

FINE SCREENING FOR RESISTANCE TO COLD-INDUCED SWEETENING IN A WILD POTATO POPULATION

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Abstract. Potato is the most important indispensable part of human food. Cold induced sweetening is a phenomenon in which reducing sugars react with free available amino acid and give brown to black color when fried at high temperature which is not acceptable at end consumers. Moreover, acrylamide a potent carcinogenic is also produced during this process. So many wild and cultivated species have been utilized for screening the best potato germplasm against many potato diseases and quality but only a small sample of the available biodiversity has been exploited. An effort was made in this study to sort out the best potato germplasm from five different wild families on the basis of fine Molecular screening at different research station against cold induced sweetening. Crosses were made between potato families and then stored at 4 °C. Total RNA was isolated for Quantification of mRNA. Protein was isolated and invertase activity was measured through Plate reader at 5.9 λ. Sucrose and reducing sugars were measured through High Performance Liquid Chromatography. After 4 months of 4 °C storage incubation families were screened on the basis of chips color, invertase enzyme activity, Sucrose, reducing sugars contents and mRNA expression level. Resistance against cold induced sweetening was genotype and trial dependent especially for chip color. Invertase activity was corresponding with genotypes and trials. Locations with good chip color were having low invertase action and reducing sugars along with low mRNA expression of invertase enzyme while at other locations vice versa. Same genotype showed variable resistance at different trial satiations and conclusively invertase activity was genotypes and trials dependent. Significant trial effects were detected; all subsequent analyses were carried by trial.

Keywords: *fine screening, mRNA, invertase, germplasm, cold induced sweetening*

Introduction

In most potato producing regions worldwide, the crop is typically harvested during a narrow window of time, but it is consumed throughout the year. Consequently, most of the potato crop is stored before it is shipped to packing facilities and processing plants. Metabolic stability of potato tubers during this storage period is one of the prime trait targets for breeding programs worldwide (Bradshaw and Ramsay, 2005; Dale and Bradshaw, 2003; Hirsch et al., 2013). Potatoes need to be cool-stored throughout the year to maintain a continuous supply to the industry, and storage at low temperature (<8 °C) is beneficial because it reduces bacterial soft rots, decreases water and dry matter loss, and prevents sprouting without the need to add sprout inhibitors (Sowokinos, 2001a). However, storage at low temperature leads to an accumulation of

the reducing sugars glucose and fructose, in a process known as cold-induced sweetening (CIS) (Herrman et al., 1996). Sugars that accumulate in non-photosynthetic tissues are recruited from the starch degradation pathway (Malone et al., 2006; Sowokinos et al., 2004). Tuber sugar content is affected by amount and activity of carbohydrate metabolizing enzymes in source (leaf) and sink (tuber) tissues and by the flux of sucrose from source (Menéndez et al., 2002). When potatoes that have undergone CIS are processed into crisps or chips, high fry temperature causes reducing sugars to react with free amino acids via the Millard reaction, resulting in an unacceptable blackening of the product (Benzing-Purdie et al., 1985). The Millard reaction also leads to the formation of the probable carcinogen acrylamide (Mottram et al., 2002).

Studies on carbohydrate metabolism in potato have shown that several enzymes contribute to low temperature sweetening. During low temperature storage, starch breakdown into sucrose is usually driven by UDP-glucose pyrophosphorylase and sucrose-6-phosphate synthase (Sowokinos, 2001b; McKenzie et al., 2005). Acid invertase converts sucrose into the reducing sugars glucose and fructose. A relationship between light chip color and low levels UDP-glucose pyrophosphorylase activity has been demonstrated (Menéndez et al., 2002; Sowokinos, 2001a). Similarly, low acid invertase activity is associated with light chip color (Bhaskar et al., 2010; Li et al., 2005; Liu et al., 2011; Wu et al., 2011). However, QTL studies have found associations between a number of additional carbohydrate metabolism genes and cold-induced sweetening (Draffehn et al., 2010). More recently, association genetics demonstrated that DNA polymorphisms in genes encoding invertase and starch phosphorylases were associated with potato chip color; starch content and starch yield. Association analysis found that SNP2746 in the *StLapN* gene was strongly associated with chip quality (Urbany et al., 2011). These genetic studies support the working model that natural variation in tuber starch and sugar content is controlled by allelic variants of enzymes that function in starch and sugar metabolism (Fischer et al., 2013; Theocharis et al., 2012).

Microarray hybridization experiments using a tomato gene chip hybridized with potato mRNA allowed the identification of known and novel genes that were differentially expressed during tuber cold storage in a potato clone. Transcript levels of known candidate genes, such as invertase, were correlated with sugar accumulation (Bagnaresi et al., 2008). Comparative proteome analysis has previously proven successful in identifying new candidate genes controlling tuber quality traits (Yang et al., 2011; Hoehenwarter et al., 2008). One approach to breeding for resistance to CIS is to reduce acid invertase activity. Silencing of the acid invertase gene has effectively reduced CIS, resulting in acceptable fry products (Bhaskar et al., 2010). Alternatively, resistance to CIS is found in wild relatives of potato. The wild diploid species *Solanum raphanifolium* is sexually compatible with diploid forms of cultivated potato. Its hybrid offspring have been shown to exhibit resistance to CIS (Hamernik et al., 2009). In fact, acid invertase activity in germplasm carrying CIS resistance genes from *S. raphanifolium* is as low as that in clones in which the gene has been silenced (Bhaskar et al., 2010). Wild potato species are useful sources of genes for potato improvement (Bradshaw and Ramsay, 2005; Herrman et al., 1996; Jansky et al., 2009; Bethke and Jansky, 2008). However, most wild germplasm is self-incompatible and maintained by intercrossing within populations. Consequently, genetic variation within and among accessions is common (Jansky et al., 2009; Cai et al., 2011; Chung et al., 2011; Jansky,

2000; Jansky et al., 2006). It is important, therefore, to identify individuals within a population carrying genes of interest. We followed for two purposes in the present study: First, we were interested in fine screening a population of *S. raphanifolium* for resistance to CIS at different stations within country. Second, we determined the relationship between chip color and potential sources of chip color variation. Since we know that low levels of acid invertase are associated with resistance to CIS in this germplasm, we focused on expression of the acid invertase gene, acid invertase enzyme activity, and levels of the sugars sucrose, glucose, and fructose.

Materials and methods

Potato plant crosses

Crosses were made between clone hap-chc as a female and five plants of *S. raphanifolium* PI 310998 as a male. Hap-chc is a hybrid between a *S. tuberosum* dihaploid (US-W730) and *S. chacoense* PI 310998 from Horticulture Lab, University of Wisconsin Madison USA. In previous work, this cross has produced clones with moderate resistance to cold-induced sweetening (Bhaskar et al., 2010; Hamernik et al., 2009). Seeds were sown on August 14, 2009, transplanted to 48-well flats three weeks later (Fig. 1a) and then 16-21 plants per family were transplanted into 10 cm square pots on October 5 (Fig. 1b). They were grown in a greenhouse at Muree, Pakistan, until maturity. Plants were harvested on January 27-29, 2010 and placed in 4 °C storage on February 2. Two tubers of each plant in each family were chipped during the first week of June and two were retained for clonal maintenance. This trial is designated M1. Thirty-five clones produced at least eight tubers each, so four tubers were planted in the field at Sahiwal, Pakistan, on May 6, 2010. Two replications of two plants each were included in a randomized complete block design. On September 10, each plant was harvested by hand and tubers were collected. The tubers were stored at 4.4 °C until the first week of June, 2011, when two tubers per plot were chipped and were named trial S1. On September 30, 2010, one tuber per clone from the M1 trial was planted again at Sahiwal, Pakistan. Tubers were harvested from mature plants on January 28, 2011. They were stored at 4 °C until June 6, when they were chipped. This was trial S2. Tubers from the S2 trial were planted on September 15, 2011 at a greenhouse in CEMB, Pakistan. Mature plants were harvested on March 8, 2012 and stored at 6 °C. On April 9, samples were collected for sugar, RNA, and invertase assays as described below. In addition, two tubers per clone were chipped. This was trial L1. Each clone was again planted in the Multan greenhouse on April 16, harvested on July 27, and tubers were stored at 6 °C for one month. Two tubers per clone were then chipped. This was trial M2.

Chipping analysis

Chip color was evaluated by taking a 1-2 mm slice from the center of a transverse tuber cut, rinsing it in tap water and frying it in 190 °C corn oil until bubbling ceased. Each chip was visually scored for color using a scale of 1 (light) to 10 (dark), at 0.5 intervals, based on the International Chip Color Institute (Cleveland, OH) color chart). In the M1 trial, each of two tubers per clone was cut into half longitudinally and one half was again cut in half longitudinally. After cutting, the samples were immediately frozen in liquid nitrogen and stored at -70 °C for gene expression analysis. The tuber

halves were used for sugar analyses. One of the quarter tuber pieces was used for the invertase enzyme activity assay and the other quarter was used for mRNA isolation. Tuber samples were passed through a Wiley freeze mill with a 40 mesh screen and stored in a -80 °C freezer.



Figure 1. *a* Potatoes varieties grown under control conditions on MS media. *b* Potatoes varieties grown in the pots under greenhouse conditions

Invertase assay

For the invertase enzyme assay, 100 g protein was extracted by using PD Midi Trap G25 column. The extraction buffer consisted of 0.50 mM HEPES-KOH pH 7.5, 5 mM MgCl₂.6H₂O, 1 mM EDTA, 1 mM EGTA, 0.1% w/v Triton X-100, and 10% w/v glycerol. Phenyl methyl sulfonyl fluoride (PMSF) was added to extraction buffer to a final concentration of 5 mM immediately before use. Before using the Midi Trap column, the cap was removed and the storage solution was poured off. The bottom cap was also removed and the column was put into 50 ml Falcon tube on ice. Then, 5 ml extraction buffer was put into column to wash it; this step was repeated thrice to a total volume ~15 ml extraction buffer and flow through was discarded. Then, 1 g tuber sample was put into a 15 ml tube along with 2 ml extraction buffer and 2-3 small glass beads. After incubating on ice for 10 min, samples were spun in a micro centrifuge for 10 min at 4 °C at maximum speed to pellet debris, and 1 ml supernatant was added to the equilibrated Midi Trap column and allowed to pack the bed completely. Flow through was discarded and the column was placed into a new Falcon tube. Then, 1.5 ml extraction buffer, along with dichlorodiphenyltrichloroethane DDT and PMSF, was added and this time the flow through was collected as it contained the protein sample. A 450 µl sample of protein was collected, frozen in liquid nitrogen, and stored at 0 °C.

Protein concentration measurements

Protein concentration was measured using the Bio-Rad assay. 10 µl sample of Bovine Serum Albumin (BSA) were used as standard. 2 µl of extracted protein along with dye was loaded into a 96 well plate. After incubating for 30 min at room temperature, absorbance at 595 nm was taken using a plate reader. Vacuolar Acid Invertase activity was measured before and after destroying invertase inhibitor proteins. The crude extract of potato tubers contains proteins which may inhibit soluble invertase activity (Pressey, 1971; Ewing and McAdoo, 1971; Barichello et al., 1990; Steel and

Whitehead, 1994). However, Ross and Davis have pointed out that technique to destroy inhibitors, such as rapid vortexing of the extract, may result in a loss of invertase (Ross and Davies, 1992). So, the protein aliquot was divided in half and one half was vortexed at low speed for 30 min in cold room (10 °C) while the remaining half was also put in cold room for 30 min, but was not vortexed. The reaction buffer was prepared for measuring enzyme activity by using 133 mM sucrose, 26.7 mM sodium acetate (NaOAc) pH 4.7, 0.1 M sodium phosphate (Napi) pH 7.4 and 50 mM NaPi pH 7.4 were prepared separately, 100 U/ml glucose oxidase (GO), 100 U/ml Horseradish Peroxide (HRP), 10 mM Amplex red (AR) and dimethyl Sulfoxide (DMSO) for preparing Amplex red was also used. 10 mM NaOAc pH 5 was also used for making up volume. For each sample two tubes of 0.2 ml were used. Each containing 20 µg total protein and 10 mM sodium acetate pH 5 was used to bring volume upto 20 µl. One set of tube was put on ice to serve as zero min control, while in other 60 µl reaction buffer was used and incubated at 30 °C for 60 min. After incubating at 30 °C the reaction buffer was added to zero min control, and 8 µl 1 M NaPi pH 7.4 to each of the zero and 60-min tubes to stop the reaction. After mixing well, the reaction was incubated at 97 °C for 3 min to deactivate the enzyme. 72 µl 50 mM NaPi was added and centrifuged to pellet the precipitated protein. Then, the enzyme assay mix was prepared using 5.3 ml 50 mM NaPi pH 7.4, 100 µl GO, 100 µl HRP and 100 µl AR. 50 µl enzyme assay mix was added into 96 well plates along with 50 µl reaction mixture. 200 µM glucose was used as standard. After incubating the plates for 30 min in dark reading of absorbance were taken at 560 nm. Activity of VInv was calculated as nmol glucose formed per minute per mg of protein.

Reducing sugars analysis

For sugar analyses (Spiro, 1966), freeze dried 5 mg samples were put into 1.5 ml tubes along with 4-5 beads and shaken in pulverize shaker for ~1 min to pulverize the sample. Samples were suspended in 4 ml 80% ethanol and then vortexes and placed in a 60 °C shaking water bath overnight. The next day, tubes were spin for 10 min at 20 °C at 4000 rpm and the supernatant was transferred into new tubes. The pellets were re-suspended in 4 ml 80% ethanol and placed in a 60 °C shaking water bath set overnight. The next day, samples were spin for 10 min at 20 °C at 4000 rpm and the supernatant was transferred added to the tubes from the first extraction. At this point, the supernatant was nearly 8 ml and the volume was brought to 10 ml with 80% ethanol. The sample was mixed by inversion and a 1.5 ml sample was pipetted into a 2 ml tube. The tube was placed in a vacuumfuge overnight to dry it without heat. For HPLC, 1 ml of distilled water was added to the dried sample and incubated at room temperature for 30 min and vortexes at high speed for 10 min to dissolve the pellet completely. Then samples were placed in centrifuge at 13,000 rpm for 10 min. The supernatant was filtered using a tuberculin syringe, carrying 0.2 µm regenerated cellulose filter with care. Then HPLC was done, glucose, fructose, and sucrose were calculated in mg/ml.

mRNA extraction and analysis

RNA was extracted using Agilent Plant RNA isolation Mini Kit (catalog no. 5155-2780). DNA was degraded using with Ambion DNase treatment Kit (Catalogue no. AM1906). Single-stranded cDNA was synthesized using the Invitrogen Super-Script III kit (Catalogue no.18080-051). Then, this cDNA was used as a template for quantitative

PCR using a Bio Rad PCR system. The PCR reaction mix consisted of 2 µl of cDNA (200 ng), short length primers 10 pico mole (Forward-AAACGGGTTGGACACATCAT, Reverse -AACCCAATTCCACAATCCAA) for Vacuolar Acid Invertase gene and Fermentas Maxima SYBP Green /fluorescein qPCR Master mix (catalog no. K0241). Actin was used as the control (forward primer 5'ATGTTCCCGGGTATTGCTGACAGA-3'; reverse 5'-CTGCCTTTGCAATCCACATCTGCT-3'). Reaction containing primers of actin and invertase samples were run in on 96-well plates, PCR cycles were run at 95 °C for 10 min, 95 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s at melt curve 55- 95 °C. One sample each positive control (genomic DNA) of actin and invertase was also run on each plate. Fluorescence data during the extension phase and melt curve temperatures were recorded. Real time PCR Data were analyzed using the general linear model in SAS. Means comparison was carried out using a least significant difference test at $p = 0.05$. Pearson correlation coefficients were used to evaluate relationships among chips scores and biochemical parameters. The data on all studied traits was recorded from all of the performed experiments, the average data from all experiments was calculated for statistical analysis by using analysis of variance technique and significance of mean differences (Steel et al., 1997) through using SPSS 23 version.

Results

The significant differences were found among all of the families under study as shown by *Table 1*. Family 15 consistently produced light chips, while Family 14 produced darker chips (*Table 2*). All five families were generated by crossing the same female clone (HC) to plants from one wild species accession. The *S. raphanifolium* clone that was crossed to hap-chc to produce Family 15 is more desirable than the others tested in PI 310998. The lightest chips were produced from Murree (M1) greenhouse tubers stored for 4 months at 4 °C, followed by Sahiwal-1 greenhouse tubers stored for 1 month at 6 °C (*Table 2*). The darkest chips were produced from tubers grown at Sahiwal-2 and stored for 9 months at 4 °C. Significant differences ($P = 0.05$) were detected among families in this trial for PCR cycle time, enzyme activity, sucrose, glucose, and fructose. There was a significant effect of clone on PCR cycle time ($P = 0.0009$), enzyme activity ($P < 0.0001$), sucrose ($P < 0.0001$), glucose ($P < 0.0001$), and fructose ($P < 0.0001$) (*Table 3*). However, there was a significant correlation between sucrose and glucose ($P = 0.0002$), sucrose and fructose ($P = 0.0213$) and glucose and fructose ($P < 0.0001$). There was no correlation between clone chip score in the M1 trial and any of the biochemical traits. Based on clone means, correlations between pairs of biochemical traits were calculated (*Table 4*). There was found a significant but negative correlation between enzymes with Multan location, sucrose with mRNA expression. Positive and significant correlation of mRNA expression with glucose and fructose was recorded.

Table 1a. ANOVA for chip color and biochemical traits

Traits/locations	CEMB (L1)	Sahiwal (S1)	Multan (M2)	Murree (M1)
Replication	1.082ns	2.123ns	0.531ns	3.7823ns
Family	7.6916*	11.8038*	1.5918*	31.4569*

ns = non-significant, * = significant at 5% probability level

Table 1b. ANOVA for chip color and biochemical traits

Traits/Locations	Sahiwal (S2)	RNA	Fructose	Glucose	Sucrose
Replication	5.608ns	0.042ns	1.028ns	1.001ns	5.123ns
Family	44.927*	0.48585*	6.82822*	7.1807*	30.7049*

ns = non-significant, * = significant at 5% probability level

Table 2. Mean chip score of five families in five cold storage trials. Chip score ratings were based on a scale of 1 (light) to 10 (dark). Chip color scores of 4 or less are commercially acceptable

Traits Family	CEMB Mean±SE	Sahiwal Mean±SE	Multan Mean±SE	Murree Mean±SE	Sahiwal 2 Mean±SE
11	4.4750±0.2311	4.1750±0.2495	4.8125±0.1932	3.4750±0.1915	6.4000±0.2350
13	4.1250±0.2311	3.9750±0.2495	4.2875±0.1932	4.1475±0.1915	4.3500±0.2350
14	4.7750±0.2311	5.0000±0.2495	4.4250±0.1932	5.8550±0.1915	4.5000±0.2350
15	3.9600±0.2311	3.9500±0.2495	4.0125±0.1932	3.1025±0.1915	4.0500±0.2350
18	4.1667±0.2255	4.2381±0.2435	4.4762±0.1886	4.9905±0.1869	4.1714±0.2293

Table 3. RNA extraction amount and biochemical analysis for different families

Traits Family	RNA Mean±SE	Fructose Mean±SE	Glucose Mean±SE	Sucrose Mean±SE
11	0.1398±0.0355	1.4443±0.2058	1.5095±0.2801	10.609±0.8381
13	1.2483±0.0355	2.6723±0.2058	2.2382±0.2801	8.433±0.8381
14	0.3337±0.0355	1.6860±0.2058	1.9653±0.2801	6.542±0.8381
15	0.0675±0.0355	0.6482±0.2058	0.4572±0.2801	9.243±0.8381
18	0.2305±0.0347	1.6902±0.2008	1.9419±0.2733	6.000±0.8179

Table 4. Correlation among various traits of potato

Traits	CEMB	Sahiwal	Multan	Murree	Sahiwal 2	RNA	Invertase enzyme	Fructose	Glucose
Sahiwal	0.0138								
Multan	-0.0546	0.0205							
Murree	0.0193	0.0631	0.0761						
Sahiwal 2	-0.0163	-0.013	-0.0279	0.0383					
RNA	-0.1026	-0.1226	0.1004	0.0075	-0.0735				
Invertase enzyme	0.0941	0.6511*	-0.1326*	0.0354	0.2175*	-0.0899			
Fructose	0.0602	0.2164	-0.0152	0.1028	0.0991	0.1409*	0.2887*		
Glucose	0.2356	0.2372	-0.0594	-0.0664	-0.0153	0.1202*	0.3352*	0.8197*	
Sucrose	0.1539	0.1284	-0.0143	0.3109	-0.0713	-0.0747*	0.1545*	0.2741*	0.2583*

* = significant at 5% probability level

Discussion

HC is a heterozygous interspecific hybrid, so it may segregate for CIS alleles. However, the set of alleles it contributes to offspring is expected to be the same among the five families. Consequently, differences among families are likely due mainly to differences genetic variability among male parents from the same *S. raphanifolium* accession within each trial. All five families were generated by crossing the same female clone (HC) to plants from one wild species accession. ANOVA revealed a significant effect of family ($P < 0.05$), but not replication, on chip score at all locations except S2 (*Table 1*). Family 15 consistently produced light chips, while Family 14 produced darker chips (*Table 2*). Individuals from a population of an outcrossing species are likely to be heterozygous and heterogeneous. While PI 310998 is a good source of CIS resistance genes, fine screening of the population for individuals that produce a high proportion of resistant offspring is likely to be productive. Fine screening of accessions for individuals carrying traits of interest has been reported in potato (Bamberg et al., 1996; Bamberg et al., 1998; Douches et al., 2001). The *S. raphanifolium* clone that was crossed to hap-chc to produce Family 15 is more desirable than the others tested in PI 310998.

The lightest chips were produced from Murree (M1) greenhouse tubers stored for 4 months at 4 °C, followed by Sahiwal-1 greenhouse tubers stored for 1 month at 6 °C (*Table 2*). The darkest chips were produced from tubers grown at Sahiwal-2 and stored for 9 months at 4 °C. We did not expect lighter chip color from tubers stored at 6 °C than at 4 °C. However, the combined effect of environmental and cultural practices can affect tuber quality, which we visualized in the finished processed products. Suboptimal environments result in an increase in internal tuber disorders (Zhou et al., 1994). Long-term storage of potato tubers (six month or more) has significant, variety-dependent effects on sugar and amino acid concentrations (Muttucumaru et al., 2014). Consequently, darker chip color in the long storage trials (M2 and L1) was expected. Tubers from trial Murree were chosen for analyses of acid invertase RNA levels (as measured by the number of PCR cycles to reach the threshold), acid invertase activity, and levels of the sugars sucrose, glucose, and fructose. Significant differences ($P = 0.05$) were detected among families in this trial for PCR cycle time, enzyme activity, sucrose, glucose, and fructose. However, since there was no difference in chip color among families in trial Murree, additional analyses were based on clones without regard to family. There was a significant effect of clone on PCR cycle time ($P = 0.0009$), enzyme activity ($P < 0.0001$), sucrose ($P < 0.0001$), glucose ($P < 0.0001$), and fructose ($P < 0.0001$) (*Table 3*).

Based on clone means, correlations between pairs of biochemical traits were calculated. There was no significant correlation between acid invertase RNA or activity and any of the three sugars. However, there was a significant correlation between sucrose and glucose ($P = 0.0002$), sucrose and fructose ($P = 0.0213$) and glucose and fructose ($P < 0.0001$). There was no correlation between clone chip score in the M1 trial and any of the biochemical traits. Based on clone means, correlations between pairs of biochemical traits were calculated (*Table 4*). There was found a significant but negative correlation between enzymes with Multan location, sucrose with mRNA expression. Positive and significant correlation of mRNA expression with glucose and fructose was recorded. It was suggested from positive correlation may be used to improve in the quality traits while improving the related traits and improvement may be fixed in next generations. The negative correlations suggested that the decrease in the trait may be

fixed in next generations. For the improvement of qualitative and quantitative traits positive correlations are used for selection (Ali and Jansky, 2015; Ali et al., 2014, 2016, 2013). The ratio of sucrose to glucose is a reflection of acid invertase enzyme activity. Again however, there was no significant difference in the sucrose: glucose ratio between the clones with high enzyme activity and those with low activity. It is interesting that acid invertase enzyme activity and mRNA production is negatively correlated with chip color in this study. In a previous study using the same accession of *S. raphanifolium*, silencing the potato vacuolar acid invertase gene resulted in light colored chips (Bhaskar et al., 2010). In that study, relatively high levels of invertase transcript were detected in hap-chc, but transcript levels were low in some hap-chc x *S. raphanifolium* 310998 hybrids. Several studies have demonstrated high sequence variability and genotype specific variants among potato protease inhibitors. These inhibitors significantly affect the Vacuolar Acid invertase activity (Bauw et al., 2006; Jørgensen et al., 2005). Effects of patatins and phospholipids in altering membrane lipid composition could explain differences between genotypes with strong (CIS-s) and weak (CIS-t) cold acclimation responses (Theocharis et al., 2012). More recently, association genetics demonstrated that DNA polymorphisms in genes encoding invertases and starch phosphorylases were associated with potato chip color, starch content and starch yield (Fischer et al., 2013).

Conclusions

Same genotype can show variable resistance in different trial satiations and in conclusive invertase activity is genotypes and trials dependent. Significant trial effects can be detected; if all subsequent analyses are carried by trial. Invertase enzyme is responsible for cold induced sweetening but not exclusively. Its activity is trial, genotype and environmental conditions dependent. Along with fine screening it is the need of time to sort out best potato germplasm based on the action of other genes involved in glycolysis pathway.

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Conflict of interests. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interests.

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