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Assessing polymorphism within common bean (*Phaseolus vulgaris* L.) mutant lines originated from variety "Mastilen 11b" using Inter Simple Sequence Repeats markers

Sibel Aziz[1,4], Velichka Spasova-Apostolova[2,5], Veselina Masheva[3], Nasya Tomlekova[1*]

[1]*Agricultural Academy, Maritsa Vegetable Crops Research Institute, Breeding Department, 4003 Plovdiv, Bulgaria*
[2]*Agricultural Academy, Tobacco and Tobacco Products Institute, Department of Breeding and Seed Production, 4108 Markovo, Bulgaria*
[3]*Agricultural Academy, Institute of Plant Genetic Resources "Konstantin Malkov", Department of Plant Genetic Resources, 4122 Sadovo, Bulgaria*
[4]*University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Developmental Biology, 4000 Plovdiv, Bulgaria*
[5]*Agricultural University, Department of Microbiology and Environmental Biotechnology, 4000 Plovdiv, Bulgaria*
[*]*Corresponding author:* nasia.tomlekova@gmail.com

Abstract

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The common bean (*Phaseolus vulgaris* L.) is an economically important crop on a global scale, including in Bulgaria as well. It is an excellent source of vegetable protein and other nutrients. One of the methods of modern plant breeding aimed at creating genetic diversity in beans is induced mutagenesis. Molecular techniques are a suitable tool for characterizing induced mutations. The objective of this work is to evaluate the genetic diversity of a collection of EMS-induced mutant bean lines and to identify specific profiles, using ISSR molecular markers. The initial variety "Mastilen 11b" and 15 mutant lines from the collection of the Maritsa Vegetable Crops Research Institute are analyzed with 14 ISSR reactions. A total of 187 fragments with lengths from 190 bp to 3100 bp are generated. Three of the reactions (ISSR 6, ISSR 8, ISSR 9) led to a generation of monomorphic profiles, and the remaining eleven reactions (ISSR 1, ISSR 2, ISSR 2T, ISSR 3, ISSR 4, ISSR 5, ISSR 7, ISSR 10, ISSR 12, UBC 807, UBC 809) amplified polymorphic profiles. The polymorphism ranged from 18.65 % to 87.50 % in the analyzed genotypes. The results showed that the applied ISSR markers are effective for assessing the existing genetic diversity of the studied bean mutant lines.

Keywords: Phaseolus vulgaris L., mutant lines, ISSR markers

Introduction

The common bean (*Phaseolus vulgaris* L.) was domesticated more than 7000 years ago in two centers of origin – Meso-America (Mexico and Central America) and the Andean region (Singh et al., 2013). *Phaseolus vulgaris* L. is a very important component of the diet of people in several continents, representing an important source of minerals and proteins (Gepts et al., 2008). Common beans are primarily grown for home or local consumption in the developing

world, while in the developed world they are primarily grown for processing uses and for exports (Smykal et al., 2015). Beans from the primary domestication centers in the Americas were transported and distributed to the Balkan Peninsula, including Bulgaria, which is considered a secondary diversity center (Gepts et al., 1986; Tomlekova et al., 2016). One effective source of solutions to problems in bean production is the great genetic diversity (Singh et al., 2013). Experimental mutagenesis is applied in crop breeding in different crops in Bulgaria. Back in 1979, a breeding programme with the application of induced mutagenesis in French bean started at the Maritsa Vegetable Crops Research Institute (Maritsa VCRI) in Plovdiv (Tomlekova, 2010). Induced mutagenesis is one way to increase genetic diversity and is used when the culture is missing the desired properties. The new varieties developed by experimental mutagenesis attain a special place in Bulgarian agriculture because of their high productivity, wide ecological adaptability, and suitability for mechanized growing and harvesting, as well as good resistance to diseases and pests and high-quality nutritional parameters. A high frequency of spontaneous mutations that exceeds other plant species was described in the common bean, *Phaseolus vulgaris* L. (Rukmanski, 2005). It was observed that most morphological characters could be mutated in common bean after applying gamma rays or chemical mutagens. The efficiency of selection is further increased with the application of molecular techniques to assess genetic potential.

Molecular markers have been shown to be effective indicators for genetic variation underlying agronomic traits with some advantages over morphological traits. Molecular markers span broader genomic areas and present different types of inheritance, so they have also been used to better estimate the levels of diversity and to understand the effects of migration and selection on the maintenance of polymorphism in the European beans (Lioi et al., 2013). In beans, molecular markers have been used in breeding programs in a variety of ways (Santalla et al., 2010; de Campos et al., 2011) including studies on the origin and diversity of common bean varieties (Blair et al., 2009; Rossi et al., 2009), genetics of resistance to important diseases (Miklas et al., 2006; Shi et al., 2011) and abiotic restrictions (Chen et al., 2010). The mutant lines included in this study were tested for resistance to halo blight *Pseudomonas savastanoi* pv. *phaseolicola* (*Psp*) and bacterial blight *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) (Sofkova et al., 2021). Some molecular studies are based on random PCR markers, such as RAPDs (Mavromatis et al., 2010), iPBS (Nemli et al., 2015, Aydin et al., 2015, Ozturk et al., 2020), ISSRs and AFLP (Svetleva et al., 2006; Šustar-Vozlic et al., 2006; Lioi et al., 2013). The most effective for the evaluation of mutant genotypes are the molecular marker systems based on the most variable regions in the genome like restriction sites, mobile elements, and microsatellites. ISSRs molecular markers have proved to be reliable, highly reproducible, and easy to develop; in addition, they are able to identify an important number of polymorphisms even in autogamous crops, such as beans, despite their high genetic homogeneity, thereby making them very useful for breeding purposes in these species (Marotti et al., 2007).

In the present study, we describe the assessment of genetic diversity among the mutant lines of bean obtained after a mutagenic treatment of initial variety "Mastilen 11b" through the use of ISSR molecular marker technique.

Materials and Methods

Plant Material

This study included 15 mutant bean lines from the Maritsa Vegetable Crops Research Institute collection, obtained after treatment with a chemical mutagen 0.0062 M Ethyl Methanesulfonate (EMS) of initial variety "Mastilen 11b", characterized by average height and sensitive to bacterial blight *Xanthomonas axonopodis* pv. *phaseoli* and halo blight *Pseudomonas savastanoi* pv. *phaseolicola* (Sofkova et al., 2021). They have been pre-phenotypically assessed and have valuable economic qualities. The developed mutant lines have valuable qualities for bean breeding such as high productivity, resistance to biotic and tolerance to abiotic factors. The genotypes included in the present study are in M_6, M_7 generations.

Molecular methods

Plant DNA was isolated from young leaves according to the CTAB protocol (Murray et al., 1980, Saghai – Maroof et al., 1984) including an RNAse A treatment. DNA quality and concentration are determined on 1 % LE Agarose gels (Lonza, Cat.No.50004, USA) containing Ethidium bromide (VWR International, Vienna, Austria). To accurately determine the DNA concentration, a lambda DNA with a known concentration (50 ng, 20 ng, 10 ng, 5 ng) was prepared and used for comparison. PCR reaction is performed in 25 µL volume mixture containing 20 ng genomic DNA, 0.2 mM dNTPs mix (Thermo Scientific, Cat. No.R0192, Lithuania), 2.5 µL of 10x Green buffer (Thermo Scientific, Cat. No B71, Lithuania), 0.2 µL of 5U Dream *Taq* DNA Polymerase (Thermo Scientific, Cat. No.EP0702, Lithuania), 2.5 µL of 10 µM primers (VBC-Genomic, Vienna, Austria and Eurofins). The conditions for amplification are: initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, Ta (47 ºC – 60 ºC) for 45 s, 72 °C for 2 min; followed by a final elongation of 72 °C for 7 min. Reactions were performed in a 96 – well Bio-Rad thermocycler (C 100 Touch Thermal Cycler with dual 48/48

fast reaction module, Cat. No.1851148, USA). The compiled profiles for each of the reactions included the lengths of the amplified fragments generated in all analyzed accessions compared to the DNA Ladder Gene Ruler 100 bp PlusDNA used (Thermo Scientific, Cat. No. SM0321, Lithuania).

The primers used in the present study are borrowed from the literature applied to tomatoes, peppers, and tobacco. The primers ISSR 2, ISSR 3, ISSR 5, ISSR 6, ISSR 8, and ISSR 9 are selected according to Tomlekova et al. (2006; 2012). The primers ISSR 1, ISSR 2T, ISSR 4, ISSR 7, ISSR 10, and ISSR 12 are selected according to Tsonev et al. (2017). The two ISSR primers at UBC (University of British Columbia) are selected according to Denduangboripant et al. (2010). The resulting PCR products are separated on 2 % LE Agarose gels, prestained/ destained with Ethidium bromide, in a standard 1×TAE buffer. The visualization of ISSR reactions results is performed using the gel-documentation system AZURE Biosystems C600 (Biosystems, Dublin, USA).

Data analysis

Data processing (percentages of polymorphic profiles and polymorphic fragments) is expressed as a percentage of a number by a formula PG % or P % =X / Y x 100 %.

Where X is the number of polymorphic profiles or fragments and Y is the total number of generated profiles or total amplified fragments, respectively. The percentage of polymorphic fragments from the eleven ISSR reactions is represented by Table 1. Band profiles generated by ISSR are completed onto a data matrix based on the presence (1) or absence (0) of selected bands. The obtained results are statistically processed by SPSS Statistics software, using Pearson correlation coefficient (r), and constructed a general UPGMA (Unweighted Pair Group Method Arithmetic Averages) dendrogram for the eleven polymorphic ISSR reactions (Fig. 3). Pearson's correlation coefficient is the covariance of the two variables divided by the product of their standard deviations. Two equations are used to calculate the distance matrix, and in our case, they are automatically generated in the program. Conditionally, the correlation coefficient ranges from minus 1 to 1. An r value of minus 1 suggested a strong negative correlation, 0 suggested no relationship at all, and 1 suggested a strong positive relationship. Other statistic method used this study generate a binary data matrix, which was analyzed using SPSS Statistics software, with multidimensional scaling option (ALSCAL). This is a graphical method for visualizing the proximity and difference between the units. The graphic show the distribution of the mutant lines, including their initial genotype based on the eleven ISSR reactions (Fig. 4). These data complement the information obtained from the molecular profiles and confirm the clear differentiation between the mutant lines bean.

Results and Discussion

The 14 ISSR markers tested generated a total of 187 fragments with lengths from 190 bp to 3100 bp. A total of 78 % of the reactions performed resulted in amplification of polymorphic profiles in the analyzed genotypes. Three reactions with primers ISSR 6, ISSR 8 and ISSR 9 generated fully monomorphic profiles. These reactions resulted in amplification of a total of 24 fragments with lengths from 300 to 2000 bp (Fig. 1).

The other eleven reactions amplified polymorphic profiles (Fig. 2 and Table 1). Table 1 summarizes the details of fragments of the polymorphic profiles.

Panel A (lanes No.1 – No.8) – are amplified by ISSR 1; panel B (lanes No.9 – No.11) – by ISSR 2; panel C (lanes No.12 – No.24) – ISSR 2T; panel D (lanes No.25 – No.27) – by ISSR 3; panel E (lanes No.28 – No.39) – by ISSR 4; panel F (lanes No.40 – No.54) – by ISSR 5; Panel G (lanes No. 55 – No.62) – by ISSR 7; lanes H (No.63 – No.73) – by ISSR 10; panel I (No.74 – No.81) – by ISSR 12; panel J (lanes No.82 – No.86) – by UBC 807; panel K (lanes No.88 – No.101) – by UBC 809. The first lane of each panel is the

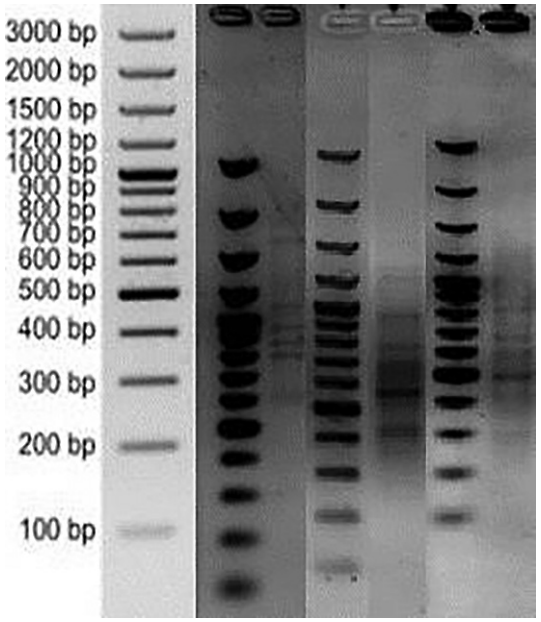

Fig. 1. Monomorphic profiles amplified by each ISSR reaction (ISSR 6, ISSR 8, ISSR 9) in mutant lines of common bean. Lane 1, 3, 5 – MMW; Lane 2 – profile ISSR 6, Lane 4 – profile ISSR 8, Lane 6 – profile ISSR 9

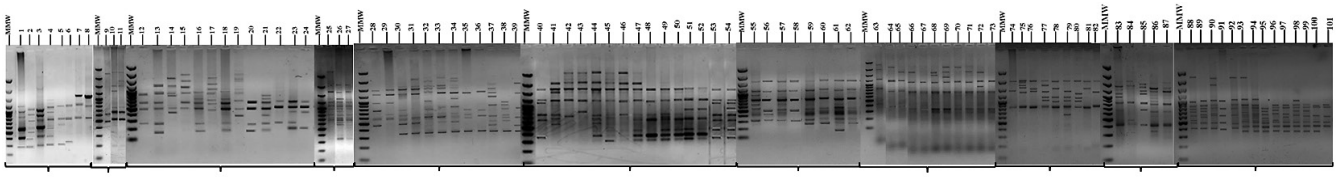

Fig. 2. Polymorphic profiles within bean mutant lines and their initial line "Mastilen 11b"

profile of the initial variety amplified by the different ISSR reactions – lanes 1, 9, 12, 25, 28, 53, 55, 63, 74, 82, 88.

The highest percentage of polymorphic profiles of the mutants is amplified by ISSR 2T, ISSR 4, ISSR 5 and UBC 809 reactions. All amplified polymorphic reactions are presented in Table 1 with values on polymorphic bands and profiles. The eleven reactions the polymorphic fragments were amplified, leading to the generation of polymorphic profiles in the studied accessions.

Eight polymorphic profiles among the studied genotypes were amplified by ISSR 1 reaction. The profile of the initial bean variety "Mastilen 11b" (Fig. 2, panel A, lane 1) is monomorphic with six mutant lines. The rest (9) genotypes possess polymorphic profiles compared to the initial one. The profile of mutant line M-195-2 (lane 2) differs from the initial genotype, as well as from the other mutant lines. Mutant lines M-197-3 and M-198-1 have monomorphic profiles and it is polymorphic and differs from the other analyzed genotypes (Fig. 2, panel A, lane 3). Profiles of M-203-1, M-205-3, M-207-2, M-207-4 (Fig. 2, panel A, from lane 4 to lane 8) are unique. Mutant lines M-212-1 and M-2012-4 are monomorphic each other, and polymorphic with all the rest of analyzed genotypes. According to the number of amplified fragments profiles 7th and 8th amplify the same number of fragments, but the fragments are with different lengths. All these lines differ from the initial line "Mastilen 11b" with ISSR1 reaction.

Three polymorphic profiles are amplified by ISSR 2 reaction. The profile of the initial genotype "Mastilen 11b" is monomorphic with eight of the mutant lines and is polymorphic with the other seven studied lines. Five of the mutant lines (M-198-1; M-203-1; M-205-3; M-207-2; M-207-4) amplify a profile shown in lane 10, and two mutant lines (M-212-1; M-2012-4) profile in lane 11 in Fig. 1, panel B.

In the reactions performed by ISSR2T, high level of polymorphism 75 % was found, which distinguishes twelve polymorphic profiles within the studied sixteen representatives (Table 1, Fig. 2). Mutant lines M-196-1 and M-197-1 amplify a monomorphic profile (lane 16 and lane 18). Two other mutant lines M-198-1 and M-203-1 are presented with profile of lane No.21. Mutant lines M-207-4, M-212-1, and M-2012-4 are described by profile of lane No.24. These monomorphic genotypes are polymorphic with the initial variety and the other studied mutant lines. Eight mutant lines (M -195-1, M-195-2, M-195-3, M-196-3, M-197-2, M-197-3, M-205-3, M-207-2) amplify unique profiles by ISSR2T reaction (Fig. 2, panel C, lanes 13, 14, 15, 17, 19, 20, 22, 23).

Table 1. Amplified polymorphic ISSR reactions with values on polymorphic bands and polymorphic profiles.

ISSR primers	G	PP	PG %	T	Fragment size (bp)	P	P %
ISSR 1	16	8	50.00	17	300-2000	15	88.00
ISSR 2	16	3	18.75	8	200-2500	3	18.75
ISSR 2T	16	12	75.00	15	150-2000	13	87.00
ISSR 3	16	3	18.75	16	350-3100	6	37.50
ISSR 4	16	12	75.00	13	350-2000	13	100.00
ISSR 5	16	14	87.50	20	320-3000	18	90.00
ISSR 7	16	8	50.00	21	150-2000	15	71.40
ISSR 10	16	11	68.75	9	400-2800	6	66.60
ISSR 12	16	9	56.25	13	700-2100	10	76.90
UBC 807	16	5	31.25	17	300-2000	7	41.20
UBC 809	16	14	87.50	14	400-2800	12	85.70

* G = Number of analyzed genotypes, PP = Polymorphic profiles, PG % = Percentage of polymorphic genotypes, T = Total number of bands, P = Polymorphic bands, P % = Percentage of polymorphic bands.

Three polymorphic profiles are amplified by ISSR 3 reaction, and only one generated a unique profile in mutant line – M-203-1 (Fig. 2, panel D, lane 27). Seven of the mutant lines amplify a monomorphic profile with the initial variety (Fig. 2, panel D, lane 25). Of the 16 genotypes studied, 6 mutant lines generated a monomorphic profile that is polymorphic with the other studied (Fig. 2, panel D, lane 26).

Another reaction amplifying highly polymorphic patterns is ISSR 4. The profiles with this reaction are presented in Fig. 2, panel E from lane 28 to lane 39. The initial genotype "Mastilen 11b" (lane 28) amplifies a unique profile that is polymorphic to all mutant lines. The profile presented in Fig. 2, lane 36 is typical for two mutant lines of common bean – M-197-3 and M-198-1, and the profile presented as lane 39 is amplified in four mutant lines – M-207-2, M-207-4, M-212-1, M-2012-4, monomorphic to each other but polymorphic to all the rest of studied genotypes, which are unique.

ISSR 5 reaction identified eleven mutant lines as well as the initial genotype with individual characteristic profiles. The genotypes in Fig. 2, panel F, lanes 51 – 52 are with a monomorphic profile, `however they are polymorphic with the other genotypes. The ISSR profile of "Mastilen 11b" differs by one fragment from M-195-1 (lane 54) and M-195-2 (lane 40).

The ISSR 7 reaction amplified eight polymorphic profiles in the analyzed bean genotypes. The initial variety "Mastilen 11b" differs from the treated representatives and amplifies a unique profile, which is presented by lane 55 (Fig. 2, panel G). The mutant lines M-195-1 and M-195-2 amplify a monomorphic profile with each other, but polymorphic with all others (lane 56), while the profile of lane 57 is amplified in M-195-3 – M-196-3. The profile of a mutant line M-196-1 is present on lane 58, and mutant lines – M-197-1 and M-197-2 are presented in lane 59. Three other bean mutant lines (M-197-3; M-198-1; M-203-1) amplify a monomorphic profile, (Fig. 2, lane 60). The profile of lane 61 is characterized three mutant lines (M-205-3; M-212-1; M-2012-4) and lane 62 is amplified in two mutant lines (M-207-2, M-207-4).

The ISSR molecular profiles of the lanes from 63 to 73 in Fig. 2, panel H, present the results obtained with the ISSR 10 reaction. The polymorphism with this reaction is generated by longer fragments, but they are reproducible in most of the mutant lines and are therefore taken into account in characterization and molecular identification. The initial genotype amplified a monomorphic profile with four of the mutant lines bean (M-195-1, M-195-2, M-196-3, M-197-1) from the analyzed population, and their profile is shown with lane 63. The other mutant lines are characterized by unique polymorphic profiles that amplify one to two additional fragments compared to the initial variety. Lane 64 is amplified profile for M-195-3 and M-207-2, lane 65 – M-196-1, lane 66 – M-197-2, lane 67 – M-197-3, lane 68 – M-198-1, lane 69 – M-203-1, lane 70 – M-205-3, lane 71 – M-207-4, lane 72 – M-212-1, lane 73 – M-2012-4.

The results show that with ISSR 12 reaction the initial genotype "Mastilen 11b" amplifies a monomorphic profile with the mutant line M-195-1, represented as lane 74 in Fig. 2. Four mutant lines from this population have a monomorphic profile, which is polymorphic with both the initial and the other lines (M-198-1, M-203-1, M-205-3, and M-207-4), shown in Fig. 2 with lane 80. Mutant lines M-212-1 and M-2012-4 are monomorphic, amplifying three fragments and the profile is shown on lane 81. The profile of lane 76 identifies the mutant lines M-195-3 and M-196-1. Six of the mutant lines with this reaction amplify unique profiles polymorphic to all others. The results described in Fig. 2, panel I, show lane 75 – M-195-2, lane 77 – M-196-3, lane 78 – M-197-1, lane 79 – M-197-2, lane 80 – M-197-3, lane 82 – M-207-2.

"Mastilen 11b" is shown in Fig. 2, panel J, lane 83, which is polymorphic to the treated accessions in the ISSR reaction with UBC 807 primer. Profile of lane 84 is characterized by two mutant lines – M-195-1, and M-195-2. Lane 85 presents the profile of M-196-1, and lane 86 – M-196-3, M-197-3, and M-203-1. The other mutant lines belong to the profile of lane 87. With the UBC 809 ISSR reaction, fourteen polymorphic profiles are distinguished. The initial genotype is polymorphic and is shown in Fig. 2, lane 88. The lane 89 is typical for M-195-1, lane 90 – M-195-2, lane 91 – M-195-3, M-197-2, M-198-1, lane 92 – M-196-1, lane 93 – M-196-3, lane 94 – M-197-1, lane 95 – M-197-3, lane 96 – M-203-1, lane 97 – M-205-3, lane 98 – M-207-2, lane 99 – M-207-4, lane 100 – M-212-1, lane 101 – M-2012-4, and differentiate the mutant lines compared to the initial genotype – "Mastilen 11b" (Figure 3).

UPGMA dendrogramme demonstrate the distribution of the studied genotypes with all the eleven polymorphic ISSR reactions. The estimated cophenetic coefficient of correlation is 0.969. This value indicates that the results obtained are statistically significant. The calculated matrix correlation value in our study is close to 1 and suggests a strong positive relationship and shows genetic distance. In the study of Svetleva et al. (2006) 78 bean genotypes, include 32 of Bulgarian origin and 46 of foreign origin analysis with thirteen ISSR primers and report a similarity matrix value of 0.895 suggesting a very good fit of the tree representation to the rough data values. The authors report also a very high similarity coefficient (0.916) expected between the varieties "Plovdiv 11M" and "Plovdiv 15M", because they are mutants obtained from the same initial variety "Dobroudjanka 2" (Svetleva et al., 2006).

For technical reasons most authors, such as Young and Harris (2004) encourage the use of dissimilarities as input to the Multidimensional Scaling (MDS) programme, because their relationship to distances is direct and positive (Giguère, 2006). MDS algorithms such as SPSS ALSCAL use the Euclidian model as a basis to compute optimal distances (Figure 4).

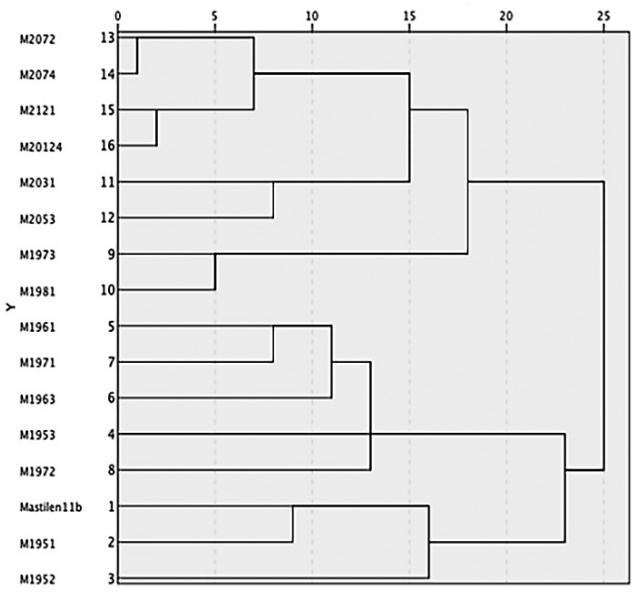

Fig. 3. UPGMA dendrogramme clustering of bean mutant lines and initial variety by using data of ISSR analyses

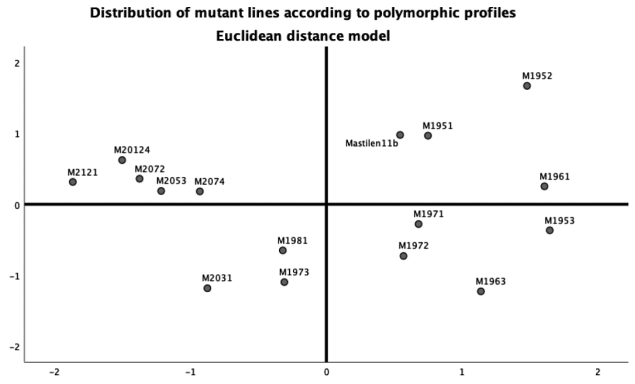

Fig. 4. Distribution of bean mutant lines and initial variety by using multidimensional scaling (ALSCAL) analysis

The correlation coefficient obtained in the present study of bean mutant collection developed at the Maritsa VCRI compared with the initial variety "Mastilen 11b" show relative high dissimilarity. The results show a significant role of the molecular markers, in particular, the ISSR markers in the assessment of genetic diversity in mutant lines of bean induced by chemical treatment. They are an important tool for the detection and practical use of polymorphisms. After the development of suitable molecular markers, their application is relatively inexpensive and leads to a very rapid result (Mohan et al., 1997) and they can be successfully applied in the breeding of vegetable crops (Michelmore et al., 1991, Powell et al., 1996). Primers UBC-807 and UBC-809 are used to study the relationship between domestic and introduced tobacco varieties (Denduangboripant et al., 2010). In tobacco, with the first reaction polymorphic fragments (680 bp and 780 bp) are amplified in nine introduced and two local varieties ("Chorlare 1" and "Chorlare 2") from the Chiang Mai Province). The effectiveness of the reaction with the second primer is also confirmed after testing all 53 introduced tobacco varieties. The present study confirms the effectiveness of the same two primers in the Bulgarian bean collection, and a total of nineteen polymorphic profiles are identified with the two primers. Zargar et al. (2016) analyzed 51 genotypes of beans from India with 15 RAPD reactions and found 100 % polymorphic fragments. Similar to our data obtained with primer UBC 807, Muthusamy et al. (2008) reported low polymorphism with this reaction in rice bean (*Vigna umbellata* L.) landraces. In a study performed on mutant tomato lines by Tomlekova et al. (2012), primer ISSR 5 amplified a polymorphic fragment in the initial genotype, which is absent in the analyzed mutant accessions. In our studies with bean mutant lines, this reaction generated high polymorphism by identifying 14 mutant lines, including initial variety "Mastilen 11b" of a total 16 analyzed genotypes. In a study by Tomlekova et al. (2012) reports for the 5 polymorphic fragments with two ISSR primers generated between the control and mutant tomato genotypes. The reactions with ISSR 2 and ISSR 3 primers in mutant tomato lines do not lead to amplification of fragments (Tomlekova et al., 2012) in contrast to beans where, although low, polymorphism is found in the analyzed genotypes. Each of these reactions is amplified by three profiles in our study. These results showed the detection ability of microsatellite variations and identify genotypes in both radiation-induced and chemically induced mutagenesis. In studies by Tsonev et al. (2017) is reported that in pepper there are two non-reproducible primers with trinucleotide repeats (ISSR 8 and ISSR 9). In our study these primers amplify monomorphic profiles. With two other reactions (ISSR 7, ISSR 12), the results obtained in mutant bean lines show

higher polymorphism, compared to the results reported by Tsonev et al. (2017) for pepper. In a study by Marotti et al. (2007) eight ISSR primers produced a total of 130 amplified fragments, and 110 of them were polymorphic (84.6 %). The highest polymorphism was reported with primer LOL9 (CAC) 3GC. This primer is not used in our study, but may be preferred in future analysis for bean genotyping.

The study demonstrates the applicability of the ISSR technique as an effective method for screening mutant bean lines and differentiating even closely related genotypes in the studied collection. For future studies, the use of more and different types of markers would enable accumulation of more detailed data to understand the genetic variability of breeding materials. They could be useful sources of novel genetic variability that have not been exploited until now. ISSR markers are effective in distinguishing genetically the evaluated mutant lines despite their expected high homozygosity due to the autogamous nature of this crop. The established polymorphic profiles are good starting point for the early identification of mutant genotypes when they are included as starting material in various cross-breeding programs.

Conclusion

Of the fourteen conducted, eleven ISSR reactions amplify polymorphic fragments selected to be high efficient markers for genotyping bean. Of the eleven polymorphic reactions, seven are efficient to characterize the initial variety "Mastilen 11b" by a unique ISSR profile different from the mutant lines. The mutant line M-207-4 can be identified by two reactions. Five of the mutant lines are identified with unique profiles by three ISSR reactions (M-195-3, M-198-1, M-207-2, M-212-1, M-2012-4), and mutant line M-195-1 is identified by four reactions. Other seven bean mutant lines (M-195-2, M-196-3, M-197-1, M-197-2, M-197-3, M-203-1, M-205-3) are identified with five ISSR reactions A genotype (M-196-1) is identified with seven of total eleven polymorphic ISSR reactions in this study.

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References

Aydin, M.F. & Baloch, F.Sh. (2019). Exploring the genetic diversity and population structure of Turkish common bean germplasm by the iPBS – retrotransposons markers. Legume Research, *42*, 18–24.

Blair, M.W., Díaz, L.M., Buendía, H.F. & Duque, M.C. (2009). Genetic diversity, seed size associations and population structure of a core collection of common beans (*Phaseolus vulgaris* L.). *Theoretical Applied Genetics*, *119*, 955–972.

Chen, L., Mi, X., Comita, L.S., Zhang, L., Ren, H. & Ma, K. (2010). Community-level consequences of density dependence and habitat association in a subtropical broad-leaved forest. *Ecologica Letter*, *13*, 695–704.

Denduangboripant, J., Setaphan, S., Suwanprasart, W. & Panha, S. (2010). Determination of local tobacco cultivars using ISSR molecular marker. *Chiang Mai Journal Scientific*, *37(2),* 293–303.

Gepts, P., Osborn, T. C., Rashka, K. & Bliss, F. A. (1986). Phaseolin protein variability in wild forms and populations of the common bean (*Phaseolus vulgaris*): Evidence for multiple centers of domestication. Economic Botany, *40*, 451–468.

Gepts, P., Francisco, J.L., Barros, A.E., Blair, M.W., Brondani, R., Broughton, W., Galasso, I., Hernández, G., Kami, J., Lariguet, P., McClean, Ph., Melotto, M., Miklas, Ph., Pauls, P., Harand, A.P., Porch, T., Sánchez, F., Sparvoli, F. & Yu, K. (2008). Genomics of Phaseolus beans, a major source of dietary protein and micronutrients in the Tropics. Springer, Genomics of Tropical Crop Plants, Chapter 5, 113–143.

Giguère, G. (2006). Collecting and analyzing data in multidimensional scaling experiments: A guide for psychologists using SPSS. Tutorials in Quantitative Methods for Psychology, *2(1)*, 26–37.

Lioi, L. & Piergiovanni, A.R. (2013). European common bean. Genetic and genomic resources of grain legume improvement, Elsevier, 11–34.

Marotti, I., Bonetti, A., Minelli, M., Catizone, P.& Dinelli, G. (2007). Characterization of some Italian common bean (*Phaseolus vulgaris* L.) landraces by RAPD, semi-random and ISSR molecular markers. *Genetic Resources and Crop Evolution*, *54*, 175–188.

Mavromatis, A.G., Arvanitoyannis, I.S., Korkovelos, A.E., Giakountis, A., Chatzitheodorou, V.A. & Goulas, C.K. (2010). Genetic diversity among common bean (*Phaseolus vulgaris* L.) Greek landraces and commercial cultivars: Nutritional components, RAPD and morphological markers. *Spanish Journal of Agricultural Research*, *8(4)*, 986–994.

Michelmore, R.W., Paran, I. & Kesseli, R.V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. National Academy of Science, USA, *88(21)*, 9828–9832.

Miklas, P.N., Kelly, J.D., Beebe, S.E. & Blair, M.W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica*, *147*, 105–131.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T. G., Yano, M., Bhatia, C.R. & Sasaki, T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding*, *3*, 87–103.

Murray, M.G. & Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research, 8(19),* 4321–4326.

Muthusamy, S., Kanagarajan, S. & Ponnusamy, Sh. (2008). Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (*Vigna umbellata*) landraces. *Electronic Journal of Biotechnology*, *11(3)*, 1–10.

Nemli, S., Kianosh, T. & Tanyolac, B. (2015). Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) accessions through retrotransposon-based interprimer binding sites (iPBSs) markers. *Turkish Journal of Agriculture and Forestry*, *39*, 940–948.

Ozturk, H.I., Dursun, A., Hosseinpour, A. & Haliloglu, K. (2020). Genetic diversity of pinto and fresh bean (*Phaseolus vulgaris* L.) germplasm collected from Erzincan province of Turkey by inter-primer binding site (iPBS) retrotransposon markers. *Turkish Journal of Agriculture and Forestry*, *44*, 417–427.

Powell, W., Morgante, M., Andre, Ch., Hanafey, M., Vogel, J., Tingey, S. & Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, *2*, 225–238.

Rukmanski, G. (2005). Role of the mutations in the living nature, Gemplant, 128.

Saghai-Maroof, M.A., Soliman, K.M., Jorgenses, R.A. & Allard, R.W. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics (ribosomal DNA spacer-length variation / restriction fragment-length polymorphisms / Rrnl / Rrn2). National Academy of Science, USA, 81, 8014–8018.

Santalla, M., De Ron, A.M. & De La Fuente, M. (2010). Integration of genome and phenotypic scanning gives evidence of genetic structure in Mesoamerican common bean (*Phaseolus vulgaris* L.) landraces from the southwest of Europe. *Theoretical Applied Genetics*, *120*, 1635–1651.

Shi, Ch., Navabi, A. & Yu, K. (2011). Association mapping of common bacterial blight resistance QTL in Ontario bean breeding populations. *BMC Plant Biology*, *11*, 1–11.

Singh, M., Upadhyaya, H. & Bisht, I. (2013). Common bean. Genetic and genomic resources of grain legume improvement, Elsevier, 2–10.

Smykal, P., Coyne, C.J., Ambrose, M.J., Maxted, N., Schaefer, H., Blair, M.W., Berger, J., Greene, S.L., Nelson, M.N., Besharat, N., Vymyslický, T., Toker, C., Saxena, R.K., Roorkiwal, M., Pandey, M.K., Hu, J., Li,Y.H., Wang, L.X., Guo, Y., Qiu, L.J., Redden, R.J. & Varshney, R.K. (2015). Legume crops phylogeny and genetic diversity for science and breeding. *Critical Reviews in Plant Sciences*, *34,* 43–104.

Šustar-Vozlic, **J., Maras, M., Kavornik, B. & Meglic, M.** (2006). Genetic diversity and origin of Slovene common bean (*Phaseolus vulgaris* L.) germplasm as revealed by AFLP markers and phaseolin analysis. *Journal of American Horticultural Science*, *131(2)*, 242–249.

Svetleva, D., Pereira, G., Carlier, J., Cabrita, L., Leitao, J. & Genchev, D. (2006). Molecular characterization of *Phaseolus vulgaris* L. genotypes included in Bulgarian collection by ISSR and AFLP™ analyses. *Scientia Horticulturae*, *109*, 198–206.

Tomlekova, N. (2010). Induced mutagenesis for crop improvement in Bulgaria. *Plant Mutation Reports, 2(2),* 4–27.

Tomlekova, N. (2006). Introduction of protocols for the establishment of ISSR markers in the *Capsicum annuum* genome. Proceedings of First International Symposium "Ecological Approaches towards the Production of Safety Food", 2006, 107–112.

Tomlekova, N., Yancheva, S., Balacheva, E. & Atanasova, B. (2012). Molecular identification of tomato mutant lines. Bioremediation, *Biodiversity and Bioavailability, 6(1),* 58–64.

Tomlekova, N., Sofkova-Bobcheva, S., Sarsu, F. & Baudoin, J.P. (2016). Genetic diversity of Bulgarian *Phaseolus vulgaris* L. based on phaseolin type and seed-coat colour. *Bulgarian Journal of Agricultural Science*, *22(3),* 447–451.

Tsonev, S., Todorova, V., Grozeva, S., Popova, T. & Todorovska, E. (2017). Evaluation of diversity in Bulgarian pepper cultivars by agronomical traits and ISSR markers. *Genetika, 49(2),* 647–662.

Young, F.W. & Harris, D.F. (2004). Multidimensional scaling. In: Norusis, M.J. (Ed.), SPSS 13.0 Advanced Statistical Procedures Companion, 287-354. Upper Saddle River, NJ, Prentice-Hall.

Zargar, S.M., Farhat, S., Mahajan, R., Bhakhri, A. & Sharma, A. (2016). Unraveling the efficiency of RAPD and SSR markers in diversity analysis and population structure estimation in common bean. *Saudi Journal of Biological Sciences, 23(1),* 139–149.

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