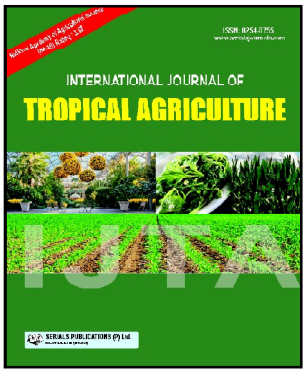

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Influence of Low and Elevated Temperature on Antioxidant Enzymes and Quality of Potato During Storage

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Abstract: The present study was conducted to evaluate effects of low (4°C) and elevated (12° ± 0.5C {CIPC, Isopropyl-N-(3-chlorophenyl) carbamate}) storage temperatures in four potato cultivars: Kufri Chipsona-1, Kufri Chipsona-3, Kufri Jyoti and Kufri Pukhraj. The potatoes were stored up to 180 days. Samples withdrawn at monthly intervals indicated that ascorbate peroxidase increased from harvest up to 60 days of storage and then decreased. Glutathione (GR) increased initially upon storing tubers at 60 days of storage at both the temperatures and declined gradually to almost initial levels at 120 days. Potatoes at elevated temperature were registered with decreased levels of catalase activity and activity slowly returned to the level that was reported initially. Malondialdehyde content increased at low temperature storage as compared to elevated temperature. Reducing sugars increased at 90 days of storage in all the varieties followed by sudden decrease upon further sampling. Potato stored at low temperature generally had higher reducing sugars concentration compared to storage at elevated temperature. It was concluded that enzymes were more sensitive to low than at elevated temperature and potato varieties responded differently.

Keywords: Antioxidant enzymes; CIPC; Potato; Quality; Temperature.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is important crop throughout the world and India is second largest producer. Potato is semi-perishable crop as it contains 80% of water and need to be stored as it can't be held outside for long period and is stored at 12 ± 0.5°C and 4 degree Celsius (°C) from processing and seed purpose point of view, respectively. Temperature

is most important factor in keeping quality of stored potatoes, as it affects respiration, sprouting, water loss, relative humidity and chemical composition. Potatoes stored at low temperature (4°C) for seed purpose reduces bacterial soft rots, decreases dry matter loss and prevents sprouting without an chemical (1). For processing, potatoes after harvest stored at 12 ± 0.5°C necessitates the use of CIPC as sprout suppressant. During storage temperatures, antioxidants and antioxidant enzymes presumed to play important role during scavenging, although potato is non-photosynthetic tissue. Potato tubers contain 9 to 25% carbohydrate but its quantity and quality changes with the changes of temperature (2). An enhanced antioxidant defense from oxidative stress is thought to be one of the mechanisms involved in response chilling temperatures. Increased activities of antioxidant enzymes like ascorbate peroxidase (AP_X), catalase (CAT) and enhanced levels of non-enzymatic antioxidants glutathione (GR) and ascorbic acid (AA) work together to create a balance between the formation and detoxification of ROS (reactive oxygen species) and is critical to cell survival during low temperature (3). Besides this other non-enzymatic antioxidants, which are generally small molecules, like ascorbic acid plays an important role in the destruction of ROS, and glutathione is essential for the regeneration of DHAA (dehydroascorbic acid), which is formed during ROS removal. In potatoes, most of the studies investigate the impact of storage on quality aspects. This study aimed to evaluate the effect and comparison of long term storage both at low and elevated temperatures on the antioxidant potential and activity of antioxidant enzymes of tubers.

MATERIALS AND METHODS

Tubers of varieties Kufri Chipsona-1, Kufri Chipsona-3, Kufri Jyoti and Kufri Pukhraj were harvested at their maturity, skin cured and stored in leno bags at 12 ± 0.5°C with two CIPC treatments (The first CIPC treatment was given on second week of april and the second treatment was given in the month of may). Another lot of samples were stored at 4°C. Stored samples were analysed (up to 180 days) for different antioxidative potential *viz.,* ascorbate peroxidase, glutathione reductase, catalase, malondialdehyde formation, dehydroascorbate were estimated by slight modifications and quality attributes like reducing sugars in potato tubers at two different temperatures. All the analysis were carried in triplicate.

ESTIMATIONS

Assay of ascorbate peroxidase activity was carried out by standardized procedure with modification (4). The sample was homogenized (3.0ml) with phosphate buffer (0.2 M pH 7.3) and centrifuged at 14000 g for 40 min at 4°C. The supernatant obtained was used as enzyme source. To 200 µl of the substrate solution (2.0 mM ascorbate in phosphate buffer, pH 7.3), 0.2 ml of the enzyme extract was added and the absorbance change at 290 nm was measured for every 30 seconds for a period of 5 minutes. One enzyme unit is equivalent to 0.01 optical density change per min.

The amount of reduced glutathione in the samples was estimated with slight modification by taking 1.0g of the sample, homogenized and suspended in tris-Hcl buffer pH 7.8 (5). After centrifugation, 2.0 ml of the protein-free supernatant was used for assay. Absorbance was read at 412 nm. GSH concentration was expressed as nanokatal $gram^{-1}$ fresh weight.

For catalase activity, tissue was homogenized in 5 ml of ice cold 0.05 M phosphate buffer pH 7.5. Homogenate was centrifuged at 10,000g for 40 min at 4°C. To 1ml of hydrogen peroxide solution, 1.8 ml of phosphate buffer and 0.1ml of enzyme extract was added. Utilization of hydrogen peroxide was recorded over maximum period of 2 min at intervals of 15 sec by measuring the change in absorbance at 240 nm on UV spectrophotometer. Reaction mixture without hydrogen peroxide was used as blank. Expressed the enzyme activity as ìmoles^{-1} min^{-1} g Fresh Weight (FW) (6).

Malondialdehyde formation was estimated by homogenising fresh tissue (0.5 g) in 5 ml of thiobarbituric acid. Content was incubated at 95°C (with modification) for 2hour in water bath and reaction was stopped by placing the tubes in ice bath for 20 minutes. Content was centrifuged at 10,000g for 1hour.Collected supernatant was used for measuring the absorbance at 240 nm (7).

DHAR activity was assayed at 25°C by following the increase in absorbance at 265 nm. The assay mixture (1 ml) for analysis was prepared with modifications containing 50 mM potassium phosphate (pH 6.8), 2.5 mM glutathione, 0.2 mM dehydroascorbate and 0.1 mM ethylene diamine tetra acetic acid (8).

Reducing sugars in potato tubers were analysed by adopting standardized procedure. Extraction of reducing sugars was done by refluxing the samples in 80% iso-propanol. Colour developed by arsenomolbdate reagent was recorded at 620 nm and concentration was calculated by preparing standard curve of glucose solution 100µg ml^{-1} (9).

Protein content was analysed by taking 1g of sample in 5ml of 0.1N sodium hydroxide. After centrifugation (10000rpm, 10 min) supernatant was collected and volume was raised to 5ml with 0.1N sodium hydroxide. Addition of 1ml of 15% trichloroacetic acid was followed by incubation at 4°C for 24 h. Content was centrifuged and residues were dissolved in 5 ml of 0.1N sodium hydroxide. To 0.5ml of aliquot, 5ml of solution C was added (prepared by dissolving

(a) 2% sodium carbonate in 0.1N sodium hydroxide and

(b) 0.5% copper sulphate in 1% sodium potassium tartarate (Solution C Prepare by mixing 50ml of solution A with 1ml of solution B). After vortexing, 0.5ml of Folin Ciocaltaue reagent was added. Incubation of samples was carried at room temperature for 60 min and absorbance read at 570nm (10).

RESULTS

Ascorbate peroxidase increased from zero day of harvest up to 60 days of storage irrespective of all the varieties. At the beginning values ranged between 83.5 to 146.8 (U min^{-1} mg^{-1} protein) in Kufri Pukhraj and Kufri Chipsona-1 respectively (Figure 1).

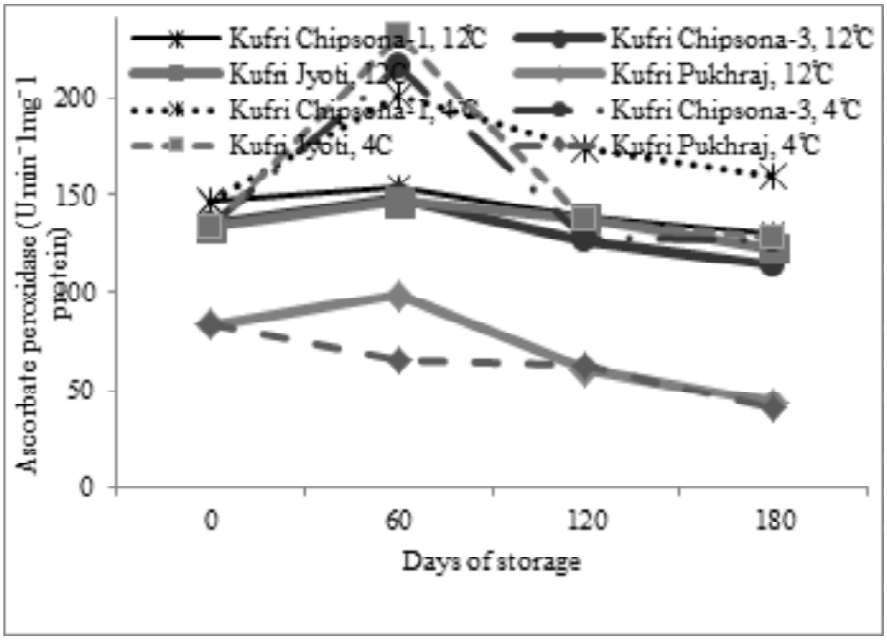

Figure 1: Effect of storage temperatures on ascorbate peroxidase

Tubers stored at low temperature had high peroxidase activity than at elevated temperature (12 ± 0.5°C). It was observed that APx activity increased significantly at lower temperature.

Glutathione reductase activity increased initially upon storing tubers at 60 days at both the temperatures and the declined gradually to initial levels. It was observed that GR activity was consistently higher in processing cultivar namely Kufri Chipsona-1 and Kufri Chipsona-3 and table Cv. Kufri Jyoti than Kufri Pukhraj with poor storability almost initial levels at 120 days of storage. (Figure 2). Likewise, APx, glutathione reductase activity was more pronounced at low temperature.

Catalase activity increased up to 60 days of sampling and indicated decrease at 90 days. Again increase was noticed at 120 days of storage.

However, values observed were more for tubers stored at low temperatures. Potato stored with CIPC treatment and at elevated temperature was registered with decreased levels of catalase activity and the activity slowly returned to the level that was indicated in the beginning (Figure 3).The trend for accumulation was found to be same as reported at low temperature.

Lipid peroxidation in tubers was measured as the content of malondialdehyde production (MDA). Compared to 12°C malondialdehyde content increased at low temperature storage.

Increase in MDA content was observed at 30 days of storage upon keeping potato at elevated temperature. Malondialdehyde content continued to augment with an increase with duration when potato stored at low temperature (Figure 4).

Dehydroascorbate levels decreased up to 30 days of storage and increased mildly up to 90 days. Dehydroascorbate content decreased at 12°C at 120 days whereas opposite trend was observed at 4°C where, content increased irrespective of all the processing and table cultivars (Fig.5). During investigation no drastic changes were found.

Reducing sugars concentrations were found to be in acceptable range up to 30 day of storage, and up to 60 days in variety Kufri Chipsona-1. Reducing sugar increased at 90 days of storage in all the varieties followed by sudden decrease upon further sampling (Figure 6).Potato stored at low temperature generally had higher reducing sugar concentration compared to storage at elevated temperature.

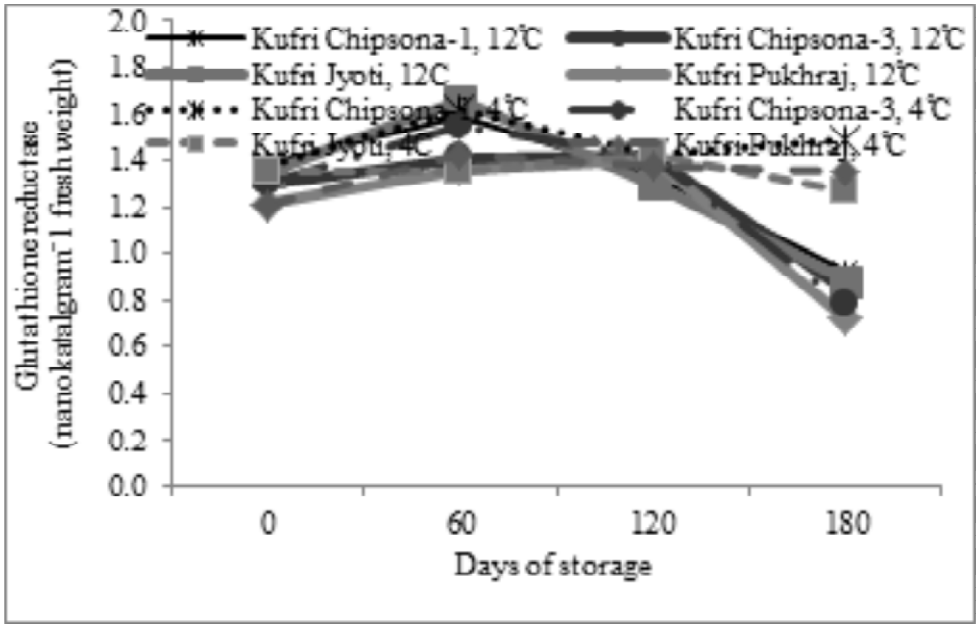

Figure 2: Effect of storage temperatures on glutathione reductase

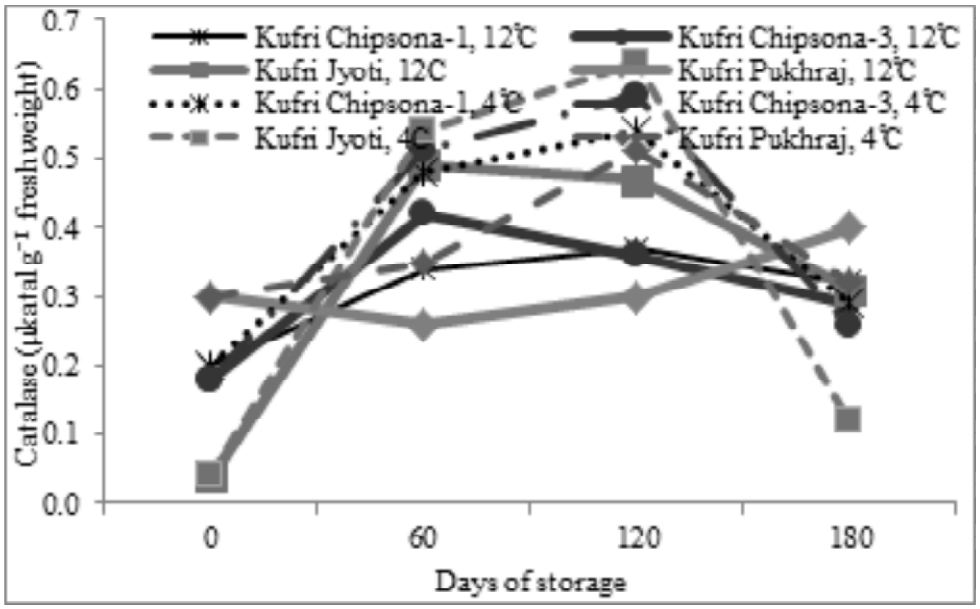

Figure 3: Effect of storage temperatures on catalase

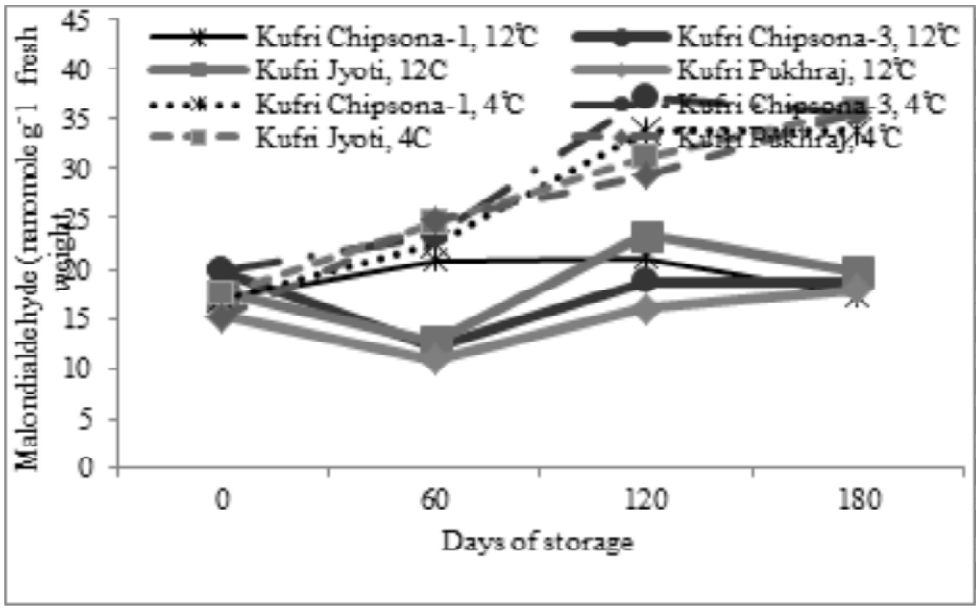

Figure 4: Effect of storage temperatures on malon-dialdehyde

DISCUSSION

Increase in APx activity at low temperature indicates that active oxygen are perhaps more readily generated than at high temperature storage and with storage duration. Compensatory increase in the expression of APx in transgenic tobacco plants was observed suggesting that APx was induced in order to protect (11) against hydrogen peroxide generated by superoxide dismutase (SOD). Although an increase in SOD activity at low temperature could induce increased levels of hydrogen peroxide that could diffuse freely between organelles and cytosol. Thus, the accumulated hydrogen peroxide in turn could trigger a mechanism that increases the activities of APx or hydrogen peroxide at low temperature stress.

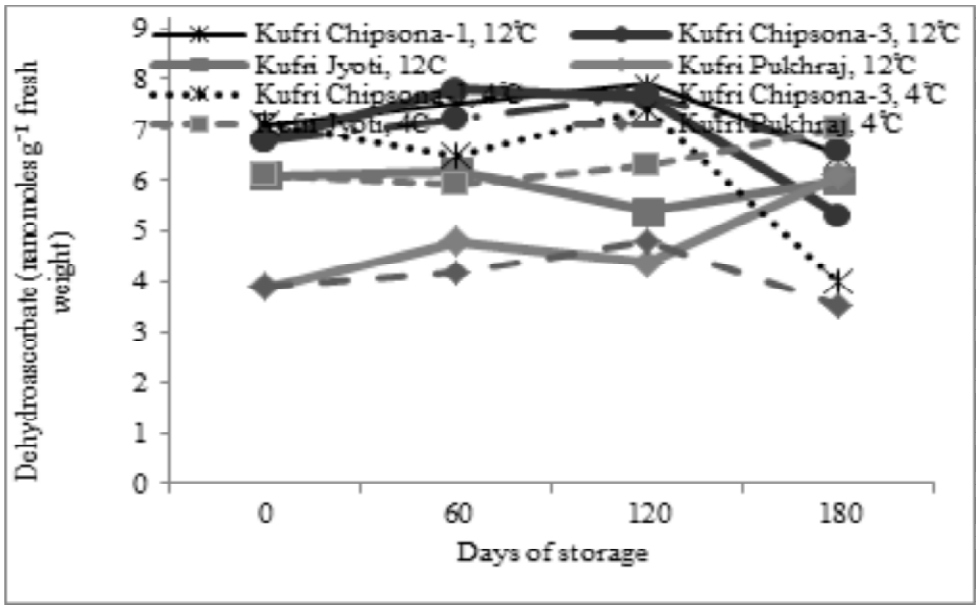

Figure 5: Effect of storage temperatures on dehydro-ascorbate

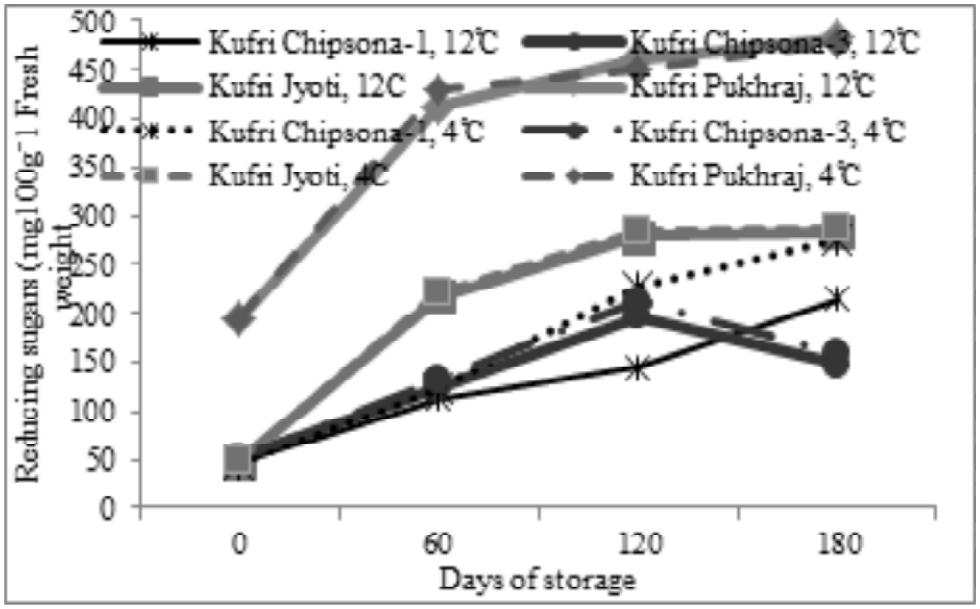

Figure 6: Effect of storage temperatures on reducing sugars

CAT activity decreased first until APx activity tended to decrease and then increased slowly to initial levels during the storage at low temperature. Same results have been reported in potato tubers that catalase activity of were more when stored at low temperatures of 3 or 9 degree Celcius (12). This could be due to that CAT possesses a very low affinity for hydrogen peroxide.

DHAR at long term storage storage led to a general decrease of DHA, a higher level of DHA was found at 4°C than at 12°C. Moreover, the DHA level also reached a maximum after about 30 days of initial stage. This may be due greater utilization of ascorbate by ascorbate peroxidase at low temperature (4).

Reducing sugars accumulated more at low temperature as compared to elevated temperature and the effect of storage was significant. Similar results have been reported in potato, where reducing accumulation was more pronounced at 4°C (13). It was noticed that varieties under test behaved differently in accumulating sugars. These results are in accordance with achievements made in recent experiment conducted (14).

CONCLUSION

Enzymes were more sensitive to low than at elevated temperature. APx activity increased more at low (4°C) than at higher temperature. Ascorbate peroxidase, decreased immediately within 30 days and then decreased gradually until 180 days of storage. Catalase activity increased more slowly than APx as this activity might have compensated for the loss of Apx activity. Positive co-orelation has been found between Apx and catalase activity indicating functional co-operation between APx and catalase activity is important for protection against the hydrogen peroxide generated during low temperature storage. Reducing sugars too accumulated more at low temperature.

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