RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
52 (3) 271-291, 2020

ASSESSMENT OF THE GENETIC STRUCTURE AND SALT TOLERANCE OF *Phaseolus vulgaris* L. LANDRACES

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SUMMARY

The common bean (*Phaseolus vulgaris* L.) is one of the most important crops in the world. Landraces represent great importance as a gene resource for developing varieties. For this reason, revealing their genetic structure and introducing them into breeding programs are necessary. In this study, the genetic structure and salt stress tolerance levels of common bean landrace genotypes were investigated. This work aimed to contribute to breeding studies by revealing the relationship between genetic structure and tolerance to salt stress. For this purpose, the population structure of 124 common bean landrace genotypes was revealed by using 30 simple sequence repeat markers. Furthermore, the salt tolerance levels of these genotypes were determined in accordance with their vegetative development under control and salt stress conditions. For this purpose, the salt stress index was used. As a result, the genotypes were clustered into six populations. The growth habits of genotypes were the determining factor in clustering. STRUCTURE analysis supported this result, and allele sharing between genotypes with different growth habits was found to be limited. Only one genotype was identified as tolerant. However, 10 genotypes were classified as moderately tolerant. All of the tolerant and moderately tolerant plants were climber genotypes. The limited allele sharing detected between the bush and climber genotypes suggested that alleles related to salt tolerance accumulated in climber genotypes. These results showed that hybridization between bush and climber genotypes should be conducted to not only create variation for breeding but also to contribute to salt stress tolerance and to increase stress-related allele sharing.

Keywords: Common bean, growth habit, microsatellite markers, salt stress index, STRUCTURE analysis

Key findings: Allele sharing between climber and bush common bean genotypes is limited and contributes to genetic bottlenecking. This situation is also reflected in the distribution of genotypes with salt stress tolerance. Hybridization between genotypes with different growth habits in landrace varieties with moderate genetic diversity, such as the populations evaluated in this study, is increasingly important

for overcoming genetic bottlenecking and to increase the alleles shared by related stress-tolerant genotypes in breeding studies.

Manuscript received: May 17, 2020; Decision on manuscript: June 15, 2020; Accepted: June 25, 2020.

Communicating Editor: Dr. Naqib Ullah Khan

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the second most important green vegetable; it is grown with 24.752.675 tons of production (FAO, 2020) worldwide and is an important source of proteins, calories, minerals, and vitamins for developing states and developed countries. Turkey is the fourth largest producer of green beans (580.949 tons) in the world (FAO, 2020). The common bean is sensitive to salt stress, which is a factor that limits the yield of legumes especially in arid and semiarid regions. For this reason, soil salinity is either absent or very low in cultivation areas of the common bean (Maas and Hoffman, 1977; Lluch *et al.,* 2007). However, the sensitivity of the common bean to abiotic stress prevents the average yield from reaching the desired level in regions with stress conditions. Yield loss in the common bean starts to occur beyond the 1 dSm^{-1} salinity threshold, and yield decreases by 19% with each unit increase in salinity (Hoffman, 1992). Approximately 20% of irrigated lands in the world are affected by salinity stress (Chemura *et al.,* 2014).

The capability of plants to grow incessantly under salt stress conditions is described as salinity tolerance (Munns, 2002). Bayuelo-Jiménez *et al.* (2012) reported that 20 days of exposure to salt causes approximately 56% biomass reduction in the common bean. Studies have demonstrated that the root length of common beans is significantly inhibited and vegetative growth parameters are suppressed by NaCl (Beltagi *et al.,* 2006; Assimakopoulou *et al.,* 2015; Cokkizgin, 2012). As revealed in their studies, salt stress suppresses the development of the common bean, and the data obtained due to the decrease in development can help determine tolerance to salt stress. The stress tolerance index (STI) is based on the stress suppression that occurs in plants. The STI (Negrão *et al.,* 2017) is a fast and easy method for selecting genotypes with superior performance under stress conditions. The most important advantage of this method is that it can perform very well in the selection of tolerant genotypes.

Landrace varieties are important breeding materials that enrich biodiversity, are highly adaptable to specific environmental conditions, and provide new alleles for the development of commercial varieties (Azeez *et al.,* 2018). National varieties of corn, sorghum, and pearl millet that were developed by using landrace varieties as starting materials are only a few examples that can be counted quickly (Ceccarelli, 2012). Landrace genotypes, which are important for the maintenance of genetic diversity, food safety, and breeding studies, are subject to genetic erosion due to the replacement of modern varieties with landraces and the effects of breeding

methods (Rauf *et al.,* 2010). First knowing the details of the distribution and structure of genetic diversity is necessary in the use of genetic resources. These data will help breeders create hybridization combinations. Currently, SSR markers are often preferred for revealing genetic diversity because they are highly polymorphic even among closely related lines. They are also found in all eukaryotic genomes, require a small amount of DNA, easily automated, stable between laboratories, and codominant (Aitken *et al.,* 2005; Uncuoğlu, 2010).

Considering the forecasts of climate change, yield reductions due to salt stress will adversely affect agriculture, especially agriculture in arid–semiarid regions. Material exchange through gene banks is a current issue encountered in developing new varieties in a globalizing world. For this reason, revealing the genetic structure of landrace varieties, which are an important gene source, and correlating biotic–abiotic stress tolerance levels are important. Although common bean landrace cultivars are grown widely in the western Mediterranean region, their genetic structure and salt stress tolerance levels have not been studied. This study was carried out for the following purposes: *i)* to reveal and understand the amount and distribution of the genetic variation of the collected landrace genotypes, *ii)* to determine the salt tolerance levels of landrace genotypes for future breeding, and *iii)* to examine the relationship between the genetic structure and salt stress tolerance of the genotypes.

MATERIALS AND METHODS

Plant materials and location

In total, 124 common bean landraces genotypes were collected in accordance with their seed color, seed shape, and growth habits (bush- Type I and climber- Type III) from three major cities (Antalya, Isparta, and Burdur) in the western Mediterranean region of Turkey (Ulukapi *et al.,* 2018). We also interviewed local authorities and farmers. The collections were sampled from different altitudes (11–1299 m).

Screening and evaluation of salt tolerance

Plants were treated with various salt (NaCl) concentrations of 2, 4, and 6 dSm^{-1} NaCl. Regular irrigation water (containing 0.5 dSm^{-1} NaCl) was used as the control to determine the salt tolerance levels of the genotypes. Salt concentrations were determined in accordance with the salinity threshold value for common beans reported previously as 1 dSm^{-1} (Hoffman *et al*., 1992). The experimental design was conducted in accordance with a completely randomized design with three replicates. The experiment was established in April–May 2017 with 15 plants from each genotype with five plants per repetition. The genotypes were grown in 4.86 m^3 plastic chambers that were 2.40 m long × 0.45 m wide × 0.45 m deep. Cultivation was carried out through the direct seed sowing method. Plants were grown with 50% peat + 50% perlite medium. Basic fertilization was performed at the beginning of the experiment by using $CaCl_2$, $MgSO_4.7H_2O$, urea (NH_2CONH_2), KCl, and H_3PO_4 with 140 mg/kg Ca, 23.3

mg/kg Mg, 90 mg/kg N, 230 mg/kg K, and 46.7 mg/kg P, respectively. Salt applications were started 25 days after sowing seeds. $CaCl_2$ and NaCl salts were used, and sodium adsorption rate was taken as SAR < 5 to prepare saltwater. The experiment was ended when all plants reached the first flowering stage. Plant fresh and dry weights (PFW and PDW, g), plant length (PL, cm), leaf width (PLW, cm), leaf length (PLL, cm), and root depth (PRL, cm) were measured at the end of the experiment to compare the vegetative development of plants under salt stress conditions. For all characters, the salt tolerance trait index (STTI) values of genotypes were calculated by using the obtained values in accordance with the formula of Ali *et al.* (2007).

STTI = (Value of character under salt stress/value of character under control treatment) × 100.

The salt tolerance index (STI) was obtained by calculating the averages of STTI data. Then, by using the standard error of the mean of these data, the genotypes were divided into four groups as tolerant, moderately tolerant, moderately susceptible, and susceptible in accordance with Ahmad *et al* (2013).

Genomic DNA extraction and SSR amplification

Young leaflet samples (0.5 mg) were taken from the plants and ground in liquid nitrogen for genomic DNA isolation, which was performed by using the modified manual method of Doyle and Doyle (1988). The primers used in this research were obtained from recent literature, and only primers with polymorphism information content (PIC) higher than 0.60 were selected (Yu *et al.,* 2000; Gaitan-Solis, 2002; Blair *et al.,* 2006; Benchimol *et al.,* 2007; Zhang *et al.,* 2008; Kwak and Gepts, 2009; Burle *et al.,* 2010). PCR amplification was performed with a 25 µL volume containing approximately 50 ng of template DNA, 1 µm of each forward and reverse primer, 10 µL of DreamTaq Green PCR Master Mix containing DreamTaq DNA polymerase, and 2× DreamTaq Green buffer. The amplification conditions had an initial denaturing step of 3 min at 94 °C then were followed by 10 cycles of 30 s at 94 °C (denaturation), 45 s at 51 °C–60 °C (annealing), and 2 min at 72 °C (extension) (pre-PCR). Then, 30 cycles were performed at 94 °C for 30 s, at 61 °C for 45 s, and at 72 °C for 2 min. PCR was terminated at 72 °C for 10 min. The PCR reaction products were evaluated for polymorphism on 3% agarose gel. After staining with 1 µg mL^{-1} ethidium bromide for 3 h, the gels were photographed by using a gel documentation system. The results were repeated twice. A third repetition was performed for monomorphic and nonworking primers.

Statistical analysis

Clear allele bands obtained for each SSR marker locus were manually separated and coded as binary characters. These data were converted by using the Convert version 1.31 program (Glaubitz, 2004) for further analysis. The PIC values of each locus were calculated by using the Genpop input format with the Molecular Kinships (MolKin) v.3.0 program (Gutierrez *et al.,* 2005). The population genetic differentiation level was estimated through AMOVA by

using GenAlEx version 6.5 software (Peakall and Smouse, 2012). The genetic diversity of loci and populations was determined through descriptive analysis. The mean number of alleles (*Na*), total number of alleles, effective number of alleles (*Ne*), expected heterozygosity (*He*), observed heterozygosity (*Ho*), Wright's fixation indices (*Fit, Fis*, and *Fst*), coefficient of genetic differentiation (*Gst*), gene flow (*Nm*), and Shannon's information index (*I*) were determined. The statistical significance of the variances was tested by using 999 random permutations at 1%. Population structure was determined by using STRUCTRE v2.3.3 software (Pritchard *et al.,* 2000). STRUCTURE software assigns individuals into clusters on the basis of genotypic data by using a Bayesian clustering algorithm. The K value for STRUCTURE analysis was determined to range from 2 to 7. The length of the burn-in period was set to 100,000 to determine the optimum population number (K). Markov Chain Monte Carlo after the burn-in period was set to 250,000, and 35 independent runs were performed. Structure Harvester program (Earl and Vonholdt, 2012) was used to assign the optimal K value on the basis of maximum likelihood and delta (Δ) K values (Evanno *et al.,* 2005). The results of CLUMPP version 1.1 (Jakobsson and Rosenberg, 2007) analysis were visualized by using DISTRUCT analysis (Rosenberg, 2004).

A total of six parametric characters were measured to determine the vegetative development of plants under stress conditions. These measurements were made by using a ruler, digital caliper, tape measure, and weighing machine. Descriptive statistics, principal component analysis (PCA), regression, and clustering analyses were performed with SAS 9.1 and Minitab 17.0 statistical software.

RESULTS

Salt stress tolerance

Plants that were irrigated in accordance with solar radiation values were exposed to salt stress until the first flowers were obtained. During the termination of the experiment, no phenotypic difference was detected between treatments. Stress effects (drying and yellowing) were detected only under 6 dSm^{-1} stress. Therefore, the vegetative development of the control group was compared with that of plants under 6 dSm^{-1} stress. STI, which was based on vegetative growth parameters, varied among the 124 common bean landrace genotypes with a range of 38% to 91% and an average of 64%. In accordance with STI values, the genotypes were classified into four groups: tolerant (89% and above), moderately tolerant (79%–88%), moderately susceptible (68%–78%), and susceptible (below 67%) (Table 1). Only one genotype was identified as tolerant (STI = 91%). Ten genotypes were moderately salt tolerant, 39 genotypes were moderately salt susceptible, and 74 genotypes were salt susceptible. All tolerant and moderately tolerant genotypes were plants with Type III growth habit.

PCA was performed by using the vegetative growth parameters of the genotypes under salt stress conditions as variables (Table 2, Figure 1). The first four eigenvalues, eigenvectors, and % explanatory

Table 1. Salt tolerance categories of landrace genotypes of the common bean based on the salt tolerance index (STI).

Salt tolerance category	Range of STI	Genotypes	Name of Landrace Common Bean Genotypes	
			Type I	Type III
Tolerant	89% and above	1	-	BKara1-B *(58)*
Moderately tolerant	79%–88%	10	-	AGB3*(21)*, AGun12*(31)*, BK3*(62)*, BY10*(71)*, BY23*(79)*, EC2*(90)*, ISCoban2*(103)*, IAMerkz2*(104)*, IAKo2*(107)*, ISGa5*(112)*
Moderately susceptible	68%–78%	39	AGB10, BY4, ISGa1, ISGa10, IYoz10	ADA4, ADA5, AGB2, AGun2, AGun4, AGun13, AGun15, AGun18, AGun23, AS1, AS4, BH1, BG3, BK9, By7-B, BY9, BY13, BY17, BY18, BY19, EA4, EA5, EAk3, EAk6, Ec1, Ec4, Ec6, EY2, IAKo3, IYoz4, IYoz7, IYoz9-B, KU1, Gay2
Susceptible	Below 67%	74	ADY4, AGB1, AGB5, AGun6, AGun19, AGun25, BKara1-A, Bkara2, BY24, ISGa7, IYoz14	ADA3, AGB6, AGB7, AGB8, Afin2, Afin4, AGun3, AGun8, AGun20, AGun22, AGun26, AGun27, Akseki, AKs1, AP1, AP3, AS2, AS3, AS7, AT2, AT3, AT4, Bc2-A, Bc2-B, Bc4, BG2, BKara3, BKara5, BKara7, BK6, BK8, BY1, BY5, BY7-A, BY14, BY15, BY20, BY27, BY30, BY31, EA6, EAk7, EE1, EY4, ISMerkz1, ISSarı1, ISSarı2, ISSarı4, ISSarı5, ISPınar2, ISCoban1, IAMerkz4, IAKo1, IAKo5, ISGa4-A, ISGa4-B, ISGa9, IYOz2, IYOz8, IYOz9-A, KU2, Gay6, Gay7

Italic numbers symbolize the numbers in Figure 1.

Table 2. Eigenvectors and eigenvalues obtained via PCA.

Variable	PC1	PC2	PC3	PC4
STTI-PDW	0.377	−0.428	−0.346	0.031
STTI-PH	0.344	0.214	−0.258	−0.692
STTI-PLW	0.288	0.353	−0.511	0.298
STTI-PLL	0.277	0.278	0.633	−0.310
STTI-PRL	0.313	0.396	0.254	0.565
STTI-PFW	0.311	−0.643	0.293	0.125
STI	0.621	−0.024	0.033	0.017
Eigenvalue	2.39	1.09	1.02	0.83
Proportion of variation	0.36	0.15	0.15	0.12
Cumulative variation (%)	0.36	0.51	0.66	0.78

PDW: Plant dry weight, PH: plant height, PLW: plant leaf width, PLL: plant leaf length, PRL: plant root length, PFW: plant fresh weight

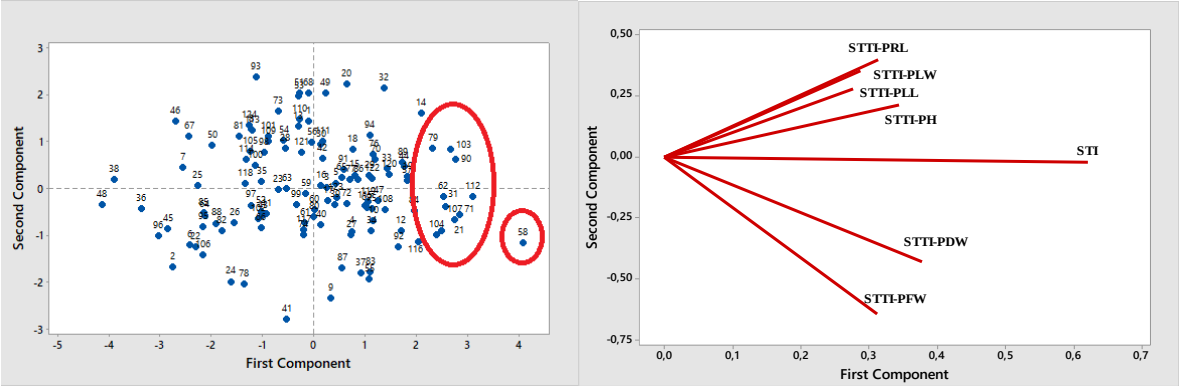

Figure 1. PCA of the distribution of landrace genotypes (left) and variables (right) based on salt stress index values.

variances obtained from PCA are given in Table 2. As shown in the table, the first two main components explained 51% of the total variation and the first four components explained 78%. According to this table, only STI (0.621) was included in PC1, and it was 36% effective in explaining the variation alone. STTI-PDW (−0.428) and STTI-PFW (−0.643) were included in PC2. STTI-PLW (−0.511) and STTI-PLL (0.633), which were related to leaf development, were included in PC3, whereas STTI-PH (−0.692) and STTI-PRL (0.565) were included in PC4. Figure 1 (left) shows the distribution of genotypes by PC1 and PC2. In accordance with their dispersion on the PCA graph, the tolerant genotype (BKara1-B) was determined to differ from other genotypes. Ten moderately tolerant genotypes were plotted on the same right portion of the PCA graph. Other genotypes with close values mostly clustered in the middle of the graph. Most of the sensitive genotypes were plotted on the left side of the diagram. The loading plot (Figure 1 right) representation of the correlation between salt stresses showed a positive correlation with the first component (36% of the explained variance). The second component (15% of the explained variance) showed that STTI-PH, STTI-PRL, STTI-PLL, and STTI-PLW were positively correlated, whereas STTI-PFW and STTI-PDW were negatively correlated.

The effects of independent variables on STI were examined through simple linear regression. A moderately positive and statistically significant relationship was observed among dependent and independent variables. As seen in Table 3, the effects of all independent variables on STI were statistically significant ($P < .0001$). The variable with the highest effect was determined as STTI-PDW, and the rate of disclosing STI was found to be 35.87% ($R^2 = 0.3587$). This was followed by STTI-PH ($R^2 = 0.2778$), STTI-PFW ($R^2 = 0.2775$), STTI-PRL ($R^2 = 0.2499$), STTI-PLL ($R^2 = 0.2105$), and STTI-PLW ($R^2 = 0.2040$). The scatter diagram is one of the most widely used visualization techniques for displaying datasets with few variants. A scatter diagram based on the values in Table 3 is shown in Figure 2. All the parameters that were obtained as a basis for vegetative changes in genotypes under stress conditions had a moderate and

Table 3. Illustrating the relationship between dependent variables (STI) and independent variables (STTI_PDW, STTI_PH, STTI_PLW, STTI_PLL, STTI_PRL, and STTI_PFW) based on regression analysis.

Variable	B	SE	t	P	R^2
Constant	49.645	1.919	25.87	<.0001	
STTI_PDW	0.2734	0.033	8.26	<.0001	0.3587
Constant	44.239	3.029	14.61	<.0001	
STTI_PH	0.287	0.042	6.85	<.0001	0.2778
Constant	45.096	3.528	12.78	<.0001	
STTI_PLW	0.269	0.048	5.59	<.0001	0.2040
Constant	46.046	3.300	13.95	<.0001	
STTI_PLL	0.255	0.045	5.70	<.0001	0.2105
Constant	47.035	2.820	16.68	<.0001	
STTI_PRL	0.261	0.041	6.38	<.0001	0.2499
Constant	51.707	1.996	25.90	<.0001	
STTI_PFW	0.235	0.034	6.85	<.0001	0.2775

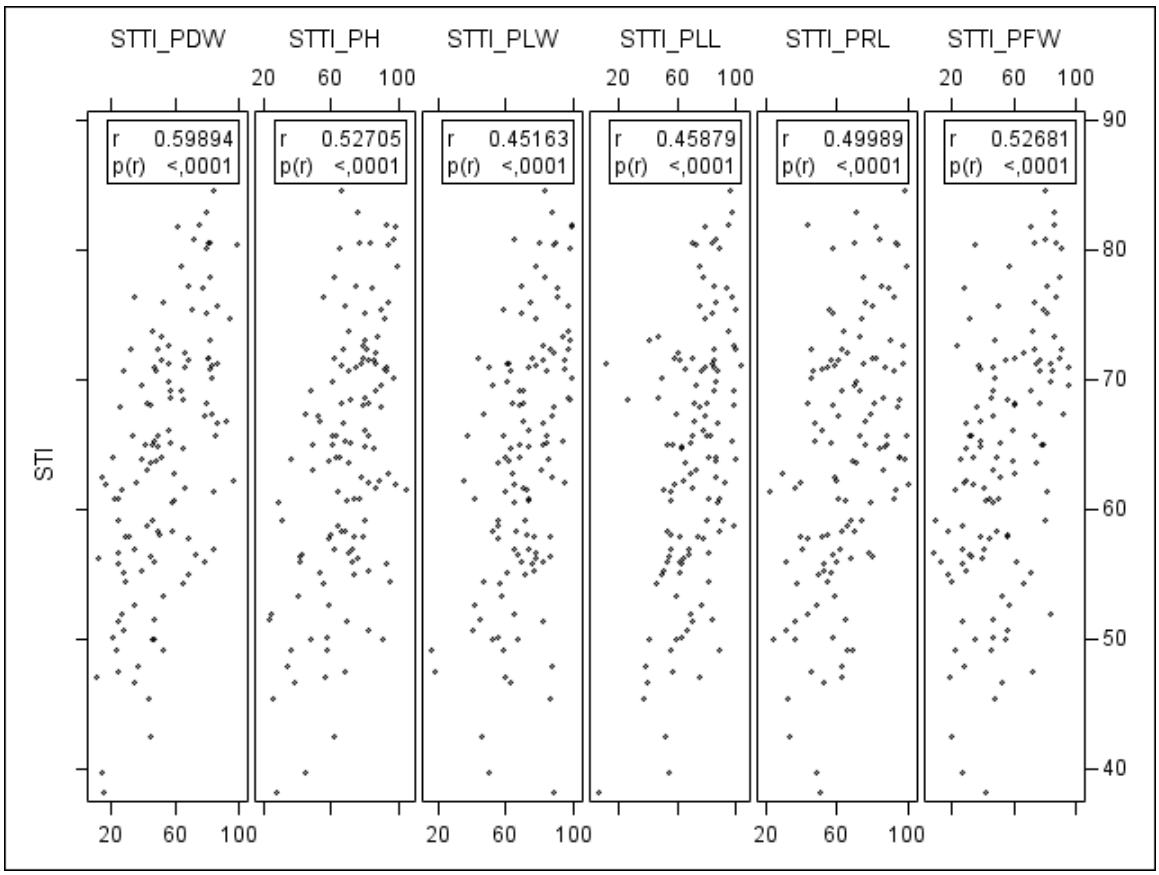

Figure 2. Scatter diagrams of the dependent variable (STI) versus independent variables (STTI_PDW, STTI_PH, STTI_PLW, STTI_PLL, STTI_PRL, and STTI_PFW).

positive statistically significant effect on the STI.

SSR marker polymorphism and genetic diversity

The genetic diversity of 124 common bean landrace genotypes was assessed by using 30 highly polymorphic SSR primers that were distributed in different linkage groups (Supplementary Table 1). Out of the 30 SSR primers used in this study, primers SSR-IAC46, SSR-IAC11, BM209, BM114, and PVat008 did not work for Type I and Type III genotypes. Four primers (GATS11, PVat007, BM199, and BM201) were determined to be monomorphic for all genotypes. In addition, some primers only worked for Type I genotypes (BM171, BM187, and PVgccacc001) but did not produce any bands for Type III genotypes. Among these primers, the PIC value of BM187 was found to be 0.77, that of Pvgccacc001 was 0.70, and that of BM171 was 0.46. According to all primer results, 21 primer pairs for the molecular characterization of Type I genotypes and 19 primer pairs for Type III genotypes provided a polymorphic result. The number of alleles ranged from 2 to 5 with a mean of 2.49. The PIC values for the primers varied from 0.46 to 0.86. The highest PIC values were recorded for BM152, BM160, and SSRIAC10 for Type I genotypes and for BM175, BM152, and BM160 for Type III genotypes (Table 4). Primers with a PIC value of less than 0.50 included BM202, BM211, and SSRIAC10 for Type III genotypes and PVctt001 and BM171 for Type I genotypes. Given that some primers only provided bands for Type I genotypes and PIC values, BM152 and BM160 primers were important for common bean genotypes.

The allelic diversity of the populations of landrace common bean genotypes collected from the western Mediterranean region is given in Table 5. The highest genetic diversity was observed in population 5 (*Na*: 3.000 and *I*: 0.841), followed by that in population 1, population 6, population 4, population 3, and population 2. *H* values ranged from 0.505 (pop 5) to 0.362 (pop 3), and *Ho* values ranged from 0.268 to 0.118. The highest *Ne* appeared in population 5. Two private alleles each were found for pop1 (GATS91 and SSRIAC10) and pop5 (BM154 and BM152).

Statistical analysis (Table 6) revealed that the genetic diversity of the Type III populations (*I*: 0.767) was higher than that of Type I (*I*: 0.618). Moreover, Type I populations had a higher rate of self-pollination than other populations (*Fis*: 0.787). Genetic differentiation among populations was 15.4% in Type I populations and 6.7% in Type III populations. Although gene flow was high in populations with both growth types, it was considerably higher in Type III populations (*Nm*: 7.383).

Among populations, the components of the total genetic variation within and among individuals of all common bean genotypes were estimated via AMOVA (Table 7, Figure 3). When all genotypes were examined, most of the variation (45%) was found within individuals. The variation between populations explained only 16% of the total variation. The distribution graph of all genotypes showed that the populations were divided into two main groups in accordance with their growth Types, not on the basis of the locations from which they were

Table 4. Genetic diversity parameters for polymorphic microsatellites in 124 landrace genotypes of the common bean.

Locus	Means		*Ho*	*Hs*	*Fst*	*Gst*	*PIC*-Type III (%)	*PIC*-Type I (%)	*Nm*
	Na	Ne							
BM205	2.833	2.629	0.657	0.612	0.071	0.025	71	63	3.270
BM141	2.833	2.055	0.191	0.504	0.250	0.182	73	77	0.750
GATS91	2.500	1.918	0.242	0.421	0.331	0.277	70	78	0.505
BM53	3.000	2.578	0.436	0.501	0.172	0.120	77	60	1.205
BM164	3.000	2.286	0.508	0.541	0.229	0.183	75	66	0.841
BM175	2.500	2.235	0.045	0.535	0.134	0.045	81	71	1.609
BM181	1.833	1.698	0.056	0.378	0.168	0.084	70	63	1.234
BM185	1.833	1.707	0.006	0.371	0.203	0.116	70	59	0.980
PVctt001	2.000	1.336	0.027	0.205	0.644	0.604	58	46	1.138
BM202	2.000	1.460	0.098	0.278	0.240	0.171	47	68	0.790
BM211	2.000	1.465	0.034	0.286	0.401	0.337	49	67	0.373
BM189	2.500	1.691	0.060	0.370	0.109	0.021	70	59	2.045
SSRIAC10	2.500	2.078	0.490	0.495	0.168	0.112	47	81	1.235
BM154	3.000	2.220	0.065	0.534	0.212	0.130	76	73	0.932
BM184	1.833	1.586	0.010	0.329	0.338	0.264	64	63	0.490
BM152	3.167	2.181	0.139	0.469	0.302	0.233	80	86	0.577
BM160	3.000	2.401	0.118	0.540	0.223	0.146	78	83	0.873
Mean	2.490	1.972	0.187	0.433	0.246	0.180			1.050

Na: number of allele *Ne*: number of effective allele *Ho*: observed heterozygosity *Hs*: expected heterozygosity *Fst*: genetic differentiation index *Gst*: genetic differentiation coefficient *PIC*: Polymorphic Information Content *Nm*: gene flow

Table 5. Genetic diversity of populations in accordance with locations and growth habits.

Populations	Province	Growth habit	*Na*	*Ne*	*I*	*Ho*	*Hs*
1	Antalya	Type I	2.647	2.112	0.788	0.118	0.483
2	Burdur	Type I	1.941	1.708	0.544	0.137	0.362
3	Isparta	Type I	2.000	1.838	0.569	0.118	0.369
4	Antalya	Type III	2.824	1.867	0.690	0.242	0.408
5	Burdur	Type III	3.000	2.254	0.841	0.240	0.505
6	Isparta	Type III	2.529	2.052	0.756	0.268	0.476
Means			2.490	1.972	0.698	0.187	0.433

Na: number of allele *Ne*: number of effective allele *I*: Shannon's index *Ho*: observed heterozygosity *Hs*: expected heterozygosity

Table 6. Genetic diversity statistic for SSR locus in accordance with growth habits.

Growth habit	*Fis*	*Fst*	*I*	*Nm*
Type I	0.787	0.154	0.618	1.939
SE	0.081	0.019	0.046	0.286
Type III	0.482	0.067	0.767	7.383
SE	0.112	0.010	0.041	1.897

Fis: fixation index *Fst*: genetic differentiation index *I*: Shannon's Information Index *Nm*: Gene flow

Table 7. Summary AMOVA showing the variability patterns of the collection relative to populations.

Source of variation	d.f.	S.S.	M.S.	Total variance (%)	P
Populations					
Among Populations	5	163.901	32.780	16	<0.001
Among Individuals	118	735.687	6.235	45	<0.001
Within Individuals	124	234.000	1.887	39	<0.001
Type I Populations					
Among Populations	2	41.089	20.545	3	<0.001
Within Populations	13	227.536	17.503	97	<0.001
ΦPT= 0.032					
Type III Populations					
Among Populations	2	139.598	69.799	11	<0.001
Within Populations	105	1356.041	12.915	89	<0.001
ΦPT= 0.114					

d.f.: degrees of freedom. SS: sum of squares. MS: mean square

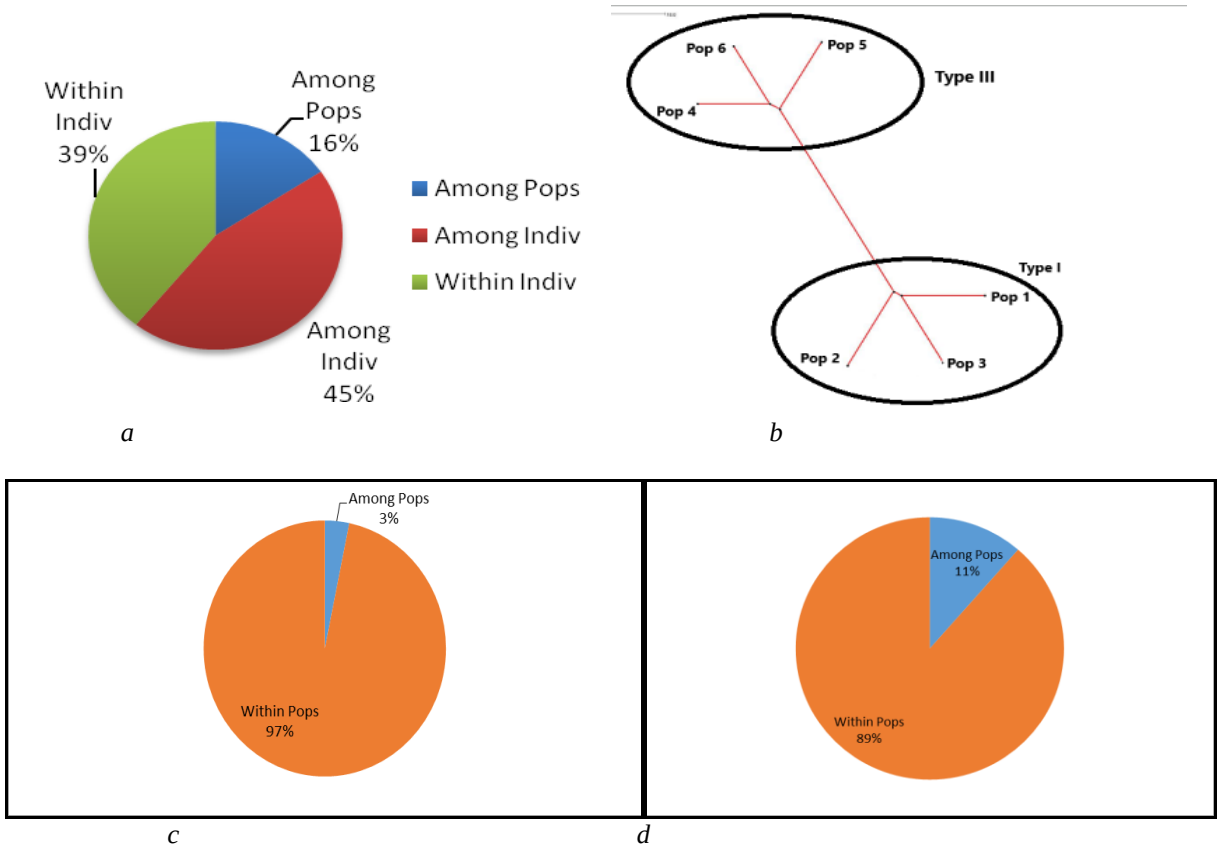

Figure 3. Percentages of molecular variance for all populations (a). Type I populations (c). Type II populations (d) and distribution of all populations (b).

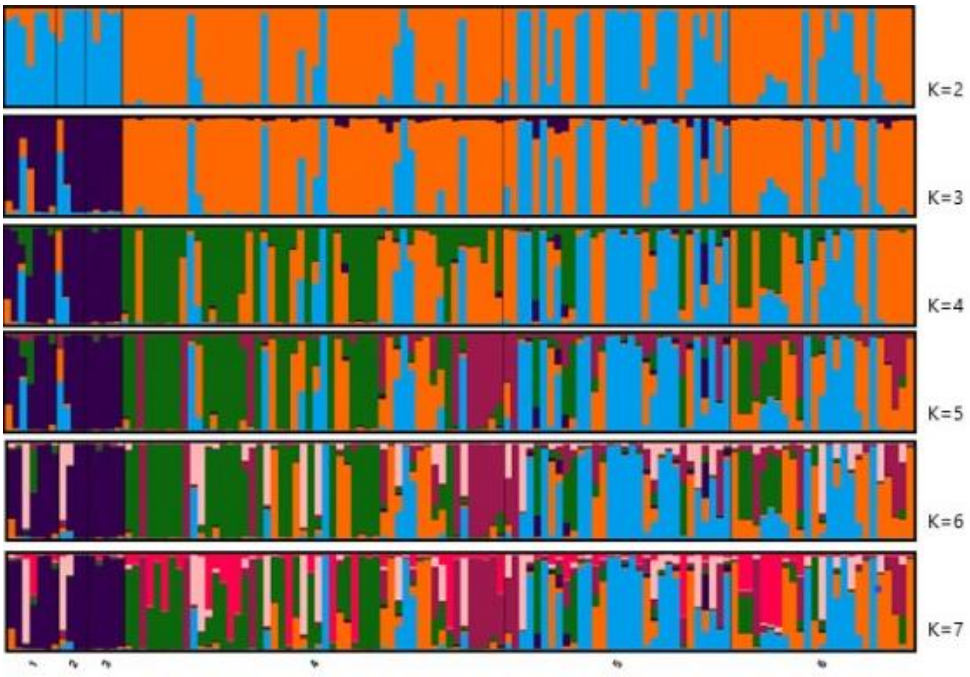

Figure 4. Bayesian STRUCTURE bar plot based on probabilities for 124 genotypes of six populations of *P. vulgaris* L. Lines separate populations.

collected. On the basis of this result, the Type I and Type III genotypes were analyzed separately to reveal variation within each. The analysis performed in accordance with growth habit showed that the variation within populations for Type I and Type III (97% and 89%, respectively) was higher than the variation among populations (3% and 11%, respectively).

Population structure

A model-based clustering method was applied in the STRUCTURE program to explain the population structure of 124 landrace genotypes. ΔK values were calculated to assess the optimal number of genetic clusters (K) in the population structure. On the basis of maximum likelihood and delta K values, the number of optimum groups was 4. As seen in Figure 2, populations were not clustered in accordance with geographical region. CLUMPP results also supported this data. STRUCTURE analysis revealed that the landrace genotypes were subdivided into two main clusters (Figure 4). Although allele exchange among the cluster was observed, in general, the populations were preserved. Populations with significant admixtures were separated in accordance with their growth habits.

DISCUSSION

Salt tolerance evaluation

Salt stress is one of the abiotic stresses that significantly reduce the plant yield of the common bean, and cultivated bean germplasm lacks wide variation in terms of salt tolerance (Gama *et al.,* 2007). Thus, landrace

varieties, which are important gene sources, for use in breeding studies should be examined morphologically and molecularly. The effects of salt stress can be observed primarily on plant growth. Reductions in plant biomass, shoot, leaf, and root growth are the first regressions that can be detected. The STI obtained from the data of parameters, such as yield, plant growth, or biomass, under stress and nonstress conditions is a supportive method for the selection of genotypes with superior performance (Negrão *et al.,* 2017). This method, which is widely used for field crops, is rarely used for vegetables. Many studies have shown that salt stress suppresses the growth of bean plants (Stoeva and Kaymakanova, 2008; Bayuelo-Jiménez *et al.,* 2012; Assımakopoulou *et al.,* 2015). Similarly, in this study, the development of genotypes exposed to salt stress was inferior to those of the control groups. The STI values calculated on the basis of the reduction in plant development provided the classification of genotypes in accordance with tolerance levels. The parameter that most affected STI was STTI-PDW. Root and shoot lengths were also important parameters for salinity stress studies. Suppressed cytokinesis and cell expansion, decreased growth-stimulating hormone levels, and elevated growth-inhibiting hormone levels restrain root and shoot growth. In addition, the toxic effects of salts and the increase in osmotic pressure around roots can prevent water intake by the roots, thus reducing root and shoot length. A study on tomato (Albacete *et al.,* 2008) reported that salt stress has an indirect effect on the accumulation of abscisic acid and decreases the contents of indole-3-acetic acid and cytokines; these processes support senescence. Indirect and direct influences affect plant development, and the severity of these effects varies in accordance with the tolerance level of genotypes. Collado *et al.* (2015) classified high-yielding maize accessories by using STIs based on vegetative growth parameters. Studies on wheat (Al-Ashkar and El-Kafafi, 2014), *Vigna* species (Win *et al.,* 2011), radish, turnip (Noreen and Ashraf, 2008), and rapeseed (Wu *et al.,* 2019) reported similar results. In another study, only dry weight was used to calculate STI, and a wide variation was found among wheat genotypes (Khatun *et al.,* 2013). Plant biomass is the result of all the physiological and biochemical activities of a plant. Consistent with this study, a study by Al-Ashkar and El-Kafafi (2014) reported that fresh and dry shoot weights have a very high direct effect on STI and are crucial parameters for selection criteria. This effect may be due to biomass and the accumulation of organic and inorganic solutes for osmotic adjustment. Consistent with these studies, plant fresh weight, plant dry weight, and plant height were identified as the first three criteria that were effective in determining genotypes. This method, which is widely used for field crops, is rarely used for vegetables. Salt stress negatively affects plant growth. STI, which is a method based on plant growth suppression, is a method that is easy to apply, calculate, and repeat. This method, which is thought to facilitate plant selection, should be used more widely in vegetable research because it is applicable even in the early stages of plant development. In this study, as a result of statistical analysis, a single

genotype was classified as tolerant, and 10 genotypes were identified as moderately tolerant. The better yield of Durango, a common bean landrace, under drought conditions than that of the varieties developed in the last 30 years (Beebe *et al.,* 2013) clearly shows the importance of landrace genotypes as genetic resources.

Genetic diversity and population structure

In this study, 30 markers were used to reveal the genetic diversity and population structure of 124 common bean landrace genotypes with Type I and Type III growth habits. Among these markers, 56.6% were polymorphic for all genotypes, 70% for Type I genotypes, 63.3% only for Type III genotypes, and 16.6% (5) did not exhibit amplification at all. In addition, the number of alleles ranged from 2–5. A study on Ethiopian and Kenyan local varieties found that the rate of polymorphism was 100% and the number of alleles ranged from 2 to 35 (Asfaw *et al.,* 2009). In another study conducted on local Brazilian bean genotypes, 67 out of 80 microsatellite markers were evaluated, the number of alleles ranged from 2 to 37, and the PIC values of the loci were between 0.01–0.96 (Burle *et al.,* 2010). Similar to these studies, many studies, especially studies on core collection and gene pools, have determined high numbers of alleles per locus (Blair *et al.,* 2009; Kwak and Gepts, 2009). For the varieties collected from different regions of Turkey or selected populations, the number of alleles per locus ranged from 1–9 (Madakbaş *et al.,* 2016; Ahmad, 2018; Ekbic and Hasancaoğlu, 2019). The effectiveness of markers may vary in accordance with genotypes. Therefore, the primers that had shown high polymorphism value in many studies were selected. However, some of these primers are monomorphic for common bean landrace genotypes from the western Mediterranean region. Similarly, the BM152 marker, which was determined as polymorphic (PIC > 0.80) in this study, provided a monomorphic result in the study of Mhlaba *et al.* (2018). The same marker was reported by Valentini *et al.* (2018) as one of the most informative markers. On the other hand, the BM201 marker gave monomorphic results for all genotypes in this study but was reported as polymorphic by Pereira *et al.* (2019). Sampling can be reproduced, showing that although markers were carefully selected, different results can be obtained depending on the genotype or accession. The makers used in the study are also important in terms of their results for Types I and III genotypes. Three of these markers (BM171, BM184, and Pvgccaacc001) did not produce bands for Type III genotypes. In this case, the use of BM187 and Pvgccacc001 primers will be useful, especially in characterization studies on common beans with Type I growth habit. In total, six highly informative SSRs were determined for all genotypes with PIC value > 0.70 (BM141, GATs91, BM175, BM154, BM152, and BM160). This finding, which was also supported by many other studies, suggested that these SSRs may be distinguishable even for genotypes collected from close regions. This will help breeding programs.

Generally, long repeats are more polymorphic than short repeats (Ellegren, 2004). Although this situation has been demonstrated in studies on different plant species

(Burstin *et al.,* 2001; Xu *et al.,* 2008;Zhao *et al.,* 2012), Yu *et al.* (1999) showed that SSRs with short repeats may also have a high rate of polymorphism. Although SSRs longer than 20 bp are considered as class I and long and SSRs less than 20 bp are considered as class II and short (Temnykh *et al.,* 2001), in this study, highly polymorphic loci generally have long repeats and lowly polymorphic loci (less than 0.50) generally have short repeats. However, the SRIAC10 marker provided a high polymorphism rate for Type I genotypes and low polymorphism rates for Type III genotypes, whereas BM152 and BM160 markers provided high polymorphism for both growth habits. As previously stated by Yu *et al.* (1999) and Masi *et al.* (2009), the number of repetitions is not always related to the number of alleles and the polymorphism ratio. In general, the ratio of polymorphism varies depending on the genotype rather than the primary length.

Populations with the highest genetic distance were common beans with different growth types (Types I and III) and were collected from different locations (Antalya and Isparta). However, although they were collected from different locations, the genetic distance was the lowest among pop 2 and pop 6 with Type I growth characteristics. Evaluating all tables and graphs related to genetic distance revealed that the main determinant factor was the growth habit of common beans. Permutation tests (based on 999 permutations) suggested that *ΦPT* was not significant for Type I (*ΦPT* = 0.032) but was significant for Type III (*ΦPT* = 0.114). This result showed that differences among location were not significant for Type I genotypes but were significant for Type III genotypes. Comparing the populations on the basis of Table 3 revealed that the average allele numbers (*Na*) of the populations ranged from 1.94 to 3.00. The loci had the same number of alleles in all populations. When the loci were examined separately, no significant difference was found between the number of alleles and the number of effective alleles. Although the total number of alleles detected in a locus was sometimes high, these alleles might have low efficacy for determining the variation in a population. Mhlaba *et al. (*2018) reported 12 alleles for the GATS 91 locus, but the number of the effective allele was 8.7. Fisseha *et al.* (2016) obtained 14 alleles for the same locus and determined that the number of effective alleles was 6.57. In the same study, similar results were obtained for the GATS54, BM205 (used in this study), BM156, BM187, BM140, BM143, and BM139 loci. Therefore, in determining loci, allele numbers, as well as the number of effective alleles in these loci, should be considered. In accordance with the results of the analysis, 1.97 alleles were effective for the differentiation of populations in terms of 17 different microsatellite loci in landraces of common beans from the western Mediterranean region.

Ho was obtained from the highest BM205 locus (0.657) and the lowest BM185 locus (0.006). Although a wide range of *Ho* values was found, the values obtained per locus were generally high. The obtained *Ho* (0.187) and I (0.698) values revealed moderate genetic diversity in populations due to the self-pollination structure of the plant, human, and natural factors. In particular, the specific features of economic importance by farmers contributed to

limited genetic variation. Masi *et al.* (2009) determined the *Ho* of the landrace varieties of Italy as 0.008 by using SSR markers. Similarly, other studies on the landrace varieties of the common bean from Italy (Raggi *et al.,* 2013; Scarano *et al.,* 2014) and Croatia (Carović-Stanko *et al.,* 2017) provided *Ho* values that were very low (0.05, 0.06 and zero). Comparing this value (only for these regions) with the *Ho* obtained from the populations in this study (0.187) showed that the heterozygosity of the landrace genotypes of the western Mediterranean was higher than those of Italian and Croatian landrace varieties but lower than those of Chinese (*Ho* = 0.100–0.954) (Xu *et al.,* 2014) and Northern Portugal (*Ho* = 0.100–0.029) (Coelho *et al.,* 2009) landrace varieties. This diversity is an important finding for evaluation in breeding studies and for in situ conservation studies. The rate of migration among populations is related to the distance between populations. Many geographic features may limit the flow of genes between populations (Whitlock and Mccauley, 1999). Populations generally differ genetically by distance isolation (similarity increases as the distance decreases) (Balloux and Lugon-Moulin, 2002). In contrast to allogamous species, some autogamic species show exceptional local genetic differentiation that is consistent with theoretical expectations (Heywood, 1991). Reliable estimates of the differentiation of populations are important to understand the link between populations and to develop conservation strategies (Balloux and Lugon-Moulin, 2002). The critical Nm value is 1.0, and rates above this value indicate that gene flow is sufficient to prevent genetic shifts (Wright, 1951). The value of gene flow (*Nm* = 1.050) has played an important role in the origin of the similarities of genotypes grown in a close geographical region. The mean *Nm* value was calculated as 1.050. *Nm* values indicated sufficiently strong gene flow that prevents regional genetic differentiation. However, the loci BM152 (0.577), BM184 (0.490), BM211 (0.373), and GATS91 (0.505) differed from the other loci in terms of *Nm* value, and these loci or the loci connected to these loci are subject to strong natural selection as described by Slatkin (1987). Evaluating *Gst* (0.180) and *Nm* (1.050) values together revealed weak genetic differentiation and frequent gene flow. The gene flow within Type I (1.939) and Type III (7.383) populations was found to higher than the gene flow among all populations. Specifically, the gene flow between Type III populations was found to be much higher than the general gene flow and the gene flow of Type I populations. This finding was supported by the results of STRUCTURE analysis.

Cluster analysis showed that the genotypes were divided into two main groups in accordance with their growth habits, not their collection locations. Raggi *et al.* (2013) and Xu *et al.* (2014) reported that landrace varieties are clustered in accordance with altitudes/regions, whereas landrace genotypes collected from different altitudes (11–1299 m) are clearly separated into clusters only in accordance with growth habits. Another study (Mavromatis *et al.,* 2010) on the landrace and commercial common bean varieties of Greece determined that growth habit is positively related to molecular classification, although genetic similarity cannot be related to the

seed characteristics and agronomic characteristics of common beans. However, as seen from the STRUCTURE analysis, admixture was present between Type I and Type III genotypes. Although populations were very clearly separated in the dendrogram, the increased admixing seen in STRUCTURE could be attributed to seed exchange among locations and natural hybridization. Similarly, Masi *et al.* (2009) reported that common bean landraces with different growth habits shared a small number of alleles. STRUCTURE analysis, which supports this finding, clearly showed limited allele sharing between Type I and Type III genotypes. The third population is seen as the most isolated population. Populations with the most admixture were the fifth and sixth populations. In these populations, alleles were shared with other populations, and the admix ratio was different from that in other populations. The preference of local farmers to cultivate Type III common beans may be one of the reasons for this situation. Mhlaba *et al.* (2018) reported that the genetic groups of tepary beans are based on geographical origin. They proposed to work on the genetic grouping of varieties with different geographical origins against genetic bottleneck. In this study, growth habit, not geographical origin, was effective for grouping, and the hybridization of genotypes with different growth habits is recommended to overcome the genetic bottleneck that may occur due to limited allele sharing between plants with different growth habits.

CONCLUSIONS

Revealing the genetic structures of landrace genotypes, as well as screening for salt stress and other stress factors, storage in gene banks, and inclusion in breeding programs should be among research priorities considering global climate predictions. This study found that growth habit plays a dominant role in genetic clustering and that allele sharing between genotypes with different growth habit is quite limited. This situation is thought to be one of the causes of bottlenecks in the common bean. In addition, the results of this research illustrate that the salt tolerance levels of genotypes are affected by allele sharing. Considering these results, hybridization between genotypes with different growth habits is necessary to increase genetic diversity, overcome genetic bottlenecking, or specifically create a new gene pool for salt stress breeding research all over the world.

ACKNOWLEDGEMENTS

The study was supported by Akdeniz University Scientific Research Projects Unit under Grant [2013.01.0104.001]. Part of this study was presented in the congress entitled "Global Conference on Plant Science and Molecular Biology" on 11–13 September 2017 and published as an abstract.

REFERENCES

Ahmad KMS (2018). Genetic diversity of common bean (*Phaseolus vulgaris*) cultivars from different origins revealed by microsatellite markers. *J. Adv. Biol. Biotechnol*. 1-9.

Ahmad M, Shahzad A, Iqbal M, Asif M, Hirani AH (2013). Morphological and molecular genetic variation in wheat for salinity tolerance at germination and early seedling stage. *Australian J. Crop. Sci*. 7(1): 66.

Aitken KS, Jackson PA, Mcintyre CLA (2005). Combination of AFLP and SSR markers provides extensive map coverage and identification of homo(eo) logous linkage groups in a sugarcane cultivar. *Theor. Appl. Genet.* 110: 789–801.

Al-Ashkar IM, El-Kafafi SH (2014). Identification of traits contributing salt tolerance in some doubled haploid wheat lines at seedling stage. *Middle East J. Appl. Sci.* 4(4): 1130-1140.

Albacete A, Ghanem ME, Martínez-Andújar C, Acosta M, Sánchez-Bravo J, Martínez V, Lutts S, Dodd IC Pérez-Alfocea F (2008). Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. J. Experimental Bot. 59(15): 4119-4131.

Ali Z, Salam A, Azhar FM, Khan IA (2007). Genotypic variation in salinity tolerance among spring and winter wheat (*Triticum aestivum* L.) accessions. *South. Afr. J. Bot.* 73: 70-75.

Asfaw A, Blair MW,AlmekindersC (2009). Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the East African highlands. *Theor. Appl. Genet*. 120(1): 1-12.

Assimakopoulou A, Salmas I, Nifakos K, Kalogeropoulos P(2015). Effect of salt stress on three green bean (*Phaseolus vulgaris* L.) cultivars. *Not. Bot. Horti. Agrobo.* 43(1): 113-118.

Azeez MA, Adubi AO, Durodola FA (2018). Landraces and crop genetic improvement. In Rediscovery of landraces as a resource for the future. Intech Open.

Balloux F, Lugon-Moulin N (2002). The estimation of population differentiation with microsatellite markers. Mol. Ecol. 11(2): 155-165.

Bayuelo-Jiménez JS, Jasso-Plata N, Ochoa I (2012). Growth and physiological responses of *Phaseolus*species to salinity stress. *Int. J. Agron.* 1-13.

Beebe S, Rao I, Blair M, Acosta J (2013). Phenotyping common beans for adaptation to drought. *Front. Physiol.* 4: 35.

Beltagi MS, Ismail MA, Mohamed FH(2006). Induced salt tolerance in common bean (*Phaseolus vulgaris* L.) by gamma irradiation. *Pak. J. Biol. Sci.* 6: 1143-1148.

Benchimol LL, Campos DeT, Carbonell SAM, Colombo CA, ChiorattoAF, Formighieri EF, Gouvera LRL, Souza de AP (2007). Structure of genetic diversity among common bean (*Phaseolus vulgaris* L.) varieties of Mesoamerican and Andean origins using new developed microsatellite markers. *Genet. Resour. Crop. Evol.* 54: 1747-1762.

Blair WM, Díaz LM, Buendía HF, Duque MC (2009). Genetic diversity, seed size associations and population structure of a core collection of common beans (*Phaseolus vulgaris* L.). *Theor. Appl. Genet*. 119(6): 955-972.

Blair WM, Giraldo MC, Buendia HF, Tovar E, Duque MC, Beebe SE (2006). Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet*. 113: 100-109.

Burle ML, Fonseca JR, Kami JA, Gepts P (2010). Microsatellite diversity and genetic structure among common bean (*Phaseolus vulgaris* L.)

landraces in Brazil, a secondary center of diversity. *Theor. Appl. Genet*. 121 (5): 801-813.

Burstin J, Deniot G, Potier J, Weinachter C, Aubert G, Baranger A (2001). Microsatellite polymorphism in *Pisumativum*. *Plant Breed*. 120:311–317.

Carović-Stanko K, Liber Z, Vidak M, Barešić A, Grdiša M, Lazarević B,Šatović Z (2017). Genetic diversity of Croatian common bean landraces. *Front. Plant Sci*. 8: 604.

Ceccarelli S (2012). Landraces: importance and use in breeding and environmentally friendly agronomic systems. Agrobiodiversity Conservation: securing the diversity of crop wild relatives and landraces. - *CABI Publishing*, Oxfordshire, UK, 103-117.

Chemura A, Kutywayo D, Chagwesha TM, Chidoko P(2014). An assessment of irrigation water quality and selected soil parameters at Mutema Irrigation Scheme, Zimbabwe. *J. Water Resour. Prot.* 6: 132-140.

Coelho RC, Faria MA, Rocha J, Reis A, Oliveira MBP, Nunes E (2009). Assessing genetic variability in germplasm of *Phaseolus vulgaris* L. collected in Northern Portugal. *Sci.Hort*. 122(3): 333-338.

Cokkizgin A (2012). Salinity stress in common bean (*Phaseolus vulgaris* L.) seed germination. *Not. Bot. Horti. Agrobot.* 40(1): 177-182.

Collado MB, Aulicino MB, Arturi MJ, Molina MDC (2015). Evaluation of salinity tolerance indices in seedling of maize (*Zea mays* L.). *Revista de la Facultad de Agronomía*. 114 (1): 27-37.

Doyle JJ, Doyle LH (1988). Isolation of plant DNA from fresh tissue. *Focus.*12(1): 13- 15.

Earl DA,Vonholdt BM (2012). Structure harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour*. 4: 359–361.

Ellegren H (2004). Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet*. 5 (6): 435.

Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol*. 14:2611–2620.

FAO (2020). http://www.fao.org/faostat/en/#data/QC (Available date: 20.06.2020).

Fisseha Z, Tesfaye K, Dagne K, Blair MW, Harvey J, Kyallo M, Gepts P (2016). Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L) germplasm of Ethiopia as revealed by microsatellite markers. *Afr. J. Biotechnol*. 15(52): 2824-2847.

Gaitán-Solís E, Duque MC, Edwards KJ, Tohme J (2002). Microsatellite repeats in common bean (*Phaseolus vulgaris*). *Crop. Sci*. 42(6): 2128-2136.

Gama PBS, Inanaga S, Tanaka K, Nakazawa R (2007). Physiologicalresponse of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. *Afr. J. Biotechnol.* 6(2): 079–088.

Glaubitz JC (2004). CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Mol. Ecol. Notes*. 4(2): 309-310.

Gutiérrez JP, Royo LJ, Álvarez I, Goyache F (2005) MolKin v. 2.0: A computer program for genetic analysis of populations using molecular coancestry information. *J. Hered.* 96:718-721.

Heywood JS (1991). Spatial analysis of genetic variation in plant populations. *Ann. Rev. Ecol. Syst*. 22(1): 335-355.

Hoffman GJ, Howell TA, Solomon KH (1992). Management of farm irrigation systems. ASAE Monograph Number 9 published by *ASAE*.

Jakobsson M, Rosenberg NA (2007). CLUMPP: a cluster matching and

permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinform*. 23:1801–1806.

Khaidizar MI, Haliloglu K, Elkoca E, Aydin M, Kantar F (2012). Genetic diversity of common bean (*Phaseolus vulgaris* L.) landraces grown in northeast Anatolia of Turkey assessed with simple sequence repeat markers. *Turk. J. Field. Crops.* 17(2): 145-150.

Khatun M, Hafız MHR, Hasan MA, Hakım MA, Sıddıqui MN (2013). Responses of wheat genoTypesto salt stress in relationtogerminationandseedlinggr owth. *Int. J. Bio-resource. Stress Manag.* 4(4): 635-640.

Kwak M, Gepts P (2009). Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., *Fabaceae*). *Theor. App. Genet*. 118(5): 979-992.

Lluch C, Tejera N, Herrera-Cervera JA, Lopez M, BarrancoGresa JR, Palma FJ, Gozalvez M, Iribarne C, Moreno E, Ocana A (2007). Saline stress tolerance in legumes. *Lotus Newslet.t* 37(2):76-77.

Madakbaş SY, Sarıkamış G, Başak H, Karadavut U, Özmen CY, Daşçı MG, Çayan S (2016). Genetic characterization of green bean (*Phaseolus vulgaris* L.) accessions from Turkey with SCAR and SSR markers. *Biochem. Genet*. 54(4): 495-505.

Masi P, Logozzo G, Donini P, Zeuli PS (2009). Analysis of genetic structure in widely distributed common bean landraces with different plant growth habits using SSR and AFLP markers. *Crop Sci*. 49(1): 187-199.

Maas EV, Hoffman GJ (1977). Crop salt tolerance, current assessment.*J. Irrig. Drain. Division ASCE*.103: 115-134.

Mavromatis AG, Arvanitoyannis S, Korkovelos AE, Giakountis A, Chatzitheodorou VA, Goulas CK (2010). Genetic diversity among common bean (*Phaseolus vulgaris* L.) Greek landraces and commercial cultivars: nutritional components, RAPD and morphological markers. *Span. J. Agric. Res*. (4): 986-994.

Mhlaba ZB, Amelework B, Shimelis HA, Modi AT, Mashilo J (2018). Genetic interrelationship among tepary bean (*Phaseolusacutifolius* A. Gray) genotypes revealed through SSR markers. *Aust. J. Crop Sci*. 12 (10): 1587.

Munns R (2002). Comparative physiology of salt and water stress. *Plant. Cell. Environ*. 25: 239-250.

Negrão S, Schmöckel SM, Tester M (2017). Evaluating physiological responses of plants to salinity stress. *Ann. Bot.* 119(1): 1-11.

Noreen Z, Ashraf M (2008). Inter and intra specific variation for salt tolerance in turnip (*Brassica rapa* L.) and radish (*Raphanussativus* L.) at the initial growth stages. *Pak. J. Bot.* 40(1): 229-236.

Peakall R, Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinform.* 28:2537–2539.

Pereira HS, Mota APS, Rodrigues LA, de Souza TLPO, Melo LC (2019). Genetic diversity among common bean cultivars based on agronomic traits and molecular markers and application to recommendation of parent lines. *Euphytica*. 215(2): 38.

Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genet.* 155:945–959.

Raggi L, Tiranti B, Negri V (2013). Italian common bean landraces: diversity and population structure. *Genet.Resour.CropEvol*. 60(4): 1515-1530.

Rauf S, Da Silva JT, Khan AA, Naveed A (2010). Consequences of plant breeding on genetic diversity. *Int. J. Plant. Breed.* 4(1): 1-21.

Rosenberg NA (2004). Distruct: a program for the graphical display of population structure. *Mol. Ecol. Notes*. 4:137–138.

Scarano D, Rubio F, Ruiz JJ, Rao R, Corrado G (2014). Morphological and genetic diversity among and within common bean (*Phaseolus vulgaris* L.) landraces from the Campania region (Southern Italy). *Sci.Hort.* 180: 72-78.

Slatkin M (1987). Gene flow and the geographic structure of natural populations. *Sci.* 236(4803): 787-792.

Stoeva N, Kaymakanova M (2008). Effect of salt stress on the growth and photosynthesis rate of bean plants (*Phaseolus vulgaris* L.). *J. Cent. Eur. Agric.* 9(3): 385-391.

Temnykh S, De Clerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001). Computational and experimental analysis of microsatellites in rice (*Oryzasativa* L.): frequency, length variation, transposon association, and genetic marker potential. *Genome Res*. 11:1441–1452.

Ulukapi K., Aydinsakir K, Kurum R (2018). Determination of some parameters of landrace green bean (*Phaseolus vulgaris* L.) genotypes collected from Western Mediterranean Region of Turkey. *Derim*. 35 (2): 87-95.

Uncuoğlu AA (2010). Moleküler Markerler ve Haritalama. *Modern Biyoteknoloji Uygulamaları Erciyes Ünv. Yayınları.* Yayın No: 180.

Valentini G, Gonçalves-Vidigal MC, Elias JCF, Moiana LD, Mindo NNA (2018). Population structure and genetic diversity of common bean accessions from Brazil. *Plant Mol. Biol. Rep*. 36(5-6): 897-906.

Whitlock MC, Mccauley DE (1999). Indirect measures of gene flow and migration: FST≠ 1/(4Nm+ 1). *Hered*. 82(2): 117-125.

Win KT, Aung ZO, Hirasawa T, Ookawa T, Yutaka H (2011). Genetic analysis of Myanmar Vigna species in responses to salt stress at the seedling stage. *Afr. J. Biotechnol.* 10(9): 1615-1624.

Wright S (1951). The genetical structure of populations. *Ann. Eugen*. 15: 323-354.

Wu H, Guo J, Wang C, Li K, Zhang X, Yang Z, Li M, Wang (2019). An effective screening method and a reliable screening trait for salt tolerance of *Brassica napus* at the germination stage. *Front. Plant. Sci.* 10: 530.

Xu S, Wang G, Mao W, Hu Q, Liu N, Ye L, Gong Y (2014). Genetic diversity and population structure of common bean (*Phaseolus vulgaris*) landraces from China revealed by a new set of EST-SSR markers. *Biochem. Syst. Ecol*. 57: 250-256.

Xu, Z, Gutierrez L, Hitchens M, Scherer S, Sater AK, Wells DE (2008). Distribution of polymorphic and non-polymorphic microsatellite repeats in *Xenopustropicalis*. *Bioinformatics Biol. Insights*. 2, BBI-S561.

Yu K, Park SJ, Poysa V, Gepts P (2000). Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). *J.Hered*. 91 (6): 429-434.

Yu K, Soon SJ,Poysa V (1999). Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus* and *Vigna*). *Genome*. 42:27–34.

Zhang X, Blair MW, Wang S (2008). Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) landraces assessed with simple sequence repeat markers. *Theor. Appl. Genet*. 117:629–640.

Zhao Y, Prakash CS, He G (2012). Characterization and compilation of polymorphic simple sequence repeat (SSR) markers of peanut from public database. *BMC Res. Notes*. 5(1): 362.