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Random amplified polymorphic DNA (RAPD) fingerprint and antioxidants profile as markers for Tropea red onion (*Allium cepa* L.) authenticity

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ABSTRACT

Random amplified polymorphic DNA (RAPD) and chemotype analyses were applied to characterise Tropea red onion (*Allium cepa* L.), a typical Italian variety that is cultivated in specific areas of Calabria. Seven Tropea red onion samples and three commercial cultivars of different coloured onions were evaluated. Amplification of genomic DNA using random primers highlighted a good clustering differentiating Tropea red onion cultivar from commercial reference samples used in this study. The total antioxidant activity and the content of quercetin-3,4′-*O*-diglucoside (3,4′Qdg) and quercetin-4′-*O*-monoglucoside (4′Qmg) were measured using photochemiluminescence method (Photochem®) and capillary electrophoresis, both very interesting because of their very short measuring times. The difference in antioxidant activity and quercetin contents between Tropea red onion and commercial sample and also the difference within Tropea cultivar suggested that the nutrient composition did not depend only on particular genetic characters but was probably connected to the intrinsic pedological soil and climate features.

1. Introduction

The history of the onion is well documented and can be traced to its origin in a wide area from India to Israel, where its production started in 3000 BC; it was then introduced into Europe by Phoenicians around 2000 years ago. In ancient Egypt, the onion was believed to be a sacred food, and it was also widely consumed by the Romans and Greeks, who liked its taste and knew of its curative properties. The worldwide annual production was estimated at almost 74 million tonnes in 2010; the main production area is Asia with 48 million tonnes (http://faostat.fao.org/). Consumption *per capita* differs greatly between countries and areas, but a major trend seems to be that onion consumption is increasing worldwide (Slimestad et al., 2007).

The red onion, *Allium cepa* L. var Tropea, is a typical Italian variety, cultivated in some areas of southern Italy (Calabria region). This variety is known for its distinctive bulb, lengthened or oval, red and sweet. It was awarded in 2008 with PGI certification by the European Union and named "Cipolla Rossa di Tropea Calabria PGI" (EC No 284/2008). Due to its characteristics, the Tropea red onion is a product known throughout the world, important for local and national economies.

The Tropea red onion is subject to food fraud (Furia et al., 2011). Methods to verify Protected Designation of Origin (PDO) and Protected Geographic Indication (PGI) products will help to protect regional foods and promote the economic growth of marginal rural areas by encouraging the production of high quality niche market foods (Negrini et al., 2008). Even with the introduction of many novel marker systems during the past 10 years, reports of genetic mapping in onion have been very scarce. Reasons for the delay in molecular marker studies in onion are the biennial nature of onion, its severe inbreeding depression and its huge genome. To generate a sufficient number of molecular markers with smaller amounts of less pure DNA, one can choose between polymerase chain reaction (PCR)-based methods like random amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSR), amplified fragment length polymorphisms (AFLPs) and selective amplification of microsatellite polymorphic loci (Van Heusden et al., 2000; Cramer and Havey, 1999).

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In particular, the random amplified polymorphic DNA (RAPD) technique makes it possible to obtain a multi-band genetic fingerprint useful in solving "pattern recognition" problems, like the clustering of different vegetables varieties at intraspecies level. Then, the defined clusters can be assigned and correlated to specific groups and characteristics (*e.g.*, geographical origin, nutritional composition). Many research studies have shown the usefulness of RAPD molecular approach on intraspecific polymorphisms studies, in both plant and animal foods. The RAPD markers are of fundamental importance in analytical traceability studies, particularly when combined with other chemical markers (chemotype) (Weder, 2002; Tedeschi et al., 2011), and supported by powerful multivariate statistic techniques, such as principal component analysis (PCA) (Brandolini et al., 2005, 2006).

Onions are rich in two chemical groups that have perceived benefits to human health. These are the alk(en)yl cysteine sulphoxides (ACSOs) and the flavonoids. The ACSOs are flavour precursors, which, when cleaved by the enzyme alliinase, generate the characteristic odour and taste of onion. Two flavonoid subgroups are found in onion: the anthocyanins, which impart a red/purple colour to some varieties, and flavanols, such as quercetin and its derivatives, responsible for the yellow and brown skins of many other varieties (Griffiths et al., 2002).

Quercetin is the major dietary flavonol in vegetables, specifically in onions, where, it is present mainly in its conjugated forms as a glucoside. Quercetin-3,4′-*O*-diglucoside (3,4′-Qdg) and quercetin-4′-*O*-glucoside (4′-Qmg) are the two major types of quercetin conjugates in onions, whereas quercetin aglycone is also present in the outer layers and skin and in some parts of the bulb (Lu et al., 2011). The radical-scavenging ability of quercetin inhibits lipid oxidation *in vitro*, and it is theoretically possible for dietary flavonoids to help prevent atherosclerosis *via* the same mechanism *in vivo* (Lee and Mitchell, 2011).

Onions have been the subject of numerous investigations, some of which concerned the differentiation of cultivars by analysing flavonoids composition; other works focused on the discrimination of the area of production and evaluation of authenticity using multielemental profiling approach (Furia et al., 2011; Yang et al., 2004; Yoo et al., 2010). To our knowledge, there is limited literature on the study of onion traceability, combining plant genotype fingerprint with chemical and nutrient composition. Our aim is to characterise Tropea red onion and create a knowledge network using genomic and chemical analyses to preserve the features and authenticity of this particular product in order to protect the rights and health of consumers. To achieve our goal we evaluated:

(1) genetic diversity among the Tropea red onion and three other commercial cultivars (Primula Rossa, red onion; Saratoga, yellow onion, Crystal, white onion), analysing the RAPDs patterns through principal component analysis (PCA);
(2) 3,4′-Qdg and 4′-Qmg content, using capillary electrophoresis (CE) as an attractive alternative to HPLC;
(3) the antioxidant activity of different onion ecotypes by using the Photochem system which detects superoxide anion radicals directly.

2. Materials and methods

2.1. Samples

Tropea red onions ([*A. cepa* L.], labelled from T1 to T6) were obtained from a local cultivar in the typical PGI area of production (Tropea and Amantea-Calabria). T7 sample was certified seed Tropea, purchased from Semencoop (Cesena, Emilia Romagna, Italy) and cultivated in Emilia Romagna region. A red (Primula Rossa), a white (Crystal) and a yellow (Saratoga) onion sample were purchased at the supermarket in Ferrara (Emilia Romagna region). The samples were stored at −25 °C until the analyses. The tops and bottoms of the onion bulbs, outer dry skins, and any inedible outer portions were removed; only the innermost part of the bulb was used for analysis.

2.2. Genetic analysis

2.2.1. DNA reagents and instrumentation

For DNA extraction phenol (Ultrapure buffer saturated phenol) was obtained from Gibco S.r.L (Grand Island, NY, USA). Chloroform (Chromasolv® Plus for HPLC), isoamyl alcohol and ethanol (ACS reagents) were purchased from Sigma–Aldrich (Milan, Italy).

Taq polymerase, $MgCl_2$ solution, and 10× buffer, dNTPs were obtained from M-Medical (Milan, Italy). RAPD primers were purchased from Eurofins MGW Operon (Ebersberg, Germany). Gels were prepared with agarose (Sigma–Aldrich). Molecular weight marker (O'GeneRuler™ 100 bp DNA ladder) was purchased from Thermo Scientific Molecular Biology (Milan, Italy). Amplification was carried out on a Biorad Gene Cycler machine (Biorad Laboratories, Segrate, Milan, Italy). The amplification products were separated on 2% agarose gel, using a Power Pack 300 power supply equipped with a subcell agarose gel electrophoresis system (Biorad Laboratories).

2.2.2. Genomic DNA extraction

Genomic DNA was extracted from onion samples according to the following described protocol. About 200 mg of each sample were crushed in a test tube and 300 μL of extraction buffer (1 M Tris–HCl pH 8.0, 2.5 M NaCl, 0.5 M EDTA, SDS 10% w/v) were added. The tubes were left in a thermo-mixer at 65 °C for an hour; 30 μL of sodium acetate buffer (3 M) were added and the tubes were centrifuged (10 min, 11,000 × *g*). The genomic DNA was purified by two successive extractions with phenol and chloroform:i-soamyl alcohol mixture (24:1; v/v) respectively and then precipitated with ice-cold absolute ethanol. The DNA was isolated by centrifugation and after two washing steps with 70% ethanol (v/v) in pure water, it was dried and re-dissolved in 50 μL sterilised water.

Purity of the DNA was evaluated by means of 260/280 nm (UV-vis spectrophotometer, DU 730; Beckman, Palo Alto, CA) absorbance ratio and by running the DNA on agarose gel electrophoresis with qualitative standards.

2.2.3. DNA amplification and detection

The optimised PCR amplification mixture (30 μL) contained 6 μL of ultrapure H_2O, 15 μL master mix (Taq DNA polymerase 0.05 unit per μL, 4 mM $MgCl_2$, dNTPs (dATP, dCTP, dGTP, and dTTP, 0.4 mM each, reaction buffer), 1 μL primer (0.1 μg/μL) and 8 μL of genomic DNA (0.1 ng/μL). PCR conditions were as follows: one cycle at 95 °C for 5 min (initial denaturation), followed by 45 cycles at 94 °C for 60 s (denaturation), 37 °C for 60 s (annealing), 72 °C for 90 s (extension), and ending with a final step at 72 °C for 5 min (extension).

Sixty random decameric primers (Table 1) were used to test their ability to generate reproducible amplification products. Reaction products (14 μL) were separated by electrophoresis in 2% agarose gels by use of Tris-borate-EDTA (TBE) buffer (8.8 mM Tris–HCl, 8.8 mM boric acid, and 0.2 mM EDTA; Sigma–Aldrich). The samples were added to loading buffer (3 μL) and then stained with 0.5 μg/μL ethidium bromide (Bio-Rad Laboratories). All RAPD profiles were photographed with the Sony DSC HX1 photocamera, equipped with GelQuant Express (DNR Bio-Imaging System, Jerusalem, Israel).