AgroSym 2019

BOOK OF PROCEEDINGS

X International Scientific Agriculture Symposium
"AGROSYM 2019"
Jahorina, October 03-06, 2019

BOOK OF PROCEEDINGS

X International Scientific Agriculture Symposium
"AGROSYM 2019"

Jahorina, October 03 - 06, 2019

Impressum

X International Scientific Agriculture Symposium „AGROSYM 2019“

Book of Abstracts Published by

University of East Sarajevo, Faculty of Agriculture, Republic of Srpska, Bosnia
University of Belgrade, Faculty of Agriculture, Serbia
Mediterranean Agronomic Institute of Bari (CIHEAM - IAMB) Italy
International Society of Environment and Rural Development, Japan
Balkan Environmental Association (B.EN.A), Greece
Centre for Development Research, University of Natural Resources and Life Sciences (BOKU), Austria
Perm State Agro-Technological University, Russia
Voronezh State Agricultural University named after Peter The Great, Russia
Faculty of Bioeconomy Development, Vytautas Magnus University, Lithuania
Selçuk University, Turkey
University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
Slovak University of Agriculture in Nitra, Slovakia
Ukrainian Institute for Plant Variety Examination, Kyiv, Ukraine
National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
Valahia University of Targoviste, Romania
National Scientific Center „Institute of Agriculture of NAAS“, Kyiv, Ukraine
Saint Petersburg State Forest Technical University, Russia
University of Valencia, Spain
Faculty of Agriculture, Cairo University, Egypt
Tarbiat Modares University, Iran
Chapingo Autonomous University, Mexico
Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy
Higher Institute of Agronomy, Chott Mariem-Sousse, Tunisia
Watershed Management Society of Iran
Institute of Animal Science- Kostinbrod, Bulgaria
Faculty of Agriculture, University of Banja Luka, Bosnia and Herzegovina
Faculty of Economics Brcko, University of East Sarajevo, Bosnia and Herzegovina
Biotechnical Faculty, University of Montenegro, Montenegro
Institute of Field and Vegetable Crops, Serbia
Institute of Lowland Forestry and Environment, Serbia
Institute for Science Application in Agriculture, Serbia
Agricultural Institute of Republic of Srpska - Banja Luka, Bosnia and Herzegovina
Maize Research Institute “Zemun Polje”, Serbia
Faculty of Agriculture, University of Novi Sad, Serbia

Editor in Chief

Dusan Kovacevic

Tehnical editors

Sinisa Berjan
Milan Jugovic
Noureddin Driouech
Rosanna Quagliariello

Website:

http://agrosym.ues.rs.ba

CIP - Каталогизација у публикацији
Народна и универзитетска библиотека
Републике Српске, Бања Лука

631(082)

INTERNATIONAL Scientific Agricultural Symposium "Agrosym 2019" (10)
(Jahorina)
Book of Proceedings [Elektronski izvor] / X International Scientific Agriculture Symposium "Agrosym 2019", Jahorina, October 03 - 06, 2019 ; [editor in chief Dušan Kovačević]. - East Sarajevo : Faculty of Agriculture, 2019

Način pristupa (URL): http://agrosym.ues.rs.ba/index.php/en/archive. - Библиографија уз радове. - Регистар.

ISBN 978-99976-787-2-0

COBISS.RS-ID 8490776

IDENTIFICATION OF MOLECULAR MARKERS FOR FOREGROUND AND BACKGROUND SELECTION IN *Ga1-S* INCORPORATION INTO MAIZE LINES

Marija KOSTADINOVIĆ*, Dragana IGNJATOVIĆ-MICIĆ, Jelena VANČETOVIĆ, Danijela RISTIĆ, Ana OBRADOVIĆ, Olivera ĐORĐEVIĆ MELNIK, Anika KOVINČIĆ

Maize Research Institute "Zemun Polje", Belgrade, Serbia
*Corresponding author: kmarija@mrizp.rs

Abstract

Marker assisted selection (MAS) significantly increases efficiency of conventional breeding. Molecular markers are utilized as selection markers for target genes (foreground selection) and also for identification of the genotypes (progenies) with the highest proportion of recurrent parent's genome (background selection). Maize Research Institute "Zemun Polje" has a breeding program with the aim to create lines with incorporated incompatibility dominant gene *Gametophytic Factor 1-S (Ga1-S)*, using the integrated conventional and molecular breeding approach. *Ga1-S* is the most described gene belonging to the group of genes specific to the pollen development, germination and pollen tube growth. The *Ga1-S* system is the most commonly used to prevent the pollination of sweetcorn, popcorn and white kerneld hybrids by standard maize. The objectives of this study were identification of gene-specific molecular marker for foreground selection, as well as the set of SSR markers polymorphic between parental lines to be used in background selection. Genetic variability between two donor and three recurrent parental inbred lines was analyzed with 42 SSRs distributed over the maize genom. Total number of alleles detected with 30 informative markers was 83, average being 2.77. The genetic similarity values calculated on Dice coefficient ranged from 0.47 to 0.71. Among 12 gene-specific markers tested on parental lines, two showed distinct polymorphism for *Ga1-S*. These markers will be used as foreground selection markers for the incorporation of *Ga1-S* into our inbred lines which will be used for the creation of white kernel hybrids.

Keywords: *Gametophytic Factor 1-S, Maize, Molecular markers, Foreground selection, Background selection.*

Introduction

The inability of certain maize genotypes to successfully pollinate other genotypes is attributed to genes known as *gametophyte factors (ga)*. Gametophyte factors regulate the success of pollen-pistil interactions and affect the success of fertilization by causing the aberrant Mendellian genetic ratios in certain crosses (Nelson, 1994). These genes have two functions associated with them: a female function that produces a barrier to non-self-type pollen and a male function that enables self-type pollen to overcome that barrier (Lu *et al*., 2014). When present in pistil, they discriminate against or completely exclude pollen lacking the same allele (Nelson, 1994).

The *Ga1* was the first characterized gametophyte factor (Mangelsdorf and Jones, 1926) and has been intensively studied. It was mapped to the short arm of chromosome 4 in maize (Bloom and Holland, 2011; Lausser *et al.*, 2010; Liu *et al.*, 2014; Zhang *et al.*, 2012). *Ga1-s* is considered the "strong" variant of *Ga1*. In the *Ga1-s* barrier, pollen tubes do not grow straight and demonstrate heavy accumulation of clustered callose plug deposits (Lu *et al.*, 2014). The homozygous *Ga1-S/Ga1-S* genotype confers complete nonreciprocal cross-incompatibility (or unilateral cross-incompatibility) with *ga1* pollen (Kermicle and Evans, 2010; Zhang *et al.*, 2012).

Various strategies (e.g. physical barriers, temporal and spatial isolation) have been taken to reduce and avoid cross-fertilization among adjacent maize fields. Specialty types maize, such as sweet and waxy maize, popcorn, as well as the white kernel maize, are required to be free from foreign pollen due to the xenia effect. Seed production and non-GM maize fields require foreign- and GM-free pollen to maintain high hybrid purity and avoid contamination. The *Ga1-S* might be used as a biological reproductive barrier for the containment of gene flows between different types of maize (Liu *et al.*, 2014).

The incorporation of *Ga1-S* from popcorn to non-crossable maize elite lines has been performed using marker assisted selection (MAS) that greatly improve the efficiency and reliability of the backcrossing process by eliminating difficulty of phenotyping of the segregating population (Zhang *et al.*, 2012). Also, *Ga1-S* was introgressed by MAS into parental lines of an elite white waxy maize hybrid by six generations of backcrossing and one generation of selfing (Liu *et al.*, 2014). Maize Research Institute "Zemun Polje" (MRI) has a breeding program aimed at incorporation of the incompatibility gene *Ga1-S* into parental components of the hybrids with specific traits (white kernel). The main objectives of the research presented herein were to identify: 1) the SSR marker specific to the *Ga1-S* gene that will be used in foreground selection and 2) the set of SSR markers polymorphic between parental lines that will be used in background selection.

Material and Methods

Plant material

Two inbred lines (provided by the gene bank in Illinois, USA) were used as the donor parents (D_1 and D_2) of the favourable allele of the *Ga1-S* gene. Three MRI commercial inbred lines adapted to the local environmental conditions in Serbia were used as the recurrent parents (R_1, R_2 and R_3). These lines are components of the white kernel MRI hybrids.

DNA extraction

Total DNA was isolated from the kernel bulk according to Doyle and Doyle (1987). Bulks were prepared by pooling an equal amount of flour obtained by grounding 10 kernels per sample. The DNA was quantified using biospectrometer BioSpetrometer kinetic (Eppendorf, Germany) and diluted to a working concentration of 20 ng/μL.

Identification of molecular markers for foreground selection

Twelve gene-specific primers were chosen according to Liu *et al.*, (2014) and Zhang *et al.*, (2012). Polymerase chain reaction was carried out in 25 μL reaction volume containing: 1×DreamTaq Green Buffer (Thermo Scientific, USA), 100 μM dNTP (Thermo Scientific, USA), 0.5 μM of each primer, 1U DreamTaq DNA Polymerase (Thermo Scientific, USA) and 20 ng DNA template. Amplifications were performed in thermocycler Biometra TProfessional Standard 96 (Biometra, Germany) with the following program: an initial denaturation at 95ºC/5min, followed by 35 cycles each of denaturation at 95ºC/1min, annealing at 55ºC/30s and extension at 72ºC/1min with final elongation at 72°C for 10 min. The amplified fragments were separated by electrophoresis on 8% polyacrylamide gel in 1xTBE buffer. Gels were run on small format vertical gel system (Mini Protean Tetra-Cell, BioRad, USA), at 80 V for 1.5 h. After staining with ethidium bromide, they were visualized under UV transilluminator and documented in gel documentation system BioDocAnalyze (Biometra, Germany). Allele designations were made and approximate size range for the amplification products for each SSR locus was determined based on the positions of the bands comparing to the 100 bp molecular weight ladder (Thermo Scientific, USA).

Identification of molecular markers for background selection

Simple sequence repeat (SSR) analysis was done with 42 primer pairs spanning over the whole genome, selected from the maize database (www.maizegdb.org). Polymerase chain reaction (PCR) was carried out in 25 μL reaction volume containing: 1×DreamTaq Green Buffer (Thermo Scientific, USA), 200 μM dNTP (Thermo Scientific, USA), 0.5 μM of each primer, 1U DreamTaq DNA Polymerase (Thermo Scientific, USA) and 20 ng DNA template. The following touch-down program in the thermocycler Biometra TProfessional Standard 96 (Biometra, Germany) was performed: an initial denaturation at 95ºC/5min, followed by 15 cycles each of denaturation at 95ºC/30 s, annealing at 63.5ºC/1min (-0.5ºC/cycle) and extension at 72ºC/1min; another 22 cycles of 95ºC/30 s, 56ºC/1min and 72ºC/1min with final elongation at 72°C for four min. The PCR products were separated by electrophoresis on 8% polyacrylamide gel, with 20 bp molecular weight ladder (Thermo Scientific, USA) as a marker. After staining with ethidium bromide, they were photographed under UV light using BioDocAnalyze gel documentation system (Biometra, Germany). SSR profiles were converted into a binary matrix based on the presence (1) or the absence (0) of a specific allele. Genetic similarity (GS) was calculated in accordance with Dice (1945): GS_{ij} = 2a/2a+b+c; where: a is the number of fragments present in both variety *i* and *j* (1,1), b is the number of fragments present in *i* and absent in *j* (1,0), c is the number of fragments absent in *i* and present in *j* (0,1). Marker data analyses were performed using statistical NTSYSpc2 program package (Rohlf, 2000).

Results and Discussion

The main advantages of the marker assisted selection for simply inherited traits are direct selection of target gene with specific SSR markers (foreground selection) and fast recovery of recurrent parent's genome (background selection). Properties such as high polymorphism, co-dominant inheritance, random and frequent distribution throughout the genome, simple detection at any stage of plant development, independence of environmental conditions, high reproducibility, as well as cost-effectiveness, characterize SSR markers suitable for wide implementation into modern grain breeding programs (Semagn *et al.*, 2006).

In our research, 12 gene-specific markers were tested on parental lines. Ten markers failed to give scorable bands due to absence of amplification product or poor amplification, while two markers showed distinct polymorphism for *Ga1-S* (Table1). The PR1 amplified ~410 bp fragment in donor parents (DPs) and ~390 bp fragment in recurrent parents (RPs). The PR2 amplified ~550 bp fragment in DPs and ~450 bp fragment in RPs. Amplification with these gene-specific SSR markers is given in Figure 1. These markers will be used as foreground selection markers for the incorporation of *Ga1-S* into our inbred lines which will be used for the creation of white kernel hybrids.

Table 1. Primers identified for foreground selection for the *Ga-1* gene

Primer	Reference	Sequence
PR1 F	ID2 F in Liu *et al.*, (2014)	5′-CAAATTGAGCCCATTACC-3′
PR1 R	ID2 R in Liu *et al.*, (2014)	5′-TTCATTCTATTGCGGGTC-3′
PR2 F	SD9 F in Zhang *et al.*, (2012)	5′-GAGAGCTACGCACGACTTAT-3′
PR2 R	SD9 R in Zhang *et al.*, (2012)	5′-CAAGACTTGCACAATCGAGG-3′

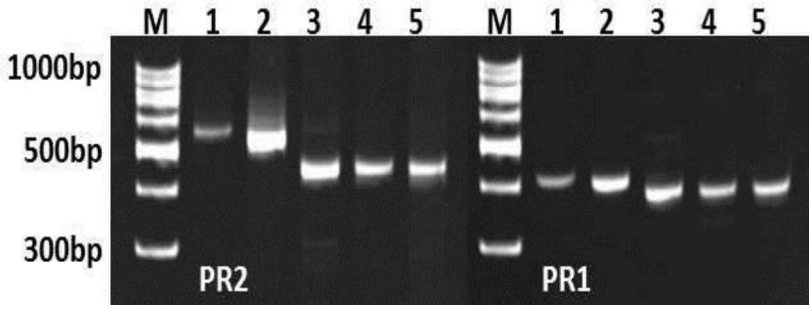

Figure 1. Parental polimorphism with the *Ga-1*-specific markers. M: 100 bp DNA ladder, 1-2: donor parents D_1 and D_2, 3-5: recurrent parents R_1, R_2 and R_3.

Genetic variability between two donor and three recurrent parental inbred lines was analyzed with 42 SSRs distributed over the maize genom. Total number of alleles detected with 30 informative markers was 83, average being 2.77, which is similar to those previously reported in maize inbreds (Bante and Prasanna, 2003; Legesse *et al.*, 2007; Kostadinovic *et al.*, 2018). These SSR markers that showed polymorphism between parental lines will be employed for identification of the genotypes with the highest proportion of recurrent parent's genome in BC_2 generation. The genetic similarity values calculated on Dice coefficient ranged from 0.47 to 0.71 (Table 2). These results can be useful for identifying the most appropriate parental lines to be crossed, i.e. predicting the yields of crosses between lines (Ribaut and Hoisington, 1998).

Table 2. The pairwise genetic similarity values calculated on Dice coefficient between donor (D_1 and D_2) and recurrent parent lines (R_1, R_2 and R_3).

	D_1	**D_2**
R_1	0.52	0.47
R_2	0.56	0.49
R_3	0.71	0.56

As concluded by Zhang *et al.*, (2012), the introduction of *Ga1-S* performed by backcrossing without markers is laborious, lengthy and inefficient because of the difficulty of phenotyping of the segregating population. Since 50% individuals of the backcrossing population do not contain *Ga1-S* allele, the tightly linked markers can greatly improve the efficiency and reliability of the backcrossing process by eliminating those without *Ga1-S* allele prior to pollination, reducing the size of the breeding population and saving both time and money. Also, background selection accelerates recovery of the recurrent parent's genome. According to Hospital *et al.* (1992), two generations can be saved by conducting marker assisted background selection.

Conclusions

Among 12 gene-specific SSR markers tested on parental lines, two markers showed distinct polymorphism for *Ga1-S*. These markers will be used as foreground selection markers for the incorporation of *Ga1-S* gene. Also, the set of 30 SSR markers polymorphic between parental lines is chosen for the background selection. The identification of these markers represents the

first step towards the incorporation of the incompatibility gene *Ga1-S* into parental components of the white kernel hybrids.

Acknowledgement

This research was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia, through the project TR31068 *Improvement of maize and soybean characteristics by molecular and conventional breeding.*

References

Bantte, K., Prasanna B.M. (2003). Simple sequence repeat polymorphism in quality protein maize (QPM) lines. Euphytica, 129: 337-344.

Bloom J.C., Holland J.B. (2011). Genomic localization of the maize cross-incompatibility gene, *Gametophyte factor 1 (ga1)*, Maydica, 56: 379-387.

Dice L.R. (1945): Measures of the amount of ecologic association between species. Ecology, 26: 297-302.

Doyle J.J., Doyle J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull, 19: 11-15.

Hospital F., Chevalet C., Mulsant P. (1992). Using markers in gene introgression breeding programs. Genetics, 132: 1199-1210.

Kermicle, J.L., Evans M.M.S. (2010). The *Zea mays* sexual compatibility gene *ga2*: Naturally occurring alleles, their distribution, and role in reproductive isolation. J. Hered. 101: 737–749.

Kostadinovic M., Ignjatovic-Micic D., Vancetovic J., Ristic D., Obradovic A., Stevanovic M., Mladenovic drinic S. (2018). Parental polymorphism analysis in marker assisted selection for β-carotene rich maize. Proceedings of the IX International Agricultural Symposium "Agrosym 2018", Jahorina, Bosnia and Herzegovina, October 4-7, 333-338.

Lausser A., Kliwer I., Srilunchang K.O., Dresselhaus T. (2010). Sporophytic control of pollen tube growth and guidance in maize. Journal of Experimental Botany 61(3): 673–682.

Legesse, B.W., Myburg A.A., Pixley K.V., Botha A.M. (2007). Genetic diversity of African inbred lines revealed by SSR markers. Hereditas, 144: 10-17.

Liu X., Sun H., Wu P., Tian Y., Cui D., Xu C. et al., (2014). Fine Mapping of the Maize Cross-Incompatibility Locus *Gametophytic Factor 1 (ga1)* Using a Homogeneous Population. Crop Science, 54: 1-9.

Lu Y., Kermicle J.L., Evans M.M.S. (2014). Genetic and cellular analysis of cross-incompatibility in *Zea mays*. Plant Reproduction 27: 19-29.

Mangelsdorf P.C., Jones D.F. (1926). The expression of Mendelian factors in the gametophyte of maize. Genetics, 11: 423-455.

Nelson O.E. (1994). The gametophyte factors of maize. In: Freeling M, Walbot V, editors The maize handbook. Springer-Verlag, Berlin. p. 496–503.

Ribaut J.M., Hoisington D.A. (1998). Marker assisted selection: new tools and strategies. Trends Plant Sci, 3: 236-239.

Rohlf F.J. (2000). NTSYS-pc Numerical Taxonomy and Multivariate Analysis System version 2.1. Owner's manual.

Semagn K., Bjornstad A., Ndjiondjop M. (2006). An overview of molecular marker methods for plants. African Journal of Biotechnology, 5 (25): 2540-2568.

Zhang H., Liu X., Zhang Y., Jiang C., Cui D., Liu H., Li D., Wang L., Chen T., Ning L., Ma X., Chen H., (2012). Genetic analysis and fine mapping of the *Ga1-S* gene region conferring cross-incompatibility in maize. Theoretical and Applied Genetics, 124: 459-465.