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Cold Induced Sweetening and Antioxidant Activity of Potato Genotypes During Cold Storage

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HIGHLIGHTS

- Starch metabolism during cold exposure relates to cold-induced sweetening (CIS).
- Tuber antioxidant activity during cold exposure relates to CIS-resistance.
- Tuber antioxidant activity during long storage relates to quality storage.
- Catalase is an important antioxidant enzyme for quality storage.

Abstract: Cold-induced sweetening (CIS) of potatoes is an industrial problem. Tuber antioxidant activity during cold storage may relate to CIS-resistance and quality storage but not well studied. CIS and antioxidant activity were measured in tubers of eleven potato genotypes during cold storage of four months. During first month, tubers were found to lose starch and produce CIS but improve starch later between 1-2 months. Loss of starch during first month was seen less and gain of starch between 1-2 months was seen high in CIS-resistant genotypes versus CIS-susceptible genotypes. Acid invertase activity increased during first month but this increase was related to CIS in CIS-susceptible genotypes as CIS-resistant genotypes also increased acid invertase. Redox state of ascorbate decreased and H_2O_2 increased during first month of storage and this change was related to CIS-susceptibility. Catalase and peroxidase levels at one month of storage related to CIS-resistance. During further storage, redox state of ascorbate decreased, H_2O_2/toxicity increased but tuber quality related to maintained antioxidant levels specially in the form of catalase. Results concluded that genotype with high tuber antioxidant activity may be beneficial for both CIS-resistance and quality storage.

Keywords: ascorbate; catalase; invertase; malondialdehyde; peroxidase; protein carbonyls; invertase, *Solanum tuberosum*.

INTRODUCTION

Cold storage of potatoes effectively extends storage life but is compromised due to the production of undesirable cold-induced sweetening (CIS). CIS occurs due to the catabolism of starch to reducing sugars which give rise to off-flavor, dark pigments and acrylamide during frying [1]. Starch degradation can either be

hydrolytic (via amylases) or phosphorolytic (via starch phosphorylases). Conversion of starch to reducing sugars by amylases is considered one of the main pathways in CIS [2, 3]. Transgenic potato plants with reduced glucan water dikinase (GWD) expression produced tubers with low phosphate content in starch and reduction in CIS [4]. Resistance to CIS is genetically controlled, some genotypes are more CIS-resistant than others [1,5].

Antioxidants, enzymatic and non-enzymatic antioxidants, detoxify reactive oxygen species which otherwise in high amount react to cellular components and produce toxicity. Antioxidants may play an important role in storage tissues like seeds and tubers [6]. Few reports [7,8] have directly or indirectly indicated that CIS-resistance may relate to tuber antioxidant activity but has not been studied in detail. In the present study, CIS and antioxidant activity has been studied in eleven potato genotypes during cold storage of four months. Lady Rosetta, Kufri FryoM, Kufri Chipsona-1 and Kufri Frysona are the known processing varieties. Kufri Pukhraj and Kufri Ganga are early bulking and table purpose varieties. Tubers of Kufri Neelkanth and Kufri Sindhuri bear colored skin. Three other genotypes MP/06 39, MS/7 645 and MS/8 1148 were included in the study.

MATERIAL AND METHODS

Plant material

Tubers of potato (*Solanum tuberosum* L.) genotypes, Lady Rosetta, Kufri Pukhraj, Kufri FryoM, Kufri Neelkanth, MS/7 645, Kufri Ganga, Kufri Chipsona-1, Kufri Frysona, MP/06 39, MS/8 1148 and Kufri Sindhuri were obtained from Department of Vegetable Science, Punjab Agricultural University, Ludhiana. Tubers were cured under field conditions for 10 days after harvest, then packed in jute bags and stored in refrigerator. For each measurement, four tubers (selected randomly) were cut longitudinally and a core (diameter of about 17 mm) was taken from the cut side of each half [5, 6]. The tissue core of each of the four tubers was consolidated into a single sample and used for biochemical measurements in three replicates.

Measurements related to CIS

These measurements were done as described [9]. Carbohydrates were extracted twice with 80% ethanol then once with 70% ethanol. Supernatant was used for sugars and dried residue was used for starch measurements. Glucose was measured by incubating extract in 1.8 U of glucose oxidase, 0.6 U of peroxidase, 0.015% o-dianisidine and 22.5% glycerol at 30°C for 90 min, reaction terminated in 1N HCL. Content was read at 540 nm and calculated using glucose standard (0.1 to 0.5 µmol). For fructose, total and bound fructose, were measured then bound fructose was subtracted from total fructose to get fructose. Total fructose was estimated by incubating extract in 0.025% resorcinol, 23.75% ethanol and 22.5% HCL at 80°C for 20 min then read at 540 nm. Bound fructose was estimated same but after destroying free fructose in 15% NaOH in boiling water bath for 10 min. Fructose amount was calculated using standard fructose (0.1 to 0.5 µmol). Starch was estimated by incubating dried residue in 30% perchloric acid in refrigerator for 20 min and then filtered. Released glucose was estimated using anthrone method. Starch amount was calculated using factor of 0.9. Acid invertase was extracted in 100 mM HEPES–KOH (pH 7.4), 5 mM magnesium chloride, 1 mM EDTA, 1 mM PMSF, 5 mM DTT, 0.1% triton-x-100, 20% glycerol, 5 mM thiourea and assayed in 0.1 M sodium acetate buffer (pH 4.3) and 0.1M sucrose at 37°C. Reaction was stopped with Nelson reagent and then reducing sugars measured by Nelson method. Glucose was used as standard.

Measurements related to antioxidants

Hydrogen peroxide (H_2O_2), redox state of ascorbate, oxidative toxicity (malondialdehyde and protein carbonyls), activities of antioxidant enzymes were measured as mentioned [10,11]. H_2O_2 was extracted in ice cold 0.1% TCA and estimated in 1.33 M KI, 0.033 M potassium phosphate buffer pH 7 in the dark at room temperature for 1 h then read at 390 nm and calculated using H_2O_2 standard (50-200 nmol). Ascorbate was extracted in ice cold 5% TCA and estimated in 0.053% H_3PO_4, 0.133% bathophenanthroline, 0.004% $FeCl_3$, 60% ethanol, 2% TCA at 37°C for 1 h, then color was read at 534 nm and calculated using standard ascorbic acid (10-100 nmol). Dehydroascorbate was extracted in ice cold 5% metaphosphoric acid and 1% thiourea and estimated in 1% 2,4-dinitrophenyl hydrazine (DNPH), 0.2% thiourea, 0.025% $CuSO_4.5H_2O$, 4.5 N H_2SO_4 at 37 ºC for 3 h, thereafter test tubes transferred quickly to ice bath and added ice cold 22.4 N H_2SO_4 and incubated at room temperature for 30 min. Color was read at 530 nm and amount calculated using dehydroascorbate (10-100 nmol). Malondialdehyde (MDA) was extracted in ice cold 0.1% TCA and estimated in 0.4% thiobarbituric acid (TBA) and 16% TCA at 95°C for 30 min, thereafter mixture cooled down,

centrifuged at 10, 000 x g for 10 min and read at 532 nm and 600 nm. Absorbance at 600 nm was subtracted from absorbance at 532 nm and amount was calculated by using molar extinction coefficient of MDA as 155 mM-1cm-1. Protein carbonyls content was extracted in ice cold 0.1 M potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM PMSF and 0.5 µg/mL aprotonin. Extract (about 0.1 mL) was reacted to 10 mM 2,4-dinitrophenyl hydrazine (DNPH) prepared in 2N HCL or 2N HCL (for control) in the dark for 1 h where it was kept vortexing after every 10-15 min. TCA (5.2%) was added to precipitate proteins, kept on ice for 10 min then centrifuged at 10,000×g for 20 min. Pellet was washed one time with 10% TCA and recentrifuged at the same speed for 20 min. Pellet was dissolved in 6 M guanidine HCL, kept at 37ºC for 15 min and centrifuged to get rid of any precipitation. Content was read at 360 nm against reagent blank and calculated using molar extinction coefficient of hydrazone of 22,000 M^{-1} cm^{-1}. Total protein content was estimated in same samples using Lowry method and carbonyl contents expressed on protein basis.

Antioxidant enzymes catalase (CAT) and peroxidase (POX) were commonly extracted in ice cold 50 mM potassium phosphate buffer (pH 7.0) with 1 mM, EDTA, 2% PVP, 0.05% triton-X-100. CAT was assayed in 50 mM potassium phosphate buffer of pH 7.0 at 240 nm where reaction was started with 25 mM H_2O_2 and decrease of absorbance was recorded for 2 min at the interval of 30 s. Activity was calculated using molar extinction coefficient of H_2O_2 of 0.0394 mM^{-1} cm^{-1}. POX was assayed in 100 mM potassium phosphate buffer (pH 6.5), 50 mM guaiacol where 16 mM H_2O_2 was added to start reaction at 470 nm and increase of absorbance was noted for 2 min at the interval of 30 s. Activity was calculated using molar extinction coefficient of tetraguaiacol of 26.6 mM^{-1} cm^{-1}.

Statistical analysis

Mean ± S.D. were calculated. Results were analyzed followed by Fisher's least significant difference (L. S. D.) at $P < 0.05$ using DSAASTAT.XLX [12].

RESULTS

Short forms of genotypes were used as LR, Lady Rosetta; KP, Kufri Pukhraj; KFM, Kufri FryoM; KN, Kufri Neelkanth; MS7, MS/7 645; KG, Kufri Ganga; KC, Kufri Chipsona 1; KF, Kufri Frysona; MP06, MP/06 39; MS8, MS/8 1148; KS, Kufri Sindhuri.

CIS

During first month of cold storage, reducing sugar (glucose+fructose) (Figure 1A) increased in all genotypes except LR and KFM. Highest level at 1 month was in KG then in MS8, KS, KC, MS7 and MP06 while lowest level was in LR and KFM. Reducing sugar level at 1 month was also low in KF. During further storage, reducing sugar level decreased in all genotypes except increased by small amount at 2 month and then decreased in LR and KFM. Results showed LR, KFM and KF can be taken as CIS-resistant genotypes.

Starch (Figure 1B) decreased between 0-1 month but increased between 1-2 month in almost all genotypes. LR showed low decrease between 0-1 and high increase between 1-2 month. KFM showed a significant decrease between 0-1 but regained level back between 1-2 month. KF also showed low decrease between 0-1 month. However, KC showed very high decrease at between 0-1 but improved level between 1-2 month. Starch levels at 1 month were seen low in MS8 and MP06. During further storage, low levels of starch were seen in KG, MS7, MS8 and KP.

Acid Inv (Figure 1C) increased between 0-1 month in all genotypes except MS7 and KC. Among CIS-sensitive genotypes, high acid Inv of KG, MS8, KS was related to high reducing sugar level at 1 month. However, acid Inv also increased by large amount in CIS-resistant genotypes LR, KFM, KF during 0-1 month. Acid Inv also increased by large amount in KP and KN during 0-1 month. After 1 month, acid Inv decreased but increase at 4 month was seen in some genotypes KS, MS7, MS8 and KP.

Results concluded that loss of starch during 0-1 month was low and gain of starch between 1-2 month was high in CIS-resistant genotypes and was best shown in Lady Rosetta. Acid invertase activity relates to CIS in CIS-susceptible genotypes.

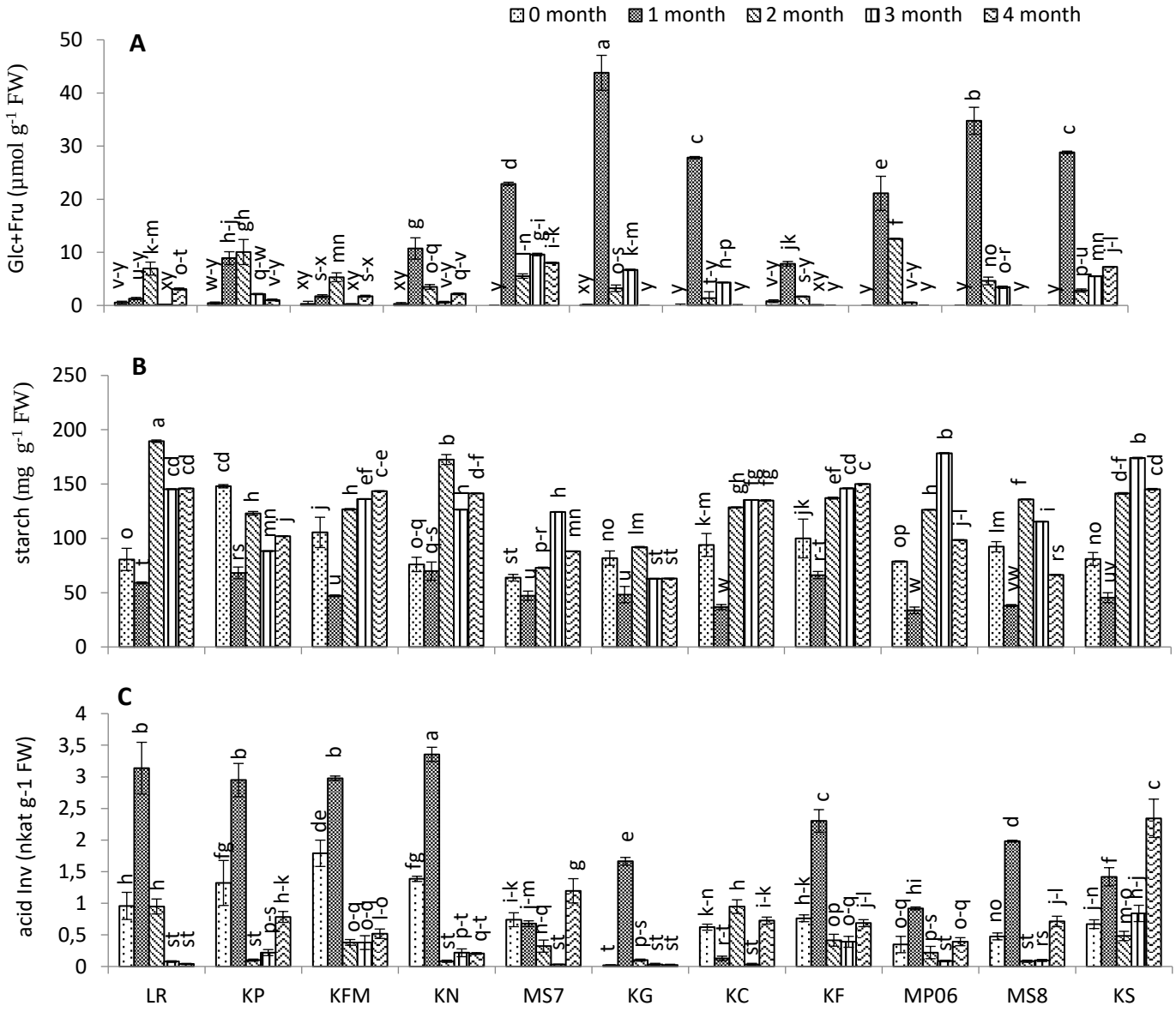

Figure 1. Reducing sugar (glucose+fructose) (A), starch (B) and acid invertase (acid Inv) activity (C) of tuber at different months of cold storage in eleven potato genotypes, LR, Lady Rosetta; KP, Kufri Pukhraj; KFM, Kufri FryoM; KN, Kufri Neelkanth; MS7, MS/7 645; KG, Kufri Ganga; KC, Kufri Chipsona 1; KF, Kufri Frysona; MP06, MP/06 39; MS8, MS/8 1148; KS, Kufri Sindhuri. Different alphabets represent significant difference (Fisher's L. S. D. $p < 0.05$).

Antioxidants

Dehydroascorbate to ascorbate ratio (Figure 2A) increased during 0-1 month in all except LR, KP, KN and MS 7 where ratio decreased. Ratio level at 1 month was low in KFM and KF besides LR, KP, KN and MS7 while high in others where highest level was in KS. During further storage, ratio increased by low amount in LR, KF, MP06 and MS7 while by high amount in KP followed by KFM, KN, KG, KC and KS.

CAT (Figure 2B) increased during 0-1 month in KF, LR, KN, MS7 while decreased in KP and KC while remained unaltered in other genotypes. At 1 month, highest CAT level was in KF but LR, KN and MS7 also had sufficient level however level was poor in other genotypes. During further storage, gradual increase of CAT was seen only in LR while in many genotypes, KS, KFM, KC, KN, MS8 and MS7, abrupt increase at 4 month was seen. Overall poor CAT levels during storage were seen in KP, KG and MP06. In KF, though CAT increased by large amount at 1 month but thereafter during storage, CAT levels were poor.

POX (Figure 2C) increased during 0-1 month in many genotypes while decreased in KS and MS7. Highest POX level at 1 month was in KC followed by LR while lowest level was in MS7. During further storage, POX decreased in KC, KP, KN while increased gradually in KFM while increase was abrupt at 3 month in KS. Overall poor POX levels during storage were seen in MS8 and MS7.

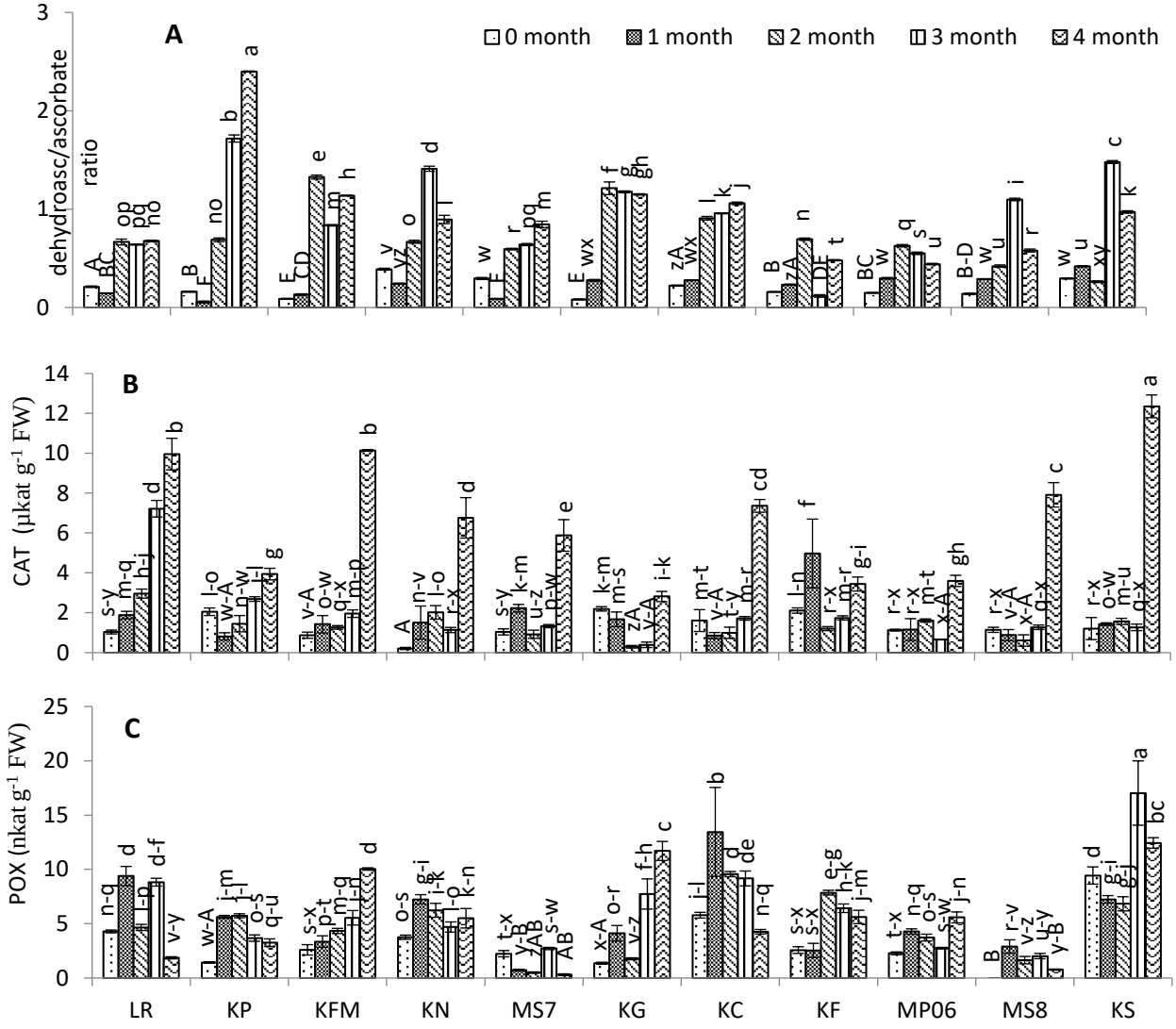

Figure 2. Dehydroascorbate to ascorbate ratio (A), catalase (CAT) (B) and peroxidase (POX) (C) levels of tuber at different months of cold storage in eleven potato genotypes, LR, Lady Rosetta; KP, Kufri Pukhraj; KFM, Kufri FryoM; KN, Kufri Neelkanth; MS7, MS/7 645; KG, Kufri Ganga; KC, Kufri Chipsona 1; KF, Kufri Frysona; MP06, MP/06 39; MS8, MS/8 1148; KS, Kufri Sindhuri. Different alphabets represent significant difference (Fisher's L. S. D. $p < 0.05$).

H_2O_2 (Figure 3A) decreased during 0-1 month in KF, KN and KG only while in rest of all genotypes except MP06, H_2O_2 increased. H_2O_2 level at 1 month was highest in KS while lowest in KF. H_2O_2 levels were also low in LR, KFM and KC at 1 month. During further storage, H_2O_2 increased by large amount in LR, KP and KFM while in other genotypes, increase of H_2O_2 was poor. MDA (Figure 3B) and protein carbonyls (Figure 3C) could not be detected at 0 and 1 month of storage. At 3 month, MDA level was low in LR, MS7 and KN while high in others where highest level was in KG and KFM. At 4 month, very high MDA level was seen in KFM and KP then in KS. Protein carbonyls level at 3 month was high in KC and MS8 while at 4 month, level was very high in MS7, KF, MS8.

Results concluded that at 1 month of cold storage, antioxidant level relates positively while H_2O_2 level relates negatively to CIS-resistance. During long storage, antioxidant activity relates to quality storage. Catalase is an important antioxidant enzyme during long storage. Among CIS-resistant genotypes, Lady Rosetta comes out to be the best genotype for quality storage.

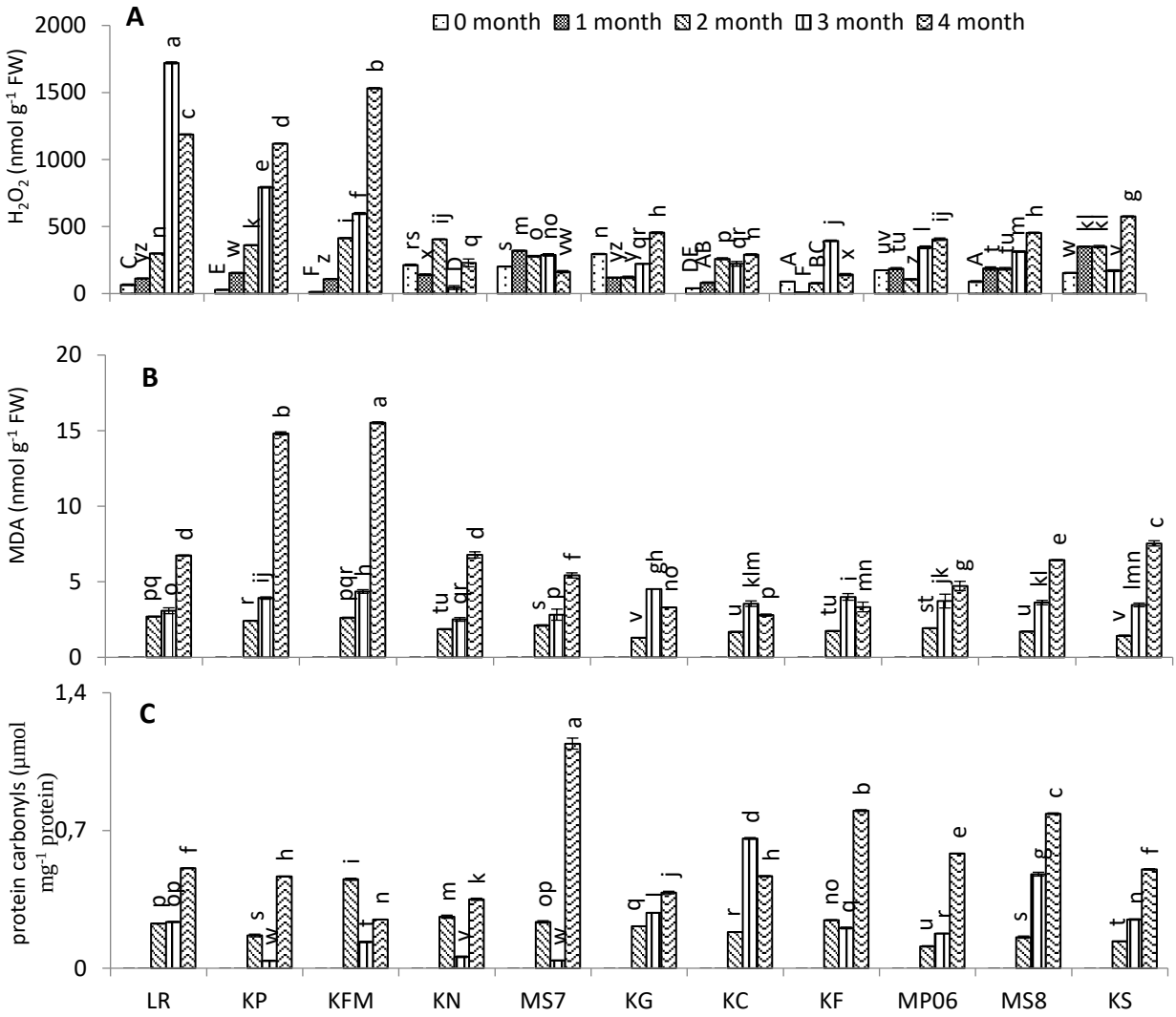

Figure 3. H_2O_2 (A), malondialdehyde (MDA) (B) and protein carbonyls (C) of tuber at different months of cold storage in eleven potato genotypes, LR, Lady Rosetta; KP, Kufri Pukhraj; KFM, Kufri FryoM; KN, Kufri Neelkanth; MS7, MS/7 645; KG, Kufri Ganga; KC, Kufri Chipsona 1; KF, Kufri Frysona; MP06, MP/06 39; MS8, MS/8 1148; KS, Kufri Sindhuri. Different alphabets represent significant difference (Fisher's L. S. D. $p < 0.05$).

DISCUSSION

Elevated hexoses during first 30-40 d of cold storage, has been reported widely in potato cultivars. Tubers produced respiratory acclimation response during this time period thus produced sugars but thereafter, tubers give low temperature acclimation in which hexoses decreased [1, 5]. The present study showed that starch metabolism may be oriented more towards synthesis than breakdown in CIS-resistant genotypes [2,13]. Orientation of starch metabolism towards synthetic direction can be a reason that in KFM, starch hydrolyzed during the first month of cold storage but reducing sugars were not increased thus soluble sugars might be present in sucrose or bound forms like UDPG/ADPG. Observed CIS-sensitivity of Kufri Chipsona 1 in the present results could be some environmental factor as pre-harvest and post-harvest environmental factors are known to affect CIS in potato genotypes [1]. Starch content increased during storage in many genotypes, thus showing starch synthesis occurring in tubers during storage [14].

Acid invertase activity was related to CIS in CIS-susceptible genotypes. CIS of Kufri Chipsona 1 was due to high starch hydrolysis but acid invertase level was low in this genotype. Besides invertases, other enzymes like amylases have been found to be involved in producing CIS [3]. Conversely, acid invertase may be playing some other important roles in CIS-resistant genotypes [2].

Potato tubers are considered with substantial amounts of antioxidants where ascorbate contributes to about 13% of total antioxidant activity during storage [15]. Present results found antioxidant level in the form of catalase, peroxidase and redox state of ascorbate at 1 month of storage was related to CIS-resistant

response of the genotype. During long storage, Lady Rosetta was the best genotype in keeping tuber quality due to high antioxidant activity while in other CIS-resistant genotypes, Kufri FryoM was poor in redox state of ascorbate and catalase levels while Kufri Frysona was poor in catalase levels thus built toxicity higher than Lady Rosetta. Catalase has been suggested for antioxidant role during tuber aging [16]. Catalase activity has been related to CIS-resistance [17].

Duality of H_2O_2 as signal and toxicity was found in the present study. H_2O_2 levels at 1 month of storage in Kufri Sindhuri and during long storage in Kufri Pukhraj and Kufri FryoM were due to low antioxidant state thus represented toxicity but H_2O_2 levels in Lady Rosetta accompanied high antioxidant state, thus represented the signal. ROS is reported to be the signal for dormancy break in tubers [18]. ROS is well known inducer of antioxidant activity. ROS production during dormancy break is not compromised with low antioxidant activity [6, 18].

Therefore, present study concludes that cold induced sweetening (CIS)-resistance may lie in low degradation of starch during cold exposure then high regain of starch during cold acclimatization. Acid invertase activity during cold exposure may relate to CIS in CIS-susceptible genotypes but activity may play some other important role in CIS-resistant genotypes. Tuber antioxidant activity in the form of catalase, peroxidase and redox state of ascorbate may play an important role in CIS-resistance and quality storage. Thus, genotype with high antioxidant activity may be beneficial for both CIS-resistance and quality storage.

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Conflicts of Interest: The authors declare no conflict of interest.

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