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EVALUATION OF AN SSR MARKER FOR MARKER-ASSISTED SELECTION IN KALE (*BRASSICA OLERACEA* var. *ACEPHALA*)

G. SARIKAMIS[1], R. YANMAZ[1], A. BALKAYA[2] and B. BELGE[1]
[1]*Ankara University, Faculty of Agriculture, Department of Horticulture, 06110, Ankara, Turkey*
[2]*Ondokuz Mayis University, Faculty of Agriculture, Department of Horticulture, 55139, Samsun, Turkey*

Abstract

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The objective of the current study is to test an SSR marker (Ol12FO2) associated with methylsulphinylalkyl glucosinolates and its potential application for marker assisted selection (MAS) in kale (*Brassica oleracea* var. *acephala*). Methylsulphinylalkyl glucosinolates such as glucoiberin and glucoraphanin are known to have health promoting properties and to protect against various forms of cancer. Markers associated with such important traits are important to expedite breeding programs aimed to select for desired traits for further use in generating improved novel lines.

The findings of the present study revealed that successful amplifications with genomic DNA from kale genotypes were observed with the SSR marker Ol12FO2 and bands of expected sizes were obtained. It was determined that Ol12FO2 was polymorphic among kale genotypes. The results obtained in the present study demonstrated successful amplification of markers within different vegetable brassicas.

Key words: Brassica oleracea, SSR, kale

Introduction

Kale (*Brassica oleracea* var. *acephala* L.) is one of the oldest forms of the cabbage family, originating in the eastern Mediterranean which is thought to have been used as a food crop as early as 2000 BC. Kale is mainly used as a green vegetable, widely grown in the Black Sea Region (Balkaya and Karaagac, 2005).

The importance of the genetic resources for the continuous improvement of crops is universally admitted nowadays (Ordas and Cartea, 2008). The intensive activity of genetic improvement, together with the technological development of agricultural inputs, has led to the replacement of many local varieties by a few uniform modern cultivars in developing countries. For this reason, preservation of populations and landraces are very important (Balkaya and Yanmaz, 2005). In this study, a population of kale genotypes collected from the Black Sea Region of Turkey was used. The seeds of the kale population are currently preserved at -20° C for long term storage in the Turkish seed gene bank (AARI) for future breeding purposes (Balkaya et al., 2004).

The availability of marker technologies has brought

E-mail: golges@yahoo.com

new insights towards better understanding of *Brassica* genetics mainly by genetic mapping using various DNA marker systems in segregating populations generated for investigations of particular traits of interest and subsequently by locating several quantitative trait loci (QTLs). Most analysis of QTLs in *Brassica* has focused on disease resistance, oil quality, flowering time and glucosinolate content in several brassica species.

Glucosinolates are sulfur containing plant secondary metabolites, mainly synthesized in crucifers, primary role in plants believed to be defense against herbivore attacks. Inclusion of cruciferous vegetables in the diet is reported to significantly reduce the risk of several forms of cancer such as breast (Fowke et al., 2003) stomach and colorectal (Seow et al., 2002; Hara et al., 2003; Zickute et al., 2005), kidney (Moore et al., 2007), bladder (Munday et al., 2008) and prostate (Traka et al., 2008). The protective effect of crucifers is attributed to the activity of isothiocyanates, derived from the corresponding glucosinolates.

Several studies have demonstrated the positioning of QTLs associated with glucosinolates in *Brassica napus* (Toroser et al., 1995; Howell et al., 2003; Basunanda et al., 2007; Gao et al., 2007) in *Brassica rapa* (Lou et al., 2008) and *Brassica oleracea* (Mithen et al., 2003). Synthesizing the most prominent glucosinolates, broccoli, has been studied extensively for the elucidation of the influence of genetics on aliphatic glucosinolate production (Mithen et al., 2003; Sarikamis et al., 2006). For the evaluation of the genetic determinants of this trait an RFLP linkage map locating three QTLs linked with methylsulphinylalkyl glucosinolates on linkage groups designated as 2, 5 and 9 was generated from a backcross population between a wild and commercial broccoli (Mithen et al., 2003). Genetic and molecular approaches were used to identify the nature of these QTLs. An SSR marker (Ol12FO2 http://brassica.bbsrc.ac.uk/cgi-bin/ace/generic/tree/BrassicaDB?name=SSR%3AOl12-F02&class=Microsatellite) from QTL-2 was identified to be linked with methylsulphinylalkyl glucosinolates and that the allelic variation at QTL-2 altered the ratio of glucoiberin and glucoraphanin, but, not the overall levels of the methylsulphinylalkyl glucosinolates (Sarikamis et al., 2006).

A recent study reported the development of gene-linked SSR markers associated with total seed glucosinolate content in genetically diverse oilseed rape (*Brassica napus*) germplasm (Hasan et al., 2008) and that the marker Gi31_387 was the marker significantly associated with seed glucosinolate content in *Brassica napus* among many other putative SSR loci evaluated also shown to be associated with seed glucosinolates (Hasan et al., 2008).

The aim of this study was to assess the potential of an SSR marker Ol12F02 associated with methylsulphinylalkyl glucosinolates, for marker-assisted selection within a collection of kales from the Black Sea region of Turkey.

Materials and Methods

Plant Material

A total of 101 kale genotypes belonging to the collection of *Brassica* germplasm from the Black Sea region collected from Samsun, Ordu, Giresun, Trabzon, Zonguldak provinces of Turkey were used as the plant material. Genotypes were grown in field trials at the experimental plots of Ankara University, Faculty of Agriculture, Department of Horticulture in 2006 and 2007. Young leaf tissue were collected, immersed immediately in liquid nitrogen and brought to the laboratory for DNA extraction.

Morphological characterization and evaluation of the genetic diversity of the collection was performed (Balkaya et al., 2004) and glucosinolate content of genotypes were determined (Sarikamis et al., 2008) previously.

DNA extraction

Genomic DNA was extracted from young leaves using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) following the instructions provided by the manufacturer. Subsequently an RNAse treatment was performed on the eluted DNA samples. Purity and concentration of the DNA were checked both on 1% (w/v) agarose gel and Nano-

Drop® ND-1000 Spectrophotometer (NanoDrop Technologies).

SSR Analysis

Primers for the microsatellite locus Ol12FO2

Forward primer: GGCCCATTGATATGGAGATG; reverse primer: CATTTCTCAATGATGAATAGT; http://brassica.bbsrc.ac.uk/cgi-bin/ace/generic/tree/BrassicaDB?name=SSR%3AOl12-F02&class=Microsatellite associated with methylsulphinylalkyl glucosinolates at QTL-2 in broccoli, amplifying a single allele of about 250 bp from broccoli cultivars Marathon and Green Duke, and an allele of 200 bp from *B. villosa*, a wild brassica (Sarikamis et al., 2006) was used for the analysis of kale genotypes. This marker has also been shown to be linked to pW114a on linkage group N15 of *Brassica napus* (http://www.ukcrop.net/perl/ace/pic/BrassicaDB?name=N15_BBSRCSSR&class= Map; (Lowe et al., 2004).

PCR reaction volume was 10µl; containing 1µl 10x Buffer, 1.5mM $MgCl_{2,}$ 0.08mM dNTPs, 2µM each primer (reverse and forward), 50ng DNA, 0.1Unit *Taq* DNA polymerase (Promega) and 7.32µl molecular grade water. Reactions without DNA were included as negative controls. PCR amplification was performed using Biometra® PCR System. The amplification conditions were 35 cycles; 20 sec at 94°C for denaturation, 30 s at 54°C for annealing and 1 min at 72°C for extension followed by 10 min at 72°C.

PCR products were separated on a 1.7 % (w/v) agarose gel stained with ethidium bromide at a concentration of 10 mg/ml, and run at 80 volts for an hour. The running buffer used was 0.5xTBE. Each gel included lanes of a DNA ladder of 100bp in size (Promega). The products were visualized under UV light (Gene Genious, Bioimaging System, Syngene®) and sized relative to the DNA ladder. The analyses were repeated at least twice to ensure reproducibility of the results.

Results

The findings of the current study revealed that successful amplifications with genomic DNA from a total of 101 kale genotypes belonging to the collection of *Brassica* germplasm from the Black Sea region collected from Samsun, Ordu, Giresun, Trabzon, Zonguldak provinces were obtained with the SSR (Ol12FO2) marker. The results showed that Ol12FO2 was polymorphic among kale genotypes. The amplification of genotypes with the marker revealed bands of expected sizes, some individuals possessing a single allele of about 250 bp and some of about 200 bp and some possessing both, determined as heterozygotes (Figure 1).

These results are consistent with broccoli; revealing that cultivars Marathon, Green Duke and some of the broccoli breeding lines amplifying an allele of about 250 bp while *Brassica villosa* and some other breeding lines amplifying an allele of 200 bp (Sarikamis et al., 2006). The results obtained in the present study demonstrated successful amplification of markers within different vegetable brassicas.

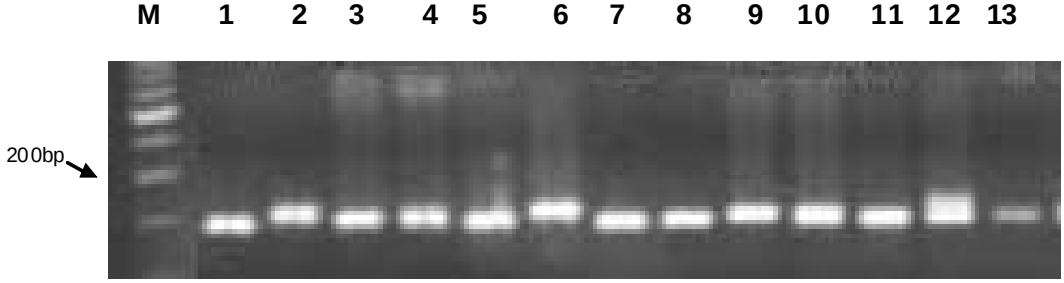

Fig. 1. Amplification of kale genotypes with Ol12FO2. M: Marker; 1-13: A group of kale genotypes representing genotypes possessing a single band of 200bp (1, 3, 4, 5, 7, 8, 11, 13) and a single band of 250bp (2, 6, 9, 10) and a heterozygote (12)

Discussion

SSR markers, or microsatellites, are one of the most prominent molecular marker of choice in marker-assisted plant breeding and marker-based genetic analysis Many microsatellite markers have been developed for related species in *Brassicaceae*, particularly from *Arabidopsis thaliana* L. and *Brassica* species (Szwec-McFadden et al., 1996; Uzunova and Ecke, 1999; Smith and King, 2000; Saal et al., 2001; Suwabe et al., 2002; Lowe et al., 2004) currently available in the public domain. Close genetic relationships between *Brassica* species lead the SSRs to be transferred. Several studies have shown the ability of SSRs developed for one species to be amplified in related species or genera (Westman and Kresowich, 1998; Hasan et al., 2008).

The use of markers with putative linkage to genes playing a vital role in trait of interest from well studied *Brassica* species might enable the utilization of these markers in other brassicas and integration of the markers into breeding programs for crop improvement. The marker used in the current study, was previously reported to be associated with methylsulphinylalkyl glucosinolates in broccoli, known to possess anticarcinogenic properties. When glucosinolate content of kale genotypes previously reported (Sarikamis et al., 2008) are taken together with the marker data obtained in the current study, it can be speculated that Ol12FO2 linked with QTL-2 can potentially be used for marker assisted selection within the collection of kale genotypes.

Conclusions

These findings are important first steps revealing the potential of the marker for marker assisted selection. Further analysis testing the co-segregation between Ol12FO2 and QTL2 in a breeding population (testcross or F2) of kale could be useful to show the effectiveness of this molecular marker for practical MAS uses.

Hence, our findings should be of considerable interest for breeding purposes and are important first steps towards better germplasm management and breeding efforts for the collection of kale germplasm.

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