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Research Article

Fine Screening for Resistance to Cold-Induced Sweetening in Potato Hybrids Containing *Solanum raphanifolium* Germplasm

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Potato is an indispensable part of human food. Many wild and cultivated potato relatives have been screened to find the best germplasm to improve productivity and quality, but only a small sample of the available biodiversity has been exploited. Most wild relatives are self-incompatible diploids. Genetic variability exists within and among populations, even within a species. Therefore, it is necessary to carry out fine screening to identify individuals carrying traits of interest. This study was carried out to quantify phenotypic variability for resistance to cold-induced sweetening, an important processing trait. Five families were evaluated for potato chip (crisp) color following cold storage of tubers harvested from four greenhouse trials and one field trial. The families were generated by crossing a single diploid clone to five plants from one accession of the wild potato relative *Solanum raphanifolium*. Analysis of variance revealed that resistance against cold-induced sweetening was dependent on family and trial. This study underscores the importance of fine screening to select individuals in potato accessions for use in potato improvement.

1. 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 [1–5]. 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 [6]. However, storage at low temperatures leads to an accumulation of the reducing sugars glucose and fructose, in a process known as cold-induced sweetening (CIS) [7]. Sugars that accumulate in nonphotosynthetic tissues are recruited from the starch degradation pathway [8, 9]. Tuber sugar content is affected by abundance and activity of carbohydrate metabolizing enzymes in source (leaf) and sink (tuber) tissues, and by the flux of sucrose from source tissues [4]. When potatoes that have undergone CIS are processed into crisps or chips, high fry temperatures cause reducing sugars to react with free amino acids via the Maillard reaction, resulting in an unacceptable blackening of the product [10]. The Maillard reaction also leads to the formation of the probable carcinogen acrylamide [11].

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 [5, 12]. Acid invertase converts sucrose into the reducing sugars glucose and fructose. A relationship between light chip color and a low level of UDP-glucose pyrophosphorylase activity has been demonstrated [4, 6, 12]. Similarly, low acid invertase activity is associated with light chip color [12–16]. However, QTL studies have found associations between a number of additional carbohydrate metabolism genes and CIS. More recently, association genetics studies have demonstrated that DNA polymorphisms in genes encoding invertase and starch phosphorylases are associated with potato chip color, starch

content, and starch yield [17, 18]. Association analysis found that SNP2746 in the *StLapN* gene was strongly associated with chip quality [19]. 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 [20].

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 [21]. Comparative proteome analysis has previously proven successful in identifying new candidate genes controlling tuber quality traits [22, 23]. 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 [13]. Alternatively, resistance to CIS is found in wild relatives of potato.

Wild potato species are useful sources of genes for potato improvement [1, 7, 24, 25]. 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 [26]. 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 [13]. However, *S. raphanifolium* and most diploid wild germplasm are self-incompatible and are maintained by intercrossing within populations. Consequently, genetic variation within and among accessions is common [24, 27–30]. It is important, therefore, to identify individuals within a population carrying genes of interest.

The purpose of this study was to carry out fine screening for resistance to CIS on a population of *S. raphanifolium*. Since tubers of wild species are typically very small, individuals in the population were crossed with a clone that would allow for the production of offspring with larger tubers suitable for CIS evaluation.

2. Materials and Methods

Crosses were made between clone hap-chc (HC) as a female and five plants of *S. raphanifolium* PI 310998 as a male. HC is a hybrid between *S. tuberosum* dihaploid (US-W730) and the potato wild relative *S. chacoense* PI 310998. In previous work, HC x PI 310998 hybrids have exhibited exceptional resistance to cold-induced sweetening [13, 26]. Seeds were sown on August 14, 2009, and transplanted to 48-well flats three weeks later, and then 16–48 plants per family were transplanted into 10 cm square pots on October 5. They were grown in a greenhouse at Arlington, WI, until maturity. Plants were harvested on January 28, 2010, and placed in 4°C storage on February 2. Two tubers of each plant in each family were chipped on June 3 (121 days in storage); two tubers were retained for clonal maintenance. This trial is designated A1. On September 30, 2010, one tuber per clone from the A1 trial was planted again at Arlington. Tubers were harvested from mature plants on January 28, 2011. They were stored at 4°C until June 6 (129 days in storage), when they were chipped. This was trial A2.

Thirty-five clones in trial A1 produced at least eight tubers each, so four tubers were planted in the field at Rhinelander, WI, 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 June 8, 2011, when two tubers per plot were chipped (271 days in storage). This was trial (R).

One tuber from each clone in the A2 trial was planted on September 15, 2011, at a greenhouse in Madison, WI. Mature plants were harvested on March 8, 2012, and stored at 6°C until April 9, when two tubers per clone were chipped (32 days in storage). This was trial M1. Each clone was again planted in the Madison greenhouse on April 16 and harvested on July 27, and tubers were stored at 6°C for 30 days. Two tubers per clone were then chipped. This was trial M2. All greenhouse trials used supplemental high intensity light to support plant growth and maintain an 18-hour photoperiod. Photoperiod was shortened to 12 hours to induce tuberization one month before harvest. Plants were grown in soil-less peat-based potting mix and fertilized weekly. The number of clones in each family is presented in Table 1. Storage conditions are summarized in Table 2.

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.

Analysis of variance was carried out using a general linear model in SAS (version 9.3; SAS Institute, Cary, NC). Means separation was carried out using Fisher's protected least significant difference (LSD) test at $P = 0.05$. Error variances were homogeneous, so no transformation of the data set was necessary.

3. Results and Discussion

Analysis of variance revealed highly significant effects of family, trial, and the family by trial interaction on chip color (Table 3). Consequently, subsequent evaluations considered each family in each trial, rather than averaging over trial or family. Not all clones tuberized in each trial, so the number of individuals evaluated varied from trial to trial. However, within each trial, a similar number of clones per family was evaluated. Many clones did not tuberize in the field, so the Rhinelander trial contained the smallest number of individuals.

Within each trial, ANOVA revealed a significant effect of family ($P < 0.05$), on chip score at all locations except M1 (Table 4). The effect of tuber sample and replication was not significant. Family 15 consistently produced light chips, while family 14 produced darker chips. All five families were generated by crossing the same female clone (HC) to plants from one wild species accession. HC is a heterozygous interspecific hybrid, so it may segregate for CIS alleles. However, the set of

Table 1: Number of clones evaluated for chip color in five families (F11–F18) across five trials (M1–R).

	M1	M2	A1	A2	R
F11	19	15	36	36	13
F13	25	15	44	44	4
F14	28	19	41	40	4
F15	22	19	34	34	8
F18	20	14	44	44	4

Table 2: Storage conditions for each of the five trials.

Trial	Storage temperature	Days in storage
M1	6°C	32
M2	6°C	30
A1	4°C	121
A2	4°C	129
R	4°C	271

Table 3: ANOVA table for effect of family and trial on chip color.

Source	Df	Sum of squares	F value	Pr > F
Family	4	135.18	16.29	<0.0001
Trial	4	63.91	7.70	<0.0001
Family ∗ trial	16	89.54	2.70	0.0004
Error	602	1249.12		

Table 4: 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).

	M1	M2	A1	A2	R
F11	4.82	4.00 b*	4.49 ab	4.25 b	6.77 ab
F13	4.38	4.43 b	4.11 bc	3.93 bc	4.75 bc
F14	4.69	6.18 a	4.76 a	5.00 a	8.00 a
F15	4.45	4.03 b	3.53 c	3.53 c	4.38 c
F18	5.03	3.93 b	4.11 bc	4.14 bc	4.00 c

*Within a column, means followed by different letters are different at p = 0.05.

alleles it contributes to offspring is expected to be the same among the five families. Consequently, differences among families are likely due mainly to genetic variability among male parents from the same *S. raphanifolium* accession. 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. For example, the use of the clone that produced family 15, rather than the one that produced family 14, would allow a breeder to make more progress toward breeding for reduced CIS. Fine screening of accessions has been reported for other traits in potato [31–33].

The darkest chips were produced from tubers grown at Rhinelander and stored for 271 days at 4°C (Table 4). This is an extreme storage environment for both temperature and duration. Long-term storage of potato tubers has significant, variety-dependent effects on sugar and amino acid concentrations [34]. However, in this study, nine clones produced chips with commercially acceptable color, illustrating the value of this germplasm for potato cultivar improvement.

Table 5: Number (percent) of clones in each family and trial with acceptable chip color scores (4.5 or less).

	M1	M2	A1	A2	R
F11	9 (47.36%)	12 (80%)	28 (77.7%)	28 (77.7%)	3 (23.07%)
F13	21 (84%)	9 (60%)	40 (93.1%)	40 (90.9%)	2 (50%)
F14	19 (71.4%)	8 (42.10%)	28 (68.29%)	26 (65%)	0
F15	16 (72.7%)	28 (84%)	34 (100%)	34 (100%)	7 (87.5%)
F18	9 (45%)	10 (71.4%)	37 (84.09%)	37 (84.09%)	4 (100%)

Because potato cultivars are clones rather than populations, it is most important for breeders to identify parental combinations that will produce a large proportion of offspring carrying the trait of interest when introgressing wild germplasm into breeding lines. Then, after selecting clones expressing that trait, the breeder still has a large number of individuals with which to select for other important agronomic traits. The parents of families 13 and 15 consistently produced families in which at least 50% of the offspring had acceptable resistance to CIS (Table 5). These would be desirable parents to use in a breeding program to improve processing quality after cold storage in potato.

Conflict of Interests

The authors have no competing interests.

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