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ORIGINAL ARTICLE

Genetic Characterization of Green Bean (*Phaseolus vulgaris* L.) Accessions from Turkey with SCAR and SSR Markers

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Abstract Characterization, conservation, and utilization of genetic resources is essential for the sustainability in agriculture. Plant genetic resources are important for breeding efforts designed for the generation of new cultivars or for the improvement of existing ones. Green bean has been cultivated extensively in Turkey giving rise to local accessions through selection over time and adaptation to various environmental conditions. The objective of the present study was to determine the genetic relationships of green bean accessions collected from Kırşehir Province of Turkey, located at the central Anatolia. Within a population of 275 green bean accessions, 50 accessions were selected on the basis of morphological observations for further evaluation with SSR and STS/SCAR markers together with 4 reference cultivars of Andean and Mesoamerican origin. SSR markers selected on the basis of high polymorphism information content revealed the genetic relatedness of selected green bean accessions. STS/SCAR markers associated with bean anthracnose, common bacterial blight, white mold, halo blight, and phaseolin protein demonstrated the inheritance of resistance traits of local accessions at the selected loci. These findings may help better utilize genetic resources and furthermore are expected to facilitate forthcoming breeding studies for the generation of novel cultivars well adapted to the region.

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Introduction

Horticulture is concerned with plants that are used for food, either as edible products or as culinary ingredients, for medicinal use or ornamental and esthetic purposes. They are genetically a very diverse group and play a major role in modern society and economy (Bajpai et al. 2014; Feng et al. 2014; Kaczmarska et al. 2015; Mishra et al. 2015). Among horticultural plants, beans are important in human nutrition as a rich source of vegetable proteins and other nutritional properties. Green bean is produced and consumed extensively in Turkey. The production has reached 632 301 t (FAO 2013) in the country, holding the third place in the world after China and Indonesia in terms of production. Beans have been reported to originate from two major gene pools: the Mesoamerican and the Andean (Gepts 1998) that have spread to other parts of the world giving rise to local accessions through selection over time and adaptation to various growing conditions. Local accessions may be valuable genetic sources for the improvement or development of novel cultivars. Characterization of local accessions may provide the opportunity to assess the genetic background of individuals and to search for important traits for subsequent breeding efforts. Like many other plant species, morphological, biochemical, and molecular approaches are being used for characterization of beans (Belletti and Lotito 1996; Durán et al. 2005; Meza et al. 2013). Molecular markers have been widely used to genetically characterize bean populations (Kumar et al. 2008; Asfaw et al. 2009; Kwak and Gepts 2009; Sarıkamış et al. 2009). Several reliable marker systems including SSR and SNP markers have been developed and used to assess genetic structure and diversity in common bean from different geographical locations (Blair et al. 2009; Burle et al. 2010; Cortés et al. 2011; Bitocchi et al. 2013; Raggi et al. 2013; Sharma et al. 2013; Song et al. 2015). Green bean and common bean accessions collected from different geographical regions in Turkey have been evaluated using morphological and molecular approaches (Balkaya 1999; Sarıkamış et al. 2009; Khaidizar et al. 2012; Ceylan et al. 2014).

Markers associated with certain agronomic traits are being successfully utilized for the selection of desired traits and hence accelerate breeding programs. Genetic linkage maps in common bean provided the positioning of QTLs and identified markers tightly linked to the target loci (Blair et al. 2003; Miklas et al. 2014; Keller et al. 2015). Reliable markers for resistance against biotic and abiotic stress factors provided successful application of MAS (marker-assisted selection) in common bean (Fourie et al. 2004; Kelly and Vallejo 2004; Miklas et al. 2014).

In the present study, a total of 275 green bean accessions were collected from Kırşehir Province (39°8′45N, 34°9′50E) located in central Turkey, forming part of the central Anatolian region standing on the north Anatolian fault. Among 275 accessions revealing diverse morphological features, 50 accessions were selected on the basis of morphological observations including seed, leaf, flower, pod characteristics, and plant growth habit for further evaluation with molecular markers. The

objectives of the current study were to determine the genetic relationships among green bean accessions at the selected SSR (Simple Sequence Repeat) loci. Obtaining an overall insight into the inheritance of resistance traits and phaseolin protein of green bean accessions using SCAR (Sequence Characterized Amplified Region) markers linked to the desired traits that may be utilized in future breeding programs.

Plant genetic resources are important for breeding efforts designed for the generation of new cultivars or for the improvement of existing ones. Breeding objectives may vary depending on the crop, growing conditions, and consumer demands. The maintenance and utilization of genetic resources is important to serve these breeding objectives. The findings of the current study are expected to help utilize, improve, and protect local accessions that are well adapted to the growing environment.

Materials and Methods

Plant Material

In the present study, 50 green bean accessions within a collection of 275 accessions were selected on the basis of morphological characteristics for molecular assays. The cultivars Michigan Dark Red Kidney (MDRK), Kaboon, and Perry Marrow (PM) of Andean origin and Widusa of Mesoamerican origin were included as reference cultivars.

DNA Extraction

Genomic DNA was extracted from young leaf tissue using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI). RNase treatment was performed on the eluted DNA samples which were visualized on 1 % (w/v) agarose gel. The purity and concentration of the DNA were determined using NanoDrop® ND-1000 Spectrophotometer.

SSR Analysis

For genetic analysis of green bean accessions, ten SSR primer pairs were selected within a list of bean SSR primers presented by Yu et al. (2000) based on their high polymorphism information content as reported by Yu et al. (2000). The primers were PV-ag001, PV-ggc001, PV-at002, PV-18791, PVMEIG, PVGLND5, PV-ctt001, PVU70530, PV-ag003, and PVSBE2 belonging to a previous study used for the characterization of green bean genotypes from eastern part of Turkey (Sarıkamış et al. 2009). The polymerase chain reactions (PCRs) were performed in a 10 µl reaction mixture containing 15 ng DNA, 5 pmol of each primer, 0.5 mM dNTPs, 0.5 units of GoTaq DNA polymerase (Promega, Madison, WI, USA), 1.5 mM $MgCl_2$, and 2 µl 5X buffer. The forward primers were labeled with WellRED fluorescent dyes D2 (black), D3 (green), and D4 (blue) (Proligo, Paris, France).

Reactions without DNA were included as negative controls. PCR amplification was performed using Biometra® PCR System. The amplification conditions had an initial denaturing step of 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C (denaturation), 1 min at 52–56 °C (annealing), and 2 min at 72 °C (extension), with a final extension at 72 °C for 10 min. The PCR amplification products were first separated on a 2 % (w/v) agarose gel stained with ethidium bromide at a concentration of 10 mg/ml and run at 80 volts for 1 h. DNA Ladder (100 bp) (Promega, Madison, WI, USA) was used for the approximate quantification of the bands. The amplification products were visualized under UV light and sized relative to the ladder. For further determination of polymorphisms, the PCR products were analyzed with CEQ™ 8800 GEXP Capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). For fragment analysis, the amplification products were diluted with Sample Loading Solution (SLS), followed by the addition of Genomelab DNA Size Standard Kit-400, and electrophoresed in the CEQ™ 8800 GEXP capillary analysis system. Allele sizes were determined for each SSR locus using Beckman CEQ™ fragment analysis software. In each run, Michigan Dark Red Kidney (MDRK), Kaboon, Widusa, and Perry Marrow (PM) were included as reference cultivars.

Genetic Analysis of SSR Markers

The genetic analysis program "IDENTITY" 1.0 (Wagner and Sefc 1999) according to Paetkau et al. (1995) was used for the calculation of the number of alleles (*n*), allele frequency, expected (He) and observed (Ho) heterozygosity, estimated frequency of null alleles (*r*), and probability of identity (PI) per locus. Genetic dissimilarity was determined by the program "MICROSAT"(version 1.5) (Minch et al. 1995) using proportion of shared alleles which was calculated using ps (option 1-(ps)) as described by Bowcock et al. (1994). The results were then converted to a similarity matrix and a dendrogram was constructed with the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) method (Sneath and Sokal 1973), using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (Version 2.0) (Rohlf 1988).

SCAR Analysis

SCAR markers *Phs* related to common bacterial blight, white mold (*Sclerotinia sclerotiorum*), and phaseolin protein (Miklas 2007); *SAP 6* associated with common bacterial blight (Miklas et al. 2000); *SB 10* with halo blight (Fourie et al. 2004); and *SBB 14* linked to anthracnose Co-4 gene were used (Awale and Kelly 2001) together with an STS marker $SE_{act}M_{cca}$ associated with common bean anthracnose (Kelly and Vallejo 2004).

The polymerase chain reactions (PCRs) were performed in a 25 µl reaction mixture containing 25 ng DNA, 0.5 µM each primer, 200 µM dNTPs, 0.1 µl Promega® Taq Polymerase, 2.5 mM $MgCl_2$, and 5 µl 5X Buffer. The amplification conditions had an initial denaturing step of 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C (denaturation), 1 min at 54–56 °C (annealing), and 1 min at 72 °C

(extension) with a final extension at 72 °C for 10 min. The amplicons were separated on a 2 % (w/v) agarose gel run at 80 volts and visualized under UV light. DNA Ladder (100 and 1000 bp) was used to estimate band sizes.

Results and Discussion

Genetic relationships within 50 selected local green bean accessions from Kırşehir region of Turkey were determined using SSR markers. Initially, 10 SSR markers were selected within a list of bean SSR primers presented by Yu et al. (2000) based on their high polymorphism information content. However, two of the primers, PVSBE2 and PV18791, did not yield consistent results within accessions and were excluded from the study. The remaining eight primers produced successful amplifications of expected sizes. However, two markers PV-U70530 and PV-ag003 were monomorphic among accessions. Therefore, six polymorphic markers were used to assess the genetic relationships among accessions that generated a total of 28 alleles among accessions and reference cultivars. The number of alleles per locus ranged from two (PV-ggc001) to nine (PVGLND5). Based on the number of alleles generated and probability of identity (PI) values, the most informative loci were determined as PVGLND5, PVMEIG, and PV-ctt001 (Table 1). These findings were consistent with our previous results on green bean accessions from Eastern part of Turkey (Sarıkamış et al. 2009). Similarly, Lioi et al. (2005) reported high levels of polymorphisms with PVGLND5 and PVMEIG loci on some Italian common bean landraces. For all loci, the expected heterozygosity was higher than the observed heterozygosity. Heterozygotes were not observed for most loci except PV-ggc001 (Table 1).

The genetic relationships within accessions were determined using Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) dendrogram. Accordingly, the genetic distance within accessions and reference cultivars varied between %17 (0.17) and %100 (1.00). Two major clusters were obtained. While 8 of the accessions were grouped with two reference cultivars Widusa and Kaboon, 42

Table 1 Number of alleles, allele range (bp), expected heterozygosity (He), observed heterozygosity (Ho), estimated frequency of null alleles, and probability of identity (PI) values of green bean accessions calculated at six SSR loci

Locus analyzed	Allele range (bp)	Number of alleles	Expected heterozygosity (He)	Observed heterozygosity (Ho)	Estimated frequency of null alleles	Probability of identity (PI)
PV-GLND5	152–182	9	0.855	0.000	0.460	0.070
PV-MEIG	194–224	7	0.795	0.000	0.442	0.128
PV-at002	246–250	3	0.580	0.000	0.367	0.403
PV-ag001	150–160	3	0.642	0.000	0.391	0.356
PV-ggc001	230–234	2	0.317	0.132	0.140	0.616
PV-ctt001	154–164	4	0.419	0.000	0.295	0.465

accessions formed a separate group together with the cultivar Perry Marrow. Among these two major groups, several subgroups were formed. According to the UPGMA dendrogram, identical accessions at the analyzed SSR loci were determined (Fig. 1). The SSR findings revealed the genetic relatedness of green bean accessions that may ultimately help design crosses that maximize genetic diversity in breeding generations and/or combine desirable traits in future breeding studies.

Evaluation of green bean accessions with STS/SCAR markers associated with bean anthracnose, common bacterial blight, white mold, halo blight, and phaseolin protein revealed the inheritance of resistance traits of the accessions at the analyzed loci (Fig. 2).

Anthracnose, caused by *Colletotrichum lindemuthianum,* is one of the most important and widespread fungal diseases affecting green bean production worldwide. Resistance to anthracnose is conditioned by independent genes *Co-1 to Co-10*. STS marker $SE_{act}M_{cca}$ associated with *Co-1* locus conditioning resistance to anthracnose (Kelly and Vallejo 2004) was used to characterize green bean accessions. Successful amplifications were obtained with the marker, all samples yielding a band of expected size around 80 bp. For exact quantification of the amplicons, the samples were further analyzed with fragment analyzer (Beckman CEQ 8800 GEXP). Fragment analysis results revealed multiple banding patterns of 70, 73, 75, 78, and 80 bp among accessions. While the reference cultivars Perry Marrow (PM) carried 71, 74, and 79 bp alleles, Kaboon carried 71, 74, 78, and 79 bp alleles, Widusa 70, 73, and 78 bp alleles, and Michigan Dark Red Kidney (MDRK)

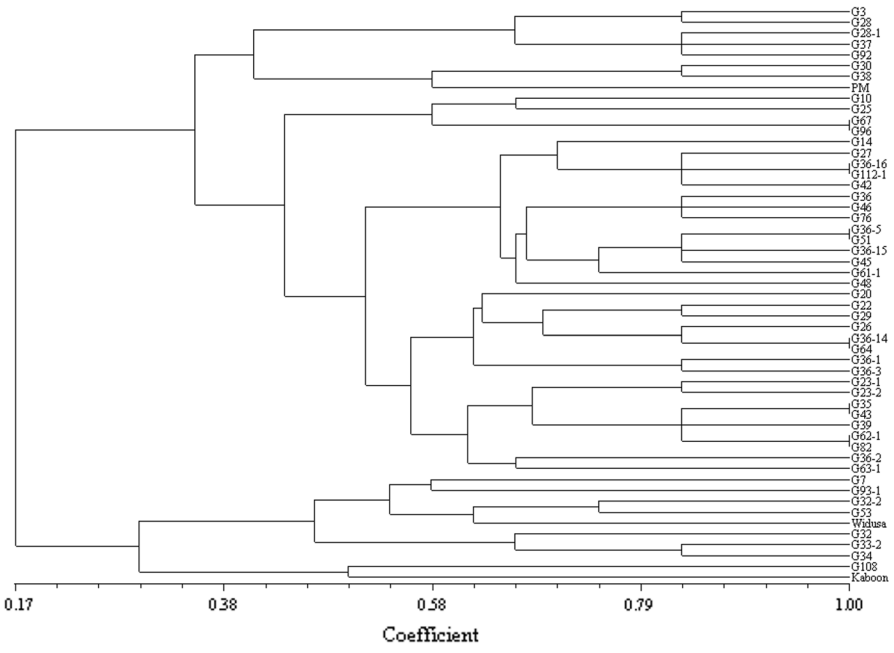

Fig. 1 UPGMA dendrogram of 50 green bean accessions with reference cultivars at six SSR loci

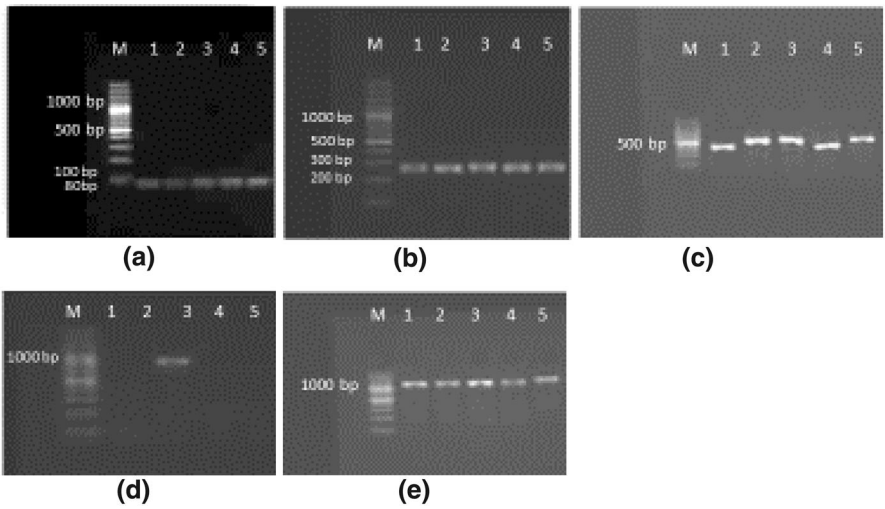

Fig. 2 Amplification of STS/SCAR markers: **a** $SE_{act}M_{cca}$, **b** Phs marker, **c** SB 10 marker, **d** SAP 6 marker, and **e** SBB 14 marker. *M* Marker; *1–5* green bean accessions representing the population

carried 72, 75, and 80 bp alleles, the majority of green bean accessions revealed a profile of 70, 73, and 78 bp alleles, except a single accession (G30) carrying 75 and 80 bp alleles similar to MDRK.

These findings were consistent with Kelly and Vallejo (2004) reporting that three Andean cultivars MDRK, PM, and Kaboon carry different alleles at the *Co-1* locus. Such that, in addition to the *Co-1* allele at MDRK, Co-1^2 and Co-1^3 alleles are reported in Kaboon and PM and probably Co-1^5 allele in the Mesoamerican origin cultivar Widusa (Kelly and Vallejo 2004). MDRK of Andean origin was reported to carry 80 bp resistance allele of *Co-1* gene designated as the first gene determined for resistance to anthracnose present in Andean origin gene pool (Madakbaş et al. 2013). In the light of this evidence, it can be concluded that different allelic forms of *Co-1* gene were present in all accessions analyzed and that a single accession (G30) revealed a similar profile to MDRK of Andean origin carrying 80 bp allele associated with resistance trait. Considering the interaction of different alleles in determining resistance traits in plants, it should be noted that the genes differ in their effectiveness in controlling the highly variable races of the pathogen.

SCAR marker SBB 14 is associated with resistance to anthracnose Co-4^2 (Awale and Kelly 2001) and is reported as a codominant marker while a 1150 bp allele delivering resistance, 1050 bp allele is related to susceptibility. Among cultivars, PM carried 1150 bp allele conditioning resistance at Co-4^2 locus together with 11 green bean accessions of the total. Remaining accessions and reference cultivars carried 1050 bp allele at Co-4^2 locus.

The codominant SCAR marker Phs (Phaseolin seed protein) (Kami et al. 1995) associated with phaseolin protein, common bacterial blight, and white mold (Miklas et al. 2007) was used to assess genetic background of green bean accessions together with reference cultivars. Multiple band patterns were determined using Beckman

CEQ 8800 fragment analyzer as 252, 266, 273, 288 bp as well as 252, 273 bp as a result of fragment analysis at Phs locus.

These findings were consistent with literature suggesting that the presence of multiple alleles 249, 264, 285 bp at Phs locus is linked to T-phaseolin protein, also providing partial resistance to white mold. The presence of 249 and 270 bp alleles at Phs locus is linked to S-phaseolin protein and susceptibility to white mold (Miklas 2007). The findings of the present study demonstrated that reference cultivars PM, MDRK, and Kaboon of Andean origin carry 252, 267, 274, and 288 bp alleles at Phs locus and thus are associated with T-phaseolin protein and partial resistance to white mold. Widusa of Mesoamerican origin carries 252 and 273 bp alleles and is associated with S-phaseolin and susceptibility to white mold. In the light of this evidence, the majority of green bean accessions carried multiple alleles of 252, 267, 274, 288 bp and is associated with T-phaseolin and partial resistance to white mold similar to cultivars of Andean origin. Few accessions (G32, G32-2, G33-2, G34, G53, and G108) carried 252 and 273 bp alleles and thus are associated with S-phaseolin and susceptibility to white mold similar to Widusa of Mesoamerican origin. Madakbaş et al. (2013) reported that 59 % of bean accessions in Turkey are of Andean origin carrying T- and C-type phaseolin, while 41 % are of Mesoamerican origin carrying S-type phaseolin. Mesoamerican originating white-seeded bean cultivars are mostly preferred for common bean production, while Andean origin beans with green pods are preferred for green bean production in Turkey. This is also consistent with our findings and that the accessions with S-type phaseolin (G32, G32-2, G33-2, G34, and G108) except G53 are used for common bean production.

SCAR markers linked with QTLs for common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* are available for marker-assisted selection (Miklas et al. 2000). SAP 6 associated with common bacterial blight (Miklas et al. 2000) was used to determine the resistance pattern of green bean accessions and reference cultivars. According to the findings of the study, while a 820 bp fragment was amplified in some accessions, this fragment was absent in others and reference cultivars. This finding was also consistent with Fourie and Herselman (2011) linking the presence of the 820 bp fragment with resistance to common bacterial blight. According to the findings accessions carrying the 820 bp fragment were determined.

Halo blight is a bacterial disease caused by *Pseudomonas syringae* pv. *phaseolicola* affecting common bean worldwide. SCAR marker SB 10, originally an RAPD marker $B10._{520}$ linked with *Pse-1* gene that conditions resistance to race 1 (Fourie et al. 2004), was used in the present study. The expected band size for SCAR marker linked to common bean halo blight (SB 10) was reported as 520 bp. While some of the green bean accessions carried 520 bp, some had a band of <500 bp size. Among reference cultivars, only MDRK carried 520 bp allele, while the others carrying <500 bp band. Recently, Miklas et al. (2014) reported SB10.550 originating from the previously mapped RAPD marker B10.525 tightly linked (1.4 cM) with Pse-6 as a potential marker for resistance to halo bacterial blight.

The UPGMA dendrogram was plotted using data from five SCAR loci mostly associated with resistance traits (Fig. 3). The dendrogram generated two major groups, one group including ten accessions and the reference cultivar PM while the remaining accessions and reference cultivars forming a second major group in terms

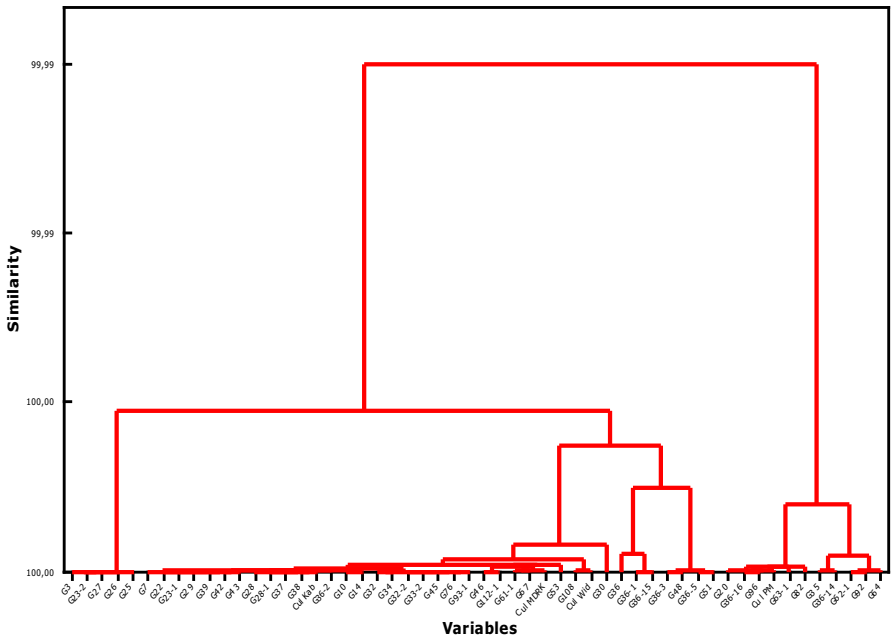

Fig. 3 UPGMA dendrogram of 50 green bean accessions with reference cultivars at five SCAR loci

of resistance traits and phaseolin content. The dendrogram demonstrated the relationship of accessions at the analyzed SCAR loci.

Evaluation of resistance in the field is hard and complicated by physiological mechanisms. QTLs identified with stable and reliable expression across environments may provide an opportunity for marker-assisted selection for breeding objectives. According to the STS/SCAR analysis results, the genetic background of green bean accessions on selected loci was determined. Together with the SSR data, these findings can contribute to a better use of genetic diversity and, furthermore, are expected to facilitate forthcoming breeding studies for the generation of novel cultivars well adapted to the region.

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