

## Genome-wide association studies revealed DArTseq loci associated with seed traits in Turkish common bean germplasm

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**Abstract:** Seed traits are very important agronomic traits that contribute significantly into crop yield and are considered useful indicators to monitor the inheritance of quantitative traits influenced by genotype and environmental conditions. To investigate genomic regions useful for breeding high-yielding and highly nutritious common bean (*Phaseolus vulgaris* L.) cultivars, a mini core collection of Turkish common bean germplasm was screened under five environments and two locations for seed traits (seed width, seed yield/plant (SYP), and hundred seed weight). The results of analysis of variance (ANOVA) across the environments revealed significant effects of genotypes and genotype by environment interaction ( $G \times E$ ) for all studied traits except hundred seed weight (HSW), where  $G \times E$  was found nonsignificant. During this study, mean seed width, SYP, and HSW were 7.038 mm, 21.815 g, and 42.1 g, respectively. Pearson's correlation coefficient revealed a significant and positive correlation ( $p = 0.01$ ) of seed width with SYP and HSW. At the provinces level, minimum and maximum HSW was reflected by Elazığ and Bolu provinces, respectively. Studied germplasm was grouped into two populations in constellation analysis based on their HSW. A total of 7900 DArTseq markers identified through genotyping by sequencing (GBS) were used for the investigation of marker-trait association. DArT-3373385 marker present on chromosome Pv08 showed a significant association for seed width. DArT-8215545 marker showed a significant association for SYP and this marker was also present on chromosome Pv08. A total of four DArTseq markers were determined to be associated with HSW, and DArT-3372052 contributed maximum (11.32%) to phenotypic variations. We believe that the reported genomic regions will be helpful for future marker-assisted breeding regarding yield-related traits in common bean.

**Key words:** *Phaseolus vulgaris*, seed width, yield/plant, 100-seed weight, GWAS

### 1. Introduction

It is believed that the world population will be more than 9 billion and this increase in population is becoming a major concern to the present-day's world regarding how to feed this high population (Alexandratos and Bruinsma, 2012; Baloch et al., 2023). Climate change is predicted to become more severe in the coming years and there is a need to take urgent steps to lower the emission of greenhouse gases into the atmosphere (Stagnari et al., 2017). Biotic and abiotic stresses associated with climatic change individually or as a combination are heavily threatening the agriculture production system (Teshome et al., 2020). Keeping these challenges in view, there is a present-day requirement to develop strategies that can bring sustainability to the production and consumption of food (Stagnari et al., 2017). Under this pressing scenario for agriculture, the universally acclaimed contribution of legumes in cropping system by improving biological nitrogen fixation, reduction in energy and input cost, and improving soil

physical conditions is needed more than ever (Courty et al., 2014; Peix et al., 2014). Therefore, legumes are considered an important pillar of our agriculture system that can bring sustainability under changing climatic conditions. Legume seeds are full of high-quality nutrition (proteins, carbohydrates, water-soluble vitamins, and minerals) and their utilization on daily basis may prevent various chronic diseases (Amarowicz, 2020).

There are more than 50 edible legumes; however, the common bean is one of the most important food legumes cultivated all over the world and serving a source of nutrition for millions of people (Nadeem et al., 2021a,b). It is considered a "grain of hope" due to its nutritional potential (Nadeem et al., 2021a). Mesoamerica is considered the origin center of this crop, while its domestication resulted in the formation of two gene pools, i.e. Mesoamerican and Andean gene pools (Bitocchi et al., 2012). Common bean was introduced to the old world during Colombian Exchange, and Europe is considered

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the second genetic diversity center of this crop (Santalla et al., 2002). The Ottoman traders played a significant role in the further distribution of this crop to other parts of the world (Nadeem et al., 2018). Currently, common bean is cultivated all over the world and in 2019, around 27 million tons of green beans and 29 million tons of dry beans were produced globally, indicating a 36% increase in total common bean production in the last 10 years (FAO, 2019). In the year 2020, common bean production all over the world reached 27,545,942 tons (FAO, 2020).

Turkey is considered a “country of continents”, the center of origin, domestication, and distribution of various crops due to its key geographic position (Nadeem et al., 2018; Baloch et al., 2017). Turkey received common bean from Europe and harbor a good number of common bean germplasm that is present in its actual form compared to Europe (Nadeem et al., 2018). In 2019, annual common bean production was 212.758 tons making Turkey the 3rd largest producer of the bean in the world and the 1st producer in the Mediterranean region (Yeken et al., 2019). Common bean becomes an integral part of Turkish cuisine and it is used in the form of fresh pods or dry seeds (Nadeem et al., 2020a,b).

It is very important to understand the effect of genotype, environment, and interaction of both factors as the same plant can be affected hugely by its surroundings (Shrestha et al., 2012; Misra et al., 2020). The information obtained can be helpful for the effective breeding of crops with better phenotypic performance (Falconer et al., 1996). Screening of germplasm facilitates the breeding community to identify the most superior genotypes and to explore the novel variations that can be very helpful for the breeding perspectives (Nadeem et al. 2020a,b). Common bean represents huge variations in their phenotype, especially in pod and seed traits. Seed traits are considered important key traits that contribute significantly to common bean yield (Lei et al., 2020). A good number of studies have been conducted to explore the agronomic trait diversity in common bean including seed traits (Ekbic et al., 2019; Yeken et al., 2019; Mazhar et al., 2013; Boros et al., 2014; Bozoglu and Sozen, 2011). Karikari et al. (2020) stated that yield traits, especially seed traits, are polygenic traits and more than 200 quantitative trait loci (QTLs) have been reported for seed traits in common bean. Keeping this in view, the present investigation aimed to investigate marker-trait association for seed traits in common bean and to confirm whether the identified markers in our study fall within the same genetic region or whether identified markers are new QTLs for seed traits available in Turkish common bean germplasm.

## 2. Materials and methods

### 2.1. Plant materials

During this study, a total of 177 common bean landraces were collected from 19 provinces of Turkey and six commercial cultivars (Akman, Goynuk, karacasehir, Onceler, Goksun, Akdag) were used as plant material. Detailed information about studied germplasm can be traced from our previous studies (Baloch et al. 2022, Nadeem et al., 2020a; Nadeem et al., 2018)

### 2.2. Phenotypic trait evaluation

This study was performed according to augmented block design at Bolu and Sivas provinces of Turkey. A total of three experimental years in Bolu (2016, 2017, and 2018) and two in Sivas (2017, 2018) were taken as five environments for analytical purposes, as this is a common approach in agricultural experimentations (Gomez and Gomez, 1984). Detailed information about experimental design, sowing dates, and agronomic practices performed during this study can be obtained from our previous study (Nadeem et al., 2020a). Harvesting was performed at 90% pod maturity. Seed width (cm) was recorded for randomly selected ten seeds from each accession using a digital Vernier caliper. Seed yield/plant (SYP) was evaluated by weighing total seeds resulting from a single plant and recorded in grams. Hundred seed weight (HSW) was measured with the help of electronic seed counter taking by randomly selected fully matured and undamaged seeds in triplicate.

### 2.3. Statistical analysis

Detailed information about how the analysis of variance and heritability was calculated is provided in our previous study (Nadeem et al., 2020a). Mean, range, Pearson correlation coefficients, and province-based diversity for studied traits were calculated through XLSTAT software ([www.xlstat.com](http://www.xlstat.com)). Most stable accessions for seed traits were evaluated through “STABILITYSOFT” (Pour-Aboughadareh et al., 2019). The constellation plot for 183 common bean accessions was constructed through JMP 14.1.0 statistical software (2018, SAS Institute Inc., Cary, NC, USA).

Marker-trait association (MTA) analysis was performed similarly to what we performed in our previous studies (Baloch and Nadeem, 2022, Nadeem et al. 2021). During MTA analysis, the kinship (K) matrix reported by Bradbury et al. (2007) was investigated through TASSEL 5.0.5 (<https://tassel.bitbucket.io>) software. The population structure of the studied germplasm was reported in our previous study (Nadeem et al. 2018), and Q-matrix for each sample was evaluated from our reported structure analysis.

A mixed linear model (MLM, Q + K) methodology was used to investigate the DArTseq loci having an association

with seed traits. To investigate highly significant associated DarTseq loci, both FDR and Bonferroni thresholds ( $p = 0.01$ ) were used during the MTA analysis. Manhattan plots were developed through R 3.4.1 statistical software (<http://www.r-project.org/>) using the qq-man R Package (Turner, 2014). Detailed information about the development of physical map and functional analysis for the investigation of putative candidate genes is provided in our previous studies (Baloch and Nadeem, 2022; Nadeem et al. 2021).

### 3. Results

During this study, ANOVA was computed within and across the environments for all studied traits to understand the effect of genotypes and genotype by interaction ( $G \times E$ ) on studied traits. During this study, genotypic and environmental effects were found significant within each environment (data not shown). The results of ANOVA across the environments revealed significant effects of genotypes and  $G \times E$  for all studied traits except HSW, where  $G \times E$  was found nonsignificant (Table 1). The present investigation reflected a good range of variations for studied traits in Turkish common bean germplasm (Tables 2 and 3). Seed width ranged from 5.110 to 9.730 mm for Goksun and Malatya-18 accessions, respectively, while the mean seed width during this study was 7.038 mm (Table 3). A good range of variation was observed for SYP which varied between 5.88 and 59.24 g for Bitlis-69 and Bitlis-76, respectively, while the mean SYP during this study was 21.815 g (Table 3). Hundred seeds weight

(HSW) ranged from 24.97 to 67.140 for Malatya-13 and Bingol-53, respectively, while the mean HSW was 42.1 g (Table 3). Frequency distribution revealed normal distribution for all studied traits (Figure 1). Pearson's correlation coefficient revealed a significant and positive correlation of seed width with SYP and HSW (Figure 1).

It was very important to understand the seed trait diversity at the provinces level. Therefore, variations for agronomic traits were evaluated and accessions from Elazığ were found poor in seed width, while accessions from Tokat reflected maximum seed width (Figure 2). Accessions from Kahramanmaraş and Bilecik were found poor and rich in SYP, respectively. Accessions from Elazığ and Bolu reflected minimum and maximum HSW, respectively. During this study, stability analysis was also performed to investigate the most stable common bean accessions for seed traits and a total of 10 accessions were evaluated as the most stable for seed traits (Table 4). The constellation plot was constructed to understand the relationship among studied germplasm. The whole germplasm was divided into two populations A and B. Population A was found bigger than population B (Figure 3).

#### 3.1. Marker-trait association, physical map of identified markers and putative genes for seed traits

During the present investigation, DArT-3373385 present on chromosome Pv08 showed a significant association for seed width (Table 5; Figure 4) DArT-8215545 showed a significant association for SYP and this marker was also

**Table 1.** Summary of Analysis of variance (ANOVA) for seed traits in Turkish common bean germplasm.

Analysis of variance for seed width					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotypes	182	988.1950717	5.284465624	6.795030475	6.15E-35****
$G \times E$	183	548.9493399	2.919943298	3.754609284	1.00E-18***
Residuals	183	146.2067817	0.777695647	NA	NA
Analysis of variance for seed Yield/plant					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotypes	182	55491.1242	296.7439797	9.763840281	1.61E-46****
$G \times E$	183	20302.22329	107.9905494	3.553239656	1.98E-17**
Residuals	183	5713.721914	30.39213784	NA	NA
Analysis of variance for 100-seed weight					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotypes	182	169482.6711	906.3244445	8.427789402	1.08E-41****
$G \times E$	183	18896.66046	100.5141514	0.934667607	0.678152268
Residuals	183	20217.51938	107.5399967	NA	NA
Statistically significant ( $p < 0.05$ ), $\neq$ Environment, ** ( $p < 0.01$ ), *** ( $p < 0.001$ ), **** ( $p < 0.0001$ ).					

**Table 2.** Seed traits variations in Turkish common bean germplasm.

Genotype	SWd (mm)	SYP (g)	HSW (g)
Bingol-1	5.64	16.28	35.3
Bingol-6	6.95	8.32	40.74
Bingol-7	6.82	16.21	33.3
Bingol-11	6.58	22.2	34.39
Bingol-16	7.4	24.4	51.01
Bingol-18	8.34	36.41	56.93
Bingol-25	7.76	28.65	54.72
Bingol-33	6.64	13.78	66.33
Bingol-36	6.68	33.2	38.69
Bingol-44	6.69	20.97	61.05
Bingol-45	6.91	40.75	40.09
Bingol-52	5.53	30.98	50.17
Bingol-53	6.34	17.71	67.14
Bingol-58	6.59	12.82	43.93
Bingol-60	6.32	30.22	42.28
Bingol-61	6.09	20.84	28.91
Bingol-63	7.3	13.25	42.19
Bingol-65	6.5	17.5	57.59
Hakkari-7	7.2	17.07	54.8
Hakkari-11	5.51	41.99	29.78
Hakkari-12	6.81	45.63	40.65
Hakkari-13	7.21	36.72	38.92
Hakkari-16	5.78	28.41	28.07
Hakkari-20	7.15	14.37	38.76
Hakkari-23	5.98	10.87	26.19
Hakkari-28	8.05	17.25	61.6
Hakkari-31	6.71	13.75	45.07
Hakkari-37	6.59	28.76	37.45
Hakkari-38	7.22	16.58	41.04
Hakkari-39	8.77	22.09	54.46
Hakkari-43	7.51	24.94	48.12
Hakkari-44	7.13	22.07	37.17
Hakkari-51	6.92	17.4	37.86
Hakkari-55	7.29	12.54	30.59
Hakkari-63	5.83	10.18	34.92
Hakkari-65	7.15	21.59	32.62
Hakkari-69	7.17	27.89	30.92
Hakkari-71	8.76	36.28	44.01
Hakkari-76	7.21	24.05	38.62
Tokat-83	8.44	17.32	51.78
Maras-92	7.09	9.83	48.21
Bitlis-5	9.49	41.06	46.57

**Table 2.** (Continued)

Bitlis-14	7.43	19.62	42.13
Bitlis-16	6.98	8.58	47.98
Bitlis-22	7.83	39.78	55.96
Bitlis-25	6.93	20.21	33.75
Bitlis-35	5.88	9.3	26.38
Bitlis-40	7.27	34.7	43.74
Bitlis-46	6.33	21.3	36.81
Bitlis-48	6.28	10.61	44.21
Bitlis-53	6.58	14.29	54.44
Bitlis-66	8.44	24.24	38.45
Bitlis-69	6.76	5.88	51.46
Bitlis-76	7.94	59.24	46.25
Bitlis-79	6.71	14.72	32.92
Bitlis-81	6.96	35.5	42
Bitlis-90	6.44	16.47	33.68
Bitlis-94	7.39	12.59	39.96
Bitlis-97	6.87	26.64	43.09
Bitlis-103	5.41	16.69	33.57
Bitlis-105	7.48	20.77	41.87
Bitlis-111	7.81	31.73	41.18
Bitlis-114	6.15	23.45	45.58
Bitlis-115	6.59	30.6	34.28
Bitlis-117	7.08	30.54	37.49
Bitlis-118	6.48	28.81	33.79
Bitlis-119	5.88	34.38	39.03
Bitlis-120	5.86	37.4	31.86
Bitlis-121	7.49	28.28	46.09
Bitlis-124	8.72	28.26	39.14
Malatya-3	6.96	18.04	51.72
Malatya-13	5.75	14.78	24.97
Malatya-14	6.89	14.23	34.98
Malatya-18	9.73	43.64	44.81
Malatya-25	5.66	7.5	32.13
Malatya-28	8.72	24.1	38.12
Malatya-32	7.06	28.29	38.53
Malatya-33	6.16	35.4	38.63
Malatya-45	6.18	23.53	35.56
Malatya-50	7.7	20.49	42.55
Malatya-51	7.25	23.73	37.36
Malatya-52	8.41	27.25	45.98
Malatya-59	5.43	10.08	33.33
Malatya-71	8.24	28.2	39.32
Tunceli-1	8.04	26.81	42.56

**Table 2.** (Continued)

Tunceli-5	8.65	26.39	46.04
Tunceli-11	7.56	22.42	44.25
Van-1	5.92	29.87	31.65
Van-11	7.07	18.53	49.4
Van-13	6.21	32.63	29.63
Van-17	8.87	46.41	41.45
Van-19	7.09	17.63	37.24
Van-25	8.03	33.45	57.44
Van-27	7.74	22.31	51.24
Van-33	6.11	26.44	38.82
Van-36	8.04	30.65	52.36
Van-42	7.98	45.66	47.47
Van-47	6.14	13.88	43.65
Van-51	6.29	29.39	28.91
Van-59	8.02	26.2	53.85
Van-64	6.64	15.63	54.92
Van-65	8.74	19.06	59.16
Van-68	6.32	20.76	36.01
Elazig-2	6.97	38.26	44.75
Elazig-7	5.82	17.35	30.46
Elazig-9	5.36	12.22	33.71
Elazig-10	6.14	10.62	41.79
Elazig-14	7.7	31.15	44.54
Elazig-16	5.99	13.7	37.41
Elazig-25	5.7	42.68	30.2
Elazig-27	5.61	10.16	32.94
Elazig-29	5.57	21.77	31.23
Elazig-30	6.28	17.1	40.7
Elazig-34	5.97	12.98	37.96
Elazig-36	7.42	25.91	43.59
Elazig-39	6.64	22.22	32.06
Mus-1	7.58	29.96	33.16
Mus-2	7.81	19.42	57.59
Mus-7	7.03	22.39	36.38
Mus-10	7.47	18.55	40.38
Mus-15	7.56	19.14	36.34
Mus-18	8.73	34.32	45.55
Mus-22	8.5	26.13	53.23
Mus-27	8.32	25.58	43.23
Mus-28	7.07	12.81	33.99
Mus-34	7.48	25.68	37.36
Mus-39	6.54	28.65	30.22
Mus-41	7.89	17.43	53.59

**Table 2.** (Continued)

Mus-42	6.88	19.75	40.75
Mus-43	6.79	28.85	38.9
Mus-46	9.02	20.15	40.85
Mus-48	7.49	30.58	36.05
Mus-49	6.55	14.51	30.13
Mus-50	6.88	20.19	29.3
Mus-51	5.8	12.55	34.96
Mus-52	7.57	26.03	44.8
Mus-53	8.73	19.94	45.82
Sivas-3	7.97	9.81	43.33
Sivas-4	6.81	12.15	41.48
Sivas-7	7.65	10.71	44.95
Sivas-12	7.28	13.41	30.92
Sivas-13	6.69	11.78	38.47
Sivas-16	5.84	7.59	35.26
Sivas-17	7.28	13.27	49.3
Sivas-18	8.16	20.17	59.09
Sivas-44	7.9	14.23	38.66
Sivas-62	7.27	12.17	51.98
Sivas-68	5.66	9.56	31.66
Sivas-69	7.29	12.54	49.1
Sivas-70	7.12	10.87	42.17
Bilecik-1	7.39	28.6	30.39
Bilecik-2	6.06	22.92	30.84
Bilecik-6	7.95	38.47	40.57
Bilecik-7	6.63	12.62	49.16
Bilecik-10	8.88	52.67	44.56
Balikesir-3	7.15	7.64	33.53
Balikesir-4	6.36	13.66	43.54
Balikesir-5	8.45	12.58	50.53
Balikesir-6	5.93	10.62	47.49
Balikesir-17	6.6	34.13	31.8
Balikesir-18	6.61	10.07	48.15
Balikesir-19	7.39	20.72	43.81
Balikesir-20	7.5	15.29	49.31
Duzce-1	7.26	13.85	48.11
Duzce-9	8.04	23.2	46.57
Yalova-13	7.37	11.8	56.21
Yalova-20	7.13	21.93	38.42
Yalova-21	7.41	30.51	45.41
Erzincan-1	6.47	12.48	43.18
Erzincan-3	6.75	16.15	55.63
Erzincan-4	6.5	9.25	49.92



Table 2. (Continued)

Erzincan-5	6.04	12.83	44.3
Bursa-1	6.61	15.52	49.38
Bursa-22	6.55	12.2	46.52
Nigde- Dermasyon	7.15	14.35	42.25
Nigde-Derinkiyu	8.68	29.69	67.13
Civril-bolu	7.32	17.77	59.9
Akman	6.62	13.34	34.97
Goynuk	6.4	14.24	44.96
karacasehir	5.32	19.32	36.22
Onceler	6.7	14.29	48.29
Goksun	5.11	18.74	26.89
Akdag	6.26	12.69	52.07

SWd: Seed width, SYP: Seed yield/plant, HSW: 100-seed weight

Table 3. Range of studied traits in Turkish common bean germplasm.

Trait	Minimum	Maximum	Mean	Std. deviation
SWd (mm)	5.110	9.730	7.038	0.921
SYP (g)	5.880	59.240	21.815	9.871
HSW (g)	24.970	67.140	42.100	8.773

SWd: Seed width, SYP: Seed/per plant, HSW: 100-seed weight

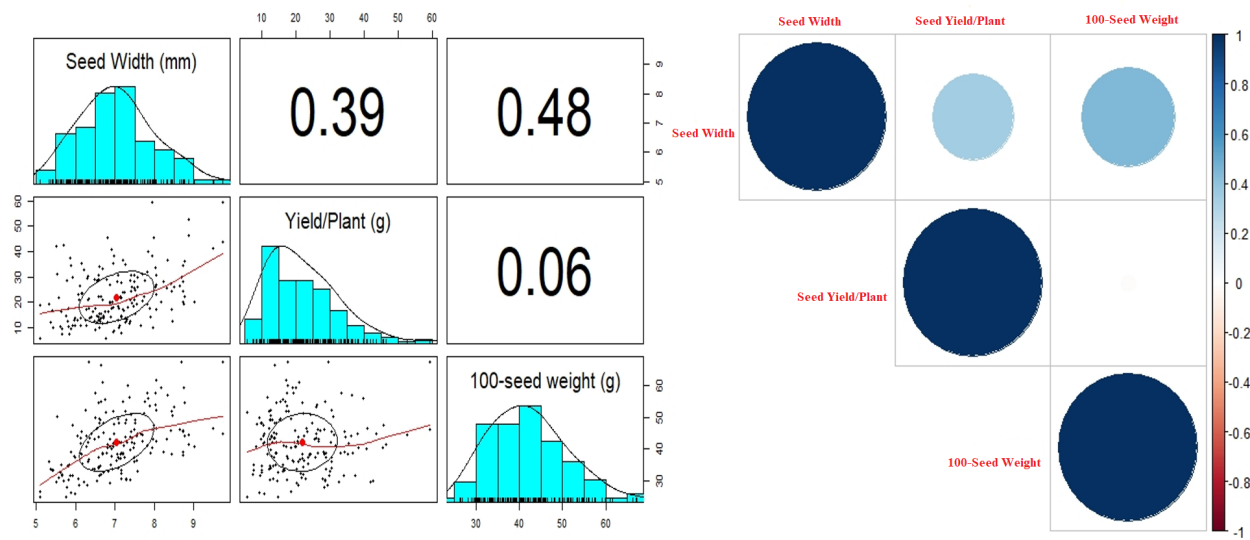
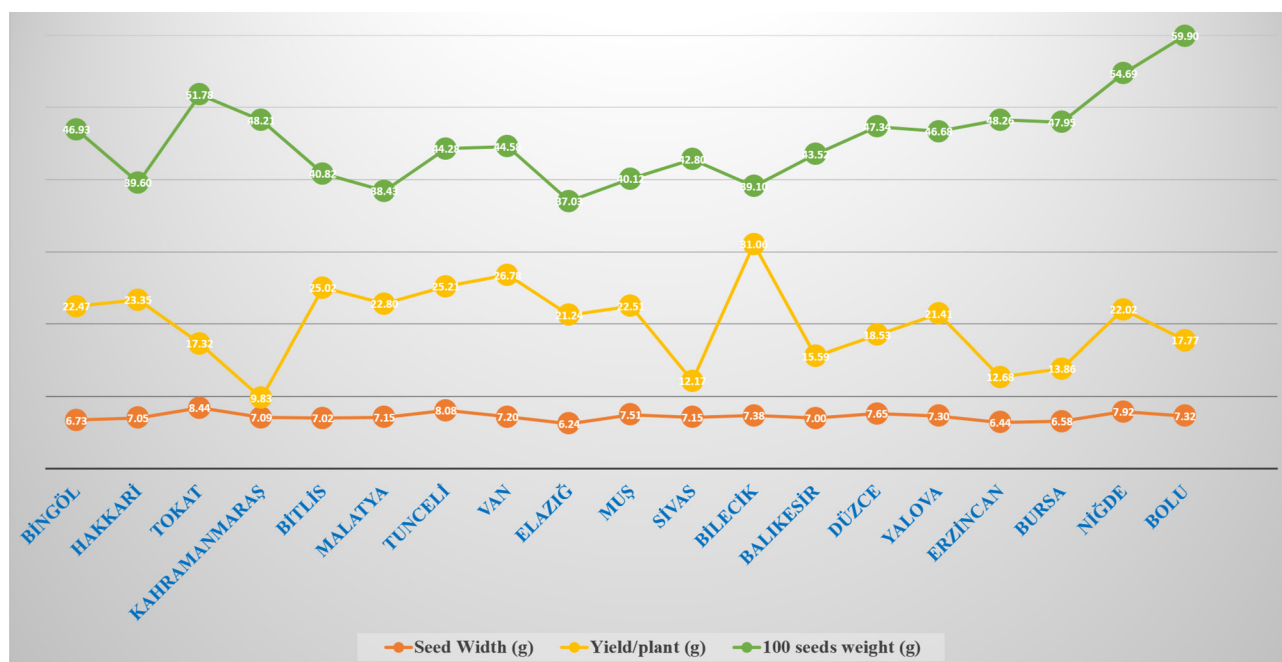


Figure 1. Frequency distribution and Pearson's correlation of seeds traits in Turkish common bean germplasm.





**Figure 2.** Seeds traits variations at provinces level in Turkish common bean germplasm.

**Table 4.** Most stable common bean landraces for seed traits evaluated from five environments and two locations.

Genotype	$W_i^2$	$\sigma_i^2$	$s^2d_i$	$b_i$	CVi
Bitlis-105	1.109	0.248	0.137	0.984	74.196
Tunceli-11	1.681	0.537	0.034	1.048	74.586
Mus-42	1.857	0.626	0.186	0.970	76.121
Malatya-50	2.382	0.891	0.340	1.000	74.764
Bitlis-14	3.925	1.671	0.560	0.997	76.340
Balikesir-19	3.935	1.676	0.385	1.045	76.863
Mus-27	4.377	1.900	0.613	0.988	67.893
Tunceli-5	4.867	2.147	0.371	1.061	69.202
Mus-52	6.852	3.151	0.724	1.054	71.232
Mus-10	6.896	3.173	0.735	0.947	75.655

SWd: Seed width, SYP: Seed/per plant, HSW: 100-seed weight

present on chromosome Pv08 (Table 5; Figure 5). A total of four DArTseq markers showed association with HSW, and all of these markers were present on different chromosomes (Table 5; Figure 6). A physical map was constructed for the identified markers for the studied traits (Figures 7–9). A total of seven putative genes were predicted from the sequences

reflecting homology to identified DArTseq markers for seed traits (Table 5).

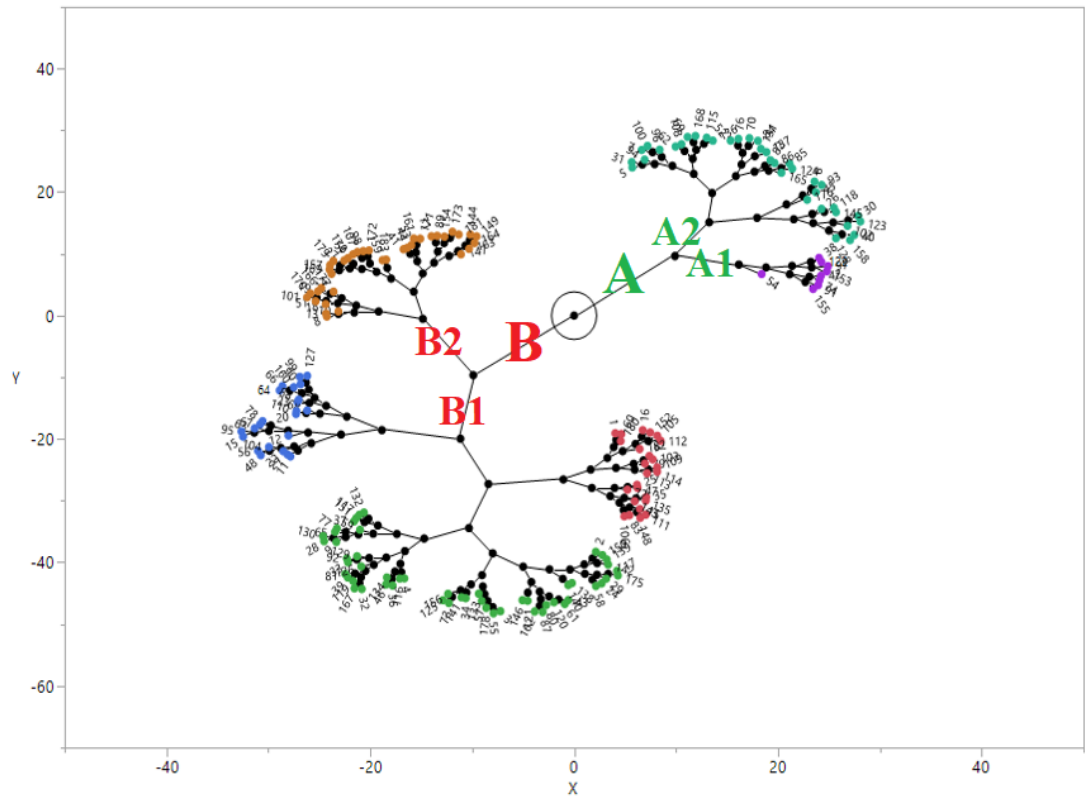
#### 4. Discussion

In recent years, climate change has become a serious threat and the world is witnessing extreme weather and climatic events that are contributing to a significant decrease

**Table 5.** Marker-trait association for seed traits in Turkish common bean germplasm.

Trait	Marker	Chromosome	Position	<i>p</i> -value	MarkerR2	Genetic variation	Putative gene
SWd	3373385	8	54980534	3.26E-04	0.08267	8.26	Phvul.008G202600 and Vigun08g145100
SYP	8215545	8	56563358	1.21E-04	0.08807	8.80	Glyma.02g232900
	3372052	9	20132762	1.29E-05	0.11326	11.32	Phvul.009G133400
HSW	3367130	3	52018089	4.15E-05	0.10331	10.33	Vigun03g184800
	8214418	11	46685857	5.40E-05	0.10081	10.08	Phvul.008G155800
	3375893	1	3824172	8.67E-05	0.09374	9.37	Phvul.001G045100

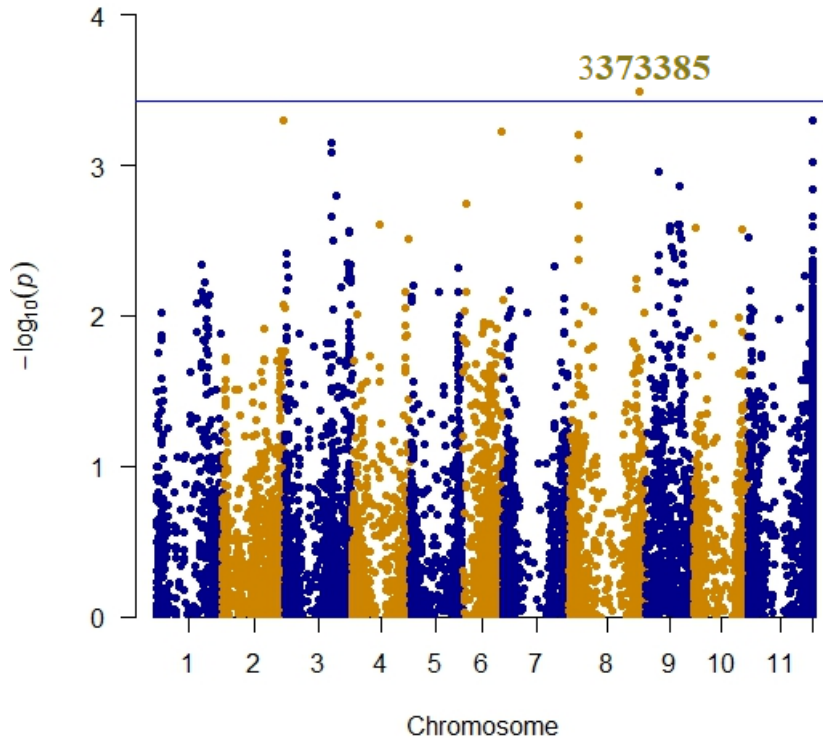
SWd: Seed width, SYP: Seed/per plant, HSW: 100-seed weight



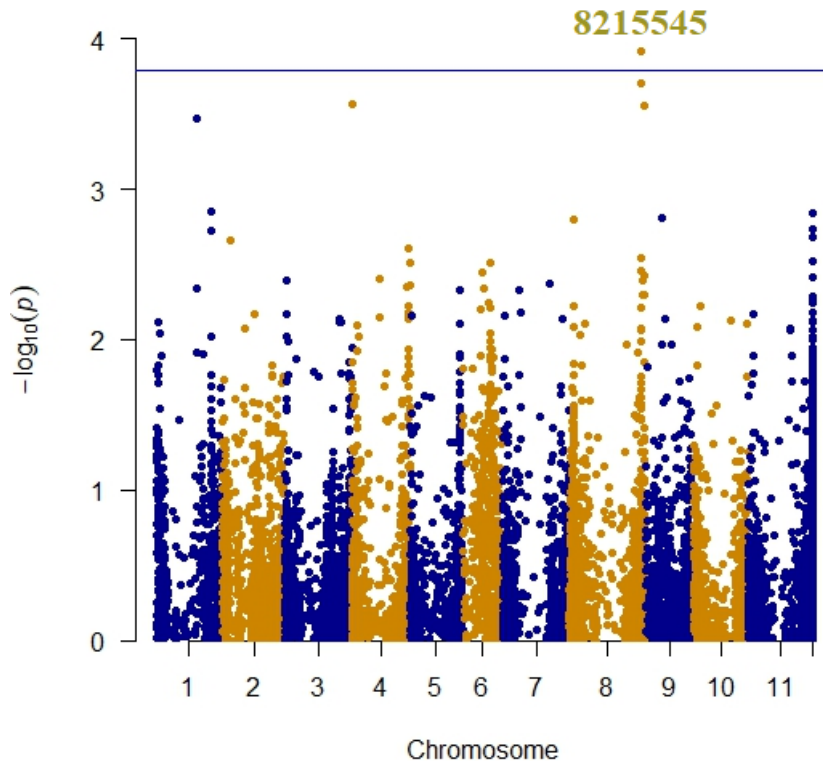
**Figure 3.** The constellation plot exploring the relationship among evaluated common bean germplasm upon seed traits.

in global production. If global production will be not improved under these climatic conditions, present and upcoming generations will face food insecurity. Therefore, it is very important to develop cultivars having better adaptation to climate change. Keeping these scenarios in view, there is a need to characterize the germplasm under multiple environments and locations. Such type of

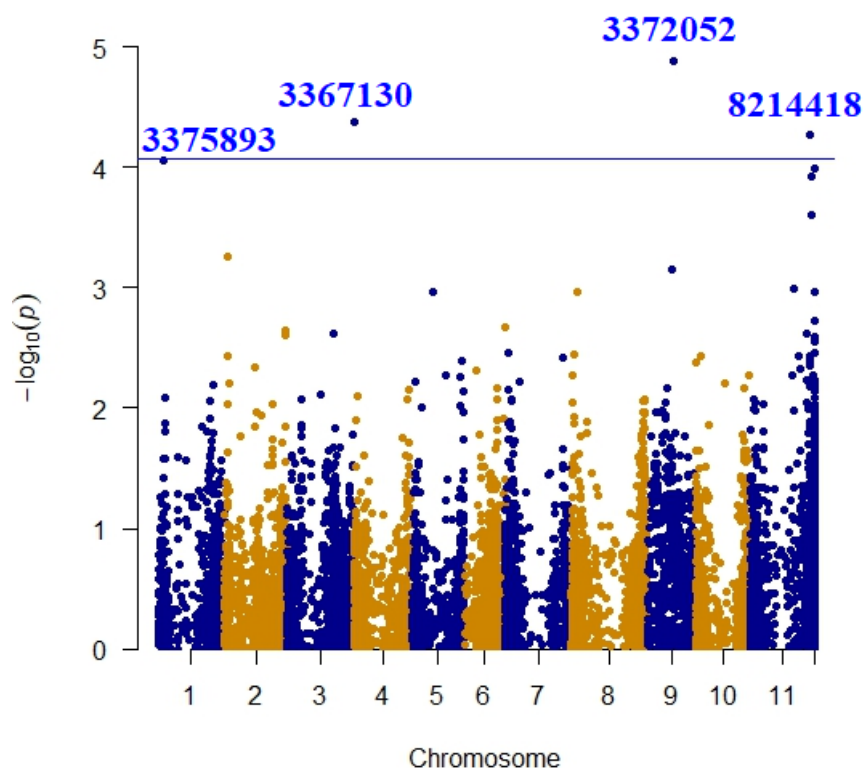
experiment can be helpful to investigate genotypes having better performance and stability in various environmental conditions (Nadeem et al., 2021b). Common bean is a very important legume crop full of nutrients essential for human growth and development. Common bean shows diversity in their phenotype and genotype. Common bean is sensitive to high temperature due to mid- to high-altitude



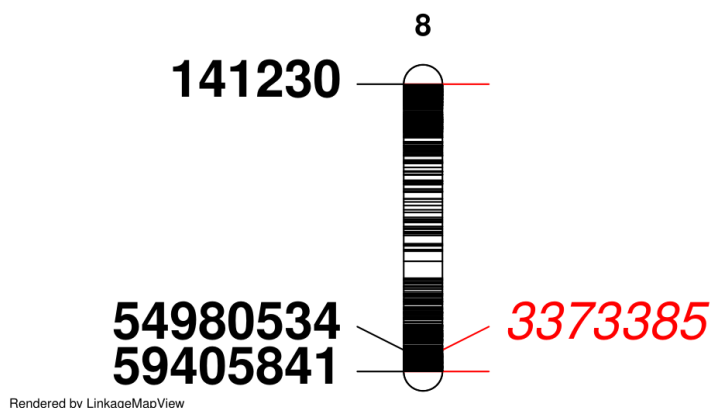
**Figure 4.** Manhattan plot for seed width in Turkish common bean germplasm using DArTseq markers.



**Figure 5.** Manhattan plot for seed yield/plant in Turkish common bean germplasm using DArTseq markers.



**Figure 6.** Manhattan plot for 100-seed weight in Turkish common bean germplasm using DArTseq markers.

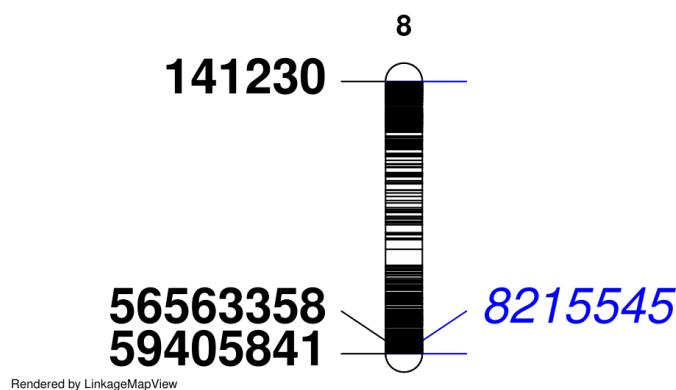


**Figure 7.** Physical map displaying the significantly associated marker with seed width in Turkish common bean germplasm.

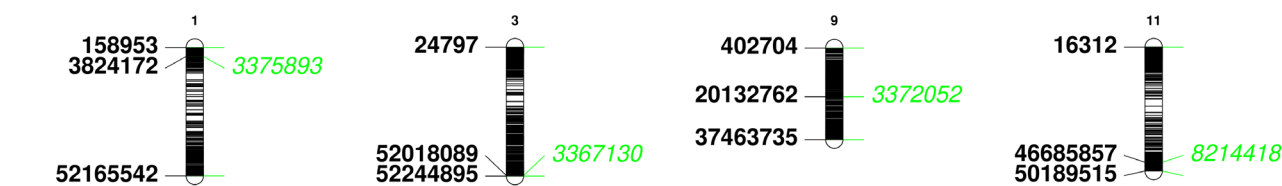
origin, and their productivity is limited by various biotic and abiotic stresses existing in the different environments where it is grown (Beebe et al., 2013). Previous studies also confirmed that common bean will face a decline in production under changing environmental conditions (Caffrey et al., 2013; CIAT, 2015). A good number of efforts are ongoing at the national and international levels to investigate the adaptation of common bean germplasm to the changing environment (Nhemachena & Hassan, 2007; Di Falco et al., 2011; Teklewold et al., 2013). Besides

confirming the adaptation to changing climate, it is very important to investigate genomic regions associated with traits of interest for marker-assisted breeding. Seed traits are very important as they contribute significantly to yield. Therefore, it is very important to investigate marker-trait association for seed traits. The present investigation aimed to explore the best-performing and most stable common bean genotypes and to identify DArTseq markers associated with seed traits.

Plentiful variations were observed for studied traits in



**Figure 8.** Physical map displaying the significantly associated marker with yield per plant in Turkish common bean germplasm.



**Figure 9.** Physical map displaying the significantly associated marker with 100 seeds weight in Turkish common bean germplasm.

Turkish common bean germplasm. Analysis of variance revealed statistically significant effects of genotype within each environment. Genotype and  $G \times E$  interaction was found significant for all traits during this study except HSW in which  $G \times E$  interaction was found nonsignificant (Table 1). Previous studies also reported a highly significant effect of genotype and  $G \times E$  interaction for seed traits (Gonçalves et al., 2010; Torga et al., 2013). A good range of variations was observed for all studied traits (Tables 2 and 3) and variability among common bean accessions confirmed the differences in their performances under five environments. These results were found in line with previous studies by Barili et al. (2015), Ashango et al. (2016), and Bassa et al. (2019). Investigation and selection of best-performing genotypes under multienvironmental conditions are important goals of agronomic and plant breeding studies (Ahmadi et al., 2015; Vaezi et al., 2018). Information about phenotypic stability can be very beneficial in the selection of best-performing genotypes. The performance of genotype can be changed under diverse and multienvironmental conditions (Ali et al., 2003). This study also aimed to investigate the most stable common bean accessions for seed traits. We used various parameters like  $W_i^2$ : Wricke's ecovalence,  $\sigma_i^2$ : Shukla's stability variance,  $s^2d_i$ : Deviation from regression,  $b_i$ :

Regression coefficient, CV<sub>i</sub>: Coefficient of variance, and these parameters helped us in the selection of most stable landraces. A total of 10 most stable and best-performing common bean landraces for studied traits were evaluated. Importance should be given to these identified landraces for future common bean breeding activities (Table 4).

During the present investigation, a good range of variation was observed for all studied traits. Range and mean seed width obtained in this study was greater than those reported by Lei et al. (2020) and Borji et al. (2007) and lesser than those reported by Palilo et al. (2018) and Yeken et al. (2019). Range and mean HSW was found much higher than in previous studies (Bozoğlu et al., 2011; Borji et al., 2007) and in line with Sinkovič et al. (2019) and Singh et al. (1991). Previous studies confirmed that changes in HSW are due to genetic differences in common bean genotypes (Merga et al., 2020; Masa et al., 2017). Frequency distribution revealed normal distribution for all studied traits (Figure 1). Pearson's correlation coefficient revealed a significant and positive correlation of seed width with SYP and HSW (Figure 1) and these results were found in line with the findings of Lei et al. (2020), Yeken et al. (2019), and Kazai et al. (2019).

As studied, germplasm was collected from 19 provinces having different geographic and topographic features.

Therefore, we analyzed the germplasm at the provinces level (Figure 2). It was observed that accessions from Elazığ were poor in seed width, while accessions from Tokat reflected maximum seed width. Regarding SYP, accessions from Kahramanmaraş and Bilecik were found poor and rich, respectively. Regarding HSW, accessions from Elazığ and Bolu were found poor and rich. It was quite understandable for the accessions from Elazığ Province to have minimum HSW because they reflected minimum seed width. Lei et al. (2020) reported a highly significant and positive correlation between seed width with HSW. Nadeem et al. (2020a) proposed selection criteria for the selection of best-performing common bean genotypes and stated that if two traits have a positive correlation, the selection of genotypes upon one trait will automatically select the other associated traits due to their gene linkage or epistatic effects between different genes. It can be confirmed from our correlation results that seed width has a significant correlation with HSW and these results are further confirmed at the province level as Elazığ Province has minimum seed width that contributed to low HSW in this province.

To explore the relationship among 183 common bean accessions, a constellation plot was constructed and the whole germplasm was divided into two populations A and B (Figure 3). Population A was further divided into subpopulations A1 and A2. Subpopulation A1 was smaller than subpopulation A2, but clustered accessions having HSW >40g. Population B was subdivided into subpopulations B1 and B2. Subpopulation B1 clustered more number of accessions compared to B2. Subpopulation B1 clustered accessions having greater HSW compared to subpopulation B2. For example, Bingol-53 reflected a maximum HSW, which was also present in subpopulation B1.

The present investigation reported one chromosomal region (DART-3373385) present at Pv08 chromosome for seed width (Table 5; Figure 4). A previous study reported the presence of genomic regions having an association with seed width at Pv01, Pv03, Pv04, Pv05, Pv06, Pv07, and Pv09 (Lei et al., 2020). Geravandi et al. (2020) reported one QTL for seed width on chromosome Pv02, and Yuste-Lisbona et al. (2014) reported QTLs for seed width on chromosomes Pv02, Pv07, and Pv09. Pérez-Vega et al. (2010) reported the presence of QTL for seed width on chromosome Pv07. During this study, only one marker DART-8215545 present on chromosome Pv08 showed an association for SYP (Table 5; Figure 5). However, Sedlar et al. (2020) reported two QTLs for yield per plant on chromosome Pv01. Trapp et al. (2015) identified QTLs for seed yield per plant on chromosomes Pv01 and Pv02. Their findings were contrary to those of this study. The present investigation reported a total of four DARTseq markers for

HSW and these markers were present in Pv01, Pv03, Pv09, and Pv11 (Table 5; Figure 6). Lei et al. (2020) also reported one marker (CBS83) on chromosome Pv03. Sedlar et al. (2020) reported a total of three putative QTLs for hundred seed weight distributed on Pv01 and Pv02. Elias et al. (2021) reported QTLs for HWS on Pv07 and Pv08 and their findings were contrary to ours. Recently, Delfini et al. (2021) investigated two SNPs having an association for HSW on Pv03 chromosome and we also identified one marker (DART-3367130) having an association for HSW on Pv03 chromosome. Mir et al. (2020) reported nine SNPs having an association for HSW on various chromosomes of common bean. They identified BM154 as a stable SNP for HSW on Pv03 and confirmed that this SNP has already been reported by Blair and Izquierdo (2012) for HSW. During this study, we identified DARTseq markers having an association with seed traits and all of the identified markers were found novel to previously reported markers. A physical map was constructed for the identified markers and it revealed all identified markers within the separate region that most probably only belongs to seed traits (Figures 7–9). Keeping this in view, we recommend the scientific community to conduct studies aiming to validate the markers identified previously and, through this study, investigate marker-assisted breeding of common bean.

The BLAST search against the 3373385 DARTseq marker resulted in *Phvul.008G202600* and *Vigun08g145100* genes. Both genes encode F-box family protein and nucleolar complex protein. Malik et al. (2020) revealed that F-box proteins (FBPs) constitute large families of regulatory proteins. They comprehensively explored their role in anther and pollen development. Jain et al. (2007) investigated the role of this protein family in panicle and seed development in rice. *Glyma.02g232900* was found to be a putative gene for the 8215545 DARTseq marker and this gene encodes for zinc finger proteins. Zinc fingers are considered one of the largest and most diverse protein families playing a vital role in plant development and provide resistance to various stresses. Seok et al. (2016) identified *AtC3H17* as a zinc finger protein and concluded that zinc finger protein has a vital role in various vegetative stages like seed germination and seedling growth, flowering, and seed development, and functions as a nuclear transcriptional activator in Arabidopsis. The BLAST search of 3372052 DARTseq marker's sequence revealed *Phvul.009G133400* as a putative gene. This gene encodes for albumin-2 protein. Souza (2020) published a review article in which they comprehensively explored the role of albumin-2 protein in plants. He stated that this protein is an important group of seed storage proteins that is essential for seeds at early and late developmental stages. The BLAST search of the 3367130 DARTseq marker's sequence resulted in *Vigun03g184800* as a putative gene that encodes for RNA-



binding protein. Lou et al. (2020) published a review article in which they provided the detailed role of RNA-Binding Proteins in seed development in various plant species. The BLAST search of 8214418 DArTseq marker's sequence revealed *Phvul.008G155800* as a putative gene that encodes for GDSL-like Lipase/Acylhydrolase superfamily protein. According to Chepyshko et al. (2012), this is a newly discovered family of lipolytic enzymes that contributes significantly to plant growth and development (Watkins et al., 2019; Ma et al., 2018). Ding et al. (2018) revealed that *B. napus* seed germination and oil contents can be improved by regulating the GDSL transcriptional level. *Phvul.001G045100* was found to be a putative gene against the sequence blast of the 3375893 DArTseq marker and this gene encodes for protein kinase family protein. Protein kinase is among the largest gene family in the plant genome and acts as a major regulatory component for various cellular functions (Wang et al. 2020). Zhang et al. (2020) stated that seed size contributes significantly to plant yield and they revealed a key role of protein kinase in seed size.

## 5. Conclusion

The present investigation aimed to explore the phenotypic diversity of seed traits in Turkish common bean germplasm and to investigate genomic regions associated with studied traits. Malatya-13 and Bingol-53 were found to be phenotypically diverse landraces for HSW. Accessions from Elazığ and Bolu reflected minimum and maximum HSW, respectively. A total of 6 DArTseq loci were identified for the studied traits. It is very important to validate all of the identified genomic regions (including markers identified previously and in this study) using an independent population to identify stable chromosomal regions for seed traits for marker-assisted breeding of common bean.

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## References

- Ahmadi J, Vaezi B, Shaabani A, Khademi K, Fabriki Ourang S et al. (2015). Non-parametric measures for yield stability in grass pea (*Lathyrus sativus* L.) advanced lines in semi-warm regions. *Journal of Agricultural Science and Technology* 17 (7): 1825-1838.
- Alexandratos N, Bruinsma J (2012). World agriculture towards 2030/2050: the 2012 revision. *Research in Agricultural and Applied Economics* 146: 1-154. <http://dx.doi.org/10.22004/ag.econ.288998>
- Ali N, Javidfar F, Mirza Y (2003). Selection of stable rapeseed (*Brassica napus* L.) genotypes through regression analysis. *Pakistan Journal of Botany* 35 (2): 175-180.
- Amarowicz R (2020). Legume seeds as an important component of the human diet. *Foods* 9(12):1812 <https://doi.org/10.3390/foods9121812>
- Ashango Z, Amsalu B, Tumisa K, Negash K, Fikre A (2016). Seed yield stability and Genotype x Environment interaction of common bean (*Phaseolus vulgaris* L.) lines in Ethiopia. *International Journal of Plant Breeding Crop Science* 3:135-144.
- Baloch FS, Alsaleh A, Shahid MQ, Çiftçi V, E. Sáenz de Miera L et al. (2017). A whole genome DArTseq and SNP analysis for genetic diversity assessment in durum wheat from central fertile crescent. *PloS One* 12 (1): e0167821. <https://doi.org/10.1371/journal.pone.0167821>
- Baloch FS, Altaf MT, Bedir M, Nadeem MA, Tatar M et al. (2023). iPBS-retrotransposons variations: DNA fingerprinting and the evaluation of genetic diversity and population structure in international cowpea germplasm. *Genetic Resources and Crop Evolution*, 1-11. [Doi.org/10.1007/s10722-023-01542-7](https://doi.org/10.1007/s10722-023-01542-7)
- Barili LD, Vale NM, Prado AL, Carneiro JE, Silva FF et al. (2015). Genotype-environment interaction in common bean cultivars with carioca grain, recommended for cultivation in Brazil in the last 40 years. *Crop Breeding and Applied Biotechnology* 15: 244-250. <https://doi.org/10.1590/1984-70332015v15n4a41>
- Bassa D, Gurmu F, Mohammed H (2019). Comparison of univariate and multivariate models to analyze the stability of common bean (*Phaseolus vulgaris* L.) genotypes in Ethiopia. *Agrotechnology* 8 (188): 2. 1000188 <https://doi.org/10.4172/2168-9881.1000188>
- Beebe S, Rao I, Blair M, Acosta J (2013). Phenotyping common beans for adaptation to drought. *Frontiers in physiology* 4: 35. <https://doi.org/10.3389/fphys.2013.00035>
- Bitocchi E, Nanni L, Bellucci E, Rossi M, Giardini A et al. (2012). The mesoamerican origin of the common bean (*Phaseolus vulgaris* L.) is revealed by sequence data. *Proceedings of the National Academy of Sciences* 109 (14): E788-E96. <https://doi.org/10.1073/pnas.1108973109>
- Blair MW, Izquierdo P (2012). Use of the advanced backcross-QTL method to transfer seed mineral accumulation nutrition traits from wild to Andean cultivated common beans. *Theoretical and Applied Genetics* 125 (5): 1015-1031. <https://doi.org/10.1007/s00122-012-1891-x>
- Borji M, Ghorbanli M, Sarlak M (2007). Some seed traits and their relationships to seed germination, emergence rate electrical conductivity in common bean (*Phaseolus vulgaris* L.). *Asian Journal of Plant Sciences* 6 (5): 781-787.



- Boros L, Wawer A, Borucka K (2014). Morphological, phenological, and agronomical characterization of variability among common bean (*Phaseolus vulgaris* L.) local populations from The National Centre for Plant Genetic Resources: Polish Genebank. *Journal of Horticultural Research* 22 (2): 123-130. <https://doi.org/10.2478/johr-2014-0029>
- Bozoglu H, Sozen O (2011). A sample for biodiversity in Turkey: Common bean (*Phaseolus vulgaris* L.) landraces from Artvin. *African Journal of Biotechnology* 10 (63): 13789-96. <https://doi.org/10.5897/AJB11.942>
- Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y et al. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23: 2633-2635. <https://doi.org/10.1093/bioinformatics/btm308>
- Caffrey P, Finan T, Trzaska S, Miller D, Laker-Ojok R et al. (2013). Uganda Climate Change Vulnerability Assessment Report. Report to the United States Agency for International Development by Tetra Tech ARD, Washington DC, USA.
- Chepyshko H, Lai CP, Huang LM, Liu JH, Shaw JF (2012). Multifunctionality and diversity of GDSL esterase/lipase gene family in rice (*Oryza sativa* L. japonica) genome: new insights from bioinformatics analysis. *BMC Genomics* 13(1): 1-9. <https://doi.org/10.1186/1471-2164-13-309>
- CIAT, 2015. Climate change research. Available at <http://ciat.cgiar.org/climate-change-research>. (Accessed 1 February 2022).
- Courty PE, Smith P, Koegel S, Redecker D, Wipf D (2015). Inorganic nitrogen uptake and transport in beneficial plant root-microbe interactions. *Critical Reviews in Plant Sciences* 34 (1-3): 4-16. <https://doi.org/10.1080/07352689.2014.897897>
- Delfini J, Moda-Cirino V, dos Santos Neto J, Zeffa DM, Nogueira AF et al. (2021). Genome-wide association study for grain mineral content in a Brazilian common bean diversity panel. *Theoretical and Applied Genetics* 134 (9): 2795-2811. <https://doi.org/10.1007/s00122-021-03859-2>
- Di Falco S, Veronesi M, Yesuf M (2011). Does adaptation to climate change provide food security? A micro-perspective from Ethiopia. *American Journal of Agricultural Economics* 93 (3): 829-846. <https://doi.org/10.1093/ajae/aar006>
- Ding LN, Guo XJ, Li M, Fu ZL, Yan SZ et al. (2019). Improving seed germination and oil contents by regulating the GDSL transcriptional level in *Brassica napus*. *Plant cell reports*. 38 (2): 243-253. <https://doi.org/10.1007/s00299-018-2365-7>
- Ekbic E, Hasancaoglu E (2019). Morphological and molecular characterization of local common bean (*Phaseolus vulgaris* L.) genotypes. *Applied Ecology and Environmental Research* 17(1): 841-853. [http://dx.doi.org/10.15666/aeer/1701\\_841853](http://dx.doi.org/10.15666/aeer/1701_841853)
- Elias JC, Gonçalves-Vidigal MC, Ariani A, Valentini G, Martiniano-Souza MD et al. (2021). Genome-Environment Association Analysis for Bio-Climatic Variables in Common Bean (*Phaseolus vulgaris* L.) from Brazil. *Plants* 10 (8): 1572. <https://doi.org/10.3390/plants10081572>
- Falconer IR (1996). Potential impact on human health of toxic cyanobacteria. *Phycologia* 35: 6-11. <https://doi.org/10.2216/i0031-8884-35-6S-6.1>
- FAO – Food and Agriculture Organization of the United Nations (2022) Faostat. <http://faostat3.fao.org/browse/Q/QC/E> (accessed on 15 Jan. 2022).
- Geravandi M, Cheghamirza K, Farshadfar E, Gepts P (2020). QTL analysis of seed size and yield-related traits in an inter-gene pool population of common bean (*Phaseolus vulgaris* L.). *Scientia Horticulturae* 274: 109678. <https://doi.org/10.1016/j.scienta.2020.109678>
- Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research. John Wiley & sons; 1984 Feb 17.
- Gonçalves JG, Chiorato AF, Morais LK, Perina EF, Farias FL et al. (2010). Estudo da estabilidade fenotípica de feijoeiro com grãos especiais. *Ciência e Agrotecnologia* 34: 922-931 (in Portuguese). <https://doi.org/10.1590/S1413-70542010000400018>.
- Jain M, Nijhawan A, Arora R, Agarwal P, Ray S et al. (2007). F-box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. *Plant Physiology* 143(4): 1467-1483. <https://doi.org/10.1104/pp.106.091900>
- Kazai P, Noulas C, Khah E, Vlachostergios D (2019). Yield and seed quality parameters of common bean cultivars grown under water and heat stress field conditions. *AIMS Agriculture and Food* 4 (2): 285-302. <https://doi.org/10.3934/agrfood.2019.2.285>
- Lei L, Wang L, Wang S, Wu J (2020). Marker-trait association analysis of seed traits in accessions of common bean (*Phaseolus vulgaris* L.) in China. *Frontiers in Genetics* 11: 698. <https://doi.org/10.3389/fgene.2020.00698>
- Lou L, Ding L, Wang T, Xiang Y (2020). Emerging roles of RNA-binding proteins in seed development and performance. *International Journal of Molecular Sciences* 21 (18): 6822. <https://doi.org/10.3390/ijms21186822>
- Ma R, Yuan H, An J, Hao X, Li H (2018). A *Gossypium hirsutum* GDSL lipase/hydrolase gene (GhGLIP) appears to be involved in promoting seed growth in *Arabidopsis*. *PLoS One* 13 (4): e0195556. <https://doi.org/10.1371/journal.pone.0195556>
- Malik A, Gul A, Amir R, Munir F, Babar MM et al. (2020). Classification and Computational Analysis of *Arabidopsis thaliana* Sperm Cell-Specific F-Box Protein Gene 3p. AtFBP113. *Frontiers in Genetics* 11: 609668. <https://doi.org/10.3389/fgene.2020.609668>.
- Masa M, Tana T, Ahmed A (2017). Effect of plant spacing on yield and yield-related traits of common bean (*Phaseolus vulgaris* L.) varieties at Areka, Southern Ethiopia. *Journal of Plant Biology and Soil Health* 4 (2): 1-3.
- Mazhar KA, Sayinci B, Elkoca E, Öztürk İ, Özmen T (2013). Seed size and shape analysis of registered common bean (*Phaseolus vulgaris* L.) cultivars in Turkey using digital photography. *Journal of Agricultural Sciences* 19 (3): 219-234. [https://doi.org/10.1501/Tarimbil\\_00000001247](https://doi.org/10.1501/Tarimbil_00000001247)
- Merga JT (2020). Evaluation of common bean varieties (*Phaseolus vulgaris* L.) to different row spacing in Jimma, South Western Ethiopia. *Heliyon* 6 (8): e04822. <https://doi.org/10.1016/j.heliyon.2020.e04822>

- Mir RR, Choudhary N, Bawa V, Jan S, Singh B, Bhat MA et al. (2021). Allelic Diversity, Structural Analysis, and Genome-Wide Association Study (GWAS) for Yield and Related Traits Using Unexplored Common Bean (*Phaseolus vulgaris* L.) Germplasm from Western Himalayas. *Frontiers in Genetics* 11: 1797. <https://doi.org/10.3389/fgene.2020.609603>
- Misra G, Joshi-Saha A, Salaskar D, Reddy KS, Dixit GP et al. (2020). Baseline status and effect of genotype, environment and genotype × environment interactions on iron and zinc content in Indian chickpeas (*Cicer arietinum* L.). *Euphytica* 216 (9): 1-6. <https://doi.org/10.1007/s10681-020-02673-z>
- Nadeem MA, Gündoğdu M, Ercişli S, Karaköy T, Saracoğlu O, Habyarimana E et al. (2020b). Uncovering phenotypic diversity and DArTseq marker loci associated with antioxidant activity in common bean. *Genes* 11 (1): 36. <https://doi.org/10.3390/genes11010036>
- Nadeem MA, Habyarimana E, Çiftçi V, Nawaz MA, Karaköy T et al. S (2018). Characterization of genetic diversity in Turkish common bean gene pool using phenotypic and whole-genome DArTseq-generated silicoDArT marker information. *PloS One* 13 (10): e0205363. <https://doi.org/10.1371/journal.pone.0205363>
- Nadeem MA, Habyarimana E, Karaköy T, Baloch FS (2021b). Genetic dissection of days to flowering via genome-wide association studies in Turkish common bean germplasm. *Physiology and Molecular Biology of Plants* 27 (7): 1609-1622. <https://doi.org/10.1007/s12298-021-01029-8>
- Nadeem MA, Karaköy T, Yeken MZ, Habyarimana E, Hatipoğlu R et al. (2020a). Phenotypic characterization of 183 Turkish common bean accessions for agronomic, trading, and consumer-preferred plant characteristics for breeding purposes. *Agronomy* 10 (2): 272. <https://doi.org/10.3390/agronomy10020272>
- Nadeem MA, Yeken MZ, Shahid MQ, Habyarimana E, Yilmaz H et al. (2021a). Common bean as a potential crop for future food security: an overview of past, current and future contributions in genomics, transcriptomics, transgenics, and proteomics. *Biotechnology & Biotechnological Equipment* 35 (1): 758-786. <https://doi.org/10.1080/13102818.2021.1920462>
- Nhemachena C, Hassan R (2007). Micro-level analysis of farmers' adaption to climate change in Southern Africa. International Food Policy Research Institute 2033 K Street, NW, Washington DC 20006-1002 USA.
- Owusu EY, Mohammed H, Manigben KA, Adjebeng-Danquah J, Kusi F et al. (2020). Diallel Analysis and Heritability of Grain Yield, Yield Components, and Maturity Traits in Cowpea (*Vigna unguiculata* (L.) Walp.). *The Scientific World Journal* 1-9. <https://doi.org/10.1155/2020/9390287>
- Peix A, Ramírez-Bahena MH, Velázquez E, Bedmar EJ (2015). Bacterial associations with legumes. *Critical Reviews in Plant Sciences* 34 (1-3): 17-42 <https://doi.org/10.1080/07352689.2014.897899>
- Pérez-Vega E, Pañeda A, Rodríguez-Suárez C, Campa A, Giraldez R, Ferreira JJ (2010). Mapping of QTLs for morpho-agronomic and seed quality traits in a RIL population of common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 120(7): 1367-1380. <https://doi.org/10.1007/s00122-010-1261-5>
- Pour-Aboughadareh A, Yousefian M, Moradkhani H, Poczai P, Siddique KH (2019). STABILITYSOFT: A new online program to calculate parametric and non-parametric stability statistics for crop traits. *Applications in Plant Sciences* 7 (1): e01211. <https://doi.org/10.1002/aps3.1211>
- Santalla M, Rodiño A, De Ron A (2002). Allozyme evidence supports southwestern Europe as a secondary center of genetic diversity for the common bean. *Theoretical and Applied Genetics* 104 (6): 934-944. <https://doi.org/10.1007/s00122-001-0844-6>
- Sedlar A, Zupin M, Maras M, Razinger J, Šuštar-Vozlič J et al. (2020). QTL mapping for drought-responsive agronomic traits associated with physiology, phenology, and yield in an Andean intra-gene pool common bean population. *Agronomy* 10 (2): 225. <https://doi.org/10.3390/agronomy10020225>
- Seok HY, Woo DH, Park HY, Lee SY, Tran HT et al. (2016). AtC3H17, a non-tandem CCCH zinc finger protein, functions as a nuclear transcriptional activator and has pleiotropic effects on vegetative development, flowering and seed development in Arabidopsis. *Plant and Cell Physiology*. 57 (3): 603-615. <https://doi.org/10.1093/pcp/pcw013>
- Shrestha S, Asch F, Dusserre J, Ramanantsoanirina A, Brueck H (2012). Climate effects on yield components as affected by genotypic responses to variable environmental conditions in upland rice systems at different altitudes. *Field Crops Research* 134: 216-228. <https://doi.org/10.1016/j.fcr.2012.06.011>
- Singh SP, Gepts P, Debouck DG (1991). Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* 45 (3): 379-396. <https://doi.org/10.1007/BF02887079>
- Sinkovič L, Pipan B, Sinkovič E, Meglič V (2019). Morphological seed characterization of common (*Phaseolus vulgaris* L.) and runner (*Phaseolus coccineus* L.) bean germplasm: A Slovenian gene bank example. *BioMed Research International* 16: 1-3. <https://doi.org/10.1155/2019/6376948>
- Souza PF (2020). The forgotten 2S albumin proteins: Importance, structure, and biotechnological application in agriculture and human health. *International Journal of Biological Macromolecules* 164: 4638-4649. <https://doi.org/10.1016/j.ijbiomac.2020.09.049>
- Stagnari F, Maggio A, Galieni A, Pisante M (2017). Multiple benefits of legumes for agriculture sustainability: An overview. *Chemical and Biological Technologies in Agriculture* 4 (1): 1-3. <https://doi.org/10.1186/s40538-016-0085-1>
- Teklewold H, Kassie M, Shiferaw B (2013). Adoption of multiple sustainable agricultural practices in rural Ethiopia. *Journal of Agricultural Economics* 64 (3): 597-623. <https://doi.org/10.1111/1477-9552.12011>
- Teshome DT, Zharare GE, Naidoo S (2020). The threat of the combined effect of biotic and abiotic stress factors in forestry under a changing climate. *Frontiers in Plant Science* 1-19. <https://doi.org/10.3389/fpls.2020.601009>
- Torga PP, Melo PG, Pereira HS, de Faria LC, Del Peloso MJ et al. (2013). Interaction of common bean cultivars of the black group with years, locations, and sowing seasons. *Euphytica* 189(2): 239-248. <https://doi.org/10.1007/s10681-012-0793-y>

- Trapp JJ (2015). Genetics of drought tolerance in common bean (*Phaseolus vulgaris* L.). Washington State University 3717506.
- Turner SD (2014). qqman: An R package for visualizing GWAS results using QQ and manhattan plots. Biorxiv 005165. <https://doi.org/10.1101/005165v1>
- Vaezi B, Pour-Aboughadareh A, Mehraban A, Hossein-Pour T, Mohammadi R et al. (2018). The use of parametric and non-parametric measures for selecting stable and adapted barley lines. Archives of Agronomy and Soil Science 64 (5): 597-611. <https://doi.org/10.1080/03650340.2017.1369529>
- Wang P, Hsu CC, Du Y, Zhu P, Zhao C, Fu X et al. (2020). Mapping proteome-wide targets of protein kinases in plant stress responses. Proceedings of the National Academy of Sciences 117(6): 3270-3280. <https://doi.org/10.1073/pnas.1919901117>
- Watkins JL, Li M, McQuinn RP, Chan KX, McFarlane HE et al. (2019). A GDSL esterase/lipase catalyzes the esterification of lutein in bread wheat. The Plant Cell 31 (12): 3092-3112. <https://doi.org/10.1105/tpc.19.00272>
- Wodajo D, Admassu S, Dereje B (2021). Geometric Characteristics and Mass-Volume-Area Properties of Haricot Beans (*Phaseolus vulgaris* L.): Effect of Variety. International Journal of Food Properties 24 (1): 885-894. <https://doi.org/10.1080/10942912.2021.1937210>
- Yeken MZ, Nadeem MA, Karaköy T, Baloch FS, Çiftçi V (2019). Determination of Turkish common bean germplasm for morpho-agronomic and mineral variations for breeding perspectives in Turkey. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi 22: 38-50. <https://doi.org/10.18016/ksutarimdog.vi.563740>.
- Yuste-Lisbona FJ, González AM, Capel C, García-Alcázar M, Capel J et al. (2014). Genetic analysis of single-locus and epistatic QTLs for seed traits in an adapted× nuña RIL population of common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics 127 (4): 897-912. <https://doi.org/10.1007/s00122-014-2265-3>
- Zhang Y, Yao W, Wang F, Su Y, Zhang D et al. (2020). AGC protein kinase AGC1-4 mediates seed size in Arabidopsis. Plant Cell Reports 39 (6): 825-837. <https://doi.org/10.1007/s00299-020-02533-z>