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Starch viscosity measured with RVA as a tool for early selection of progenies for chip quality in a potato breeding program

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The development of potato cultivars with acceptable chipping quality is a key aspect of potato breeding as potato consumption is shifting from fresh potatoes to added-value processed potato products. In most cases, the assessment of chipping quality is still performed at advanced stages by using conventional time consuming, high sample demanding destructive methods. However, during the last decades, several studies have focused on applying alternative selection markers that could be used at early stages. In order to accelerate the process of evaluation and selection of potato genotypes with good chipping quality, a set of parental genotypes and progenies following an incomplete diallel mating design were selected from the breeding program of APPACALE SA (Burgos, Spain) and analyzed using a screening method based on starch viscosity measured with RVA (Rapid Visco Analyser). Significant correlations between Pasting Temperature, a starch viscosity parameter, and some chipping quality traits, such as Specific Gravity and Chip Colour, were found. Although the correlations between Pasting Temperature and chipping quality traits were not high enough to be a definitive selection tool, this approach could be considered a first step in the process of screening for quality in breeding programs, providing a solution to early selection for chip quality.

Keywords: Starch Gelling Temperature, Chip Colour, Dry Matter, Selection Procedure

Abbreviations:
RVA: Rapid Visco Analyser
SG: Specific Gravity
DM: Dry Matter

INTRODUCTION

The potato (*Solanum tuberosum* L.) is a starchy tuber-bearing crop with multiple commercial uses, including the production of processed food products, such as French fries or chips (*crisps* in England) and the manufacture of starch. Chip production uses specific cultivars defined by special characteristics, including high-solids and low reducing sugar contents after cold storage, resulting in light-coloured chips, whose appearance, flavour and texture can be determined by the manufacturer

(Bradshaw et al., 1998a).

Both chipping quality and starch content are the main objectives of potato breeders for processing cultivars, along with resistance to diseases and pests and agronomic performance. Breeders use different strategies to achieve these targets in a minimal time frame, since in terms of commercial interest, tuber quality should be considered a market-limiting trait (Douches et al., 1996). One of them comprises the use of improved selection methods with the aim of identifying parental lines with an appropriate extent of genetic differentiation able to transmit the desired quality characters to the progeny (Bradshaw et al., 2003).

The traditional breeding process of obtaining new cultivars is based on the individual analysis of the offspring derived from crosses after discarding undesirable genotypes by visual selection for traits with high heritability, such as tuber shape and eye depth (Schaalje et al., 1987). The quality analysis are first performed at a relatively advanced stage of the breeding program (4th or 5th year), since it is an expensive and time-consuming process with large infrastructure requirements (Mackay et al., 1997). In order to make a judicious selection of clones at an early stage, an alternative non-destructive and storage-independent testing method is required. With this aim, an approach based on a possible correlation between starch related parameters and frying quality traits was suggested.

Although levels of reducing sugars are used as predictors for chip colour (Jakuczun and Zimnoch-Guzowska, 2004) this method is not always suitable because varying levels of reducing sugars can produce chips with similar colours (Rodriguez-Saona and Wrolstad, 1997). Several mechanisms have been proposed for starch degradation and accumulation of sugars, but the process it is not completely known. Nevertheless, results of QTL analyses on some starch properties, chipping colour and cold induced sweetening indicate a strong correlation between candidate genes on starch properties and chipping quality (Werij et al., 2012) so that selection on starch properties could be a feasible approach to an indirect selection for chipping quality.

The existence of a correlation between starch and frying quality has been suggested based on the fact that changes in the activity of various enzymes involved in starch biosynthesis can alter the starch structure and consequently, its response during the frying process (Sowokinos, 2001; Lorberth et al., 1998; Cottrell et al., 1993). Like starches from other plant species, potato starch consists of two types of glucose polymers, amylose and amylopectin, with contributions of approximately 25% and 75%, respectively. These two polymers differ greatly in their physicochemical properties: amylopectin gives viscosity to solutions, while amylose provides gelling capacity (Zaidul et al., 2007). The higher value of pasting viscosity of potato starch compared with other commercial starches may be due to a high phosphate monoester content and the absence of lipids and phospholipids (Singh et al., 2003), allowing it to achieve a higher viscosity and unique texture at lower temperatures than starches from other sources (Noda et al., 2004).

The pasting properties of starch can either be determined rapidly by a standard procedure with a Brabender visco amylograph or with a RVA (Rapid Visco Analyzer). Results using both methods show a very high correlation (Haase et al., 1995; Wickramasinghe et al., 2009). The analysis of starch with RVA provides viscosity data when heated in the presence of water. In this method, test conditions like sample weight, heating rate, cooling time can affect the result. Several methods for the analysis of potato starch viscosity have been published after evaluating results using different sample weights, peak temperatures and heating times, in order to define a standard system that could be able to differentiate potato cultivars (Higley et al., 2003). The results appear to be suitable for determining varietal differences based on starch properties.

This study examines whether certain starch quality parameters measured with the RVA, are correlated with standard chip quality parameters, in order to use them as an early and accurate selection procedure in breeding programs to identify individuals that may have good chip quality traits. These correlations could help to make the selection process more efficient.

MATERIALS AND METHODS

Plant Materials

The breeding program of APPACALE (Ortega and Lopez-Vizcon, 2012) develops a specific line of clones selected for use in chip production. Four commercial cultivars and three advanced clones from APPACALE were selected as a parental set considering their pedigree data, behaviour in crosses and field performance. The cultivars and advanced clones were rated from ‘Very Good’ to ‘Very Bad’ in terms of quality results in industrial chip processing, based on the behaviour of these cultivars after chip analysis in our quality laboratory (Table 1). Moreover, advanced clones had excellent field performance as well as immunity to the PVY virus (Ortega and Lopez-Vizcon, 2012).

In 2001, twenty-one crosses were performed between cultivars and advanced clones’, following an incomplete diallel design (Griffing, 1956) without reciprocals and self-crosses (Table 2). Seeds of 17 families were obtained, as four crosses did not produce berries or seeds as a result of poor pollen viability, either due to sterility of the male or both parents.

Following the standard procedure of the program, up to 200 seeds for each cross were sown in trays (March 2002). The best seedlings were transplanted to individual

Table 1: Parents used in crosses

Variety / Clone	Female Parent	Male Parent	Origin	Year	Chip quality
ATLANTIC	Wauseon	Lenape	USDA –USA	1976[a]	Very good
CAESAR	Monalisa	Rop B 1178	HZPC – Netherlands	1990[a]	Good
HERTHA	Dijkhuis 61-133-3	Konst 62-374	P&J Dijkhuis - Netherl.	1980[a]	Very good
TOMENSA	ST 155	Taiga	Boehm-Nordkart-Agrar – Germany	1989[a]	Very good
94APP-3	Optima	Valetta	APPACALE –Spain	2000[b]	Very bad
95P17-3	Iroise	V-2	APPACALE – Spain	2001[b]	Very bad
95P87-4	Spunta	V-2	APPACALE – Spain	2001[b]	Bad

[a] year of release in the country of origin
[b] year of classification as advanced clone after tests

Table 2: Families obtained from crosses

	CAESAR	HERTHA	94APP-3	95P17-3	ATLANTIC	95P87-4	TOMENSA
CAESAR	X	2001D-12	2001D-7	2001D-26	2001D-16	2001D-1	2001D-4
HERTHA		X	2001D-8	2001D-11	2001D-3	2001D-14	2001D-5
94APP-3			X	NO SEEDS	2001D-20	NO SEEDS	NO SEEDS
95P17-3				X	2001D-19	NO SEEDS	2001D-24
ATLANTIC					X	2001D-23	2001D-13
95P87-4						X	2001D-25
TOMENSA							X

pots after a month and placed in an aphid-proof greenhouse. At the end of the growing season the tubers of each plant were collected individually. After tagging and visual evaluation, at least five tubers of each clone were kept separate from the others in every progeny. After multiplication of seed tubers in 2003, two experimental field trials were carried out in 2004. Tubers analyzed in this study came from the SG01-2004 field trial carried out in Quintanadueñas (Burgos, Spain) where 828 individuals from all the 17 families and 7 parents were planted following a randomized complete block design with two replications.

Quality Analysis

At harvest, tubers of all individuals of every progeny and parents from the SG01-2004 field trial were collected plant by plant. A randomly selected sample of 200 individuals of all families and parents were submitted to quality tests. Two sets of quality parameters were analyzed, one related to chip characteristics and the other related to starch properties.

Chip quality tests: Chip characteristics were analysed at APPACALE's quality laboratory, with laboratory-grade

equipment. Various quality parameters were evaluated, with tuber density, also known as Specific Gravity (SG), and chip colour considered being the most descriptive and significant. SG is considered an important trait for the chips processing industry since most potato product manufacturers prefer tubers of higher specific gravity to get more chips (Haase, 2003). Dry matter (DM) content directly influences yield of the processed product and it also affects the oil absorption rate in fried products. Costs of frying oil are major components in the processing costs and therefore the processor tries to obtain potatoes with optimum DM levels to maximize processed yield and to minimize oil costs. The acceptable SG values for chip processing industry are between 1.075 and 1.090 (Fernando and Slater, 2010).

SG was evaluated by calculating the difference between dry weight and underwater weight for tubers of each individual. The standard procedure involves washing tubers of each clone at room temperature, drying them carefully and weighing them in air and under water at room temperature. The SG of tubers was calculated using the formula: SG = weight in air / [(weight in air) - (weight in water)] (EAPR, 1987).

For assessing chip quality, two tubers of each clone were machine-cut into two slices 0.1 mm thick in the direction navel to apex, trying to imitate the industrial method as far as possible, resulting in 4 chips per clone. The chips were then fried for 3 minutes in sunflower oil heated to 180°C in an electric fryer. Under these conditions the oil temperature dropped to 155-160ºC, depending on the slice size, with little variation during the first minute, and then rose slowly towards the end of the process, finishing at around 170°C (Haase, 2003). Chip colour data were obtained by comparison with standard colour charts from IBVL Colour Card for Evaluation of Chips, (IBVL- Institute of Storage and Processing of Agricultural Produce, Netherlands, EAPR, 1987), in which the colour of the sample is rated on a scale of 1 (very dark) to 9 (very clear).

Starch pasting properties: Small bars (cross-section of about 1 cm^2, length from 4 to 10 cm) were obtained from all tubers of the sample, lyophilized (Labconco Freezone 12, Kansas City, USA) and ground in a laboratory grinding mill (Foss Tecator, Eden Prairie, USA) with a sieve of 0.5 mm.

The starch viscosity analyses were performed using a Rapid Visco Analyser (RVA-3D, Newport Scientific Pty. Ltd., Warriewood, Australia) following the standard procedure for potato starch by preparing a suspension of 3 g of tuber lyophilized powders in 25 mL of distilled water in a cylindrical canister. Within this canister is a stirrer that constantly stirs the suspension. The canister is inserted into the apparatus, which heats and subsequently cools the sample following a specific program (Table 3). The starch viscosity data are recorded graphically in a Pasting Curve, hence parameters are recorded describing the starch behaviour of the samples (Higley et al., 2003).

The total time of each test was 13 minutes and the samples were analysed in duplicate. The following parameters were evaluated:

- Peak Viscosity: indicates the water binding capacity of the starch, which gives an indication of the viscosity of the cooked product (RVA units: RVU).
- Minimum Viscosity (RVU).
- Peak Viscosity Time**:** time taken to reach peak viscosity (minutes).
- Pasting Temperature: temperature at it paste starts to gellify (ºC).
- Holding Strength**:** decrease in viscosity calculated as the difference between peak and minimum viscosity (RVU).

Statistical Data Analysis

Chip colour, dry matter content, and starch characteristics from the RVA analysis of each individual were analysed statistically to identify significant correlations.

All variables were submitted to an analysis of variance using the SAS System statistical software for Windows (Cary, NC) using a GLM (General Linear Model) procedure allowing for multiple comparisons with Duncan's Multiple Range test. For the sake of clarity, standard errors of the mean values were not included in the tables. Pearson's R Correlation Test was used to identify correlations between chip quality and RVA variables.

RESULTS

Table 4 shows the Analysis of variance (ANOVA) for the chip quality parameters (tuber SG and chip colour) carried out to detect significant differences between families, individuals (within families) and tubers (within individuals and families). Significant differences ($p<0.01$) were found between the SGs of families and individuals within families. Significant differences ($p < 0.01$) were also detected for chip colour between families, individuals (within families) and tubers (within families and individuals).

Comparisons of means between families or parents were performed by Duncan's Multiple Range test for each quality parameter for progenies and parents (Table 5). These values were plotted in a bi-plot graph (Figure 1) to aid the interpretation of the data. Mean Specific gravities were in the range between 1.05930 and 1.11779 g/mL, corresponding to 15.85 to 28.19 in terms of percentage dry matter using the von Scheele formula (EAPR, 1987), while mean chip colour ranged from 2.17 and 7.00.

Table 3: RVA Temperature and stirring program (Higley et al., 2003)

Time (min:sec)	Type	Value
00:00	Temperature	50ºC
00:00	Speed	960 rpm
00:10	Speed	160 rpm
01:00	Temperature	50ºC
04:42	Temperature	95ºC
07:12	Temperature	95ºC
11:00	Temperature	50ºC

Table 4: Analysis of variance (mean squares) for chip quality variables

Source	d.f.	SG	Chip colour
Family	23	0.00322**	21.36686**
Individual (Family)	111	0.00097**	3.86054**
Tuber (Fam*Ind)	134	0.00000	0.40111**
Error	304	0.00002	0.18257
R (%)		(96.14)	(94.36)

***P* values significant at 0.01

Table 5: Means of Chip Colour (1 to 9) and Specific Gravity (g/mL)

Family / Parental	Chip Colour		Specific Gravity (Density)	
HERTHA	7.0000	a	1.11779	a
2001D-11	6.1000	ab	1.08068	ef
2001D-12	5.9722	Abc	1.09298	bcdef
2001D-3	5.8889	abcd	1.10727	abcd
2001D-14	5.8000	abcd	1.08838	cdef
2001D-23	5.5789	abcde	1.10257	abcdef
2001D-16	5.5000	bcde	1.09817	abcdef
ATLANTIC	5.5000	bcdef	1.10495	abcde
2001D-25	5.4375	bcdef	1.08324	def
95P17-3	5.0625	bcdefg	1.10483	abcde
95P87-4	5.0500	bcdefg	1.09346	bcdef
2001D-5	4.8333	bcdefg	1.10254	abcdef
TOMENSA	4.8000	bcdefg	1.11376	ab
2001D-13	4.7500	bcdefg	1.09078	bcdef
2001D-7	4.7000	bcdefg	1.05930	g

Table 5 Cont'd

2001D-24	4.5714	bcdefg		1.10906	abc
2001D-4	4.5500	cdefg		1.09031	bcdef
2001D-1	4.3571	defgh		1.08965	bcdef
CAESAR	4.1250	efgh		1.09138	bcdef
2001D-26	4.1250	efgh		1.09343	bcdef
2001D-19	4.0000	fgh		1.08816	cdef
2001D-8	3.9000	gh		1.09256	bcdef
2001D-20	3.0000		hi	1.08314	def
94APP-3	2.1667	i		1.07898	fg

Different letters indicate significant differences ($p \leq 0.01$) within the same column according to the Duncan's test

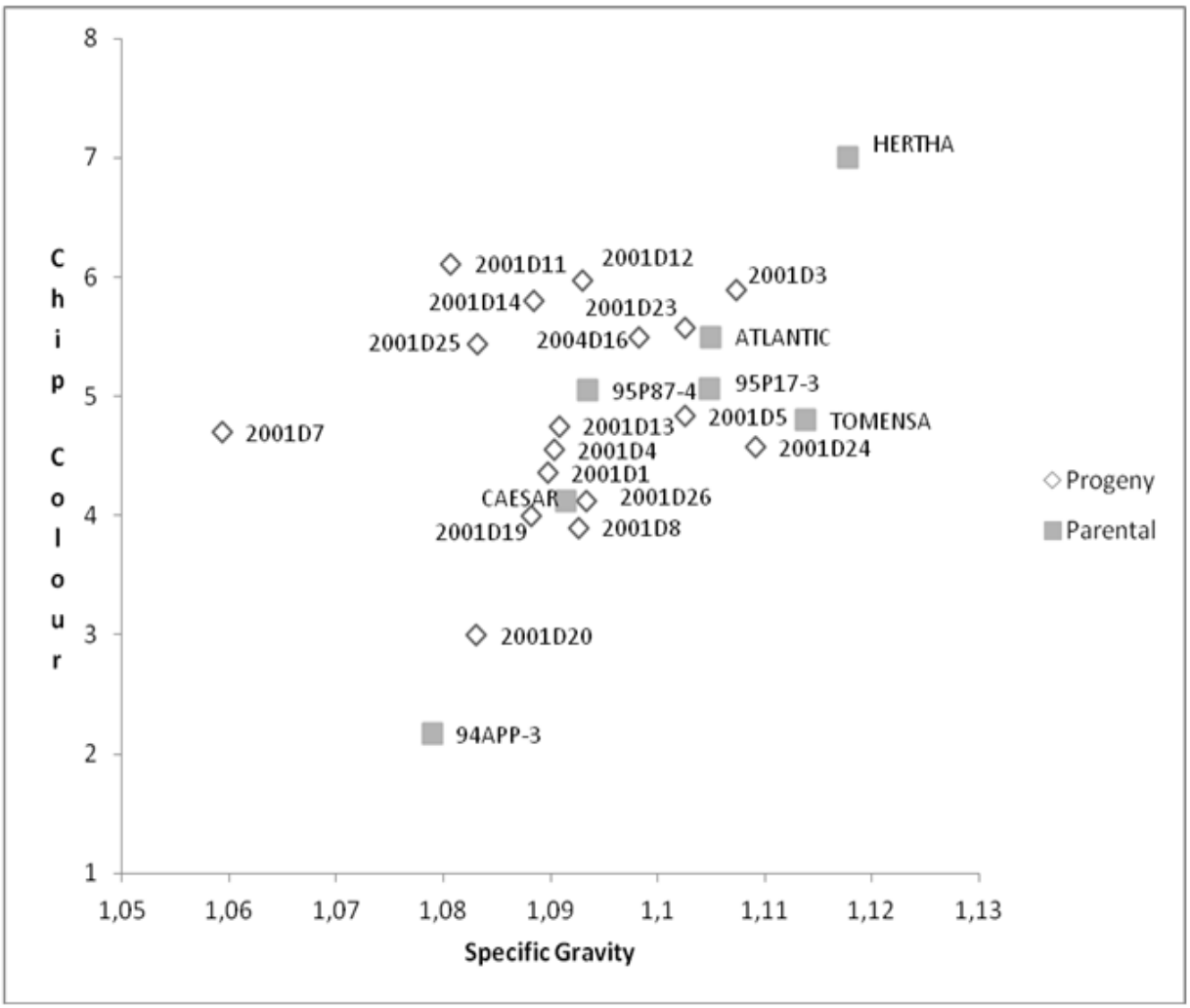

Figure1: Bi-plot graph of Chip Colour (y axis) vs. Density (x axis) for Progenies and Parents

The first step of the analysis of the RVA parameters was to look at the curves of different individuals of each progeny. The variability of pasting curves obtained from trial clones was quite high but consistent within each family. Figure 2 shows an example of starch viscosity RVA curves of different individuals of the same family (2001D-3).

The ANOVA results for RVA viscosity variables indicated that differences between Families were significant ($p < 0.01$) for all of the starch viscosity parameters (Peak Viscosity, Minimum Viscosity, Final Viscosity and Pasting Temperature) except Peak Time (Table 6).

Mean values of viscosity parameters of progenies and parents are shown in Table 7.

The Correlation Analysis identified relationships between chip quality traits (SG and Chip colour) and RVA starch viscosity parameters (Table 8).

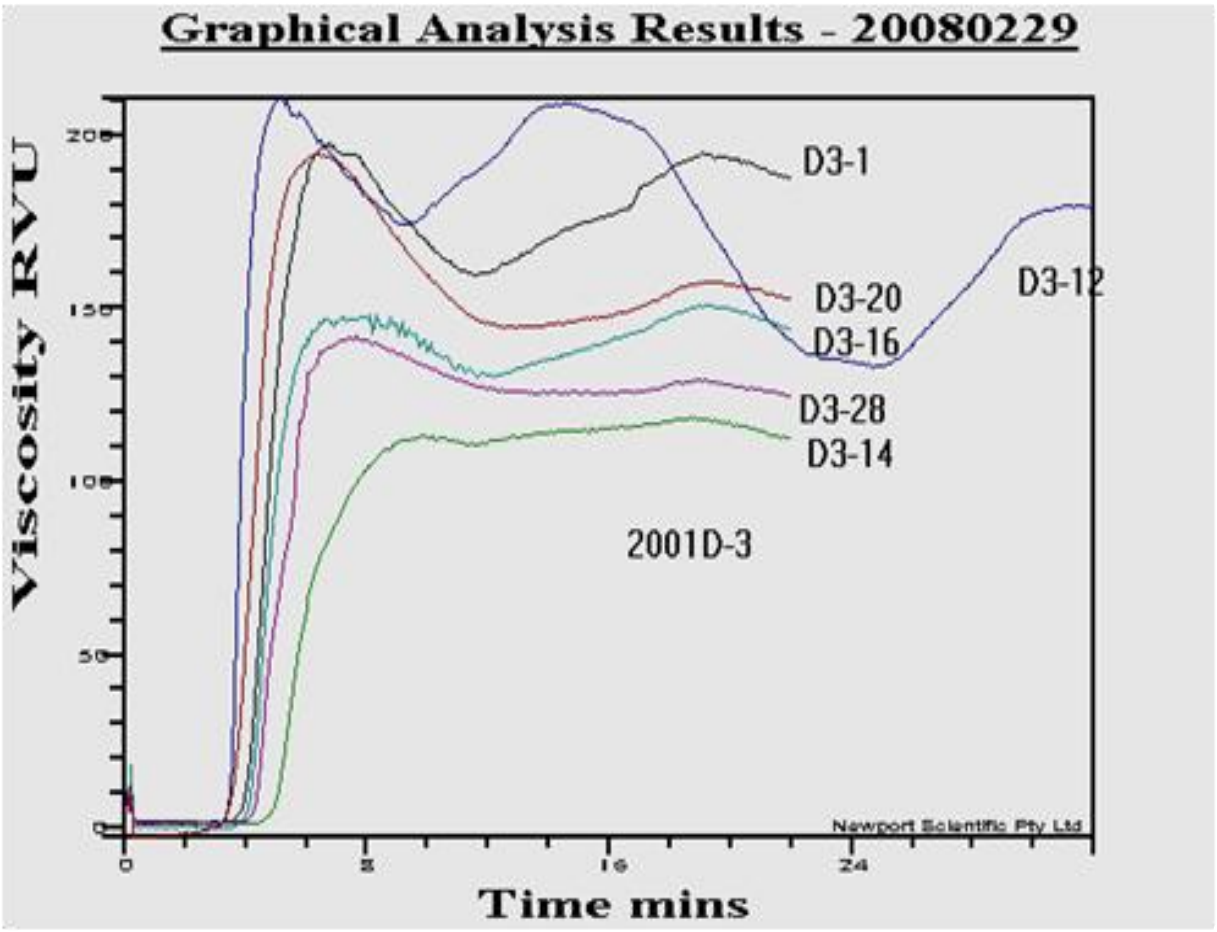

Figure 2: RVA graphs from different individuals of family 2001D-3

Table 6. Analysis of Variance (mean squares) of starch quality data obtained with RVA

Source of variation	d.f.	Peak Viscosity	Minimum Viscosity	Final Viscosity	Peak Time	Pasting Temperature
Family	20	219325.26**	102754.50**	114478.09**	10.88	216.62**
Error G(F)	54	180760.87	104292.21	125575.05	18.27	122.35
R (%)		(54.81)	(49.63)	(47.68)	(37.33)	(63.90)

** *P* values significant at 0.01

Table 7: Mean values of RVA parameters

Family / Parental	Pasting Temperature	Peak Viscosity	Minimum Viscosity	Final Viscosity
2001D-13	68.12 a	216.03 cdefg	191.50 bcd	206.16 cd
2001D-12	67.22 a	221.72 cdefg	215.97 bcd	201.80 cd
2001D-23	67.19 a	175.81 fg	161.21 cd	166.08 cd
2001D-5	66.93 ab	199.75 defg	160.46 cd	182.21 cd
95P17-3	66.49 abc	158.83 g	136.88 d	221.87 cd
2001D-3	66.42 abcd	177.85 efg	158.98 cd	157.41 d
CAESAR	66.23 abcd	218.00 cdefg	194.96 bcd	206.25 cd
2001D-1	65.98 abcde	189.33 defg	156.04 cd	179.96 cd
HERTHA	65.80 abcdef	233.71 bcdefg	207.12 bcd	224.79 bcd
2001D-19	65.55 abcdefg	217.43 cdefg	198.23 bdc	207.15 cd
2001D-11	65.53 abcdefg	228.43 cdefg	200.75 bcd	209.75 cd
ATLANTIC	64.25 bcdefgh	362.00 a	308.87 a	325.00 a
2001D-25	64.07 bcdefgh	252.50 abcdefg	214.83 bcd	210.92 cd
2001D-24	63.90 cdefgh	290.83 abcde	225.32 bc	245.18 abcd
2004D-16	63.67 defgh	321.44 abc	256.83 ab	315.75 ab
2001D-7	63.53 defgh	206.33 defg	189.97 bcd	188.77 cd
2001D-26	63.21 fgh	256.69 abcdefg	230.71 abc	236.71 abcd
TOMENSA	63.04 fgh	342.62 ab	226.75 bc	247.47 abcd
2001D-8	62.87 gh	296.98 abcd	251.45 ab	256.61 abc
94APP-3	62.53 h	284.36 abcdef	239.80 bc	254.38 abc
2001D-4	62.35 h	285.46 abcdef	231.17 abc	237.67 abcd

Different letters indicate significant differences ($p \leq 0.01$) within the same columnaccording to the Duncan's test

Table 8: Correlation Analysis – Pearson Correlation Coefficient values (R)

	Chip Quality		RVA			
Parameter	**SG**	**Chip Colour**	**Peak Viscosity**	**Minimum Viscosity**	**Final Viscosity**	**Peak Time**
SG	-	-	-	-	-	-
Chip Colour	0.25**	-	-	-	-	-
Peak Viscosity	N.S.	N.S.	-	-	-	-
Minimum Viscosity	N.S.	N.S.	0.90**	-	-	-
Final Viscosity	N.S.	N.S.	0.84**	0.88**	-	-
Peak Time	N.S.	N.S.	-0.70**	-0.50**	-0.48**	-
Pasting Temperature	0.24*	0.39**	-0.73**	-0.64**	-0.57**	0.63**

P values significant at 0.05 (*) and 0.01 (**) respectively. N.S. = not significant

DISCUSSION

The significant differences found between tubers of the same individual and progeny for Chip Colour, may be due to differences in the degree of maturity of tubers of the same plant. The progenies obtained displayed a great diversity, especially in the growth cycles, and in many individuals the tuberization process was very long, resulting in an uneven distribution of tubers with different stages of maturity. Although we tried to process tubers with a similar maturity and size, unavoidable variation could be the cause of the observed differences in chip colour between tubers of the same individual.

These results indicated a weak but significant positive correlation between chip colour and the Specific Gravity of tubers, both of them chip quality parameters ($R = 0.25$, $p \leq 0.01$), highlighting the importance of selecting clones with high SG to get good chip quality varieties (Bradshaw et al., 2003). A positive significant correlation ($R = 0.24$, $p \leq 0.5$) was also found between pasting temperature and SG, and between pasting temperature and chip colour ($R = 0.39$, $p \leq 0.01$). This may indicate the existence of a relationship between starch-related traits and chip quality, as suggested by Werij et al. (2012) who found a significant positive correlation between chip colour and starch content, indicating that most of the starch-related traits (including chip colour) are interrelated.

It is clear that significant correlations between chip colour, SG and pasting temperature have relatively low coefficients, so that this parameter could be considered poorly representative as quality character to be used in the selection process. However, environmental effects and genotype x environment interactions can reduce the efficiency of selection and genetic gain in a context of a breeding program by changing the phenotypic response in different ways. Bisognin and Douches (2002) indicate that correlation coefficients for Chip Colour trait for all families combined between first and second clonal generation is significant but low (0.42**), and generally speaking, for breeders implicated in phenotypic selection of early generations, intensive selection of individual clones was found to be very ineffective (Caligari, 1992; Tarn et al., 1992; Bradshaw et al., 1998b). The development of progeny tests led to most potato breeders from year 2000 to apply a progeny based breeding strategy by selecting families instead individuals (Bradshaw et al., 2003). The need of a great number of analyses for individuals of each progeny makes necessary the development of a cheap and rapid technique to tackle the task, so the RVA analysis, and particularly Pasting Temperature values, could be an efficient tool – combined with other as markers – to use as a chip quality predictor avoiding the over estimation of visual selection.

Of all the starch viscosity parameters tested, Pasting Temperature seems to be the only parameter to use in the selection process, because it is the only one that correlates with chip quality parameters. This result is in agreement with that of O'Donoghue et al. (1996) who suggested that the gelatinization temperature of fresh tissue could be a good parameter for evaluating the potential chipping quality of potatoes. This result is also in agreement with those obtained by Werij et al. (2012), indicating that several candidate genes may play a putative role in controlling tuber starch properties and chip quality. In particular, StPho1a which encodes a starch phosphorylase, is considered a candidate gene for onset of starch gelling, chipping and starch granule size (Werij et al., 2012), thus playing an active role in both aspects.

Pasting properties of starch can be strongly influenced by cultivar and environment, as reported by Zaidul et al. (2007) and Noda et al. (2004), but the significant correlations found in this study highlights the importance of the genetic basis on starch properties, specifically the starch gelling temperature. QTL co-segregating with StPho1a is chipping colour, so marker-trait correlations suggest a genetic relationship between starch phosphorylation and chipping quality (Werij et al., 2012), which could have important consequences for breeders.

The analysis of starch viscosity parameters could, therefore, improve the efficiency of the breeding process by simplifying and reducing the costs of screening clones for good frying quality in the early stages of the selection process.

CONCLUSIONS

RVA analysis of potato starch could be used as quality predictor to improve the efficiency of early generation's selection of progenies for chip quality. Even with weak significant positive correlations between chip quality traits and the Pasting Temperature, some kind of relationship between sugars, starch structure and characteristics of fried chips could be suspected, as described by other authors. As a result of these correlations, RVA measurements of Pasting Temperature could be a good tool, which can be combined with other helping to discriminate breeding materials for chip quality.

Competing interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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