenol oxidase activity assayed according to Mayer and Harel (1979). The data obtained from three replications were statistically analyzed. Standard error (S.E.) and critical difference (C.D.) values of both season and stage at 5% and 1% levels were calculated from the respective analysis of variance (ANOVA).

Results and Discussion

The duration of the experimental period was from November 2003-October 2004 - a period characterized by considerable variations in temperature, photoperiod, light intensity, relative humidity and rainfall. The meteorological data (Table 1) were obtained from Regional Meteorological Centre, Kolkata. The data indicated the monthly mean values, which have been finally expressed as seasonal mean values for the suitability of this work.

Carotene (Fig. 1), xanthophyll (Fig. 2) and pectic substances (Fig. 3) were the highest in winter. Their amounts dropped significantly in summer while the rainy season recorded the minimum concentration of all these constituents. The pre-flowering stage (35 days) recorded a higher carotenoid content as compared to post- flowering (75 days) in all three seasons. Pectic substances on the other hand exhibited an opposite trend - a higher amount was recorded in the post- flowering stage as compared to pre-flowering in all the three seasons.

IAA oxidase enzyme activity (Fig. 4) peaked in rainy and dropped significantly in summer and finally winter. The post- flowering stage of all three seasons recorded significantly higher activity as compared to pre-flowering. Polyphenol oxidase activity (Fig. 5) showed the exact trend as IAA oxidase.

Carotenoid pigments such as beta-carotene or xanthophylls such as lutein and zeaxanthin are very widely distributed in nature, where they play an important role in protecting cells and organisms against the harmful effects of light, air, and sensitizer pigments. The primary mechanism of action of this phenomenon appears to be their ability to quench excited sensitizer molecules as well as $^1O_2$. In addition to this protection, and potentially of even greater biological importance, is the fact that carotenoids can also serve as antioxidants under conditions other than photosensitization (Krinsky, 1998). Carotenoid cation radical formation in light harvesting complexes may provide a novel mechanism for excitation energy dissipation as a means of photo protection (Frank and Brudvig, 2004; Wormit and Dreuw, 2007).

Carotenoid content exhibited seasonal variation in *Picea sitchensis* with a winter maximum (Lewandowska and Jarvis, 1977). While studying seasonal variations in antioxidant components of cherry tomato it was reported by Raffo *et al*. (2006) that carotenoid concentration did neither show definite seasonal trends, nor correlation with solar radiation or average temperature.

However, our results exhibit significant seasonal changes in carotenoid (both carotene and xanthophyll) content which imply that the tomato plants grown in the rainy season are greatly prone to oxidative damage (least carotenoid content in this particular

Table - 1: Meteorological data from November 2003 to October 2004

	Summer	Rainy	Winter
Temperature (°C)	33.8	31.5	14.6
Sunshine hr (hr)	13.5	12.5	10.5
Light intensity (lux)	75000	68000	34000
Relative humidity (%)	81	88	67
Rainfall (mm)	117	339	10.5

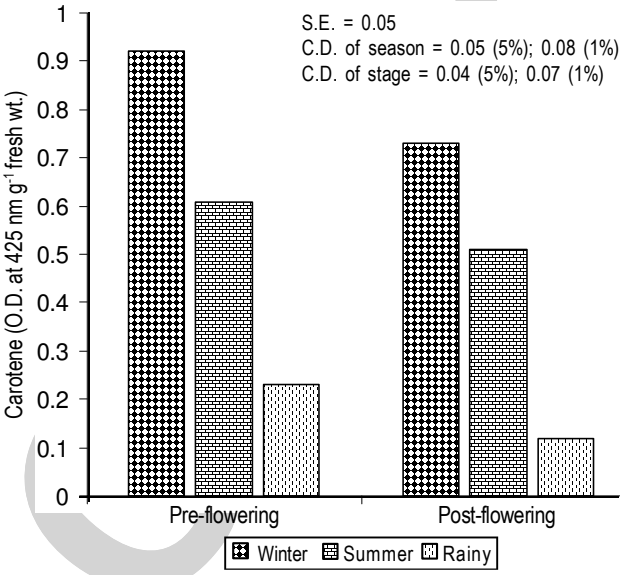

Fig. 1: Carotene content expressed as optical density at 425 nm per gram fresh weight

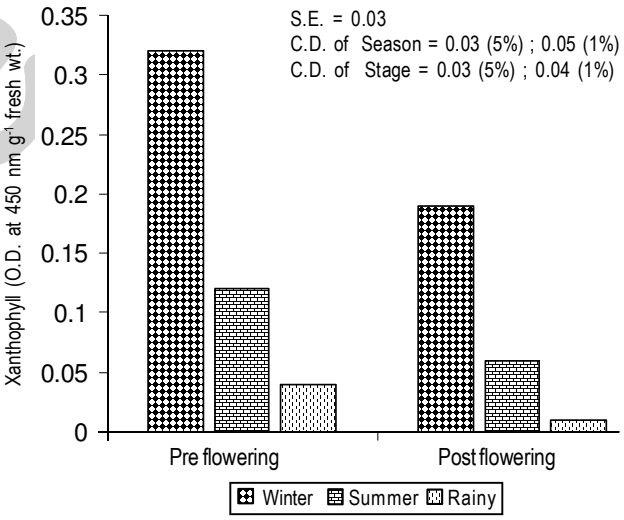

Fig. 2: Xanthophyll content expressed as optical density at 450 nm per gram fresh weight

season) due to poor scavenging and detoxification of the active oxygen species. Carotenoids, especially lycopene (present in tomato), are efficient scavengers of reactive oxygen species (Heinrich *et al*., 2003). Raffo *et al.* (2006) reported that tomatoes harvested in mid-summer were characterized by lowered lycopene (carotenoid) levels. In an earlier work, Sen and Mukherji (2000) reported minimum lycopene content in the rainy season in tomato fruits. It may thus be implied that tomato fruits of the rainy season are poor in nutritional attributes as well.

Pectic substances exhibited strongly distinctive climate influence in radish (Schreiner *et al.,* 1989), *Pyrethrum balsamic* (Rupasova *et al*., 2000) and grapes (Robertson *et al*., 1980). Present in middle lamella of cell walls, pectic substances consist of pectic acids, pectin and protopectin. During fruit ripening, protopectin converts into pectic acid and pectin. Annual and seasonal variations in pectin content have been reported by Kaaber *et al*. (2007).

In our present study, content of pectic substances was significantly altered by seasonal changes with maximum amount in winter and declining amounts in summer and rainy. Pectic substances could be correlated with high yield quality in *Abelmoschus* in an earlier work by Sen and Mukherji (1999).

The enzyme IAA oxidase along with peroxidase oxidizes the plant growth hormone IAA. As the end products of IAA oxidation are physiologically inactive, IAA oxidation is an effective way of removing the hormone IAA once it has accomplished its purpose. IAA oxidative catabolism is the chemical modification of the indole nucleus or side chain resulting in the loss of auxin activity and is the only irreversible output regulating IAA levels which may be very important in regulating IAA-mediated responses (Hopkins, 1997).

IAA oxidase activity is positively correlated with growth inhibition in the rainy season in *Lycopersicon* (Sen and Mukherji, 1998a) and it would be pertinent to suggest that growth inhibition results from reduction in the auxin level owing to enhanced auxin destruction due to higher IAA oxidase activity during these particular months. Since growth is highest in winter, it can be assumed that auxin requirement is the highest in this season. In the winter season IAA oxidase activity was found to be the lowest indicating low auxin destruction. Pre- flowering stages (35 days),when growth is higher (Sen and Mukherji, 1998a), had higher auxin requirement in all seasons as compared to older post- flowering (75 days) and this correlated with the lower IAA oxidase activity in the pre- flowering stages. Ebrahimzadeh and Abrishamchi (2001) reported flower formation in *Crocus sativus* to be accompanied by enhanced activities of IAA oxidase but lower levels of IAA. This record is in conformity with our findings – the reproductive stage in all seasons had higher IAA oxidase activity than the vegetative, indicating lower growth rate.

p-diphenols, *o*-diphenols and polyphenols generally inhibit IAA oxidation. It has been suggested that these compounds serve a regulatory function in IAA peroxidative oxidation. It was observed in this work that a low IAA oxidase activity in the winter season of

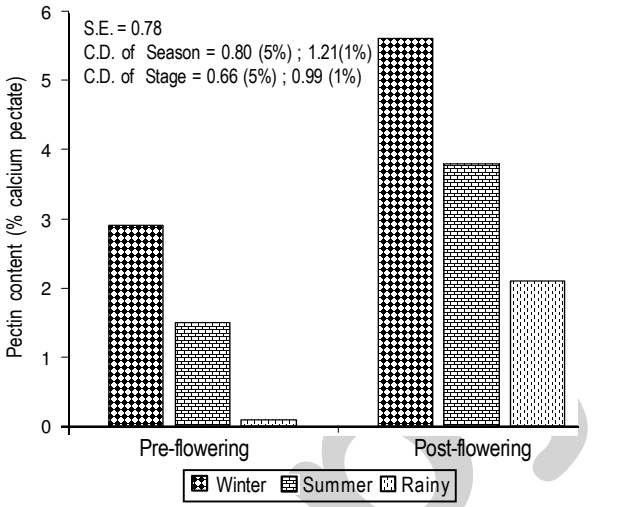

Fig. 3: Pectin content expressed as percent calcium pectate

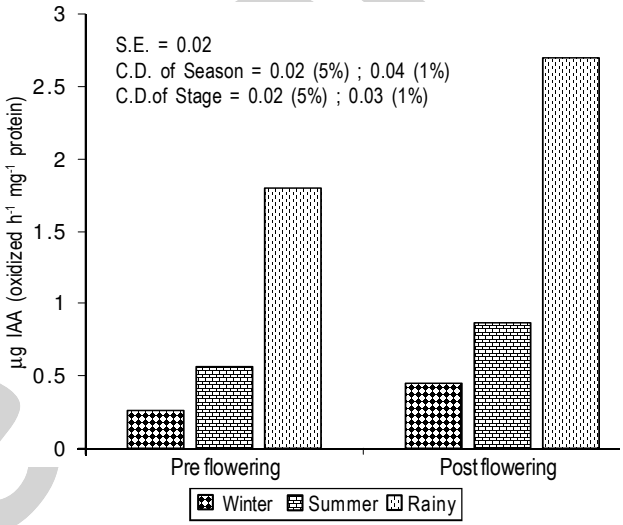

Fig. 4: Indole-3- acetic acid oxidase expressed as mg IAA oxidized per hour per mg protein

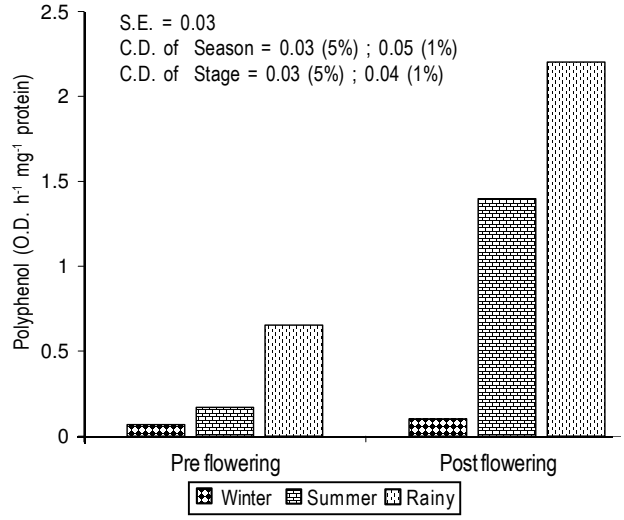

Fig. 5: Polyphenol oxidase enzyme activity expressed as change in optical density at 480 nm per hour per mg protein

Lycopersicon could be correlated with a high phenolic content in the same season reported in an earlier work (Sen and Mukherji, 1998b), indicating that phenolic compounds were responsible for suppressing IAA oxidase activity in that particular season. An opposite trend was observed in the rainy months. Similarly the pre-flowering stage- 35 days had low IAA oxidase activity accompanied by a higher phenolic content while the post -flowering stage showed the opposite trend in all seasons.

Polyphenol oxidase (PPO) enzyme functions as a phenol oxidase in higher plants. PPO oxidizes phenolic compounds which have been associated with antioxidant activity. During periods of stress, this plastidial enzyme is released into the cytoplasm and it oxidizes phenols to produce quinones which are quite toxic in nature (Mayer and Harel, 1979) and helps in prevention of chlorophyll bleaching.

An increase in the activity of PPO in the summer and rainy seasons possibly indicates a protective measure adopted by the plant in response to the prevailing conditions of environmental stress. An increase in PPO activity always resulted in lowering of phenol content in the summer and rainy seasons as revealed by an earlier work by Sen and Mukherji (1998b). PPO was found to be more active in the post- flowering stage in all seasons as compared to the young pre- flowering stage thus indicating that older tissues are more susceptible to prevailing environmental stress conditions.

PPO activity exhibited seasonal variation as reported by Ravichandran and Parthiban (1998), Szecskg *et al*. (2004). Anh-Thu *et al*. (2004) recorded peak activity of PPO in summer corresponding to periods of high solar irradiance and herbivorous insect populations, both of which are stresses against which PPO has been demonstrated to be effective. In our work, PPO activity was also found to be high in the summer months.

Ebrahimzadeh and Abrishamchi (2001) reported flower formation to be accompanied by enhanced activities of PPO. According to them transition to flowering is correlated with PPO and IAA oxidase activities as these enzymes might exert their roles in the regulation of flowering through their participation in IAA catabolism. This finding too is in conformity with our work – the reproductive stage in all three seasons exhibited higher PPO activity than the vegetative along with IAA oxidase activity suggesting a possible role in the process of flowering.

To conclude, it is evident from this work that winter season environmental conditions are the most favourable for the growth of tomato plants characterized by high quantities of carotenoids and pectic substances and low activity of IAA oxidase and PPO enzymes. Environmental conditions of the rainy season on the other hand are unfavourable (stressful) as evidenced by low content of carotenoid and pectic substances and enhanced activity of IAA oxidase and PPO, all of which adversely affect plant metabolism. Winter season proved to be the most optimum for *Lycopersicon* characterized by high photosynthetic efficiency (Sen and Mukherji, 1998c), increased photosynthates (Sen and Mukherji, 1998b), high phosphorus and respiratory metabolism (Sen and Mukherji, 2006, 2007), increased ion uptake (Sen and Mukherji, 1997), and consequently a high growth rate and yield (Sen and Mukherji, 1998a) with good yield quality (Sen and Mukherji, 1998d,e, 2000, 2002).

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