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Genetic markers for processing traits in potato

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ABSTRACT - The success of an improvement program depends on the correct parent choice and an effective selection of superior genotypes in segregating populations. The use of genetic markers can allow an early identification of plants with a better combination of favorable alleles and make the selection process more effective. This study aimed to evaluate potato clones for processing traits and to identify genetic markers associated to them. Forty-two genotypes were separated in two contrasting groups for tuber specific gravity and chip color and evaluated by three enzymatic systems, 82 RAPD's and one PCR marker. It was possible to detect polymorphisms in the markers associated to tuber specific gravity and chip color. When considering the partial sum of squares most markers were not significant, indicating the existence of redundant information among them. The best markers *were selected by backward analysis. Together they explained 73.5% and 41.9% of the phenotypic variation for tuber specific gravity and for chip color, respectively.*

Key words: *Solanum tuberosum* L, tuber specific gravity, chip color, potato processing

INTRODUCTION

Potato quality for processing as chips or frozen french fries depends directly on two factors tuber contents of dry matter and reducing sugars (Porter et al. 1964, Woodbury and Weinheimer 1965, Kleinkoff et al. 1987). High dry matter contents are associated to greater productivity and quality of the processed products due to the lower oil absorption during frying, thus reducing the costs and improving taste (Melo 1999, Xiong et al. 2002). Dry matter content is a trait that depends mainly on tuber starch content. Dry matter synthesis, transport and accumulation are quantitative traits controlled by several genes (Haynes and Haynes 1983) and affected by different environmental factors (Smith, 1975, Dale and Mackay 1994, Chen et al. 2001), especially the locality, crop season and the presence of pests and diseases.

Tuber dry matter content can be indirectly determined through the specific gravity based on the weight of a certain tuber volume in the air and in water. The other processing quality factor is the concentration of sugars, mainly of glucose and fructose which affect the coloration of the processed products and therefore condition the consumers' acceptance of these products (Dale and Mackay 1994, Pereira and Costa 1997). According to Melo (1999) and Pereira (2000) reducing sugars content in potato tubers for industry must be less than 0.2%. The tendency of accumulating higher or lower reducing sugar content in the tubers is a trait determined by complex genetic control and influenced by the environment (Stevenson et al. 1954, Melo 1999)

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In other words, it is a quantitative trait for which gains with selection can be obtained stepwise (Love et al., 1998).

The modern molecular techniques have opened up expectations for enhanced experimental precision and the selection of superior genotypes for potato processing. Among these techniques the molecular markers are most promising as they make the identification and localization of QTL ("Quantitative Trait Loci") with considerable individual effects possible. Such markers could be used to obtain direct information on plant genotypes and be of support in selection, in a process called marker-assisted selection (MAS). The objective of this study was to identify genetic markers that explain the phenotypic variability for potato tuber processing traits.

MATERIAL AND METHODS

In this study we tested 42 potato genotypes previously evaluated by Amaro et al. (2003) that presented stability for tuber specific gravity and frying color over several generations. These genotypes were separated in two contrasting groups , according to their high or low values of tuber specific gravity and acceptable ($\leq$ 2.5) or non-acceptable color standards ($\geq$ 3.0) after frying (Douches et al., 1996). The trial was performed in the winter crop of 2002, on an experimental field of the Department of Biology of the Universidade Federal de Lavras, at 918.80 m asl, lat 21° 14'S and long 40° 17'W.

Tuber weights, were obtained in hydrostatic balance, and tuber specific gravity (TSG) determined by the expression:

$$TSG = \frac{\text{weight in air}}{\text{weight in air - weight in water}}$$

Reducing sugars contents were indirectly estimated through the chip color, determined in large (ϕ >45 mm) and healthy tubers. The tubers were washed and cut in thin slices (2-3 mm) and fried right away in hydrogenated plant oil at an initial temperature of 180 ºC, until bubbling ceased. The color was evaluated based on the Table of the *Potato Chip and Snack Food Association* (USA), from 1-light to 5-dark (Douches et al. 1996).

Genotypes were characterized by the analysis of polymorphic bands of three enzyme systems a-esterase, peroxidase and malate dehydrogenase, according to Alfenas (1998). Homogenized, top leaves of plants in two developmental stages were used: i) leaf tissue of the intermediate plant part before flowering, from any of the leaflets at the 3rd or 4th leaf, ii) leaf tissue of the intermediate plant part at full flowering, from the leaflets of the 4th or 5th leaf. At least five plants of each clone were used for each one of the three analyzed enzyme systems. In the extraction procedure 500 mg of leaf tissue of each sample were used, 0.5 mg polyvinylpyrrolidone - PVP and 0.1 mL solution buffer nr. 1 of Alfenas et al. (1998), enriched with 0.15% 2-mercaptoethanol. The leaf material was ground by hand and cooled for extraction. 10 µl of the supernatant were automatically pipetted into the gel channel for electrophoresis. The electrophoresis was conducted using 4.5% polyacrylamide gel as support, and 12% for separation. In the electrophoretic run a voltage of 10 mA was used for each gel and the total running time was 3 hours at 4 °C.

DNA was extracted according to Rogers and Bendich (1988), with modifications. Approximately 2g young leaves of each plant were ground with sterilized sand, together with 10 mL of extraction buffer preheated to 65 °C [2% cetyltrimethylammonium bromide (CTAB), 100mM TRIS (pH 8.0); 20mM EDTA (pH 8.0); 1.4M NaCl; 1% polyvinylpyrrolidone (PVP); 8.6mL pure water] and 40 ml of b-mercaptoethanol. The mixture was incubated at 65 °C for 30 minutes, followed by chloroform:isoamyl alcohol extraction (24:1) for protein denaturation and to make phase separation easier. The DNA was precipitated with 30mL of the solution (6:1) 95% alcohol and 7.5M ammonium acetate. The nucleic acids were then rehydrated in TE (1mM of TRIS and 0.1 mM of EDTA) buffer. Thereafter, the second extraction with chloroform: isoamyl alcohol (24:1) was performed and the nucleic acids were precipitated by the addition of at least three volumes of the mixture 3M sodium acetate: 95% ethanol (1:20). The alcohol - sodium acetate solution was eliminated, the DNA dissolved in 300 mL TE and quantified on a Hoeffer Scientific TKO 100 fluorometer and then diluted to a concentration of 10 ng/mL, used in the amplification reactions.

A total of 82 decamer primers (Operon Technologies Inc., Alameda, CA,USA) were chosen at the GenBank. This selection occurred on the base of the 70-80% homology of the constituent bases, with primers used by Chen et al. (2001) for the detection and mapping

of genes related to the carbohydrate metabolism in potato tubers and with one PCR primer pair synthesized by the Laboratório MWG Biotec to detect the starch synthase gene, based on specific DNA sequences. The sequence of these primers were 5' TCT CTT GAC ACG TGT CAC TGA AAC 3' and 5' TCA CCG ATT ACA GTA GGC AAG AGA 3'.

Each RAPD reaction was performed with a volume of 12ml containing 200mM dNTP (equitable mixture of ATP, GTP, CTP and TTP); 0.6 units of *Taq* DNA polymerase, 0.4mM of primer, reaction buffer (50mM of TRIS; 2,0mM $MgCl_2$; 20mM KCl; 250mg/mL bovine serum albumin; 1% Ficol 400; 1mM tartrazine). The DNA amplification was run on an Eppendorf MasterCycler Gradient 5331 thermocycler in 42 cycles; in the first two cycles the DNA was denatured at 94 °C for 2 minutes and elongated at 72 °C for one minute. In the other cycles the denaturation temperature was 94 °C during 15 seconds followed by 15 seconds at 37 °C for annealing and 72 °C for 1 minute for the elongation. A last period of 3 minutes at 72 °C was programmed for final elongation. The amplification products were separated by electrophoresis in 1% agarose gels for 2.5 hours at 3 V cm^{-1} and stained with 0.5mg/ml ethidium bromide. The gels were photographed under 254 nm light with a digital camera (KODAK DC 290 Zoom).

In the case of the PCR the reactions consisted of 30ng of DNA; 200mM dNTP; 0.6 units of *Taq* DNA polymerase; 0.2mM of each primer; reaction buffer (50mM TRIS, 2mM $MgCl_2$, 20mM KCl, 250mg/mL bovine serum albumin, 1% Ficol 400. 1 mM tartrazine) and pure water up to a volume of 14mL. The amplification reaction included an initial denaturation at 94°C for two minutes, followed by 28 cycles with denaturation at 94 °C for 30 seconds, annealing at 60 ºC for 30 seconds and elongation at 72 °C for one minute, with a final extension step for 5 minutes at 72 °C. The fragments obtained by PCR were separated in 2.5% agarose gel. Electrophoresis was conducted in TBE 1X buffer at a constant voltage of 100V, during a variable period of 3.5 to 4 hours. The gel was visualized under UV light and electronically stored by a digital camera (KODAK DC 290 Zoom).

Single point ANOVAs were run for tuber specific gravity and chip color for the 42 genotypes and each marker using the statistical software SISVAR version 4.5 (Ferreira, 1999). The F test was used to test the statistical differences ($P < 0.05$ and $P < 0.01$) between the marker classes. A significant difference for tuber specific gravity or chip color was interpreted as QTL-marker linkage. Epistatic interactions or gene linkage between the significant loci were tested by pairwise input analysis of variance. The multiple linear regression analysis of the markers was performed according to procedures of Edwards et al. (1987). The total phenotypic variance explained by the markers was estimated by the determination coefficient (R^2). The loci with highest R^2 for the QTL were combined in a multiple analysis of variance to predict the total variation for tuber specific gravity and chip color explained by the identified QTL (Keim et al., 1990). The percentage of phenotypic variation explained by the markers of greater importance obtained through the significant values of the F test of the analysis of variance ($P < 0.05$ and $P < 0.01$) was determined by the partial determination coefficient (Draper and Smith 1981). Multiple linear regression analysis of the type partial sum of squares or of type II was also carried out. This methodology consists in the reduction of one parameter (marker) at a time beginning with the complete model (all significant markers) of evaluated parameters, to determine the statistical significance of each marker, when it is considered jointly with the effects produced by the other markers on the phenotypic variation. The software SISVAR version 4.5 (Ferreira 1999) was used to perform this analysis. For the elimination of non-informative markers the selection process of regressing variables was used backwards (Draper and Smith 1981). Initially all significant markers were included in the model for the backward process. The marker with the least partial F that did not present significance ($P < 0.05$) was eliminated. The remaining markers made up a new model, in which the process of elimination of variables was once more performed. The process went on until all markers of the model presented significant partial F. To obtain the partial F it was necessary to calculate the sum of squares of a marker (i) eliminating the effects of the remaining markers of the actual model.

RESULTS AND DISCUSSION

The RAPD and PCR primers produced 53 polymorphic bands that underwent the analysis of variance, while the three enzymatic systems (a-esterase, peroxidase and malate dehydrogenase,) presented 33 polymorphic bands in the evaluated material, before flowering as much as during flowering. The results of the analysis of variance of the 86 polymorphic bands

indicated that one PCR locus, three RAPD loci and nine polymorphic enzymatic bands showed significant association with tuber specific gravity, whereas for chip color the analysis of variance indicated significant association of one PCR locus, 12 RAPD loci and one enzymatic locus (Table 1).

When all markers were considered again for a multiple linear regression analysis of the type of partial sum of squares (SS type II), where the effects of each marker in relation to the other markers were evaluated, we observed that the results did not coincide with the ones obtained through the analysis of variance of each marker individually. In this case, results included nearly all non-significant markers, except for PCR-2, A10.4, and Est-4 (sampled during flowering) and Prx-1 (sampled before flowering) for tuber specific gravity and of A10.2, C01.3, and G05.2 for chip color (Table 1).

The proportion of total phenotypic variance explained for all markers was 15.53% for tuber specific gravity and 6.28% for chip color. These values are considerably different from those observed in the multiple linear regression analysis performed only with significant F loci that were obtained through single point analysis of variance. This analysis indicated that the significant markers explained 74.53% of the phenotypic variance for tuber specific gravity and 47.84% for chip color in the set. This result is similar to that of Douches and Freyre (1994) who estimated a total phenotypic variance of 43.5% for chip color using 12 RAPD markers and one isoenzymatic marker explaining these QTL. The most likely cause for the difference between the determination coefficients is that markers of small effect on the phenotypic variation of the traits considered for the analysis contribute equally to increase the experimental error and consequently diminish the experimental precision in the estimation of the phenotypic

Table 1. Genetic markers with significant association with tuber specific gravity and potato chip color

Marker[a] Specific gravity	**Partial R^2 (%)**	**SS type II[b]**	**Marker Chip color**	**Partial R^2 (%)**	**SS type II[b]**
PCR-1	12.50**	*	PCR-2	0.52 **	
A07.1	0.70 *		A07.3	1.48 *	
A10.4	9.99 **	*	A10.2	4.27 **	*
G03.2	2.87*		AE05.2	3.71 *	
Est-4[1]	8.03 **		AT02.2	12.60 *	
Est-4[2]	16.65 *	*	AT10.2	3.65 *	
Est-5[2]	0.09 *		C01.3	3.30 *	**
Prx-1[1]	33.90 *	**	E06.1	5.36 *	
Prx-6[1]	11.64 **		F13.1	1.52 *	
Prx-10[1]	4.58 *		F13.5	4.27 *	
Prx-2[2]	4.61 *		G03.1	0.70 *	
Prx-5[2]	17.21 **		G05.1	0.62 *	
Mdh-2[2]	0.08 *		G05.2	4.11 **	*
			Mdh-3	7.89 *	
R^2 (%)[c]	74.53		R^2 (%)[c]	47.84	
total R^2 (%)	15.53		total R^2 (%)	6.28	

[a] enzymatic loci in italic, the others are RAPD markers obtained with decamer primers (Operon Technologies Inc.). The nomenclature for RAPD was used as proposed by Quiros et al. (1993).
[b] Sum of squares of type II
[c] Determination coefficient explained by the markers with significant F values ($P < 0.05$ and $P < 0.01$) of the analysis of variance of a single point.
**, * significant at 1% and 5% probability, respectively and ns (non-significant) by the F test,
[1]Sampled before flowering
[2]Sampled during flowering

variation. The proportion of individual phenotypic variance of the markers was determined by the partial R^2 values, derived from the multiple linear regression analyses of markers whose F values were significant. They varied from 0.08% to 33.90% for tuber specific gravity and from 0.52 to 12.60% for chip color. These values exceeded those found by Freyre and Douches (1994), who obtained R^2 values of 4 to 15.8% in the evaluation of individual loci.

Only the interactions between the RAPD F13.1 marker and the enzymatic marker *Est-4* product sampled during plant flowering and G03.2 together with *Mdh-2,* sampled at the same period, showed significance ($P < 0.05$). This was demonstrated by the significance of the F test in the multilocus variance analyses of the pairwise markers considered to estimate epistatic or gene linkage interactions that provide redundant information. Still, the significance obtained by the analysis of variance for each variable between F13.1 (marker for chip color) and *Est-4* (marker for tuber specific gravity) indicates that these markers are close to a QTL that would be important in the metabolic route to determine starch accumulation as well as reducing sugar concentrations.

To solve the problem of redundant information and simultaneously evaluate the effect of two or more markers, the multiple regression analysis with marker elimination was carried out by the backward process (Drapper and Smith 1981). The significance of the analysis of variance and partial F are presented in Table 2.

It was observed that several non-significant markers included in the initial model were discarded. The remaining and more important markers were closer to the QTL. With the removal of markers with redundant information, the importance of the remaining markers increased, as expressed by the increased estimate of the partial F and the increment in the proportion of phenotypic variance explained by each one of them. The remaining markers for tuber specific gravity explained 73.51% of the phenotypicvariance. For chip color, two remaining markers, G05.2 and A10.2 were detected, which were able to explain 41.93% of the total phenotypicvariance. This result is in line with the estimates of Douches and Freyre (1994), who found in chromosome 2, a highly significant polymorphic locus for chip color, associated to primer G05.

The identification of several primers associated to the different QTL supports the idea of the polygenic control involved in the traits that determine the potato processing quality. The results of Pereira et al. (1993) who examined chip color in tetraploid clones also demonstrated the existence of the polygenic model.

Several molecular and enzymatic polymorphic markers were identified as responsible for the variation of tuber specific gravity and chip color in this study. The identification of these QTL related to potato processing can be highly valuable for potato breeders, with a view to an early selection of superior genotypes.

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Table 2. Remaining markers selected by the backward regression variables associated with tuber specific gravity and potato chip color

Marker Specific gravity	Partial R^2 (%)	Marker Chip color	Partial R^2 (%)
PCR-2	16.47 *	G05.2	20.78 **
A10.4	15.27 *	A10.2	20.67 **
Est-4[1]	13.18 *		
Est-4[2]	22.74 **		
***Prx-1*[1]**	35.99 **		
Prx-2[2]	20.60 **		
Mdh-2[2]	15.64 *		
R^2 (%) total	73.51	R^2 (%) total	41.93

*, ** significant at 5% and 1% probability by the F test, respectively.
[1]Sampled before flowering.
[2]Sampled during a flowering.

Marcadores genéticos associados a características de qualidade industrial da batata

RESUMO - *O sucesso de um programa de melhoramento depende da correta escolha de genitores e da eficiente seleção de genótipos superiores em populações segregantes. O uso de marcadores genéticos pode permitir a identificação precoce e precisa dos indivíduos com melhor combinação de alelos favoráveis tornando o processo seletivo mais eficiente. O presente trabalho objetivou avaliar clones de batata para características de processamento industrial e identificar marcadores genéticos associados a elas. Quarenta e dois genótipos foram divididos em dois grupos contrastantes para peso específico dos tubérculos e cor de chips e avaliados para três sistemas isoenzimáticos, 82 marcadores RAPD e um marcador de microssatélite. Foi possível detectar polimorfismos dos marcadores associados ao peso especifico e à cor de chips. Quando considerada a soma de quadrados parcial, a maior parte dos marcadores foi não significativa indicando a existência de informações redundantes entre eles. Pela análise backward foram detectados e selecionados os melhores marcadores que conjuntamente explicaram 73,51 e 41,93% da variação fenotípica para o peso específico e para a cor de chips, respectivamente.*

Palavras-chave: *Solanum tuberosum* L., peso específico, cor de chips, processamento da batata

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