

# Assessment of the usability of four molecular markers to identify potato genotypes suitable for processing

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## Abstract

The development of processing potato cultivars through a conventional breeding program requires a detailed analysis of post-harvest traits, which is a process that demands high labor and is often time-consuming. Visual selection by breeders is biased and difficult in the field, particularly for quality traits, which shows the importance of marker-assisted selection over conventional techniques. In this study, four allele-specific markers, *AGPs5-9a*, *Stp23-8b*, *StpL-3e*, and *Pain1-8c*, developed from tuber quality-related genes, were used to screen a breeding population of the NOHU for processing traits to check the efficiency of these markers in processing trait selection. Marker association with tuber quality trait results showed that *AGPs5-9a* (0, absent) and *StpL-3e* (0) individually were associated with increased chips quality, yet their individual presence improved the reducing sugar content. Further, *Pain1-8c* presence was associated with high levels of reducing sugar accumulation and lower dry matter content, specific gravity, and starch content. The marker combination *Stp23-8b* (0) and *StpL-3e* (0) reached statistical significance ( $P \leq 0.05$ ) for better chips quality in the NOHU population. However, the markers (individual and combination) showed poor selection efficiency as a diagnostic marker, possibly reasoning from the multigenic inheritance of tuber quality traits, population structure, and environment.

## Introduction

Potato breeding using conventional approaches is a laborious and time-consuming process because of its dependence on many years of selection, along with a means of measuring desired traits such as tuber yield, processing quality, disease resistance, and abiotic stress tolerance in the laboratory and field ([Milczarek et al., 2014](#)). Molecular marker studies evaluating the quality properties of potato tubers after harvest are very limited compared to other marker studies in potato because of their multigenic inheritance. However, the development and validation of markers related to processing quality in potato will largely decrease labor

and provide a reliable selection approach for breeding programs ([Li et al., 2013](#)).

Labor-intensive quality traits, such as dry matter, starch, reducing sugar content, and French fry/crisp quality, are screened in routine potato breeding programs, and scientists argue the applicability of molecular markers for selection in breeding programs. The selection process during a potato breeding program requires visual evaluation in order to keep the promising breeding line for the next year; however, the measurement of tuber quality traits is possible only at the post-harvest stage. Unfortunately, this latter

circumstance results in the loss of several promising processing breeding lines that are selected based on appearance. In addition, many of the methods currently used to measure tuber quality traits rely on destructive screening. It is, therefore, very critical, especially regarding breeding efforts, to develop a non-destructive mechanism that allows selection of breeding lines for processing purposes during the vegetation period (before the harvest). Therefore, implementing marker assisted selection for tuber quality traits in potato is one of the main concerns of a few scientists today.

There are a limited number of molecular mapping studies regarding dry matter content, and they have reported that the relevant loci are located on chromosomes 2, 5, 8, 9, and 11 ([Manrique-Carpintero et al., 2015](#)). In particular, an earlier study observed that a QTL region on chromosome 5 had a minor effect on increasing dry matter content; interestingly, the same QTL region was also associated with maturity and tuber shape ([Bradshaw et al., 2008](#)). Unfortunately, the application of the genomic selection model in another study did not produce a reliable and predictable selection approach for dry matter content across different breeding MASPOP populations in potato ([Sverrisdóttir et al., 2017](#)). Another work screening specific gravity using marker assisted selection (isozymes, RFLP, and RAPD) in a diploid population had an achievement in different trial locations, and several QTLs were mapped on different chromosomes (1, 2, 3, 5, 7, 11). However, this study had restriction on application in tetraploid level ([Freyre and Douches, 1994](#)). The use of novel systems, such as the SolCAP SNP array in breeding programs, has exploited the association of several genomic regions with several tuber quality traits, starch content ([Schönhals et al., 2017](#)), and browning after fry ([D'hoop et al., 2014](#)). Although there are more studies on these particular tuber quality traits, they were not found to be statistically significant ([Sharma et al., 2018](#)). However, several novel QTLs, such as *cPHO1B-1b*, *PHO1B-1a*, *StI024-e*, *PHO1A-b*, *StI013-a*, and *SSR327-a*, have been identified for starch content and specific gravity ([Urbany et al., 2011](#)). Several SNPs, later related to starch content, have been known to have loci primarily on chromosome 10, revealed using genotyping by sequencing strategy in potato ([Sverrisdóttir et al., 2017](#)). These regions are related to a role in carbohydrate metabolism and making the main task of major studies for processing traits in marker development studies ([Li et al., 2008, 2013](#); [Schreiber et al., 2014](#)). Novel QTL regions have been investigated for several other processing traits, such as cold-induced sweetening during storage and fry color ([Sołtys-Kalina et al., 2020](#)).

Four SSCP markers, *Pain1-8c*, *AGPs5-9a*, *StpL-3e*, and *Stp23-8b*, have been associated with tuber yield, starch content, starch yield, crisp color, and French fry color stored at 4°C for three-four months and verified as diagnostic markers for these traits ([Li et al., 2013](#); [Schreiber et al., 2014](#); [Schönhals et al., 2016](#)). The effect

of *PHO1* alleles, *Stp23-8b* (*PHO1a-Ha*), and *StpL* (*PHO1b*)-*3b*, on starch content was observed independently in two different German populations, CHIPS-ALL with 243 potato cultivars and breeding populations (BNC and SKC clones). Furthermore, the aforementioned molecular markers have been found to be related to decreased sugar content (glucose and fructose) as well ([Schreiber et al., 2014](#)). The presence of *Stp23-8b* was associated with increased starch content and positively related to starch yield and chip quality ([Li et al., 2013](#)).

In this study, the NOHU population developed in the breeding program of Nigde Omer Halisdemir University was screened with molecular markers developed by [Li et al. \(2013\)](#) to test the reproducibility of these markers for the selection of promising processing breeding lines.

## Materials and Methods

### Plant material

This study was conducted at Nigde Omer Halisdemir University, Nigde, Turkiye. A breeding population named "NOHU" combining breeding lines from the crosses between four different parental combinations, *04.123 x Hermes*, *06.62 x Hermes*, *01.536 x Hermes*, and *Pomqueen x CIP 397039.51*, was used for screening. The parental potato genotypes *Hermes* (Austria), *01.536* (Hungary), and *CIP 397039.51* (Peru) were chosen for chips purposes, while *Pomqueen*, *06.62* (Hungary), and *04.123* (Hungary) were chosen for French fry purposes ([ECPD, 2017](#)). In total, 182 individuals were included in the study. Three seed tubers at the third year of the potato breeding scheme stage for each crossing combination were planted in the field by hand on May 6, 2015, with a 33 cm in-row distance and 70 cm inter-row spacing. The plants were irrigated regularly using a sprinkler irrigation system. Standard agricultural practices for potato were applied during the growth period. Tubers were harvested by hand 175 days after planting, and all genotypes were kept at 8°C and 90% humidity for post-harvest analysis ([Cottrell et al., 1993](#)).

### Specific gravity, dry matter, and starch content

Specific gravity (SG) was determined by analyzing potatoes (approximately 1500 g) with a PW2050 Digital Potato Hydrometer. Breeding lines with an SG greater than 1.080 were considered suitable for processing. The dry matter content (DMC) was calculated based on specific gravity. Breeding lines with DMC greater than 20% are considered suitable for processing ([Lisinska et al., 2009](#)). Starch content (SC) was calculated according to the following formula ([Haase, 2003](#));

$$SC = (183 \times \text{Specific gravity}) - 183$$

### Reducing sugar (glucose and fructose) content

The tuber samples were placed in refrigerator bags without peeling and kept at -20°C for at least 24 h. The

samples were then freeze-dried in a lyophilizer (Labconco) at  $-80^{\circ}\text{C}$  and 0.5 mBar conditions for five days. The freeze-dried samples were homogenized using a blender to obtain powder. The tuber skin, which was difficult to break, was crushed using a mortar. The powder was filtered through a sieve (Friedman et al., 2009). Potato powder (200 mg) was weighed and placed in a 15 mL centrifuge tube. Ultrapure water (three mL) was added to the centrifuge tube. The mixture was vortexed for three min then seven mL of pure ethanol ( $\geq 99.9$ ) was added to the mix, and the mixture was gently shaken. The mixture was then centrifuged for 10 min at 1600 x g. The supernatant was collected using a syringe and passed through 0.45  $\mu\text{m}$  filters. An Inertsil  $\text{NH}_2$  (GL Science) column was used for the HPLC (Shimadzu Prominence Series) analysis. The pure glucose and fructose (HPLC grade-Merck) were used as the standards for HPLC analysis of reducing sugar content (RSC). A mixture of acetonitrile and water (80:20, V/V) was used as the mobile phase. The column conditions were determined to be one mL flow rate per minute at  $40^{\circ}\text{C}$ . Solid-phase extraction is not required (Yavuz, 2016).

#### Crisp and french fry color

Tubers meeting the criteria of tuber shape and size for processing purposes were selected for crisp and French fried potato color analysis and quantified using colorimetric measurements. The peeled tuber samples were sliced to a thickness of two mm for crisp processing. These slices were fried in sunflower oil at  $180^{\circ}\text{C}$  for three min (REMTA). The L, a, and b values were measured using a colorimeter (Konica Minolta- CR700) to analyze French fry L value (FLV) and crisp L value (CLV). The tuber samples were cut into strips of 10x10 mm for French fry analysis. The same procedures were followed for the color measurement of French fried potato samples.

#### DNA extraction

Young leaf samples were ground using TissueLyser II for three min. DNA was extracted from powdered tissue samples using the GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Scientific) according to the manufacturer's protocol. DNA quantity was measured using spectrophotometer (BioSpec-SHIMADZU).

#### Molecular screening

Four of the most promising candidate molecular markers, *StpL-3e*, *Stp23-8b*, *AGPs-9a*, and *Pain1-8c*, associated with tuber quality traits (Li et al., 2013), were tested on 182 potato breeding lines and parents, 04123, 0662, and *Pomqueen*, to determine their diagnostic precision. In addition, *Agria*, a non-parental processing variety, was also included in the study as an external control, but we lacked tubers for 01536, *Hermes*, and *CIP 397039.51*, so we could not screen these genotypes. For PCR optimization, the DNA samples of the potato cultivars *Diana*, *Satina*, *Theresa*, *Solana*, and *Leyla*

provided by Dr. Christiane Gebhardt were used as positive or negative control based on the marker in the molecular screening studies (*Satina* for *Pain1-8c* and *Solana* for three other markers as positive control and *Leyla* as negative control for all four markers). Primers were constructed as described by Li et al., 2013.

The PCR was performed in 25  $\mu\text{l}$  volume solution containing 10x DreamTaq buffer (Thermo), 0.2 mM dNTP, 1  $\mu\text{M}$  primer (for each forward and reverse primer), 1 unit Taq DNA polymerase, and 50 ng genomic DNA. While a touchdown PCR method was used to screen the NOHU population to increase the specificity of the *StpL-3e*, *Stp23-8b*, and *Pain1-8c* primers and avoid non-specific amplification, a standard PCR method was used for *AGPs-9a* because the reaction was already very specific for the target. The thermal cycling conditions of the PCR consisted of an initial incubation at  $95^{\circ}\text{C}$  for three min, followed by 35 cycles of denaturation at  $95^{\circ}\text{C}$  for 15 s, annealing, and elongation at  $72^{\circ}\text{C}$  for 30 s. For the touchdown PCR, the first annealing temperature was set to  $5^{\circ}\text{C}$  above the optimal annealing temperature. The annealing temperature was decreased by  $1^{\circ}\text{C}$  during each subsequent cycle during the five cycles. The reaction was continued for 30 more cycles at the final annealing temperature. The annealing temperatures were set as  $63^{\circ}\text{C}$ - $58^{\circ}\text{C}$  for *Pain1-8c* and *Stp23-8b*,  $65^{\circ}\text{C}$ - $60^{\circ}\text{C}$  for *StpL-3e*. A constant annealing temperature of  $63^{\circ}\text{C}$  was used for *AGPs-9a*. Depending on the size of the PCR product, the elongation time was set to 30 s for those smaller than 500 bp, and 45 s for the range of 500-750 bp at  $72^{\circ}\text{C}$ . The PCR products were run on a 2% agarose gel at 7 V/cm and produced scorable band sizes of 703 bp for *Pain1-8c*, 348 bp for *Stp23-8b*, 360 bp for *StpL-3e* and 210 bp for *AGPs-9a*. After ethidium bromide staining of the gels, images were captured using the Bio-Rad UV Transilluminator machine. Single bands were scored based on their absence (0) or presence (1).

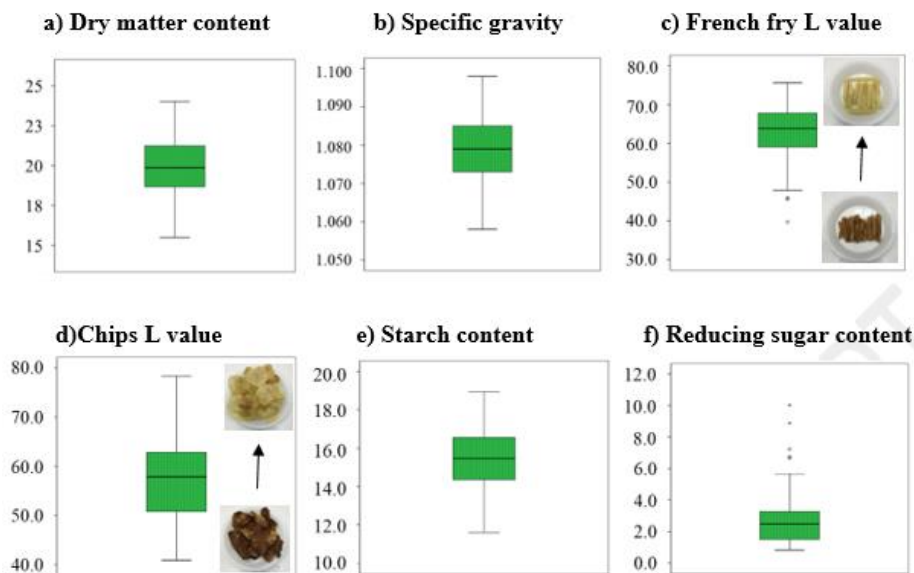
#### Statistical analysis

A total of 186 individuals (182+three parents+one non-parental external control) in the NOHU population were analyzed using the SAS Software. Single marker-trait associations were tested using t-test, and multiple marker combination (based on presence or absence in two-, three-, and four-marker relations) associations with phenotypic traits were statistically analyzed by ANOVA. Statistical significance was set at  $P \leq 0.05$ .

## Results and Discussion

#### Assessment of processing traits of potato tuber in breeding population

A total of 186 individuals along with parents and non-parental external controls in the NOHU were investigated for processing traits such as SG, SC, DMC, FLV, CLV, and RSC. The L-score values determining the brightness of the fried samples were measured using a colorimeter. A brighter chip/French fry color was



**Figure 1.** Whisker-box plots of a) dry matter content (%), b) specific gravity, c) French fry L value, d) chips L value, e) starch content (%), f) reducing sugar content (g/100 g DW) (circle and asterisk are indication of outlier values).

previously shown to be closely associated with better processing quality (Sobol et al., 2020). The whisker box plots for processing traits are shown in Figure 1. The phenotypic data showed that the mean values were uniformly distributed for all traits, and the lowest/highest values for each trait are depicted in plots. Browning in French fry was less common in comparison to chips, which was associated with better processing quality in French fry. The accumulation of this brown pigmentation in processed potatoes is not desirable, as it causes a bitter taste, as reported earlier by Roe et al. (1990). Therefore, the processing quality is inversely proportional to browning (Rodriguez-Saona and Wrolstad, 1997). In addition to its taste, color is also an important parameter that determines the final consumer preference for consumption, and a dark brown color is undesirable for consumers (Mestdagh et al., 2008). Overall, the population in this study provided a reliable and suitable system/structure in order to validate the selected molecular markers for processing traits.

Pearson correlation analysis showed that RSC was negatively correlated with all tuber quality traits in our population; however, there was a positive correlation for the rest ( $P \leq 0.05$ ) (Table 1). As the FLV and CLV levels increased, the processing quality improved. However, an increase in RSC had the opposite effect, as it reduced the accumulation of sugars following the breakdown of

starch, thereby negatively affecting tuber quality traits. The findings of our study regarding tuber quality traits were consistent for the processing population.

Reducing sugars (glucose and fructose) and several amino acids (asparagine and glutamine) form a carcinogenic compound, acrylamide, which causes browning/darkening via a reaction called Maillard in potatoes and other processed foods (Mestdagh et al., 2008). It is important to ensure that the reducing sugar level in potatoes is below 1 g/kg of average fresh weight, as higher levels of reducing sugar can negatively impact the quality characteristics of potatoes (Rodriguez-Saona and Wrolstad, 1997; Mestdagh et al., 2008).

#### Genotyping the NOHU population for processing traits

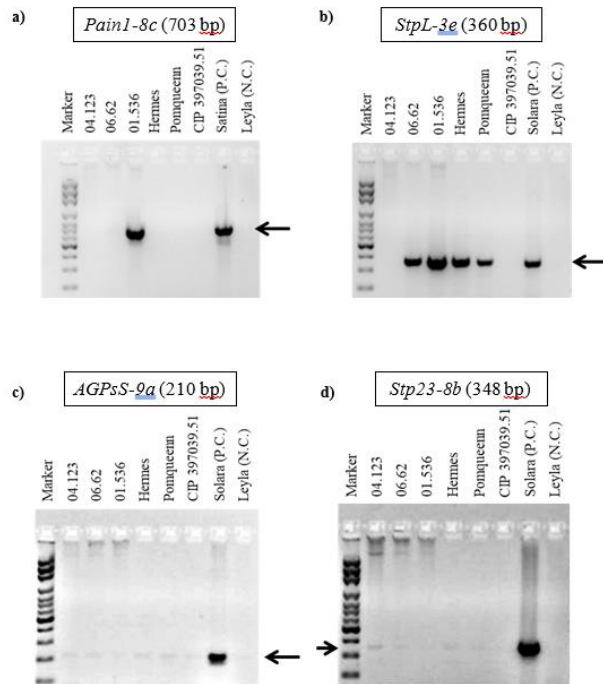
We screened the NOHU population with four diagnostic markers, *Stp23-8b*, *StpL-3e*, *AGPsS-9a*, and *Pain1-8c*, for French fry/chip quality traits, dry matter content, specific gravity, starch, and reducing sugar content. *Stp23-8b* was developed from one of the *PHO1* alleles, *PHO1<sub>a</sub>-H<sub>a</sub>* and *StpL-3e* was derived from the *plastidic starch phosphorylase* gene (Li et al., 2008). Both *Stp23-8b* and *StpL-3e* were strongly correlated with tuber quality traits. The *AGPsS-9a* marker was developed using the *ADP-glucose pyrophosphorylase* (or *glucose-1-phosphate adenyltransferase*) enzyme (Ballicora et al., 2004; Li et al., 2013). The *Pain1-8c* marker was developed using the *vacuolar acid invertase*

**Table 1.** Pearson correlation analysis of the NOHU population

Correlation	DMC	SG	FLV	CLV	SC	RSC
DMC	1	0.993*	0.376*	0.206*	0.993*	-0.485*
SG	0.993*	1	0.367*	0.201*	1.000*	-0.468*
FLV	0.376*	0.367*	1	0.433*	0.367*	-0.564*
CLV	0.206*	0.201*	0.433*	1	0.201*	-0.415*
SC	0.993*	1.000*	0.367*	0.201*	1	-0.468*
RSC	-0.485*	-0.468*	-0.564*	-0.415*	-0.468*	1



gene (Li et al., 2013). These molecular markers produced a single scorable marker size of 348 bp for *Stp23-8b*, 360 bp for *StpL-3e*, 210 bp for *AGPs5-9a*, and 703 bp for *Pain1-8c*, as suggested (Li et al., 2013); the same pattern was also observed in our study, as the gel results are shown in Figure 2. A score table for the NOHU population is provided in [Supplementary Table 1](#).



**Figure 2.** Screening of parental genotypes and positive/negative controls with a) *Pain1-8c*, b) *StpL-3e*, c) *AGPs5-9a* and d) *Stp23-8b*. P.C. : Positive control, N.C.: Negative control.

The study conducted by Li et al. (2013) found that the frequency of positive markers in the BNC population (including 76 genotypes) was 46.0% for *Stp23-8b*, 61.8% for *StpL-3e*, and 36.8% for *Pain1-8c*. The frequency rates in our study were 68.8, 88.2, and 5.9% for the three molecular markers, respectively. Despite consistent positive marker frequency in the SKC population, as

observed in a previous study by Li et al. (2013), the positive band rate was found to be higher for *StpL-3e* and lower for *Pain1-8c* in our study. The “1” frequency of *AGPs5-9a* in Li and colleagues study were 23.6 - 34.9% (BNC and SKC populations) (Li et al., 2013) as the frequency of 1 (present) was almost three times more in our study. The frequency rate is not a definite and constant parameter, which we can infer in a population, as it may be greatly affected by population size and structure; however, it provides a general banding pattern for each molecular marker, that is, *Pain1-8c* had 5.9% in the present study, unlike in previous reports. The BNC population contained individuals from different parental lines, whereas SKC was generated from the progeny of a single parental line, Diana x Candella. The frequency of *Pain1-8c* (1) changed in these two populations as well as in different years (42% in 2009 and 62% in 2010) in the SKC (Li et al., 2013). It is obvious that the marker frequency, especially *Pain1-8c*, can vary in populations with different genetic backgrounds, which might explain the reason to have low frequency of *Pain1-8c* (1) in our study. As these markers are allele specific, they are valuable for providing information about the frequency of alleles in a population. Given that our study population is a segregating population, this information will be useful for other researchers who may wish to employ these markers in future.

#### Marker associations with tuber quality traits

In the current study, it was observed that the presence (1) of *AGPs5-9a* and *StpL-3e*, unlike to *Pain1-8c* (0), was closely correlated with increased RSC (Table 2). RSC mean value was ranged between 2.76 - 2.82 in the presence of these single markers. The association of RSC with *AGPs5-9a*, *StpL-3e*, and two other allele-specific markers has not been tested by Li et al. (2013). However, a subsequent study by Schreiber et al. (2014) found that 12 different alleles were associated with RSC (reasoning from cold-induced sweetening). They generated a new population named SUGAR40, indicating that the absence of all four validation markers in the SUGAR40 population was significantly associated

**Table 2.** Correlation of single markers with several tuber quality traits

Marker	Trait	0/1	Number of Genotype <sup>a</sup>	Mean <sup>b</sup>
<i>AGPs5-9a</i>	French fry L value	0	19	*67.36↓
	Chips L value	0	19	*63.89↑
	Reducing sugar content	1	163	*2.76↑
<i>Stp23-8b</i>	Chips L value	0	54	*59.66↑
<i>StpL-3e</i>	Reducing sugar content	1	134	*2.82↑
<i>Pain1-8c</i>	Dry matter content	0	171	*19.84↓
	Specific gravity	0	171	*1.078↓
	Starch content	0	171	*15.31↓
	Reducing sugar content	0	171	*2.74↑
	French fry L value	1	11	*67.80↑
	Chips L value	1	11	*66.37↑

<sup>a</sup>Number of genotypes showing either 0/1 banding pattern

<sup>b</sup> Statistical significance by t-test, \* $P \leq 0.05$

with higher RSC. The finding of our work, as it mainly showed an increase in RSC in the presence of *AGPsS-9a* and *StpL-3e* contradicted previous reports, while the absence of *Pain1-8c* reinforced the earlier findings (Table 2).

Negative (0) banding patterns in *AGPsS-9a* and *Stp23-8b* for CLV and *AGPsS-9a* alone for FLV had significant effects on these traits in different ways. There was an increase in CLV (mean value for the individual markers: 59.66 - 63.89) thereof associated with better chips quality, whereas the direction of effect was negative for FLV (67.36). Although FLV was negatively associated with the absence of *AGPsS-9a*, the mean FLV still persisted high score for processing purpose. The FLV and CLV mean values increased in the presence of *Pain1-8c*. Previous studies on the CHIPS-ALL population showed that *AGPsS-9a* (1) had a significant positive effect on chip quality; however, the effect was the opposite in the BNC breeding population. In the same study, the presence of three other markers improved the overall chip quality (Li et al., 2013). The absence of *Pain1-8c* was associated with lower DMC, SG, and SC in this study (Table 2). *AGPsS-9a* (-) in CHIPS-ALL and *AGPsS-9a* (+) in BNC breeding population overall improved the tuber starch content yet the marker did not produce a reproducible system for the selection and moreover, *AGPsS-9a* showed a minor effect in SKC population for SC in previous work (Li et al., 2013). Furthermore, the presence of the other three markers led to an increase in mean SC, and the findings were consistent among different populations, unlike the association of *StpL-3e* with SC in two consecutive years (positive in 2009 and negative in 2010) (Li et al., 2013). The question raised after screening different populations with these candidate molecular markers for processing traits concerned the reproducibility and applicability of these markers in other populations. This is expected because tuber quality traits are multigenic. Low reproducibility restricts the use of single markers that are selective for several tuber quality characteristics. As is known from other studies, marker combinations are considered to be more decisive for marker-assisted selection (Li et al., 2013).

This study screened the NOHU population using different marker combinations for all processing traits, and we investigated the outcomes showing significant associations (Table 3). The absence of *AGPsS-9a* and *Stp23-8b* markers had a significant effect on SC; however, the direction of the effect could not be tested

in our study, and the same response was obtained in *Stp23-8b* (0)/*StpL-3e* (0) for DMC, SG, and SC (Table 3). The available knowledge is very limited, and this makes it difficult to comment further on the findings; in a previous study, the presence of *Stp23-8b* and its combination with any of the markers presence improved SC (Li et al., 2013). In the NOHU population, the mean CLV value was higher score when the population was genotyped at 0/0 for *Stp23-8b* and *StpL-3e*. This is the only marker combination which exploited a potential for being a molecular marker for screening in our study. The association of previously defined marker combinations with different traits, *AGPsS-9a* (+)/ *Stp23-8b* (+) or *AGPsS-9a* (+)/ *Pain1-8c* (-/+) or *Pain1-8c* (+)/ *StpL-3e* (-) with chips quality; *AGPsS-9a* (+)/ *Stp23-8b*(+)/ *Pain1-8c* (-) with high level of starch content was not observed in the NOHU population (Li et al., 2013). The use of marker combinations rather than single markers in MAS studies is more reliable for screening processing traits in a population. Marker combinations are shown to have higher reproducibility compared to single markers. As Li et al. (2013) suggested, the optimal marker and its combination, unfortunately, might vary with the population and environment (Li et al., 2013). The screening for tuber quality traits may show inconsistent responses for individuals at the initial phases of a breeding process, as in the NOHU (our study) and in SKC population (Li et al., 2013). The findings of previous studies in different populations, therefore, require elaborate research on marker-trait associations.

## Conclusion

This study aimed to screen the association between these four markers and tuber quality traits. The results showed that these markers and their combinations still have restrictions on the selection of processing populations in potato breeding programs. A similar situation was observed in the study by Li et al. (2013), and marker-trait associations were found not to be reproducible among different populations; therefore, these markers should be further screened in different and larger populations.

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**Table 3.** Correlation of marker combinations with several tuber quality traits

Marker combination	Trait	Number of Genotype <sup>a</sup>	Mean <sup>b</sup>
<i>AGPsS-9a</i> (0)/ <i>Stp23-8b</i> (0)	Starch content	13	*16.00
<i>Stp23-8b</i> (0)/ <i>StpL-3e</i> (0)	Dry matter content	18	*20.41
	Specific gravity	18	*1.081
	Chips L value	13	*64.30↑
	Starch content	18	*15.82

<sup>a</sup>Number of genotypes showing either 0/1 banding pattern

<sup>b</sup>Statistical significance by ANOVA, \**P*≤0.05

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### Author Contribution

CY: Investigation, Data Curation, Writing, and Editing; UD, MEÇ: Supervision, Conceptualization, Data Curation, Writing, and Editing

### Conflict of Interest

The authors declare that they have no conflict of interest.

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