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Microstructure and tuber properties of potato varieties with different genetic profiles

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ABSTRACT

The objectives of this research were to study tuber starch characteristics and chemical - thermal properties of 21 potato varieties, and to determine their genetic diversity through SSR markers. Starch granular size varied among samples, with a wide diameter distribution (5–85 μm), while granule shapes were similar. Differential Scanning Calorimeter analysis showed that the transition temperatures (69 °C–74 °C) and enthalpies of gelatinization (0.9 J/g–3.8 J/g) of tubers were also variety dependent. SSR analysis allowed the detection of 157 alleles across all varieties, with an average value of 6.8 alleles per locus. Variety-specific alleles were also identified. SSR-based cluster analysis revealed that varieties with interesting quality attributes were distributed among all clusters and sub-clusters, suggesting that the genetic basis of traits analyzed may differ among our varieties. The information obtained in this study may be useful to identify and develop varieties with slowly digestible starch.

1. Introduction

Due to its versatility, the potato (*Solanum tuberosum*) is cultivated throughout the world and represents a major food for millions of people. It is now the most important non-cereal food crop, ranking third in terms of total production after rice and wheat (Birch et al., 2012). Besides being important in the human diet, tubers are also used as animal feed and as raw material for starch and alcohol production. One of the most important aspects related potato utilization and market is its quality. Tuber quality parameters change according to the specific market utilization types, and are often referred to two major categories. The first is "external quality", based on aspects comprising skin colour, tuber size and shape, eye depth. The second category comprises "internal quality" aspects, including nutritional properties, culinary value, after-cooking attributes (Carputo, Aversano, & Frusciante, 2005). Several factors affect tuber quality of a potato variety, including the genetic background, field practices, environmental and storage conditions. Starch content and properties represent major tuber quality characteristics. They show a polygenic control, with candidate genes located on all potato chromosomes (Chen, Salamini, & Gebhardt, 2001). Potato starch has some unique physico-chemical characteristics compared to starches from other sources (e.g. high phosphate content, absence of internal lipids and proteins in granules) (Burlingame, Mouillé, & Charrondiére, 2009; Romano et al., 2016). Starch characteristics are considered to be a major factor affecting the functionality of the processed potatoes and other potato starch based food applications (Singh, Kaur, & Mccarthy, 2009). Gelatinization is one of the main functional properties of starch. It may strongly influence the rate at which starch is digested and elicits the glycemic response (Ek, Wang, Brand-Miller, & Copeland, 2014). In spite of this, only for a relatively low number of commercial varieties this aspect has been studied in details and there is still a lack of understanding over what gelatinization actually is and how the thermal transitions measured by differential scanning calorimetry relate to starch gelatinization (Wang & Copeland, 2012). Genetics strongly determine starch structure, which in turn influences properties such as gelatinization temperature range (DT) and the energy absorbed (ΔH, melting enthalpy) during starch gelatinization (Lai et al., 2016). Understanding the relationships between structural and functional properties of potato starch is very important for optimizing food and industrial applications and for the development of starchy tailored foods (Lai et al., 2016; Romano et al., 2016). It can also provide novel

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opportunities to breeders to generate novel starches for use in food and non-food products.

A first, logical criterion to assess plant characteristics and diversity is based on phenotypic analysis. Despite its limitations, this method is often employed to identify potato varieties possessing target traits and to design appropriate breeding schemes. To increase the efficiency of characterization, the use of DNA markers is particularly attractive since they provide a direct estimation of diversity and help in the selection of potato parental clones that guarantee a superior genetic combination (Bisognin & Douches, 2002). In addition, molecular fingerprints can be used to create a genetic signature for traceability purposes (Adamo et al., 2012). One particularly attractive application of molecular markers is the possibility to assist selection in breeding programs. It enables breeders to precisely introgress small genome pieces from the genotype of interest to elite varieties while reducing the incorporation of genes with undesirable effects (Gupta, Kumar, Mir, & Kumar, 2010).

The objectives of this research were to compare tuber starch characteristics of several potato varieties and to evaluate additional noteworthy properties of tuber tissues. Although there have been many studies on potato, the results usually relate to a limited number of varieties, making generalizations about its properties difficult. We also performed microsatellite (SSR) DNA analysis to highlight molecular polymorphisms and perform comparisons with the diversity detected at phenotypic level. This knowledge will help to address the commercial use of specific varieties and the choice of suitable parents in breeding programs aimed at developing potato varieties with specific starch attributes.

2. Materials and methods

2.1. Plant materials

Used in this study were 21 commercially grown potato varieties widely employed in our breeding programs (Actrice, Adora, Arielle, Arizona, Asterix, Bellini, Berber, Colomba, Crisper, Fontana, Inova, Laura, Marlene, Red Scarlet, Safrane, Sinora, Soprano, Triplo, Universa, Vivaldi and Volumia). Plants were grown in the field in Celano (central Italy) in the summer 2014 to collect leaves for DNA analysis and to produce tubers for determining characteristics and properties. Tubers were harvested 120 days after planting, when leaves started senescing. Only medium-sized tubers (approximately 5 cm in diameter as measured perpendicular to the apical-stolon end axis, regardless of orientation) were collected. The selection of tubers with similar shape and size is felt important because variations in the activity of enzymes such as granule-bound starch synthase during growth may affect starch granule morphology in potatoes (Blennow, Engelsen, Nielsen, Baunsgaard, & Mikkelsen, 2002). Tubers were stored in the dark at 7 °C for a week before evaluating starch and dry matter.

2.2. Microstructural analysis: Scanning Electron Microscopy (SEM) analysis and physical characterization

Particle size and shape of starch granules were quantified as starch surface area and roundness using a method based on image analysis of Scanning Electron Microscopy (SEM) micrographs. Potato slices (thickness of 0.1 mm) were cut from the pith of the parenchymatous region of potato tubers using a scalpel. A circular cutting mold was used to make circular slices with a diameter of 3–4 mm. This procedure minimized the large textural differences occurring between the cortex and the pith tissues (Anzaldùa-Morales, Bourne, & Shower, 1992). Slices were rinsed immediately after cutting for 1 min in distilled water to eliminate some starch adhering to the surface prior to analyze. Two slices were prepared from each potato variety and were lyophilized by means a freeze dryer (mod. Alpha 1–2 LD plus, Christ, Germany), at −50 °C for 48 h. Samples were then dried at the critical point and coated with gold particles in an automated critical point drier (model SCD 050, Leica Vienna). Microstructure of samples was examined by means of Scanning Electron Microscopy (LEO EVO 40, Zeiss, Germany) with a 20 kV acceleration voltage and a level of magnification: 1000X, secondary electron mode. Three micrographs were obtained from each slice. Three micrographs were selected randomly from the total of 6 micrographs collected per variety, giving a triplicate measurement of starch surface area and roundness. Images were processed by Image Pro Plus 6.1 for Windows® (Media Cybernetics Inc.). Computed parameters included the following:

- Granule surface area (μm^2).
- Starch granule roundness. Selection of shape descriptor (roundness) was based on its relationship to changes observed in the shapes of features. This measurement calculates the circularity of an object:

$$\text{roundness} = \frac{P_i^2}{4 \cdot \pi \cdot AF_i}$$

where AF_i is the area of the ith starch granule and P_i is the measured perimeter of the ith starch granule. A perfect circle has a shape factor of 1 and a line has a shape factor approaching to zero.

Starch granule area distribution analysis was also performed by counting the percentages of granules falling into three predefined area classes: small (<350 μm^2), medium (350–1250 μm^2) and large (>1250 μm^2).

2.3. Tuber properties

The dry matter content of tubers was determined by the AACC method (number 44–15.02, 1999). Two-3 g of sample were weighed and then dried in an oven for 24 h at 105 °C. The results were expressed as percentage on the dry matter content (%). Average values of three measurements were calculated for each sample. The pH of samples was measured by means of a digital pHmeter (MP220, Mettler, Toledo) according to the AACC method 21 (number 02–52.01, 1999). Thermal characteristics of the samples were determined using a Differential Scanning Calorimeter (DSC Q200, TA Instruments, Milan, Italy). Potato slices (thickness of 0.1 mm) were cut from the central part of potato tubers using a scalpel. Slices were rinsed immediately after cutting for 1 min in distilled water to eliminate some starch adhering to the surface prior to analyze. Samples of approximately 25 mg were placed in weighed coated aluminum pans (TA Instruments, New Castle, USA), which were immediately hermetically sealed and reweighed. Samples were heated from 20 °C to 100 °C at 10 °C min^{-1} using an empty pan as reference. The enthalpy (ΔH), onset temperature (To), peak temperature (Tp) and end temperature (Te) of endotherms were measured. Average values of three measurements were calculated for each sample.

2.4. Microsatellite (SSR) analysis

DNA extraction was carried out using the Qiagen Plant DNeasy Kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). Analyses were carried out with 23 nuclear microsatellite (SSR) primer pairs. They were chosen from Ghislain et al. (2009). All SSRs were recommended at CIP (www.cipotato.org), based on quality criteria, genome coverage, and locus-specific information content (Supplemental Table 1). PCR reactions were performed in