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RESEARCH PAPERS

Influence of High Temperature on Carbon Assimilation, Enzymatic Antioxidants and Tuber Yield of Different Potato Cultivars[1]

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Abstract—High temperature is one of the major limiting factors for cool season crops like potato in many parts of the world. This problem is more aggravated in early season planting of potato crop. This study was conducted to evaluate the performance of five potato cultivars (*Solanum tuberosum* L. cultivars Kufri jyoti, Kufri megha, Kufri pokraj, Rangpuria and Badami) under normal (mid October–mid January) and early season (mid August–late October) conditions during two consecutive years in terms of carbon assimilation, activities of antioxidant enzymes and tuber yield. Temperature during growth of early season crop remained 2–14°C higher than in the normal season crop, which imposed severe heat stress on early season crop. However, this heat stress in early season crop caused several folds increase in the activity of antioxidant enzymes, which had strong positive correlation with tuber yield. Although tuber yield of all tested cultivars was less in early season than in normal season; nonetheless cultivars Kufri megha and Rangpuria performed better in early season planting owing to higher net photosynthesis, carotenoid contents, membrane stability, and activities of enzymatic antioxidant enzymes. In crux, carotenoids, activities of enzymatic antioxidants, carbon assimilation and membrane stability may be used as physiological markers in future breeding programs aimed to improve the heat resistance in potato.

Keywords: Solanum tuberosum, antioxidant defense system, carbon assimilation, high temperature, tuber yield

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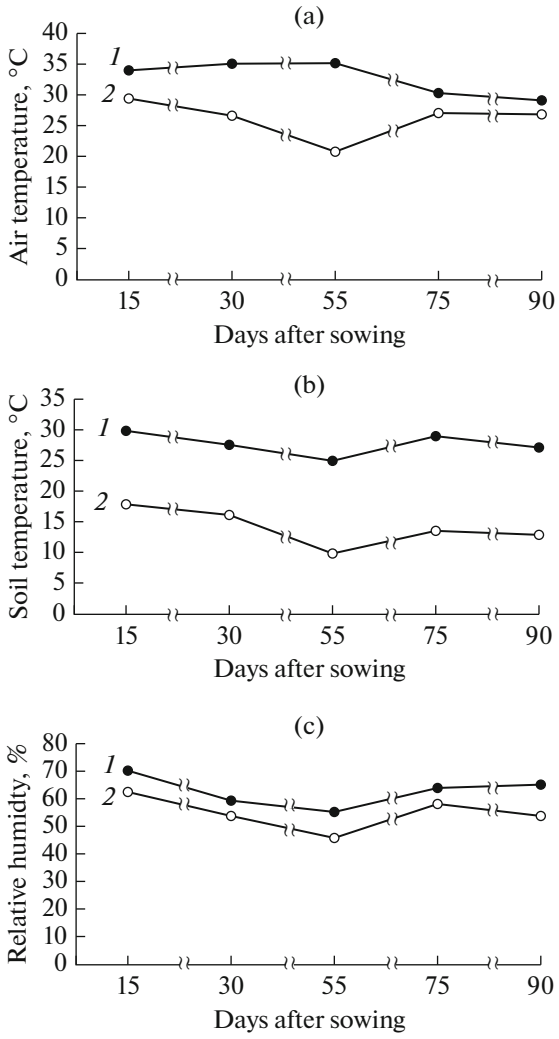
INTRODUCTION

Global average temperature of the earth's surface is predicted to rise by 1.6 to 3.8°C by the end of this century [1]. This increase in temperature may cause significant reduction in productivity of cool season crops like potato. The optimum temperature for the growth of potato crop is 17°C whereas tuberization is inhibited at 25°C [2]. High temperature may decrease the potato productivity by 3 and 14% by the years 2020 and 2050, respectively in India [3]. ROS—superoxide radicals, hydroxyl radicals, and hydrogen peroxide—are produced in the cells in a natural fashion, but heat stress increases the production of these species, which may be harmful for lipids, proteins, and other macromolecules [4, 5]. Hence their detoxification by antioxidant systems is important for protecting plants against heat stress [6, 7]. Some plants activate antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and metabolites like ascorbic acid, and carotenoids [8], under stress conditions, which help to detoxify the ROS. Among these, SOD acts as first line of defence against toxic levels of ROS by scavenging $O_2^{\bullet-}$ through dismutation whereas CAT and POD converts H_2O_2 into H_2O and O_2 [9]. Polyphenol oxidase (PPO) catalyzes the oxidation of phenol to quinone. Both POD and PPO activity is increased upon exposure to abiotic stresses in resistant plants [10]. Carotenoids are also considered important scavengers of ROS protecting plant pigments and fatty acids from oxidative damage by modulating physical properties of photosynthetic membranes with involvement of the xanthophyll cycle in this process [11].

Although several studies identified physiological and biochemical markers for use in selection and breeding programs aimed at improving resistance against abiotic stresses [12–15]; nonetheless information on the response and characterization of various potato cultivars to heat stress is lacking. This study was, therefore, conducted to characterize and evaluate the response of various potato cultivars to high temperature stress in terms of carbon assimilation, activation of antioxidant defense system and tuber yield.

[1] The article is published in the original.

Abbreviations: CAT—catalase; DAS—days after sowing; MSI—membrane stability index; POD—peroxidase; PPO—polyphenol oxidase; SOD—superoxide dismutase.



Variation in aerial (a) and soil temperatures (b), and relative humidity (c) in the normal season (*1*) and early season (*2*) sown potato crop over the sampling period.

MATERIALS AND METHODS

Experimental site and soil. The experiment was conducted in the experimental field of Tezpur University (26°14' N and 92°50' E) at Tezpur, Assam, India under two growing seasons for two consecutive years. The experimental soil was silt loam with slightly acidic pH (5.92).

Plant material and growth condition. *Solanum tuberosum* L. seeds of three high yielding cultivars Kufri jyoti, Kufri megha, and Kufri pokraj were collected from Central Potato Research Station (CPRS), Shillong, India; whereas seed of local cultivars Rangpuria and Badami were collected from local market in Sonitpur, Assam, India. The experiment was conducted in a randomized complete block design in factorial arrangement with three replications. The field was thoroughly ploughed to obtain a good tilt.

Tubers of all the five potato cultivars were sown consecutively for two growing seasons on August 17, 2013 and 2014 (early season) and October 17, 2013 and 2014 (normal growing season) in 65 cm spaced rows by maintaining plant to plant distance of 25 cm in a plot measuring 2.6 m × 2.0 m. Fertilizers were applied at 60, 100, and 100 kg/ha N, P_2O_5, and K_2O as basal dose using urea, diammonium phosphate and murate of potash, respectively as sources. Soil water was monitored with tensiometers and was maintained above –30 kPa (at 20 cm below the soil surface) to ensure that the plant did not suffer water stress. In total three irrigations were applied 30, 55, and 75 days after sowing (DAS).

Aerial temperature and relative humidity data were obtained from the meteorological tower of Krishi Vigyan Kendra (situated ~200 m from the experimental field), Napaam, Tezpur, India. Soil thermometers were placed in both the treatments to record the soil temperature data during the course of study. Air temperature, soil temperature, and relative humidity (mean of two years) are presented in figure.

High yielding and local potato cultivars, grown under normal growing season, were harvested at 90 DAS (January 14, 2014 and 2015) and 100 DAS (January 24, 2014 and 2015), respectively, whereas in early sown crop, both high yielding cultivars and local cultivars were harvested at 80 DAS (November 4, 2013 and 2014).

Observations. For analysis of various biochemical parameters, the third and the fifth leaves from the top of the potato plants were collected at 15, 30, 55, and 75 DAS during both years. However, the data recorded at four different sampling dates during two years have been pooled to represent the homogeneity of the data set during both the years.

Chlorophyll and carotenoid contents. Fresh leaf material (500 mg) was grinded with 10 mL 80% acetone at 4°C and centrifuged at 2500 *g* for 10 min. This procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube to make the volume up to 50 mL with 80% acetone. Aliquots of the extract (3 mL) were transferred to a cuvette and the absorbance was read at 645, 663, and 480 nm with a spectrophotometer (UV 1700, Pharma Spec, Japan). Total chlorophyll contents were determined using the formula given by Anderson and Broadman [16]:

$$\text{Total chlorophyll (mg/g FW)} = (20.2\,A_{645} - 8.02\,A_{663})\,V/1000\,W,$$

where FW—fresh weight, *V*—volume of the sample, *W*—fresh weight of the tissue.

Carotenoid contents were estimated using the following formula of Kirk and Allen [17]:

$$\text{Carotenoid (mg/g FW)} = A_{480} + (0.114\,A_{663} - 0.638\,A_{645}).$$

Net photosynthesis. Net photosynthesis of fully expanded young leaves of all potato cultivars in both

normal and early season was determined (between 10 am to 12 noon) by portable photosynthesis system (model LI-6400, United States) at 15, 30, 55, and 75 DAS and was expressed in μmol $CO_2/(m^2 s)$.

Superoxide dismutase (SOD) activity. Superoxide dismutase activity was estimated following the method of Dhindsa [18]. The amount of enzyme, which reduced the sample color by 50% with respect to blank (without enzyme) was considered as one unit of enzyme activity, and expressed as enzyme unit (EU)/(mg protein h). Protein was estimated with Lowry's method [19] and the amount of protein was calculated against the standard curve of BSA and expressed in mg.

Catalase (CAT) activity. Catalase activity was assayed according to Aebi [20]. The extract was prepared by homogenizing 500 mg fresh leaf material in 10 mL phosphate buffer (0.1 M, pH 7.5) and centrifuged for 15 min at 20000 *g*. Supernatant (100 μL) thus obtained was added to cuvette containing 1.9 mL of 50 mM phosphate buffer (pH 7.0). By adding 1.0 mL of freshly prepared 30 mM H_2O_2 the reaction was started. The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm using the equation for a first-order reaction.

Peroxidase (POD) activity. Peroxidase activity was determined following the method of Sadasivam and Manickam [21] by measuring the times required to increase in absorbance of oxidized guaiacol from 0.05 units to 0.1 units at 436 nm using 0.05 mL guaiacol solution (20 mM) in 3 mL phosphate buffer (0.1 M, pH 7.0), 0.03 mL H_2O_2 (12.3 mM), and 0.1 mL enzyme extract.

Polyphenoloxidase (PPO) activity. Fresh plant leaf (1 g; fully expanded the third and the fifth leaf from top) was digested in 3 mL of 0.1 M phosphate buffer, pH 7, in mortar and pestle. The homogenate was cenrifuged at 18000 *g* at 5°C for 15 min and was used as an extract and estimated as described by Sadasivam and Manickam [21] using pyrogallol as substrate.

Membrane stability index (MSI). Membrane stability index was estimated by assessing the electrical conductivity of leaf discs in double distilled water at 40 and 100°C, respectively. Leaf samples (100 mg, the third and the fifth leaf from top) were cut into discs of uniform size and taken in test tubes containing 10 mL of double distilled water in two sets. One set was kept at 40°C for 30 min and another at 100°C in boiling water bath for 15 min and their respective electrical conductivities C1 and C2 were measured by Conductivity meter (Model ME 885, Labotech Precision Instruments) and MSI was taken as a ratio of C1 and C2 in percentage.

H_2O_2 scavenging activity. The ability to scavenge hydrogen peroxide was determined according to the method of Ruch et al. [22]. At first, 1 mL of the extract prepared for enzyme estimation was dissolved in 3.4 mL of 0.1 M phosphate buffer (pH 7.4) and mixed with 0.6 mL of 40 mM H_2O_2 solution. Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged was calculated as:

$$[H_2O_2] \text{ scavenged} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 was the absorbance of the control (without sample extract) and A_1 was the absorbance in presence of the sample extract.

Statistical analysis. Data were statistically analyzed within sampling dates using two-way analysis of variance (ANOVA) by SPSS (v. 16.0) to determine the significance between treatments, cultivars and treatment × cultivar interactions. Correlation matrix was developed taking the average of all sampling dates for each parameter with crop yield at harvest using Microsoft Excel.

RESULTS

In both years, initially (15–30 DAS) the difference in air temperature between normal season sown and early season sown potato crop was 5–7°C. Later, from 30 to 75 DAS, temperature during growth of early season crop was 10–15°C higher than that of normal season sown crop. After 75 to 90 DAS the smallest temperature difference of 2–3°C was found (Fig. 1a). Soil temperature during normal season was 10–18°C while in the early season soil temperature remained 25–30°C (Fig. 1b). The relative humidity recorded during normal season was 46–63% whereas additional 7–10% humidity was recorded in early season during both years (Fig. 1c).

Carbon assimilation, activities of enzymatic antioxidants and tuber yield were significantly different among all five tested potato cultivars in both the growing seasons irrespective of sampling periods (Tables 1–3). Total chlorophyll contents were lower in early season potato cultivars Kufri jyoti, Kufri pokraj, and Badami. However, in cultivar Kufri megha chlorophyll contents in early season potato were higher than in normal season planted crop (Table 1). Cultivar Kufri megha had maximum chlorophyll contents amongst all tested cultivars in normal season crop till 55 DAS; however, cultivars Kufri jyoti, Kufri pokraj, and Badami had similar chlorophyll contents at 75 DAS (Table 1). In early season crop, cultivar Kufri jyoti had maximum chlorophyll contents at 15 DAS; cultivar Kufri megha had maximum chlorophyll contents at 30 DAS; whereas cultivars Kufri megha and Rangpuria had more chlorophyll contents at 55 and 75 DAS (Table 1).

Carotenoid contents were reduced in cultivars Kufri jyoti, Kufri pokraj, and Badami in early season crop; however, cultivars Kufri megha and Rangpuria had higher carotenoid contents in early season crop than normal season planted potato irrespective of

Table 1. Influence of sowing time on total chlorophyll content, carotenoids content and net photosynthesis in different potato cultivars

Cultivar	15 DAS		30 DAS		55 DAS		75 DAS	
	normal season	early season	normal season	early season	normal season	early season	normal season	early season
Total chlorophyll content (mg/g fr wt)								
Kufri jyoti	1.08^{b}	0.99^{bc}	2.82^{bc}	2.25^{cd}	4.25^{a}	1.57^{bc}	2.33^{a}	0.70^{b}
Kufri megha	0.83^{d}	0.97^{c}	2.40^{bc}	2.32^{bc}	1.44^{bc}	2.29^{b}	0.72^{b}	2.54^{a}
Kufri pokraj	1.54^{a}	0.68^{e}	3.65^{a}	0.91^{ef}	4.16^{a}	0.84^{c}	2.66^{a}	0.50^{b}
Rangpuria	1.00^{bc}	0.92^{cd}	2.78^{bc}	1.57^{de}	1.73^{bc}	2.00^{b}	0.86^{b}	2.43^{a}
Badami	1.12^{b}	0.63^{e}	3.00^{ab}	0.85^{f}	3.36^{a}	0.80^{c}	3.19^{a}	0.50^{b}
Carotenoids content (mg/g fr wt)								
Kufri jyoti	0.059^{bc}	0.050^{de}	0.072^{bcd}	0.049^{de}	0.096^{bc}	0.084^{e}	0.069^{d}	0.071^{d}
Kufri megha	0.022^{f}	0.055^{cd}	0.035^{e}	0.127^{a}	0.089^{cde}	0.094^{bc}	0.062e	0.075^{cd}
Kufri pokraj	0.086^{a}	0.048^{de}	0.087^{bc}	0.063^{cde}	0.113^{a}	0.084^{e}	0.096^{a}	0.059^{e}
Rangpuria	0.055^{cd}	0.059^{bc}	0.052^{cde}	0.074^{bcd}	0.090^{b}	$0.093b^{cd}$	0.075^{cd}	0.079^{c}
Badami	0.066^{b}	0.046^{e}	0.102^{ab}	0.045^{de}	0.095^{bc}	0.086^{de}	0.087^{b}	0.060^{e}
Net rate of photosynthesis (μmol CO_2/(m^2s))								
Kufri jyoti	75.52^{b}	52.79^{ef}	80.68^{a}	55.21^{e}	81.73^{a}	51.56^{g}	72.14^{b}	41.78^{h}
Kufri megha	78.15^{a}	60.69^{d}	79.46^{a}	63.15^{d}	82.42^{a}	60.63^{e}	74.86^{a}	55.27^{d}
Kufri pokraj	76.84^{ab}	53.72^{e}	78.39^{ab}	54.91^{e}	75.33^{b}	52.23^{g}	73.82^{ab}	48.24^{f}
Rangpuria	68.49^{c}	51.84^{ef}	76.74^{b}	64.86^{d}	77.00^{b}	66.24^{d}	75.31^{a}	53.20^{e}
Badami	62.89^{d}	51.40^{f}	67.61^{c}	56.95^{e}	69.17^{c}	56.70^{f}	68.23^{c}	45.49^{g}

Means showing the same case letter do not differ significantly at $p \leq 0.05$. FW = Fresh weight.

sampling time (Table 1). In early season planted crop, maximum reduction of carotenoid was observed in cultivars Kufri pokraj and Badami. All the early planted potato cultivars showed maximum carotenoid contents at 55 DAS except cultivar Kufri megha, which had maximum carotenoid contents at 30 DAS. In normal season cultivar Kufri pokraj had the highest carotenoid contents at 55 DAS (Table 1).

Early sown potato cultivars had substantially low rate of net photosynthesis than normal season crop. However, the peak net photosynthesis was observed at 55 DAS during both the seasons; where the cultivars Kufri megha and Kufri jyoti had the maximum net photosynthesis. However, only cultivar Kufri megha was able to maintain the same from normal season planting. Among the tested cultivars Rangpuria showed maximum rate of net photosynthesis when grown in early season (Table 1).

The membrane stability in early planted crop was significantly lower than in the crop sown in normal season (Table 2). In this regard, cultivar Kufri megha had the maximum MSI when grown in normal season irrespective of sampling time. In all the cultivars the maximum MSI during early season was at 55 DAS (Table 2). Likewise, hydrogen peroxide scavenging activity was highest in early season crop (Table 2). All the early season sown potato cultivars had many fold higher activity of hydrogen peroxide at 30 DAS, the highest being from cultivar Kufri megha, which then declined. Maximum hydrogen peroxide scavenging activity was observed in cultivar Badami when grown in normal season followed by cultivars Kufri megha, Rangpuria, Kufri pokraj, and Kufri jyoti at 55 DAS (Table 2). Total SOD activity was higher in all the early sown potato cultivars where the highest activity was noted in cultivar Kufri megha throughout the sampling periods which was followed by cultivars Kufri pokraj (15 and 75 DAS) and Kufri jyoti (30 and 55 DAS). Cultivars Rangpuria and Badami had maximum activity of SOD at 30 DAS. Maximum SOD activity, from normal season crop, was found in cultivar Kufri megha at 30, 55, and 75 DAS (Table 2). Maximum CAT activity was found in all the early sown potato cultivars till 55 DAS. Cultivar Kufri megha depicted the highest CAT activity irrespective of sampling periods followed by cultivars Kufri jyoti and Kufri pokraj. Cultivar Badami depicted higher CAT activity as compared to Rangpuria. Though similar trend with significantly lower CAT activity was observed in potato plants from normal season crop but cultivar Kufri megha recorded higher value till 55 DAS (Table 2).

Increased peroxidase activity was noted in potato cultivars when grown as early season crop (Table 2). Cultivar Kufri megha showed the highest POD activity at 30 DAS followed by cultivars Kufri jyoti, Kufri pokraj, Badami, and Rangpuria. From normal season crop maximum peroxidase activity was detected in cultivar Kufri megha throughout the crop growing period followed by cultivars Kufri jyoti (15 and 30 DAS) and Kufri pokraj (55 and 75 DAS) (Table 2).

Table 2. Influence of sowing time on activity of antioxidant enzymes, membrane stability index and hydrogen peroxide scavenging activity in different potato cultivars

Cultivar	15 DAS		30 DAS		55 DAS		75 DAS	
	normal season	early season	normal season	early season	normal season	early season	normal season	early season
Superoxide dismutase (SOD) activity (EU/(mg protein h))								
Kufri jyoti	5.30^{c}	8.97^{b}	23.81^{f}	46.07^{bc}	33.27^{de}	36.29^{cd}	15.63^{c}	18.53^{b}
Kufri megha	8.01^{b}	11.35^{a}	30.20^{e}	57.37^{a}	42.85^{b}	50.32^{a}	17.98^{b}	24.87^{a}
Kufri pokraj	8.84^{b}	11.17^{a}	24.30^{f}	36.42^{d}	24.60^{h}	28.56^{fg}	16.58^{bc}	23.54^{a}
Rangpuria	5.10^{c}	7.57^{b}	21.75^{f}	47.57^{b}	31.02^{ef}	35.30^{d}	6.14^{e}	11.57^{d}
Badami	5.01^{c}	7.35^{b}	23.80^{f}	44.29^{c}	27.52^{gh}	39.67^{bc}	4.85^{e}	11.80^{d}
Catalase (CAT) activity (mmol H_2O_2/(min mg fr wt))								
Kufri jyoti	12.50^{fg}	19.67^{b}	33.93^{d}	51.50^{b}	45.50^{e}	65.61^{b}	31.07^{b}	16.57^{fg}
Kufri megha	15.27^{d}	26.61^{a}	41.72^{c}	57.99^{a}	48.79^{d}	72.98^{a}	35.48^{a}	21.27^{de}
Kufri pokraj	13.77^{ef}	17.82^{c}	34.19^{d}	44.62^{c}	47.12^{de}	57.24^{c}	24.42^{cde}	14.57^{g}
Rangpuria	11.35^{g}	14.60^{de}	20.55^{f}	24.64^{e}	33.35^{h}	36.61^{g}	25.43^{cd}	20.34^{ef}
Badami	11.41^{g}	17.31^{c}	23.46^{ef}	31.94^{d}	36.37^{g}	40.57^{f}	28.52^{bc}	24.64^{cd}
Peroxidase activity (POD) (mg/g fr wt)								
Kufri jyoti	11.25^{d}	14.22^{b}	35.43^{e}	53.80^{b}	41.27^{d}	49.54^{b}	32.22^{e}	44.58^{b}
Kufri megha	13.67^{b}	15.92^{a}	37.17^{e}	61.71^{a}	45.65^{c}	52.38^{a}	36.72^{d}	47.72^{a}
Kufri pokraj	10.40^{de}	13.17^{bc}	34.64^{e}	48.91^{c}	42.20^{d}	46.84^{c}	36.43^{d}	41.87^{c}
Rangpuria	9.08^{e}	13.92^{b}	24.10^{g}	40.80^{d}	31.47^{g}	35.47^{f}	24.64^{g}	28.31^{f}
Badami	11.87^{cd}	16.63^{a}	27.19^{f}	43.22^{d}	31.38^{g}	37.77^{e}	27.84^{f}	31.20^{e}
Polyphenol oxidase activity (PPO) (mg/g fr wt)								
Kufri jyoti	3.32^{e}	5.21^{c}	3.74^{e}	11.11^{b}	6.41^{fg}	17.58^{b}	4.35^{d}	8.37^{a}
Kufri megha	5.08^{c}	10.38^{a}	5.21^{d}	15.89^{a}	8.54^{de}	21.09^{a}	5.53^{c}	8.36^{a}
Kufri pokraj	3.78^{e}	7.31^{b}	4.72^{d}	8.12^{c}	7.39^{ef}	16.31^{b}	3.28^{e}	6.57^{b}
Rangpuria	1.59^{f}	4.00^{de}	2.15^{f}	4.91^{d}	4.42^{h}	9.38^{d}	2.53^{f}	4.20^{d}
Badami	1.24^{f}	4.73^{cd}	2.72^{f}	7.29^{c}	5.60^{gh}	10.90^{c}	2.32^{f}	5.72^{c}
Hydrogen peroxide scavenging activity (%)								
Kufri jyoti	8.31^{d}	14.53^{b}	14.73^{c}	66.08^{b}	42.89^{cd}	44.83^{cd}	14.92^{f}	24.96^{cd}
Kufri megha	10.57^{c}	16.43^{a}	18.12^{c}	75.73^{a}	55.83^{b}	68.28^{a}	18.63^{ef}	28.78^{c}
Kufri pokraj	7.59^{d}	16.46^{a}	16.22^{c}	68.07^{b}	46.41^{c}	55.12^{b}	13.47^{f}	22.50^{de}
Rangpuria	5.03^{e}	16.48^{a}	18.94^{c}	69.66^{ab}	54.05^{b}	34.81^{e}	17.24^{ef}	41.71^{a}
Badami	7.65^{d}	17.83^{a}	19.37^{c}	69.37^{ab}	56.79^{b}	40.44^{d}	21.94^{de}	35.64^{b}
Membrane stability index (%)								
Kufri jyoti	75.51^{a}	52.79^{d}	80.66^{a}	55.21^{e}	81.74^{a}	51.56^{e}	72.14^{a}	41.77^{e}
Kufri megha	78.18^{a}	60.53^{c}	79.46^{ab}	64.16^{d}	82.41^{a}	60.63^{d}	74.86^{a}	55.27^{c}
Kufri pokraj	76.54^{a}	53.72^{d}	78.39^{ab}	54.91^{e}	75.32^{b}	52.22^{e}	73.82^{a}	48.23^{d}
Rangpuria	68.49^{b}	51.83^{d}	76.71^{b}	64.87^{cd}	76.99^{b}	66.23^{c}	74.56^{a}	53.19^{c}
Badami	62.86^{c}	51.39^{d}	67.61^{c}	56.96^{e}	69.17^{c}	57.69^{d}	68.23^{b}	45.49^{d}

Means showing the same case letter do not differ significantly at $p \leq 0.05$. FW = Fresh weight.

Table 3. Influence of sowing time on tuber yield (kg/ha) of various potato varieties

Cultivar	Normal season	Early season	Decrease over control, %
Kufri jyoti	7766[c]	3048[f]	60.75
Kufri megha	9025[a]	4839[d]	46.38
Kufri pokraj	8423[b]	3626[e]	56.95
Rangpuria	1621[g]	730[h]	54.96
Badami	1444[g]	600[h]	58.45

Means showing the same case letter do not differ significantly at $p \le 0.05$.

Polyphenol oxidase activity was higher in early season crop than in normal season potato crop (Table 2). From the early sown potato crop the peak polyphenol oxidase activity was noted at 55 DAS where cultivar Kufri megha maintained the highest with lowest in Rangpuria; whereas cultivars Kufri jyoti and Kufri pokraj remained at par. From normal season sown potato crops, all the studied cultivars recorded maximum activity of PPO at 55 DAS with the highest being cultivar Kufri megha followed by cultivars Kufri pokraj, Kufri jyoti and the lowest in cultivars Rangpuria and Badami (Table 2).

Normal season sown potato cultivars produced higher tuber yield compared to early season crop. High temperature was found to reduce tuber yield in all tested cultivars. This reduction was more prominent in cultivar Kufri jyoti followed by Badami. Cultivar Kufri megha produced the lowest reduction of tuber yield from early season crop (Table 3). There was strong positive correlation between carotenoid contents and tuber yield from early sown crop. Total SOD, POD, and PPO activity also had positive correlation with crop yield irrespective of the sowing time; whereas there was no correlation of CAT and H_2O_2 scavenging activities with tuber yield during both growing seasons (Table 4).

DISCUSSION

Carbon assimilation, activities of antioxidant enzymes and tuber yield were significantly lower in early sown crop than in normal season crop (Tables 1–3) as both air and soil temperatures remained 2–15°C and 12–15°C higher in early sown crop, respectively (Figs. 1a and 1b). Moreover, relative humidity was 7–10% higher in early season than in normal season (Fig. 1c). This high temperature and high relative humidity put substantial stress on the early sown crop as revealed by decrease in tuber yield (Table 3). However cultivars Kufri megha and Rangpuria performed better even when grown as early crop. Carotenoid contents were substantially increased in early season, in these cultivars (Table 1). Being a non-enzymatic antioxidant, carotenoids protect the photosynthetic apparatus from photooxidative damages [11, 23]. Strong positive correlation of carotenoid contents with tuber yield (Table 4) also supports this assumption. Protection from oxidative damages by carotenoids and other antioxidant enzymes including POD, SOD, CAT, and PPO, in these cultivars during early season sowing (Table 2) helped to maintain chlorophyll contents (Table 1) and membrane integrity (Table 2) and sustain net photosynthesis (Table 1) which was then resulted in better tuber yield (Table 3). The higher activity of SOD at 30 DAS in early sown potato cultivars ratifies the generation of more superoxide radical ($O_2^{\bullet-}$) at that period which acts as a signal for induction of antioxidant enzyme, SOD. Being the first line of defense to enzymatically change superoxide radical ($O_2^{\bullet-}$) to relatively stable H_2O_2; the activity of SOD plays an important role during stress [24]. In this experiment, significantly higher activity of SOD was noted in cultivars Kufri megha and Rangpuria which indicate their ability for superoxide radical ($O_2^{\bullet-}$) scavenging capability during the stress. This high temperature induced SOD activity is presumably due to *de novo* synthesis of the enzyme protein [25]. By triggering the conversion of H_2O_2 to water and oxygen, POD takes part in enzymatic defense of plant cells during stress [26]. Therefore, the maintenance of higher POD activity in tolerant cultivars (Kufri megha and Rangpuria) helped in cell membrane lignifications and recovery as noticed from their higher MSI value (Table 2). On the other hand, PPO catalyzed the oxidation of phenol to quinone and thereby helped in electron transport and generation of ATP and thus supported the plant to thrive under high temperature situation [27].

As the cultivars Kufri jyoti and Badami, when sown in early season, couldn't maintain enzymatic and non-enzymatic defense system namely carotenoid, SOD, POD, and PPO (Table 2), membrane integrity was disrupted (Table 2), chlorophyll was degraded (Table 1) which caused decrease in net photosynthesis (Table 1) and finally the tuber yield (Table 3). As carotenoids help in assembling the photosystems in plants [28, 29],

Table 4. Correlation coefficients (*r* values) of various parameters with tuber yield during normal and early season

Treatment	Carotenoids	SOD	CAT	POD	PPO	H_2O_2-SA
Normal season	0.457 ns	0.999**	0.416 ns	0.917*	0.835*	−0.650 ns
Early season	0.912*	0.981**	−0.484 ns	0.958*	0.828*	−0.781 ns

Ns—non-significant; **significant at $p \le 0.01$; *significant at $p \le 0.05$. SOD—superoxide dismutase; CAT—catalase; POD—peroxidase activity; PPO—polyphenol oxidase activity; H_2O_2-SA—hydrogen peroxide scavenging activity.

therefore, any decrease in carotenoids may lower the rate of net photosynthesis owing to less energy transfer to the chlorophyll [30].

In conclusion, tuber yield of all tested potato cultivars was low under early season than under normal season conditions; however cultivars Kufri megha and Rangpuria had better yield under early season sowing owing to higher net photosynthesis, carotenoid contents, membrane stability, and activities of enzymatic antioxidant enzymes. Carotenoids, activities of enzymatic antioxidants, carbon assimilation, and membrane stability may be, thus, used as physiological markers in future breeding programs aimed to improve the heat resistance in potato.

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