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Assessment of variation in seed morphological traits in *Phaseolus* sp. landraces from western Anatolia

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Abstract. Seed size, seed shape and seed colour are easily discernible phenotypic traits of beans in the market. Consumers often have specific preferences to different combinations of seed size, shape and colour. Local landraces are usually grown for their desired organoleptic and seed properties by consumers. This study investigated the variability in seed size attributes, seed shape, visual seed colour properties and seed luminosity/chromaticity properties of the germplasm of 255 landraces representing major local bean types grown in 20 provinces in Western Anatolia Region of Turkey. Measurements were made on seeds from representative single plants for seed size index, 100 seed weight, seed length, seed width, seed thickness, seed width in cross section, seed shape, seed degree of curvature in kidney types, seed shape in cross section, number of seed colors, main seed color, secondary seed colour, distribution of secondary seed color, seed veining, colour of hilum ring and values of L (Luminosity), chromaticity a*, chromaticity b*, chroma (C) and hue angle. Data were statistically analysed, principle component analysis was carried out, the germplasm was classified by UPGMA dendograms and selections were made on the bases of GGE biplot analysis. Landraces showed a considerable diversity for seed size, seed shape and seed colour trait measured. All seed size variables ranged by 2–10–fold within the germplasm. 100–seed weight ranged ten–fold from very small seeded Meso–American types (18.4 g, e.g. AYD–8, KTH–30, MGL–1, DNZ–1 and BRD–24) to very large seeded Andean types (182.3 g, e.g. MGL–37, PKS–1, ANT–33, MGL–31 and ANT–16). Seed shape also varied as kidney–shaped (96 landraces), circular to elliptic (73), circular (58), elliptic (27) and rectangular (1) shapes. Main and secondary seed colours had considerable variation within the germplasm with 151 landraces had white, 31 grey, 31 beige, 23 brown, 8 violet, 5 red and 4 black main seed colour whereas 24 landraces had violet followed by 17 black and 14 red secondary seed colour. Landraces exhibited a large variation for chromaticity and luminosity variables. L value ranged between 20.3 and 90.1 representing a 4.5–fold variation. The landraces in the germplasm were assessed and characterised on the bases of diversity in seed morphological traits. Selections were made using UPGMA dendogram and GGE–biplot for production and breeding bean cultivars for preferences of changing consumer demands.
Keyword: Beans, seed size, seed shape, seed colour, luminosity, chromaticity.

Introduction

Dry beans are importance source of proteins in the diets worldwide. Plant breeders commonly aim for higher yields and enhanced tolerance/resistance to a biotic and biotic stress factors. Farmers give also priority to achieving higher seed yields from the cultivars they grow. However, customers, hence dealers and wholesalers attach importance to quality and seed traits such as seed size, shape and colour [KELLY *et al.*, 1999, STOLERU, *et al.*, 2019, KURZBAUM *et al.*, 2019. CARUSO, *et al.*, 2019].

Depending on the country, region and location, costumers developed specific preferences for the combinations of seed size, seed shape and seed colour [BENINGER and HOSTFIELD, 2003]. In breeding programs, some high yielding cultivars were discarded because of poor

acceptance by consumers [KELLY *et al.*, 1999]. Plant breeders need a rich base of diversity for seed traits to be able address consumer demands.

Recent work concentrated, therefore, on enhancing genetic bases of common bean for seed traits [PEREZ–VEGA *et al.*, 2010; YUSTE–LISBONA *et al.*, 2014].

Local landraces are adopted and grown in their localities owing to their traits meeting consumer's tastes irrespective of their seed yields.

Local landraces with proven qualities and rich diversity up to the consumer demands may serve a good starting material in the breeding programs [RIVERA *et al.*, 2015]. In Turkey, local heirloom dry beans have been grown in response to a constant demand by costumers for their quality and seed traits.

Local landraces developed for centuries in response to consumer preferences for seed traits may have a rich source of variation and diversity that need to be exploited in breeding programs.

In Western Anatolia, in spite of highly intensive farming, local beans competed with new high yielding commercial cultivars owing to readily available consumer demand.

Local bean diversity is, hence, so far maintained in many regions.

This work investigated seed traits regarding seed size attributes, seed shape, visual seed colour properties and seed luminosity/chromaticity properties of the germplasm representing major local bean types collected from 20 provinces in Western Anatolia Region of Turkey.

Material and methods

Seeds of 255 landraces of beans (*Phaseolus vulgaris* L.) collected from 20 provinces in Western Anatolia Region in autumn–spring period of 2015 and 2016 and produced/harvested in the field in

September 2017 were investigated for seed traits. Seeds from single plants representing each landrace were harvested after which seed samples were stored at room temperature for approximately 10 days until seeds dried to about 12 % moisture content, then maintained in a cold chamber at 4 ºC until laboratory testing.Classification by seed characteristics of the landraces were made under laboratory by Voysest and DESSERT, Yuste–Lisbona and collab., Dalla Corte and collab. and the cultivar evaluation criteria developed by IPGRI (International Plant Genetic Resources Institute) and EU–CPVO (European Union Community Plant Variety Office) in October–December of 2017 [VOYSEST and DESSERT, 1991, YUSTE–LISBONA *et al.*, 2014, DALLA CORTE *et al.*, 2010].

Measurements listed in table 1 were made on seed size parameters of seed size index (1–5 scale), seed weight (100 g seed), seed length (mm), seed width (mm), seed thickness (mm) and seed width in cross section (mm); seed shape parameters of seed shape (1–5 scale), seed degree of curvature in kidney types (3–7 scale) and seed shape in cross section (3–7 scale); seed colour traits of number of seed colors (1–3 scale), main seed color (9 seed colours), secondary seed colour (7 colours), distribution of secondary seed color (4 classes), seed veining (3–7 scale) and colour of hilum ring (2 classes) and seed coat color variables of L (luminosity) value, chromaticity a* value, chromaticity b* value, Chroma value (C) and Hue Angle value. 100–seed weight was recorded on a precision electronic scale (± 0.001 g), lengths, width and thickness were measured with an electronic calliper, seed coat color was recorded against colour scales used by IPGRI and EU–CPVO and seed seed coat luminosity & chromaticity were recorded with a portable CR–400 tristimulus colorimeter (Minolta Chroma Meter CR 400, Osaka, Japan).

The colorimeter was calibrated as instructed on a white plate provided and of L (luminosity) value, chromaticity a* value, chromaticity b* value, Chroma value (C) and Hue angle values were obtained on cluster of seeds from each landraces. L value indicates luminosity [black L=0, white L=100], chromaticity a* value indicates redness–greenness (red [a*=100] and green [a*=–100]), chromaticity b* value indicates yellowness–blueness (yellow [b*=100,

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blue [b*=–100]), chroma value (C) colour saturation or intensity (c=[(a*)2+(b*)2]0.5) and hue angle value (h=arc tan b/a) indicates red, yellow, green, blue, purple, or intermediate colours between adjacent pairs of these basic colours [AYALA–SILVA *et al.,* 2005].

The colorimeter was expected to accurately identify color characteristics in seed coats and to determine color differences between the seeds of landraces.

Statistical analysis

Data made on the seed traits of 255 landraces were subjected to statistical analysis and the descriptive statistics (mean, standard deviation and ranges), the correlation coefficients and the factor loadings were calculated with the aid of Minitab version 17 statistical software (Minitab Inc., State College, PA, USA).

Correlation and principal component analyses (PCAs) were performed for all data in XLSTAT 2016 statistical software (Addinsoft, New York, USA). Components with greater than 1 Eigen value were taken into consideration.

The distribution of the seed traits of the landraces on the first two components was visualized. Additionally, XLSTAT 2016 statistical software and JMP 13.2.0 statistical software's were used for UPGMA cluster and biplot analysis.

For visual assessment of landraces, a GGE biplot is constructed by plotting the first principal component (PC1) scores of the landraces against their respective scores for the second principal component (PC2) [YAN *et al.*, 2000].

Selections of landraces were made by means of an irregular polygon and a set of lines drawn from the biplot origin and intersecting each of the sides at right angles [YAN *et al.*, 2007].

Results and discussion

Seed Size. There was a considerable variation within the germplasm of 255 landraces investigated for the parameters of seed size, seed shape, seed colour and seed luminosity & chromaticity (Table 1, Figure 1).

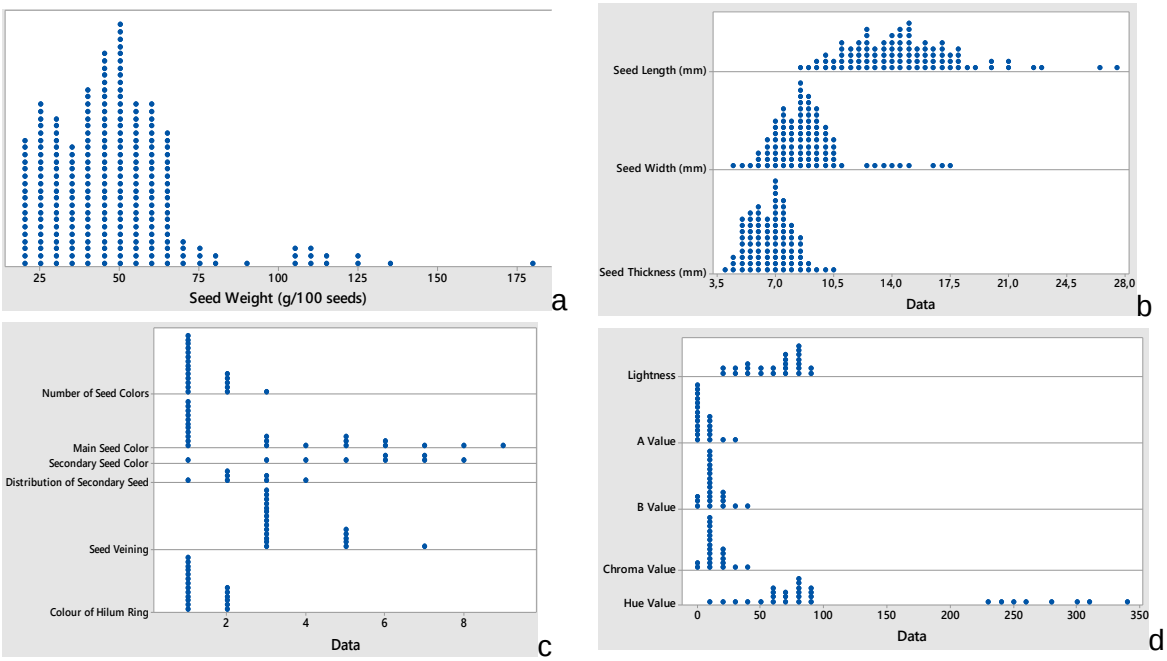


Figure 1 a, b, c, d. Frequency histogram graphs for 100 seed weight, seed size, seed color and seed luminosity & chromaticity values of the landraces investigated.

Of the seed size traits, 100 seed weight had the highest variation followed by seed size index, seed with, seed length, seed thickness and seed width in cross section (Table 1, Figure 1). 100 seed weight of the landraces ranged from

18.4 g to 182.3 g with an average of 48.2 g, which represented a ten–fold variation between the landraces (Table 1).

Landraces of MGL–37, PKS–1, ANT–33, MGL–31 and ANT–16 had the highest 100 grain weights of 182.26 g, 135.87 g, 127.25 g, 124.23 g and 113.18 g while AYD–8, KTH–30, MGL– 1, DNZ–1 and BRD–24 had the lowest 100 seed weights of 18.41 g, 18.66 g, 19.08 g, 19.42 g and 20,16 g respectively.

Of the landraces, 28 had 100 seed weight below 25.00 g, 212 landraces had 100–seed weight between 25.00 g and 75.00 g and 15 landraces had 100–seed weight over 75.00 g (Figure 1).

Table 1.

Seed size, seed shape, seed color and seed Luminosity & chromaticity values measured of 255 landraces from 20 Western Anatolia provinces

Seed Variables	Unit/explanation	Min	Max	Mean	S. Dev
Size&weight traits					
Seed size index	1–very low, 2–low, 3–medium, 4–high, 5–very high	1.0	9.0	5.1	1.77
Seed weight	g/100 seeds	18.4	182.3	48.2	21.99
Seed length	mm	8.6	27.3	14.5	2.97
Seed width	mm	4.3	17.4	8.7	1.84
Seed thickness	mm	4.2	10.4	6.7	1.23
Seed width in cross section	mm	3.0	7.0	4.8	1.04
Shape traits					
Seed shape	1– circular, 2–circular to elliptic, 3–eliptic, 4–kidney–shaped, 5–rectangular	1	5	2.6	1.20
Seed degree of curvature in kidney types	3– weak, 5– medium, 7– strong	3	7	4.0	1.16
Seed shape in cross section	1–flat, 2–narrow elliptic, 3–medium elliptic, 4–broad elliptic, 5– circular	1	5	3.2	0.67
Visual colour traits					
Number of seed colors	1– one, 2– two, 3– more than two	1	3	1.3	0.45
Main seed color	1– white, 2– green or greenish, 3–grey, 4–yellow, 5–beige, 6–brown, 7–red, 8–violet, 9–black	1	9	2.7	2.29
Secondary seed color	1– grey, 2–yellow, 3–beige, 4–brown, 5–red, 6–violet, 7–black	1	7	6.1	1.24
Distribution of secondary seed color	1–around hilum, 2– on half of grain, 3– on entire grain, 4–patchy shape	1	4	2.5	0.75
Seed veining	3– weak, 5– medium, 7– strong	3	7	3.6	1.01
Colour of hilum ring	1–same colour as testa, 2– darker than testa	1	2	1.3	0.46
Luminosity & chromaticity					
L (Luminosity) value	Lightness (black L=0, whiteL=100)	20.3	90.1	63.2	20.5
chromaticity a* value	redness–greennes (red [a=100] and green [a=–100])	–0.7	25.8	4.5	5.37
chromaticity b* value	yellowness–blueness (yellow [b=100, blue [b=–100])	–3.1	40.9	11.6	6.64
Chroma value (C)	colour saturation/intensity (c=[(a)2+(b)2]0.5)	0.08	43.2	13.1	7.50
Hue angle value	intermediate colours, (h=arc tan b/a)	5.5	342.6	89.4	59.80

Seed Shape. Seed shape traits varied also by 300%, 133% and 400% respectively in traits of seed shape, seed degree of curvature in kidney types and seed shape in cross section respectively (Table 1).

96 landraces had kidney–shaped seeds followed by 73 landraces having circular to elliptic seeds, 58 landraces having circular, 27 landraces having eliptic and one genotype rectangular seed shape.

Seed shape in cross section had the highest variation with 176 landraces having medium elliptic, 58 landraces having broad elliptic, 11 landraces having circular, 4 having narrow elliptic and 6 having flat seeds.

Seed Colour. Visual seed colour traits showed a great deal of variation within the germplasm investigated (Table 1, Figure 1).

Main seed colour and secondary seed colour had the highest variation followed by distribution of secondary seed colour, number of seed colours, seed veining and colour of hilum ring (Table 1).

Of 255 landraces, 151 landraces had white main seed colour with 31 grey, 31 beige, 23 brown, 8 violet, 5 red and 4 black main seed colour (Figure 1).

24 landraces had violet secondary seed colour followed by 17 landraces with black, 14 landraces red and the other colours represented by 1 to 8 landraces

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with no genotype having yellow secondary seed colour (Figure 1).

Seed luminosity & chromaticity. Landraces exhibited larger variation for testa chromaticity and luminosity variables than in other seed attributes (Table 1, Figure 1).

Chroma value (C) had the highest variation followed by Hue angle value, chromaticity a* value, chromaticity b* value and luminosity (L) value (Table 1, Figure 1). L value ranged between 20.3 and 90.1 representing a 4.5–fold variation (Table 1, Figure 1).

White seeded landraces usually had the highest L values (e.g. ANT–19, BLK–8, ANT–42, ANT–7, BKS–18, BRD–23, ÇNK–5 and ISPT–4) but other white seeded landraces of IZM–4, MNS–3, AYD–6, IZM–2 and KTH–16 had the lowest L values.

Hue value varied greatly from 5.5 to 342.6 within the germplasm. Landraces with coloured seed coats of DZC–6, DZC–9, BLK–10, ÇNK–8, IZM–3 and BRD–1 had the highest hue angle values of 342.6, 339.3, 310.1, 303.2, 279.9 and 264.8 respectively.

Luminocity values increased with main seed color becoming darker as white (72,79), grey (72,42), yellow (59,3), beige (47,85), brown (36,84), red (35,28), violet (29,53) and black (26,93) (Table 4).

Lighter colored seeds had higher L values.

But L value also varied depending on the secondary seed colour (Table 4).

Red and red–brown seed coats had the highest a* values (22.54 and 23.15 respectively) (Table 4). a*, C and H values varied depending on the main and secondary seed colors (Table 4).

Correlations between seed morphological traits. 100 seed weight was significantly correlated with seed length (r=0,793, P<0.01), seed with (r=0,835, P<0.01) and seed thickness (r=0,671, P<0.01) but not with seed shape parameters (Table 2).

Table 2.

Pearson's correlation coefficients between seed size, seed shape, seed color and seed luminosity & chromaticity values of the landraces investigated

	S	SW	SL	SWi	ST	SWCS	SSH	SDC	SSCS	NSC	MSC	SSC	DSC	SV	CHR	L	a	b	c	H
SS	1	0.613**	0.648**	0.496**	0.400**	0.101	0.244**	0.215**	0.022	0.191**	0.101	0.193**	0.171**	0.03	0.147*	0.04	0.132*	0.106	0.129*	−0.159*
SW		1	0.793**	0.835**	0.671**	0.245**	0.017	0.015	0.054	0.231**	0.038	0.232**	0.219**	−0.025	0.074	0.018	0.012	0.008	0.011	−0.11
SL			1	0.672**	0.421**	0.016	0.411**	0.376**	−0.156*	0.103	0.037	0.114	0.072	−0.047	0.078	0.046	0.063	0.009	0.03	−0.112
SWi				1	0.649**	0.358**	−0.149*	−0.104	0.156*	0.278**	0.136*	0.283**	0.272**	−0.099	0.174**	−0.04	0.093	0.045	0.067	−0.098
ST					1	0.275**	−0.271**	−0.185**	0.293**	0.367**	0.210**	0.348**	0.367**	−0.050	0.202**	−0.153*	0.111	0.048	0.06	−0.032
SWCS						1	−0.386**	−0.283**	0.165**	0.25**	0.069	0.261**	0.278**	0.023	0.095	0.046	0.058	0.001	0.013	−0.103
SSH							1	0.830**	−0.414**	−0.138*	−0.129*	−0.145*	−0.142*	0.005	0.027	0.171**	0.042	−0.037	−0.01	−0.102
SDC								1	−0.317**	−0.142*	−0.146*	−0.159*	−0.138*	0.073	0.078	0.259**	0.017	−0.083	−0.057	−0.125*
SSCS									1	0.163**	0.042	0.162**	0.147*	0.057	0.066	0.028	−0.003	0.129*	0.078	−0.109
NSC										1	0.299**	0.942**	0.949**	−0.15*	0.504**	−0.152*	0.364**	0.203**	0.252**	−0.155*
MSC											1	0.303**	0.227**	−0.067	0.294**	−0.666**	0.634**	0.154*	0.362**	−0.037
SSC												1	0.918**	−0.17**	0.515**	−0.156*	0.355**	0.203**	0.248**	−0.154*
DSC													1	−0.161*	0.546**	−0.117	0.319**	0.184**	0.227**	−0.161*
SV														1	−0.210**	0.033	−0.101	−0.089	−0.113	−0.064
CHR															1	−0.136*	0.502**	0.292**	0.382**	−0.227**
L																1	−0.401**	0.139*	−0.044	−0.407**
a*																	1	0.548**	0.802**	−0.429**
A*																		1	0.925**	−0.551**
C																			1	−0.582**
H																				1

Significant at P<0.05, ** significant at P<0.01, SS: Seed Size Index, SW: Seed Weight (g/100 seeds), SL: Seed Length (mm), SWi: Seed Width (mm), ST: Seed Thickness (mm), SWCS: Seed Width in Cross Section, SSH: Seed Shape, SDC: Seed Degree of Curvature İn Kidney Types, SSCS: Seed Shape in Cross Section, NSC: Number of Seed Colors, MSC: Main Seed Color, SSC: Secondary Seed Color, DSC: Distribution of Secondary Seed Color, SV: Seed Veining, CHR: Colour of Hilum Ring, L: Luminocity, A: a* Value, B: b* Value, C: Chroma Value, H: Hue Angle Value

Seed length was positively correlated with seed shape (r=0.411, p<0.01) and seed degree of curvature (r=0.376, P<0.01).

Seed weight related traits did not have high Pearson's correlation coefficients with seed colour parameters excepting for seed thickness having significant and relatively higher r values with number of seed colors (r=0.367, p<0.01), main seed color (r=0.210, p<0.01), secondary seed color (r=0.348,

p<0.01) and distribution of secondary seed colour (r=0.367, p<0.01).

Seed weight parameters were not correlated significantly with seed coat luminocity and chromaticity values (Table 2). Seed shape was correlated with seed degree of curvature in kidney types (r=0.830, p<0.01) and seed shape in cross section (r=–0.414, p<0.01) but not with colour parameters (Table 2).

Main seed colour was negatively correlated with luminosity (L) (r=–0,666, P<0.01) and positively with chromaticity a* value (r=0,634, P<0.01) while colour of hilum ring was positively correlated with chromaticity a* value (r=0.502, P<0.01) (Table 2).

Principle Component Analysis (PCA) showed that 6 components had Eigen values above 1 explaining 79.32 % of cumulative variance (Table 3).

Table 3.

Principle component analysis of seed morphological traits of the germplasm investigated

Variables	PC1	PC2	PC3	PC4	PC5	PC6
Seed Size Index	0.454	0.595	0.205	0.012	–0.118	0.114
Seed Weight (g/100 seeds)	0.536	0.747	–0.098	0.121	–0.165	–0.060
Seed Length (mm)	0.384	0.800	0.266	–0.091	–0.145	–0.062
Seed Width (mm)	0.605	0.595	–0.204	0.151	–0.198	–0.125
Seed Thickness (mm)	0.617	0.394	–0.339	0.097	–0.175	–0.007
Seed Width in Cross Section	0.356	0.053	–0.400	0.300	0.065	0.117
Seed Shape	–0.174	0.358	0.741	–0.368	0.184	0.069
Seed Degree of Curvature İn Kidney Types	–0.166	0.384	0.687	–0.290	0.232	0.163
Seed Shape in Cross Section	0.236	–0.141	–0.367	0.435	–0.021	0.165
Number of Seed Colors	0.791	–0.155	–0.156	–0.217	0.450	0.096
Main Seed Color	0.486	–0.312	–0.006	–0.415	–0.530	0.139
Secondary Seed Color	0.788	–0.154	–0.161	–0.214	0.442	0.072
Distribution of Secondary Seed Color	0.770	–0.150	–0.168	–0.194	0.507	0.074
Seed Veining	–0.187	0.080	–0.006	0.170	–0.107	0.911
Colour of Hilum Ring	0.613	–0.219	0.193	–0.211	0.206	–0.068
Luminocity (L)	–0.221	0.250	0.275	0.632	0.556	–0.081
a* Value	0.622	–0.425	0.423	–0.134	–0.342	0.064
b* Value	0.458	–0.378	0.498	0.456	–0.132	–0.135
Chroma Value (C)	0.555	–0.438	0.542	0.292	–0.259	–0.088
Hue Angle Value	–0.367	0.119	–0.531	–0.536	–0.095	–0.135
Eigenvalue	**5.204**	**3.171**	**2.791**	**1.929**	**1.733**	**1.036**
Variability (%)	**26.022**	**15.856**	**13.955**	**9.647**	**8.666**	**5.180**
Cumulative %	**26.022**	**41.877**	**55.832**	**65.479**	**74.146**	**79.325**

All the traits with Eigen values had positive contributions to the components.

Considering strongest correlations and contribution of seed traits, visual seed colour parameters excepting seed veining were positively correlated with the first component (Table 3).

The highest contribution was that of number of seed colours (0,791) and secondary seed colour (0.788).

The first component could be, hence, termed as "visual colour axis".

Apart from seed width in cross section, seed weight variables were positively correlated with the second component, termed therefore as "weight axis".

Seed shape (0.741) and seed degree of curvature in kidney types (0.687) were positively correlated with and contributed to the third component.

The third component could be then termed as "shape axis".

Measured colour luminosity and chromaticity positively contributed to the fourth component as luminosity (L) (0.632), chromaticity b* value (0.456) and hue value (–0.536) (Table 3).

The fourth component could be termed as "luminosity & chromaticity axis".

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Table 4.

Variation of luminosity (L), chromaticity a*, chromaticity b*, Chroma (C) and Hue angle (H) values of various seed main colour and secondary seed coat colour of bean landraces

Main seed color	Secondary seed coat color	X	SE	X	SE	X	SE	X	SE	X	SE
White	**White***	72.79	1.48	1.17	0.08	9.83	0.33	9.96	0.33	98.74	4.23
White	**Red**	48.73	0.79	6.02	0.32	17.32	0.22	18.35	0.11	70.83	1.18
White	**Violet**	58.57	7.48	3.98	2.33	8.61	1.04	10.35	0.92	67.09	12.84
White	**Black**	74.51	3.83	0.12	0.15	9.70	0.20	9.70	0.20	89.29	0.89
Grey	**Grey***	72.42	2.34	7.80	0.96	14.89	1.17	16.88	1.30	62.34	2.62
Grey	**Red**	71.64	1.12	6.53	0.37	12.41	0.93	14.05	0.94	61.95	1.53
Grey	**Violet**	70.98	1.20	8.85	0.63	13.20	0.59	15.95	0.81	56.41	1.11
Grey	**Black**	66.94	2.95	6.08	0.91	10.89	1.27	12.54	1.42	60.65	3.39
Yellow	**Yellow***	59.3		5.4		12.50		14.3		90.7	
Yellow	**Violet**	46.50		18.22		13.16		22.49		35.91	
Beige	**Biege***	47.85	3.74	12.60	1.87	25.78	2.54	28.98	2.85	64.46	2.69
Biege	**Red**	42.36	5.86	6.17	2.15	12.47	4.11	14.00	4.59	128.92	41.00
Beige	**Violet**	56.38	6.09	13.67	1.32	23.85	2.42	27.58	2.52	59.91	2.48
Biege	**Black**	42.86	3.59	10.25	2.20	16.78	3.36	19.73	3.97	86.39	27.68
Brown	**Brown***	36.84	1.35	10.00	0.71	16.83	1.09	19.77	0.82	59.09	3.29
Brown	**Violet**	53.12		9.88		18.09		20.67		61.23	
Brown	**Black**	43.03	6.69	8.41	3.24	16.34	5.80	18.49	6.54	112.44	49.43
Red	**Red***	35.28	6.18	22.54	1.75	7.67	2.90	24.15	2.59	16.38	5.16
Red	**Brown**	44.14		23.15		7.86		24.47		18.80	
Violet	**Violet***	29.53	2.71	4.45	1.17	0.62	1.06	5.127	1.28	154.67	52.06
Black	**Black***	26.93	0.36	1.35	0.35	0.19	0.34	1.540	0.29	166.85	90.45
Mean		52.41	3.40	8.89	1.21	13.44	1.73	16.33	1.85	79.36	17.77

*Monocolour seed coats

Biplot analysis showed visual standing of landraces relative to seed morphological traits (Figure 2). 100–seed weight and main seed colour had the longest vector lengths indicating their relative importance within the seed morphological traits investigated (Figure 2).

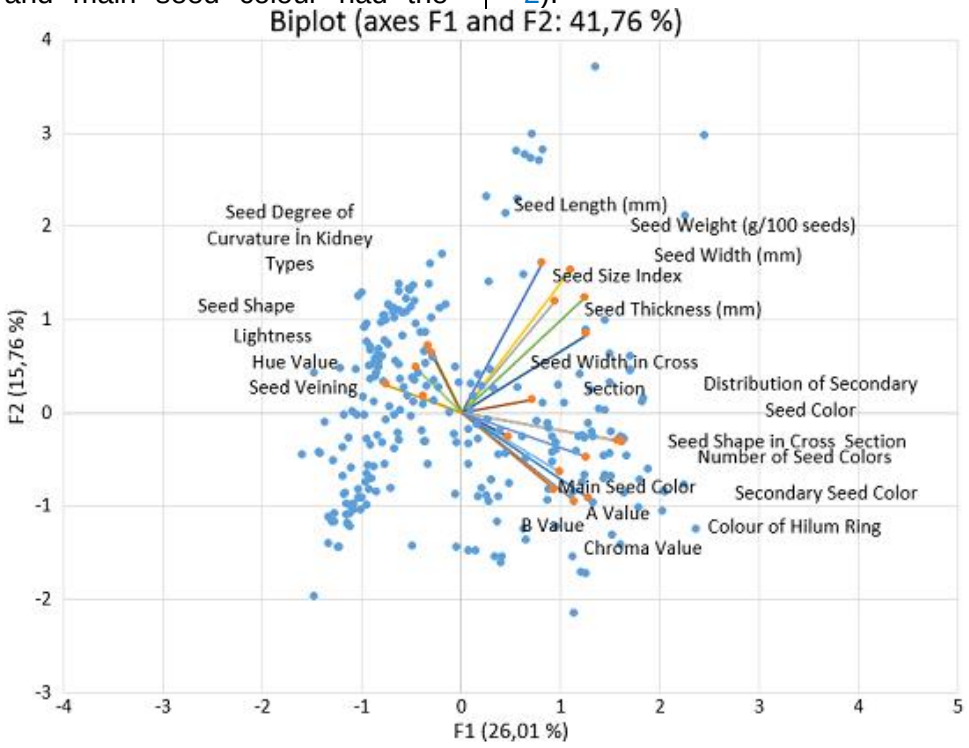


Figure 2. Biplot of seed morphoogical traits investigated of Phaseolus bean landraces

Seed size, 100–seed weight, seed length and seed thickness had narrow vector angles and significant and positive Pearson's coefficients of correlations between these variables indicating that

they are closely related characters (Table 2).

All colour parameters were represented by vectors with right angles to seed size traits (Figure 2) that were displayed on positive X axis and on negative Y axis (Figure 2).

Main seed colour had the longest vector length amongst other seed coat colour traits (Figure 2).

Seed colour traits did not have high correlation coefficients with seed weight parameters excepting seed thickness having significant correlation coefficient values with number of seed colour (r=0.367, P<0.01), main seed colour (r=0.210, P<0.01), secondary seed color (r=0.348, P<0.01) and distribution of secondary seed color (r=0.367, P<0.01) (Table 2). Seed shape, hue value and luminosity had vector values in opposite direction to seed colour traits (Figure 2).

Main seed colour was negatively correlated with L value (r=–0,666, P<0.01), but positively correlated with a* value (r=0,634, P<0.01) and C value (r=0,362, P<0.01) (Table 2).

Main seed colour and secondary seed colour had opposite direction to seed shape, luminosity and seed veining (Figure 2).

UPGMA dendogram of seed traits investigated were clustered in to two main groups (group A and B) with four sub–groups (A–1, A–2, B–1 and B–2) (Figure 3).

Main group A included colour traits while sub–group B–1 listed weight parameters. B–2–1 listed shape variables and sub–group B–2–2 listed luminosity, hue value and seed veining traits (Figure 3).

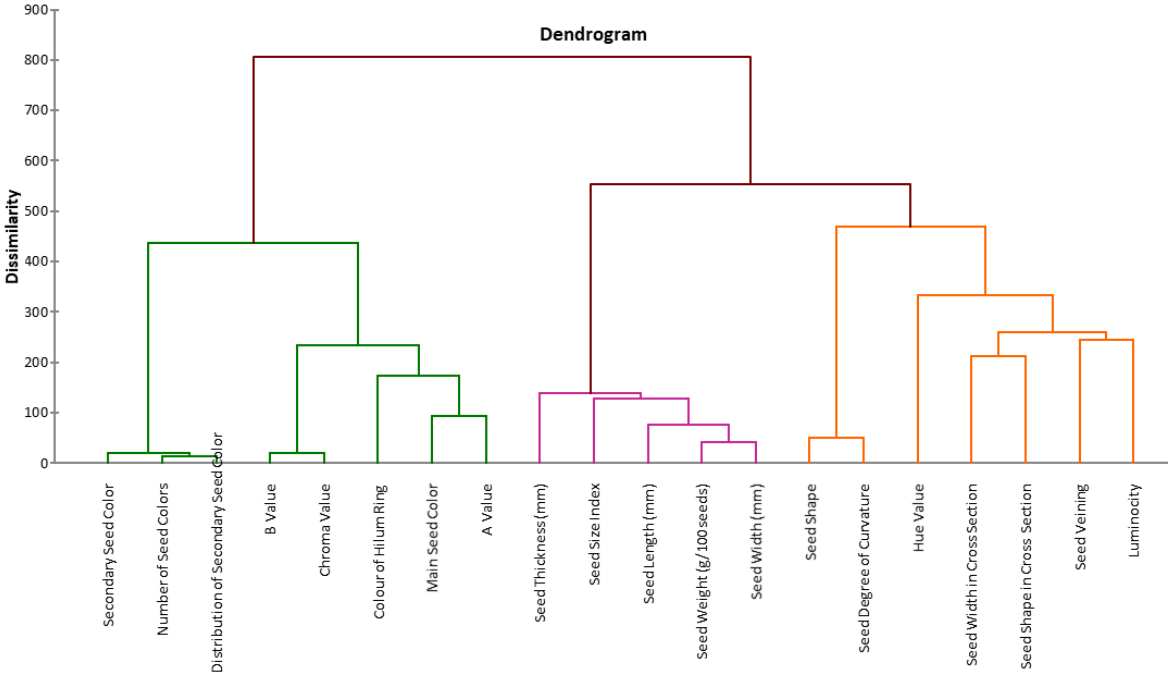


Figure 3. UPGMA dendrogram of seed morphological traits of germplasm from Western Anatolia

Of the seed morphological traits listed in UPGMA dendogram, seed thickness, seed size index, seed length and 100–seed weight were the most closely related characteristics to a* value, main seed colour, colour of hilum ring and seed shape (Figure 3).

Luminosity and seed veining were the most distant traits to secondary seed colour, number of seed colours and distribution of secondary seed colour traits (Figure 3).

Characterisation of landraces by seed traits. UPGMA dendogram on the bases of data made on seed weight, seed shape and seed luminosity & brightnes traits divided 255 landraces in to two main groups, group A and group B with several sub–groups (Figure 4).

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Group A landraces branched further in to two sub–groups, A–1 and A–2, which also further divided in to two subgroups, A–1–1 and A–2–1 with each forking in to two sub–sub–groups (Figure 4).

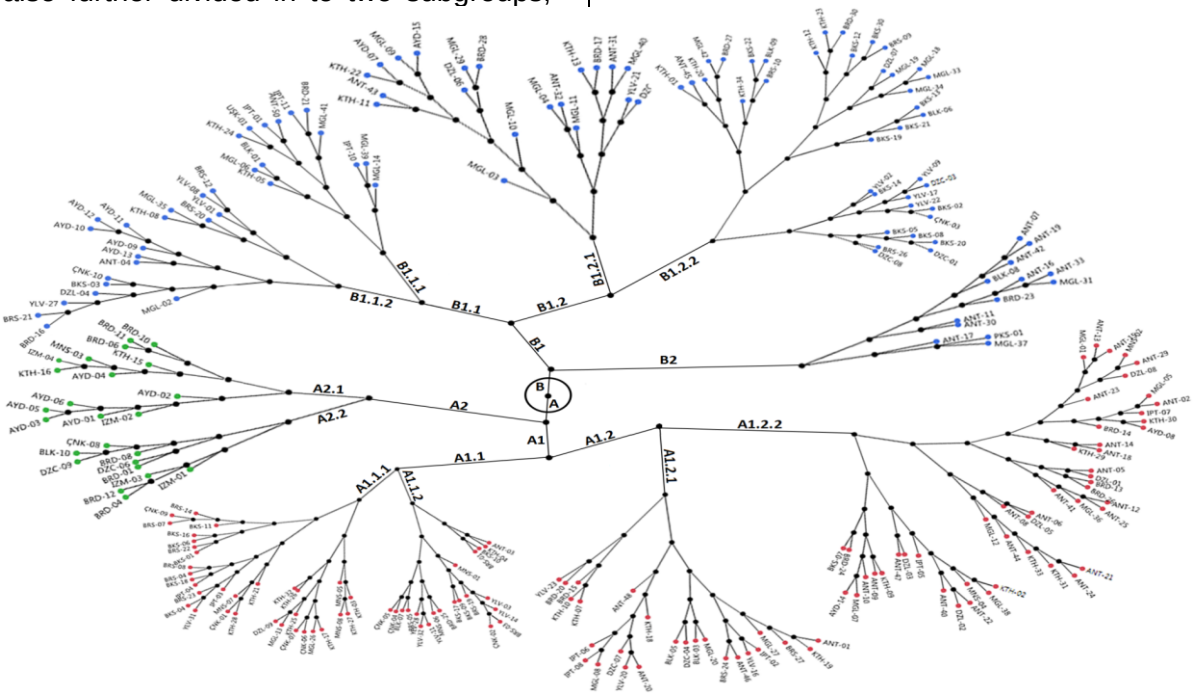


Figure 4. UPGMA dendogram using seed morphological attributes of Phaseolus sp. germplasm from Western Anatolia

Group B further branched in to two sub–groups, B–1 and B–2, with B–1 branching in to two sub–groups and each sub–group terminating in further sub groups (Figure 4).

Landraces were clustered in 11 distinct sub–groups in total (Figure 4).

Landraces with coloured seed coats were clustered in group B and with white testa in group A (Figure 4).

Large seeded landraces were grouped in B–2 on the right of the dendrogram and small seeded landraces were clustered in opposed sub–group A–2 with intermediary seed sized ranging in between.

The landraces from certain regions were predominantly clustered in some terminal groups, (e.g. B–1–2–2, A–2–1 and A–2–2). B–2 landraces of ANT–11, ANT–33, ANT–17, PKS–01, MGL–37 were the most distant to landraces of YLV–23, BRD–15, BRD–20, KTH–10, KTH–19 and MGL–27 in group A–1–2–1 (Figure 4).

Selection of landraces on the bases of GGE Biplot analysis. Biplot analysis showed that 100–seed weight and main seed colour had longer vectors than other seed traits indicating their relative importance in comparison with other variables (Figure 2).

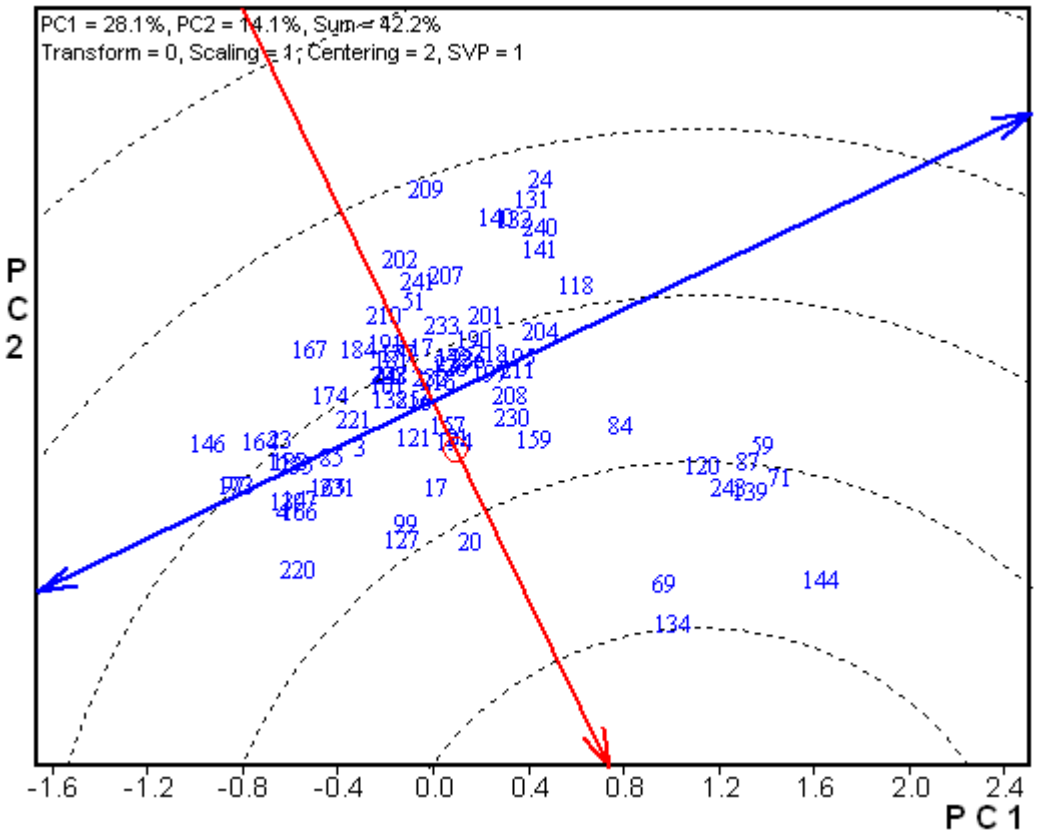
100–seed weight and main seed colour had also more variation than other traits (Table 1, Figure 1).

Narrow angles indicate closeness of certain two traits in biplot.

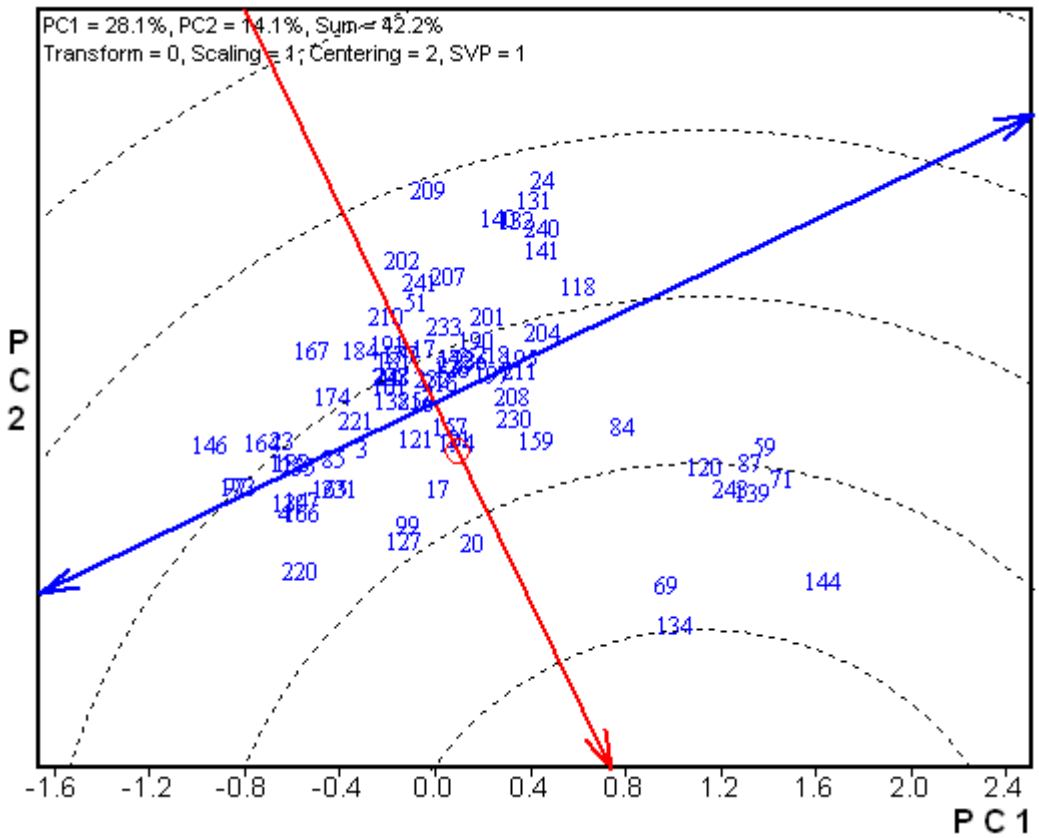
Other parameters could be, therefore, also considered depending on the angle of vectors relative to these characters.

GGE biplot displayed visual standing of landraces in relation to seed traits investigated that may be helpful for the selection of outstanding landraces (Figure 5 a, b).

Landraces of PKS–01, MGL–37, ANT–71, AT–33, ANT–07, IZM–01, MGL–18, BLK–21, MGL–39 and DZC–01 were displayed on the corners of the polygon of covariates of seed traits created by GGE biplot (Figure 5 a).



a



b

Figure 5 a,b. GGE Biplot of seed morphological traits investigated of the Phaseolus sp. bean germplasm.

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Landraces of ANT–48, DZC–01, BLK–20, BRD–28 and BLK–30 were displayed on the right of mean axis close to periphery axis (Figure 5 b). These genotypes could be selected for a start with diversity of seed morphological traits in a breeding program.

High yielding potential and tolerance to biotic and abiotic stress factors are important breeding objectives for common beans, which is of economical importance for producers.

Seed morphological traits such as seed size, testa colour and seed shape are on the other hand more important for consumers. In fact, seed size, colour and shape are the most important traits determining market value of bean cultivars. Depending on the county and region, consumers developed specific preferences for seed size, seed colour and seed shape. In breeding programs, some high yielding cultivars were known to be discarded because of poor acceptance by consumers who usually have special preference for seed size, shape, colour and quality.

Breeders need a rich base of diversity for seed traits to be able address consumer demands.

Recent work concentrated, therefore, enhancing and analysis of genetic bases of common bean for seed traits [PEREZ–VEGA *et al.*, 2010; YUSTE–LISBONA *et al.*, 2014; HEGAY *et al.*, 2014]. In our trial, common bean germplasm of 255 landraces representing important local types from 20 provinces in Western Anatolia had a great deal of diversity in seed size, colour and shape parameters investigated.

Large diversity in seed traits may enhance breeding programs in order to develop bean cultivars up to the preference of consumers.

There was a tenfold difference in seed 100–weight ranging from 18.4 g to 182.3 g (Table 1). Other attributes of size related traits namely of seed size index, seed length, seed width, seed thickness and seed width in cross section showed also a rich variation.

Of the germplasm, 28 landarces were Meso–American types with 100 seed weight below 25.00 g and 212 landraces were Andean type large seeded landraces had 100–seed weight between 25.00 g and 75.00 g (Figure 1). 15 landraces had very large 100–seed weights between 103.43 g and 182.2 g.

Common bean seeds were described as Meso–american germplasm (<25 g) and Andean counterpart (>25 g) using 100 seed weight [GEPTS *et al.*, 1986].

Seed size is a polygenic trait with a high heritability (h^2>0.5) [YUSTE–LISBONA *et al.*, 2014].

Of the seed size related traits, the highest variation was present in 100–seed weight that can be used in quantifying variability in the germplasm. Seed size traits measured namely of 100–seed weight, seed size index, seed length, seed width, seed thickness and seed width in cross section had significantly high and always positive Pearson's correlation coefficients (Table 2), positive contributions in principle component analysis (Table 3), fell on the same UPGMA dendogram group (Figure 3) and very close vector angles in biplot analysis (Figure 2).

Seed length, seed with and seed thickness can be also used in the seed industry for selection and sorting purposes.

Seed weight traits did not have high correlation coefficients and a wide vector angles with seed shape, seed colour and luminosity & chromaticity parameters.

Coloured seed coats were represented in both large and small seeded groups in UPGMA dengogram of landraces (Figure 4) although there is a genetic association between seed size and seed coat colour [YUSTE–LISBONA *et al.*, 2014].

This may give the advantage of finding desired seed sizes in different seed shapes and colours in the breeding programs in response to various consumer demands. Yuste–Lisbona and collab. using RILs evaluated nine seed size and seed coat colour traits in different photoperiod environments of Andean bean gene pool and showed a continuous distribution, a wide range of variability and high heritability that indicated a quantitative nature of

inheritance for seed size and seed colour [YUSTE–LISBONA *et al.*, 2014].

They concluded that digenic epistatic interactions played important role in genetic control of seed quality traits in Andean common beans. In our bean gene pool, 100–seed weight range was larger than their Andean gene pool.

Dalla Corte and collab. investigated genetic analysis of seed morphological traits and its correlation with grain yield and concluded that seed morphological traits were controlled by a complex of genes with predominant additive effect [DALLA CORTE *et al.*, 2010].

High and negative correlations present between seed length and thickness with grain yield suggested greater grain yields in bean plants with smaller seeds. In our experiment seed yield per plant and seed number per pod were not correlated with any of the seed attributes investigated apart from negative but low correlation between 100–seed weight and number of seeds per pod (r=–0.256, P<0.01) (data not presented).

Rivera and collab. investigated seed curvature in Spanish local Ganxet common beans and concluded that easy to measure seed curvature being under a simple genetic control could be used as a marker to transfer high protein content and mealiness to other cultivars by backcross procedures [RIVERA *et al.*, 2015].

Our germplasm representing 20 provinces in Western Anatolia Region showed a diversity of main testa colours with white (151 landraces) and violet (24 landraces) being dominant main and secondary seed colours respectively.

Seed coat colour traits had positive Pearson's coefficient of correlations with seed weight and negative with seed shape variables but they were all low (Table 2).

Seed coat colour has a complex genetic inheritance in beans controlled by the interaction of many genes [MCCLEAN *et al.*, 2002; YUSTE–LISBONA *et al.*, 2014; POSSOBOM *et al.*, 2015]. Certain colour combinations are difficult to breed and high yielding coloured cultivars are less preferred by consumers [KELLY *et al.*, 1999].

In the germplasm studied here had a wide range seed colour combination in an array of seed sizes that may support a breeding program. Seed colour traits had positive contributions to the first component in PCA analysis (Table 3) and were closely related traits in UPGMA dendogram (Figure 3).

Luminosity (L) (r=–0.666, P<0.001) and chromaticity a* value (r=0.634, P<0.01) had positive and high Pearson's coefficient of correlations with main seed colour (Table 2).

There was a fourfold variation in L value (20,3–90,14) (Table 1, Figure 1 c). L value was negatively correlated with main seed colour. When coloured landraces were excluded, L values also varied within white seed colour landraces (min. 23.99 and max. 90.08) (data not presented).

L value measures luminosity of seed coats with L=00 black and L=100 white (Table 1). Luminosity value was used for the characterisation of seed coat color differences in beans in combination of chromaticity a* and chromaticity b* values [SILVA *et al.*, 2009; SOARES JU´NIOR *et al.*, 2012].

Possobom and collab. investigated genetic control of seed coat colour heritability and gain with selection for ''L'', a* and b* values; and select recombinants with the seed coat colour required by the market demand [POSSOBOM *et al.*, 2015].

They reported high broad–sense heritability for luminosity (h2b: 76.66–95.07 %), chromaticity a* (h2b: 73.08–89.31 %), and chromaticity b* (h2b: 88.63–92.50 %) in bean seeds. 'Luminosity', a* and b* values may be used to select bean cultivar suitable for the market demand [POSSOBOM *et al.*, 2015].

Higher luminosity values (L>55) indicate higher market value and were associated with greater clarity [RIBEIRO *et al.*, 2008].

However, luminosity values were generally lower in colored and black seeded genotypes [POSSOBOM *et al.*, 2015] as also appeared in our tests.

In colored seeded types, high luminosity values may be associated with longer cooking time [POSSOBOM *et al.*, 2015].

Therefore, decisions should be made by considering also of chromaticity a*, chromaticity b*, chroma and hue angle values in order to characterize seed coat color differences between cultivars and landraces, cultivar identification and seed darkening [AYALA–SLIVA et al., 2005].

Measurement of colour reflection was also used for the purposes of visual separation of fruit cultivars.

Conclusions

The landraces and genotypes were characterised using the traits of seed weight, seed shape and seed colour with the help of advanced data analysis and visualisation techniques. The most distant and close landraces were effectively defined in terms of seed traits.

UPGMA dendograms of seed traits and landraces may be used for the selection of landraces in the breeding programs.

GGE biplot analysis may also enable selection of landraces for specific requirements for seed size, seed shape and seed colour. PKS–01, MGL–37, ANT–71, AT–33, ANT–07, IZM–01, MGL–18, BLK–21, MGL–39, DZC–01, ANT–48, DZC–01, BLK–20, BRD–28 and BLK–30 could be a core collection of landraces selected with the help of GGE–biplot from the germplasm.

Further landraces could be included in the collection with selection by targeted seed traits for the production and breeding purposes.

In conclusion, the germplasm collection of 255 landraces representing major bean types from 20 provinces in western Anatolia investigated showed a considerable diversity for seed size, seed shape and seed colour traits. 100–seed weight ranged ten–fold from very small seeded Meso–American types (18.4 g, e.g. AYD–8, KTH–30, MGL–1, DNZ–1 and BRD–24) to very large seeded Andean types (182.3 g, e.g. MGL–37, PKS–1, ANT–33, MGL–31 and ANT–16).

Seed colour also varied considerably within the germplasm with 151 landraces had white main seed colour with 31 grey, 31 beige, 23 brown, 8 violet, 5 red, 4 black whereas 24 landraces had violet secondary seed colour followed by 17 black and 14.

Seed shape also showed large variation from kidney–shaped (96 landraces), circular to elliptic (73), circular (58), eliptic (27) and rectangular (1) seed shapes.

Landraces exhibited a large variation for the measured colour chromaticity and luminosity variables with Chroma value (C) having the highest variation followed by hue angle value, a value, b value and luminosity (L) value.

The landraces in the germplasm were assessed and characterised on the bases of diversity in seed morphological traits.

Selections were made using UPGMA dendogram and GGE–biplot for production and breeding bean cultivars for preferences of various consumer demands.

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