Development of Molecular Markers for Origin Determination in Saffron

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Abstract

Measures of molecular and morphological genetic variation are often used to set conservation priorities and design management strategies for plant taxa. More recently, the certification of the origin and quality of food is another area where the use of molecular techniques is getting increased. Random Amplified Polymorphic DNAs (RAPDs) is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence been widely utilized in plant genomic studies. Saffron is considerate to be the most expensive spice in the world, due to its cultivation that requires careful handling at every step and yields so very little. Because of its great cost, many adulteration ways have been exploited and consumers through the centuries have guarded against paying for impure saffron. We investigated the distinction and variability of *Crocus sativus* from several geographic areas (Italy, Iran, Greece and Spain) using 38 random amplified polymorphic DNA (RAPD) markers and dry stigmas as plant material.

INTRODUCTION

Saffron, the dry stigmas of *Crocus sativus*, is considerate to be the most expensive spice in the world. Its high price is due to the much direct labour required for its cultivation, harvesting and handling.

Because the great cost of saffron, it has been object of adulteration by means of mixing genuine stigmas of saffron with other parts of plants (e.g. some species of grass) artificially coloured. Furthermore, the saffron produced in Spain is protected with the distinction D.O.* that is associated to a high quality of the spice which is produced in this region. In addition, the small production of the spice in this region result in an increase of the price of the saffron which is commercialized as D.O. "Azafrán de La Mancha" so there is an increasing interest in mixing it with Iranian saffron, fundamentally.

Random Amplified Polymorphic DNAs (RAPDs) is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence been widely utilized in plant genomic studies (Barazani et al., 2003; Lopez-Sese et al., 2003). We investigated the variability and the presence of markers in the molecular pattern of different *Crocus* spp. and the distinction and variability of *C. sativus* from several geographic areas (Italy, Iran, Greece and Spain) using thirty-eight random amplified polymorphic DNA (RAPD) markers on dry stigmas as plant material.

MATERIAL AND METHODS

Plant Material

The material used in this study was dry stigmas of *C. sativus*, commercial saffron from four different countries: Spain, Greece, Iran and Italy. In the first part of this work, we used several species of the genus *Crocus*: *vernus, cartwrightianus, kosaiini, hadriaticus, chrysanthus* and an unidentified wild variety from Sierra de Alcaraz (Albacete).

DNA Extraction

Genomic DNA was isolated from samples using the NucleoSpin Plant Kit of BD

Biosciences Clontech (Palo Alto, CA). DNA concentration was determined in each sample by using the spectrophotometer (Pharmacia, Biotech) and then we adjusted the different concentration to 100 ng/µl.

RAPD Analysis

Oligonucleotides primers used in this study correspond to commercial short nucleotide sequences that are able to bind to a fragment of the isolated DNA by hazard. RAPD analysis was conducted in a 20 µl final reaction mixture containing PCR buffer, 2,5 mM $MgCl_2$, 0,1 mM concentrations of each deoxynucleosides triphosphate, 0,5 µM of primer, 1 µl of target and 0,5 U of Taq polymerase (Promega). Reactions were carried out in an automatic thermocycler (Perkin Elmer) with a thermal cycling profile of 92ºC for 2 min, 45 cycles at 92ºC for 20 s, 35ºC for 20 s, 72ºC for 1 min and 10 s, and finally 72ºC for 5 min, and at that point the thermocycler maintained a constant temperature of 4ºC. Amplified fragments were analyzed by electrophoresis in a 2% agarose gel containing ethidium bromide (0,5 µg/ml) at 100 V for 1h.

RESULTS

In a first approach, we determined if commercial available RAPDs were able to discriminate among close *Crocus* species. We selected the following species: *vernus, cartwrightianus, kosaiini, hadriaticus, chrysanthus* and a wild variety from Sierra de Alcaraz (Albacete). We used two pairs of RAPD primers that allowed us to clearly discriminate among them, and therefore were considerate as suitable for identification studies (Figure 1).

In the second experiment we obtained the molecular pattern proportionate by six primers out of 38 polymorphic ones. These six primers were selected for screening of the saffron from mentioned areas and provide eight markers that conform a good system for analysis of genetic variability and determine the origin of saffron (Figure 2). The origin of saffron that present more differences with the rest of origins tested is Greece where we have found five of the eight differential molecular markers.

DISCUSSION

C. sativus is a triploid species that only can be reproduced vegetatively because of its irregular meiosis. This characteristic is consequent with the results obtained: only a low percentage of primers were suitable to differentiate among the four saffron samples from different origins. However, other studies with triploid species with apomictic reproduction offer a larger genetic variation in the genetic structure of the population (Palacios and Gonzalez-Candelas, 1997). In accord to the results obtained with the random amplified polymorphism, the origin of saffron that present more differences with the rest of origins tested is Greece (five of the eight molecular markers), probably due to the low influences that this culture have received from the other regions.

In the other cases has been difficult to find some molecular differences. Several theories indicate that saffron, which is actually cultivated in Iran, is originating from Spain. This theory could explain the difficulty to find molecular differences between these two origins. Probably the short passed time since the geographical diversification of saffron is the reason for such a results.

The analysis of the results have been used to confirm the limited genetic differences between origins in this triploid specie that, due to its sterility, only can be reproduced vegetatively.

Literature Cited

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Figures

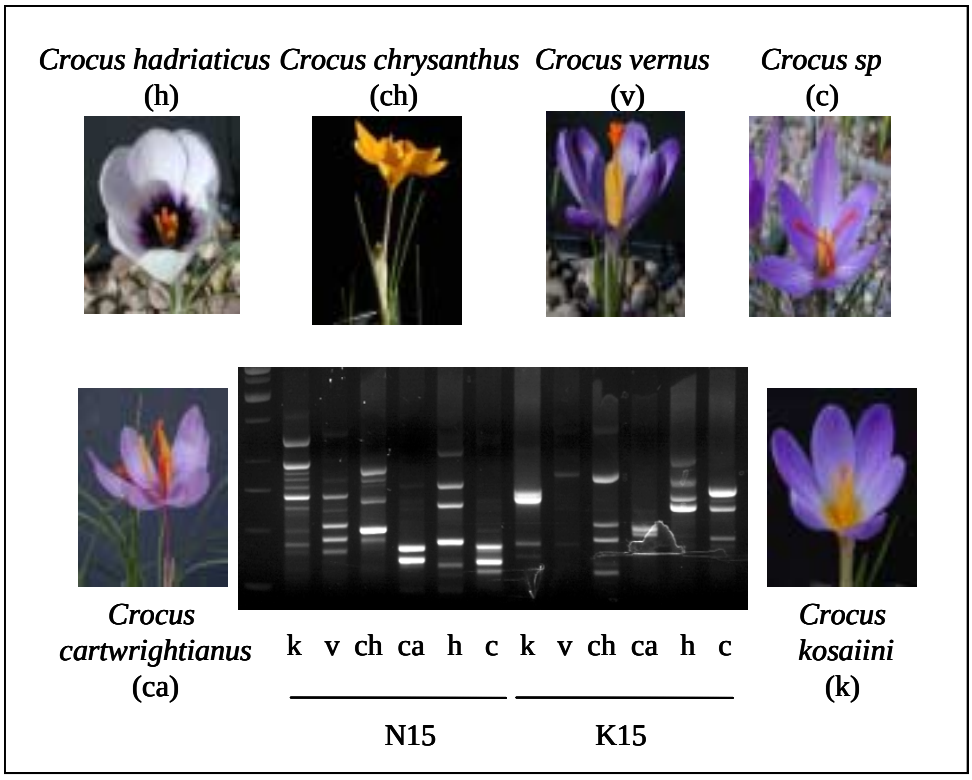

Fig. 1. Photographs of the six different species of *Crocus* used for the experiment. The electrophoresis shows that RAPD analyses using 2 primers gave polymorphic fragments.

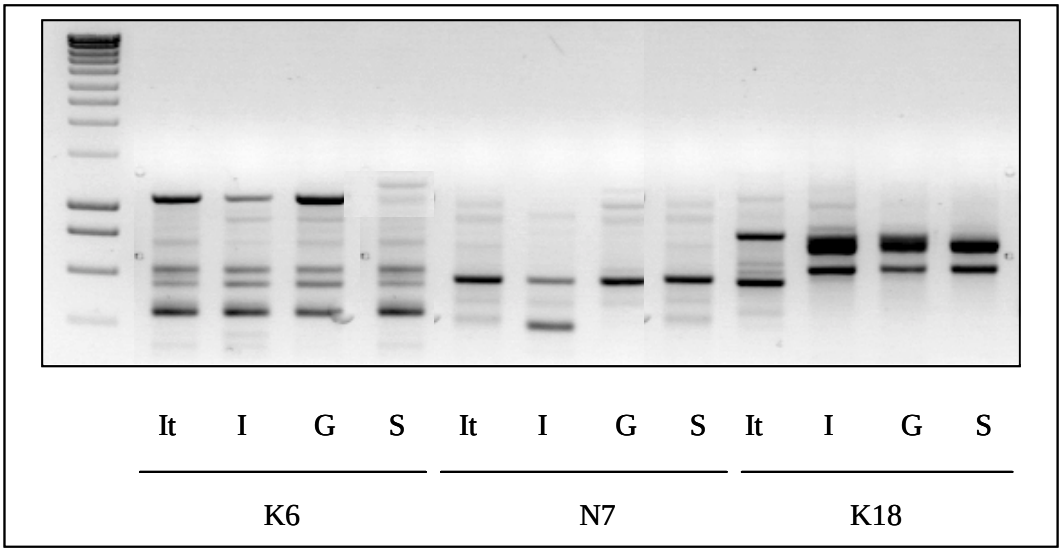

Fig. 2. K6, N7 and K18 are three of the primers tested that provided molecular markers. Italy (It), Iran (I), Greece (G) and Spain (S) is the order maintained with the samples.