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CORRIGENDUM

Ignjatovic-Micic D., D. Ristic, V. Babic, V. Andjelkovic and J. Vancetovic (2015): *Asimple SSR analysis for genetic diversity estimation of maize landraces*.-Genetika, Vol 47, No. 1,53-62. DOI: 10.2298/GENSR1501053I

The Editor in Chief has been informed that a part of the data in the article published in Genetika, vol 47 (1), 2015 was published without proper cross referencing to the date already published in the paper Ignjatovic-Micic, D., D. Ristic, V. Babic, V. Andjelkovic, K. Marković J. Vancetovic (2013): Genetic assessment of maize landraces from former Yugoslavia. Genetika *45(2)*: 405-417. Inspection of these data confirmed the abova claim. To correct these errors the correspoding author of the article submited a corrections. After further disscussion with corresponding author and reviewers, the Edithor in Chief has decided to publish a corrigendum for this article, providing correct date and correction in literature.

List of changes:

ABSTRACT

A collection of 2217 landraces from western Balkan (former Yugoslavia) is maintained at Maize Research Institute Zemun Polje gene bank.*Deleted*

Nine flint and nine dent accessions from six agro-ecological groups (races), chosen on the basis of diverse pedigrees, were analyzed for genetic relatedness using phenotypic and simple sequence repeat (SSR) markers. *Partially (because these are necessary common data) changed to:*Eighteen accessions from six races chosen on the basis of diverse pedigrees (from Maize Research Institute Zemun Polje gene bank), were analysed for genetic relatedness using morphological and simple sequence repeat (SSR) markers.

In the principal component analysis (PCA) the first three principal components accounted for 80.86% of total variation and separated most of the flint from dent landraces. *Connected with Ignjatovic-Micic 2013 and changed to:*It was shown in the previous work that the principal component analysis (PCA) could distinguish between flint and dent landraces.

Ten SSR primers revealed a total of 56 and 63 alleles in flint and dent landraces, respectively, with low stuttering and good allele resolution on the gels.*Partially deleted and changed to:*SSR primers revealed alleles with low stuttering and good allele resolution on the gels.

Both phenotypic and SSR analyses distinguished flint and dent landraces, but neither of them could abstract agro-ecological groups. *Deleted*

Genetic diversity revealed for both markers indicated that the landraces were highly adapted to specific environmental conditions and purposes and could be valuable sources of genetic variability.*Partially deleted and changed to:*Genetic diversity revealed for both markers indicated that the landraces could be valuable sources of genetic variability.

INTRODUCTION

Maize is the most diverse crop containing huge variation in morphological traits and extensive polymorphism in DNA sequences (MATSUOKA et al., 2002). *Deleted*

Carena et al., 2009). Even though this germplasm enables production of high yielding hybrids, its narrow genetic base is adverse for maize adaptation to different stresses and in this context inadequate to meet the contemporary and future major challenges - population growth and climate changes. One of the solutions to this problem lies in the utilization of diversity preserved in maize gene banks (landraces), which represent the raw material that farmers and breeders can use to improve its quality and productivity. *Partially deleted (because these are necessary common data) changed to:*Even though this germplasm enables production of high yielding hybrids, its narrow genetic base is adverse for maize adaptation to different stresses. One of the solutions to this problem lies in the utilization of diversity preserved in maize gene banks (landraces), which represent the raw material that farmers and breeders can use to improve its quality and productivity.

The collection of landraces from western Balkan (former Yugoslavia) was created in 1960-ies (PAVLIČIĆ and TRIFUNOVIĆ, 1968). Eighteen agro-ecological groups were established upon the natural classification method of ANDERSON and CUTLER (1942). A re-classification, conducted by a concurrent analysis of the widest range of morpho-biological traits of all populations in one year, confirmed the validity of the original classification (RADOVIĆ et al., 2000).*First sentence remained – it is the name of the method, Second sentence deleted – not necessary data*

These agro-ecological groups encompass early introduced flint types grown on small and isolated areas, later introduced dent types that spread on wide areas of crop production in river valleys and plains, as well as types created through hybridization between flint and dent genotypes. Today, Maize Research Institute Zemun Polje gene bank maintains a collection of 2217 landraces. The classification methods based on phenotypic traits were improved with the use of DNA markers in the last 15-odd years, with a variety of different techniques for genetic variation analysis (SPOONER et al ., 2005). *First sentence remained – relevant common data,Second sentence deleted – data not important for the research,Third sentence remained – common data that make a link between phenotypic and molecular data analysis*

The objective of this study was to evaluate genetic diversity and relationships of flint and dent accessions belonging to different agro-ecological groups of former Yugoslavia landraces. *In order to clarify the similarity, it was connected with previous work and changed to:*The objective of this study is a part of a program to evaluate genetic diversity and relationships of flint and dent accessions belonging to different agro-ecological groups of former Yugoslavia landraces (Ignjatovic-Micic *et al*., 2013).

MATERIAL AND METHODS

The 18 analyzed accessions from the Maize Research Institute gene bank belong to three flint and three dent agro-ecological groups (Table 1). Each of the group was presented with three landraces in this work. *It was connected with previous work and changed to:*The 18 analyzed accessions from the Maize Research Institute gene bank belong to three flint and three dent agro-ecological groups (Table 1). Each of the group was presented with three landraces in this work, as given in Ignjatovic-Micic *et al.* (2013).

Table 1. Agro-ecological groups, landrace abbreviated name and country of collection of the analyzed maize landraces *It was connected with previous work and changed to:*Table 1. Agro-ecological groups, landrace abbreviated name and country of collection of the analyzed maize landraces (from Ignjatovic-Micic *et al.* (2013).

The abbreviations for each landrace consists of a letter indicating flint (F) or dent (D) affiliation, number of the agro-ecological group (given in Roman numerals) and number of the landrace from the particular agro-ecological group (1, 2 and 3) *Remained, as it explains Table 1 to which the reference was added*

All accessions were sown in Zemun Polje in 2010, at two different sowing densities – 44640 and 64935 plants/ha. The experimental design applied was RCBD (Random Complete Block design) with two replicates, four rows per replicate and 20 plants per row. *Deleted and connected with with previous work* Field experiments and trait analysis are explained in IGNJATOVIC-MICIC et al. (2013).

Principal Component Analysis (PCA) was performed on the phenotypic correlation matrix of the adjusted means of the populations for the 16 traits from the descriptor. The matrix of distances between populations was calculated upon the standardized principal components with eigenvalue higher than one.*Deleted and connected with with previous work* Principal Component Analysis (PCA) was performed as given in IGNJATOVIC-MICIC *et al.* (2013).*One sentence was added before statistical analysis for better explanation:*For higher precision three PCA was performed.

All statistical analyses were performed using program package SPSS 15.0 (http://spss-for-windows-evaluation-version.software.informer.com/).*Remained – it is the name of the method*

SSR analysis was carried out using a DNA bulk analysis with 30 plants per accession. PCR amplification was initially performed with 50 SSR probes, but only ten were chosen for genetic diversity estimation. The amplification reaction was carried out in 25 µl reaction volume containing 2X DreamTaq™ Green PCR Master Mix (Fermentas), 0.5 µ M primers (LKB) and 50 ng of DNA. The amplification was performed in TProfessional Thermocycler (Biometra) with the following profiles: an initial denaturation at 950C/5 min, followed by 15 cycles each of denaturation at 95°C/30 s, annealing at 63.5°C/1 min (–0.5°C/cycle) and extension at 72°C/1 min; another 22 cycles of 95°C/30 s, 56°C/1 min and 72°C/1 min were performed. *First sentence was changed and connected with previous work* DNA isolation, amplification reaction and PCR program were performed as explained in IGNJATOVIC-MICIC *et al.* (2013).*Second and third paragraphs remained, as they explain the protocols which could be informative to the readers.*

Cluster analysis was performed with unweighted pairgroup method (UPGMA) and relationships between landraces were visualized as dendrograms. *Remained – it is the name and presentation of the method*

RESULTS

The 18 analyzed landraces displayed great variation for most traits, but flint landraces were more diverse. The first three principal components accounted for 80.86% of the total variation. In the first PC (57.10%) PH, EH, LN, HLL, NTPB, ERN, NKR, ED, KL and ASI were the most important traits. *Changed into:*Phenotypic analysis showed great variation for the majority of the traits, but dent landraces were less diverse. Eigenvectors, eigenvalues and accumulated variation of the first three principal components (PC) from the correlation matrix based on maize population means is presented in Table 2.

The analyzed landraces were plotted in the area defined by the first three PC (Figure 1). Flint and dent landraces were clearly separated. Majority of flint landrace (except FIV3 and FVII1) had negative PC1 value, while all dent landraces except DXIII3 had positive PC1 value – flint and

dent landrace separation was yet achieved by the PC1 alone. *First sentence remained – there is no other way to say this,Second sentence deleted,Third sentence (bolded part) deleted*

The third PC (7.23%) described variation in EL and KT.*Deleted*

Distribution of the 18 maize landraces on the first three principal components PC1, PC2 and PC3 of the PC performed for phenotypic data. *Remained – it is PCA with three components – no other way to name the Figure*

Most of the 50 SSR primers used for PCR amplification could not be applied for landrace analysis due to the absence of amplification product (two primers), poor amplification (15 primers) or complex band pattern (28 primers). *Deleted*

Total number of alleles found for all landraces was 63, varying from 3 (umc1418) to 9 (umc1274 and umc1492) and with the average value of 6.3. Total number of alleles for dent landraces was 62, with the average value of 6.2. Flint landraces had lower number of alleles (56), with the average value of 5.6. *Deleted*

DISCUSSION

On the other hand, dent types are the most recently introdced types that were the varieties used for development of hybrids. *Remained, relevant common data*

The results of flint and dent landraces' genetic assessment showed large heterogeneity and genetic diversity revealed by both phenotypic and molecular traits indicated that the landraces were highly adapted to specific environmental conditions and purposes through a long period of selection and local adaptation (from XVI century to the present). *Connected to previous work and somewhat changed:*The results confirmed previous findings (IGNJATOVIC-MICIC et al., 2013) that large heterogeneity is revealed by both phenotypic and molecular traits, indicating high adaptability potential throughout the period from XVI century to the present.

Phenotypic analysis showed great variation for most traits. Flint landraces were more diverse. *Connected to previous work:*Phenotypic analysis showed great variation for most traits and flint landraces were more diverse (IGNJATOVIC-MICIC et al., 2013).

It was previously shown that the diversity of flint types is greater compared to dent types, due to their earlier and predominant introduction into this region. *Remained – relevant common data:*

PCA analysis of morphological traits could separate most flint and dent types, but not agro-ecological groups. *Connected to previous work:*As it was shown with two PCA analysis (IGNJATOVIC-MICIC et al., 2013) and confirmed in this paper with three PCA, PCA analysis of morphological traits can separate most flint and dent types, but not agro-ecological groups.

It could be due the small number of landraces per agro-ecological group analyzed and/or the fact that morphological parameters are not the most reliable indicators of genetic relationships, especially when heterogeneous open-pollinated populations are considered. *Connected to previous work:*It could be due the small number of landraces per agro-ecological group analyzed and/or the fact that morphological parameters are not the most reliable indicators of genetic relationships, especially when heterogeneous open-pollinated populations are considered (IGNJATOVIC-MICIC et al., 2013).

The average number of alleles per locus was similar to the average number of alleles found in the analyses of different maize landraces presented in REIF et al. (2005) and CÖMERTPAY et al . (2012). Higher average number was found in DUBREUIL et al. (2006), MATSOUK Aet al. (2002) and SHARMA et al. (2010). Different numbers of alleles detected in different studies can be attributed to several factors,such as the type of SSR loci and whether individual plants or

bulked samples were used.*First sentence – partialy deleted,Second sentence remained,Third sentence - remained*

However, tri-nucleotide or higher repeat motifs are less polymorphic and thus reveal lower number of alleles (VIGOUROUX et al., 2002). Additionally, bulk approach might discard some alleles with frequencies < 0.02 (REIF et al., 2005). *Remained-*

Serbian dents have traits that are intermediate between these two dent types. It was assumed that they originated from Dent type of Southern areas of USA or Mexican dent that had been crossed with flints existing in Serbia at that time (PAVLICIC et al., 1976). *Remained-common data relevant for explaining differences between flints and dents*

In conclusion, both morphological and SSR analyses distinguished most flint and dent landraces, but neither of them could abstract agro-ecological groups. The results revealed a large genetic heterogeneity indicating that the analyzed landraces could be valuable sources of genetic variability. Certain incongruities between the results of morphological and molecular analyses suggest that both phenotypic and genetic studies are necessary for achieving the most accurate assessment of genetic diversity. *First sentence – partially deleted and connected to previous work:*In conclusion, it was confirmed with three PCA and SSR allele frequency analysis that both types of markers can separate flint and dent landraces, but not agro-ecological groups.*Second sentence deleted,Third sentence – remained with a small part deleted* Certain incongruities between the results of morphological and molecular analyses suggest that both phenotypic and genetic studies are necessary of genetic diversity.

References

Two references were deleted due to the deleted text:

RADOVIĆ, G., J. MUMINOVIĆ, D. JELOVAC (2000): Local maize germplasm – potentially valuable breeding material. Genetika *52*: 221-234.

SAGHAI-MAROOF, M., K. SOLIMAN, R. JORGENSEN, R. ALLARD (1984): Ribosomal DNA spacer length polymorphism in barley; Mendelian inheritance, chromosomal location and population dynamics. PNAS *91*: 8014-8018.

Three references were added:

(Ignjatovic-Micic et al., 2013 – due to the connection with previous paper Pavlicic 1973, Pavlicic et al. 1976 – these two were given in the text of the original paper, but were missing from the reference list)

IGNJATOVIC-MICIC, D., D. RISTIC, V. BABIC, V. ANDJELKOVIC, K. MARKOVIĆ J. VANCETOVIC (2013): Genetic assessment of maize landraces from former Yugoslavia. Genetika *45(2)*: 405-417.

PAVLIČIĆ, J. (1973): Prilog poznavanju evolucione srednosti domaćih tipova kukuruza iz naših brdskih krajeva. Contribution to the knowledge of evolutionary relationship between domestic maize types from hilly regions. Genetika 5:95-108.

PAVLIČIĆ, J., D. JELENIĆ and G. RADOVIĆ (1976): Study of some traits of classified Yugoslavian maize types: dent type of Southern areas of USA and Serbian dents. Proučavanje nekih osobina jugoslovenskih klasifikovanih tipova kukuruza: zubani tipa južnih predela SAD i srbijanski zubani. Savremena poljoprivreda XXIV: 5-13.

Table 3 was added as it was missing from the previous paper.(Editorial mistake)

Table 3. Polymorphism information content (PIC), total number of alleles and average number of alleles per SSR loci for all, flint and dent landraces

	Simple Sequence Repeats (SSR)			PIC	Total number of alleles			Average number of alleles		
	Probe	Bin	Repeat motif		All	Flint	Dent	All	Flint	Dent
1	umc1282	1.01	(AT)6	0.849	6	6	6	2.7	2.6	3
2	umc2047	1.09	(GACT)4	0.773	5	5	5	2.9	2.9	3
3	umc1418	4.08	(GGAAG)4	0.750	3	3	3	2	1.2	2.8
4	umc1109	4.10	(ACG)4	0.704	6	5	6	2.2	2.3	2.1
5	umc1274	5.03	(TGC)5	0.904	9	8	9	4.4	4.7	4.7
6	phi087	5.06	(ACC)	0.919	8	6	7	3.4	3.4	3.7
7	umc1393	7.02	(GTC)4	0.814	8	6	8	5.3	5	5.7
8	umc1324	7.03	(AGC)5	0.748	4	4	4	3	3	2.9
9	umc1492	9.04	(GCT)4	0.839	9	8	9	5	5.1	5
10	umc1827	10.04	(GAC)6	0.918	5	5	5	3	3.7	2.3
Average				*0.822*	*63*	*56*	*62*	*3.4*	*3.4*	*3.5*