TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2019) 43: 331-342

doi:10.3906/bot-1812-12

Forage pea (*Pisum sativum* var. *arvense* L.) landraces reveal morphological and genetic diversities

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Received: 07.12.2018 • **Accepted/Published Online:** 27.03.2019 • **Final Version:** 06.05.2019

Abstract: The objective of this study was to assess morphological and genetic diversities of 48 forage pea landraces collected from different locations at different altitudes in Turkey. Morphological, quality, and yield features were determined for the landraces and three control cultivars in three subsequent years. Genetic diversities of the landraces and cultivars were also monitored using microsatellite (SSR) markers. Our results revealed that the features of landraces are significantly different. The hay weights and the relative feed values were found to be significantly affected by altitude, with the landraces generally showing significantly higher hay weight and relative feed values at lower altitudes ($P < 0.05$). At the genetic level, 32 SSR primers led to distinct placement of one of the samples into a different clade of the dendrogram, showing that it is genetically different from the other 47 samples. This genetically different landrace had the highest forage value, suggesting that it shows higher prime forage features than the cultivars and the other landraces. Moreover, altitude and generally flower color were found to be important factors affecting the genetics of the landraces, as the landraces having white flowers or collected at similar altitudes were clustered well in the dendrogram. The results of this study reveal that the morphological and genetic diversities of forage pea landraces collected from different locations at different altitudes show variations. Such information could be used to develop forage pea landraces with improved characters that can be used in hay management.

Key words: Fodder pea, genetic differences, molecular characterization, simple sequence repeat

1. Introduction

Pisum sativum var. *arvense*, one of the oldest crops in the world, is an annual legume diploid plant and an important forage crop (Asci et al., 2015). It is a rich source of protein, fiber, slowly digestible starch, soluble sugars, vitamins, and minerals (Sarikamis et al., 2010). In 2017, globally, 16 million tons of pea grain were produced for human consumption, while 20 million tons of pea were produced for forage purpose (http://www.fao.org/faostat/en/#data/QC). Due to insufficient forage cultivation, hay production with high quality in Turkey still remains a main challenge, although Turkey is the center of the origin and the genetic pool of many wild and cultivated forms of forage crops (Açıkgöz, 2001). Such problems could be overcome by increasing the studies on natural landraces, which could contribute to forage cultivation not only in Turkey but also in the world.

Genetic diversity studies of crop species are very important to breeding programs. Generally, researchers on genetic and plant breeding have emphasized the need for further development in capturing and harnessing genetic diversity. Therefore, assessments of morphological and genetic diversities among landraces were usually utilized for their protection, conservation, and registration. Moreover, this can also be used for breeding purposes to provide abundant allelic variation in breeding material (Jain et al., 2014).

Molecular markers can be used effectively to study genetic diversity in crops (Ahmad et al., 2015). For the analysis of *P. sativum* var. *arvense* diversity, microsatellites, also known as simple sequence repeat (SSR) markers, are widely used because of their high polymorphism level, high information content, codominance, and good reproducibility (Smýkal et al., 2008a).

The development of cultivars in response to environmental challenges, including those associated with climate change, is an important goal of plant breeding (Merkouropoulos et al., 2017). Information on the regional, morphological, and genetic diversity in pea landraces is insufficient in Turkey. However, such information is needed for the development of cultivars with improved characters that can be used in hay management. The objective of this research was to assess the morphological and genetic diversities of forage pea landraces collected from different locations at different altitudes. We also

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aimed to select promising landraces for hay management in similar ecological regions.

2. Materials and methods

2.1. Plant materials

The seeds of 48 landraces were obtained from the East Black Sea Region in Turkey (Table 1). The cultivars, which are suitable for the research region, were obtained from Uludağ University. First the obtained seeds were sowed for the purpose of multiplication and then the uniform landraces that seemed to be uniform based on morphological features were sown in a field. The research was conducted at the research station of Ordu University in the northeast Turkey (6 m elevation, 40°58′N, 37°56′E) during 2013-2014, 2014-2015, and 2015-2016 in a randomized complete block design with ten replications. The seeds were planted with the spacings of 15 × 50 cm. Plots (6 m in length with 3 rows) were formed for each landrace and cultivar. The landraces and cultivars were sown in early November in all three years. Fertilizers were applied as 30 kg N ha^{-1} and 60 kg P ha^{-1}. During the trial no irrigation was done.

2.2. Soil and climatic values

The soil used in the research field was clay loam, neutral (6.87 pH), unsalted (0.04%), insufficient in phosphorus (40.88 kg ha^{-1}), high in potassium (740.76 kg ha^{-1}), medium in organic substance (2.71%), and had little lime (0.52%). According to meteorological data (https://www.mgm.gov.tr), average temperature, total precipitation, and relative humidity were measured as 13.6 °C, 632.6 mm, and 67.1% in the 2013-2014 growing period; 12.9 °C, 636.8 mm, and 68.9% in 2014-2015; 13.2 °C 651 mm, and 68.2% in 2015-2016; and 11.2 °C, 693.1 mm, and 72.2% in the long term (the average of 1960–2016), respectively. Sufficient

Table 1. Collected areas, codes, and altitudes of the landraces.

City-District	Code	Altitude (m)	City-district	Code	Altitude (m)
Ordu-Gülyalı	O1	0–400	Giresun-Çamoluk	G11	>1200
Ordu-Centrum	O2	0–400	Giresun-Şebinkarahisar	G12	>1200
Ordu-Ünye	O3	400–800	Trabzon-Akçaabat	T1	0–400
Ordu-İkizce	O4	400–800	Trabzon-Of	T2	0–400
Ordu-Perşembe	O5	400–800	Trabzon-Arsin	T3	400–800
Ordu-Fatsa	O6	400–800	Trabzon-Centrum	T4	400–800
Ordu-Çaybaşı	O7	400–800	Trabzon-Çarşıbaşı	T5	400–800
Ordu-Ulubey	O8	800–1200	Trabzon-Vakfıkebir	T6	800–1200
Ordu-Kumru	O9	800–1200	Trabzon-Çaykara	T7	800–1200
Ordu-Kabadüz	O10	800–1200	Trabzon-Maçka	T8	800–1200
Ordu-Korgan	O11	>1200	Trabzon-Tonya	T9	>1200
Ordu-Gürgentepe	O12	>1200	Trabzon-Sürmene	T10	>1200
Ordu-Akkuş	O13	>1200	Rize-Ardeşen	R1	0–400
Ordu-Mesudiye	O14	>1200	Rize-Pazar	R2	0–400
Giresun-Tirebolu	G1	0–400	Rize-Centrum	R3	400–800
Giresun-Bulancak	G2	0–400	Rize-Kalkandere	R4	800–1200
Giresun-Piraziz	G3	0–400	Rize-Çayeli	R5	800–1200
Giresun-Espiye	G4	0–400	Rize-Hemşin	R6	>1200
Giresun-Keşap	G5	400–800	Rize-Çamlıhemşin	R7	>1200
Giresun-Eynesil	G6	400–800	Rize-İkizdere	R8	>1200
Giresun-Centrum	G7	400–800	Artvin-Arhavi	A1	0–400
Giresun-Yağlıdere	G8	800–1200	Artvin-Hopa	A2	0–400
Giresun-Güce	G9	800–1200	Artvin-Ardanuç	A3	800–1200
Giresun-Dereli	G10	>1200	Artvin-Centrum	A4	800–1200

precipitation, temperature, and humidity were observed for pea cultivation in a mild climate in these three years (Açıkgöz, 2001).

2.3. Field experiment and traits

Harvesting was started at the time when the pods had started to form on the bottom. After maturity of the plants, seed shape, cotyledon color, anthocyanin coloration, auricle, spots on testa, flower color, seed coat color, and dark hilum of the landraces were detected according to the International Union for the Protection of New Varieties of Plants. After harvesting, the values of time to harvest, plant height, hay weight, hay crude protein with near-infrared reflectance spectroscopy (NIRS), and relative feed values were determined. The relative feed value index estimates digestible dry matter (DDM) of the forage from ADF and calculates the DM intake potential (as a percentage of body weight, BW) from NDF. The index is then calculated as DDM multiplied by dry matter intake (DMI as a % of BW)

Table 2. Names, codes, sequences, and melting temperatures of microsatellite primers used.

Primer name	Code	Forward sequence	Reverse sequence	T_m
PSMPSAD148	P-01	gaaacatcattgtgttgtcttctg	ttccatcacttgattgataaac	56
PSBOX13.1	P-02	gaactagagctgatagcatgt	gcatgcaaaagaacgaaacagg	54
PSGAPA1	P-03	gacattgttgccaataactgg	ggttctgttctcaatacaag	56
PSADH1	P-04	gatgtgataggcctagaacaagc	cagtcacacactacaagagatc	57
AF016458	P-05	cactcataacatcaactatctttc	cgaatcttggccatgagagttgc	55
AA430902	P-06	ctggaattcttgcggtttaac	cgttttggttacgatcgagcta	54
PSMPA5	P-07	gtaaagcataaggggttctcat	cagcttttaactcatctgaca	60
PSMPA6	P-08	cttaagagagattaaatggacaa	ccaactcataataaagattcaaa	56
PSMPA7	P-09	cttgaaatactaaggcaccata	gtgaacactctttgttttacca	56
PSMPA9	P-10	gtgcagaagcatttgttcagat	cccacatatatttggttggtca	58
PSMPB16	P-11	gcatttgtgcagtttcaatttcg	ccaattacggacaatgtttgatca	60
PSMPC20	P-12	gagttctccgtaatagaaggct	cactctgttctgcttcatcatc	60
PSMPAA67	P-13	cccatgtgaaattctcttgaaga	gcatttcacttgatgaaatttcg	60
PSMPAD134	P-14	tttatttttccatatattacagacccg	acacctttatctcccgaagacttag	60
PSMPAD141	P-15	aatttgaaagaggcggatgtg	acttctctccaacatccaacga	60
PSMPAD21	P-16	tattctcctccaaaatttcctt	gtcaaaattagccaaattcctc	54
PSMPSAA205	P-17	tacgcaatcatagagtttggaa	aatcaagtcaatgaaacaagca	56
PSMSAA473	P-18	caatcgatcagacagtccccta	aagctcacctggttatgtccct	60
sP446	P-19	atggaggttgctattgaattagatg	catcccatgtacatattcaccttt	60
PSMPSAD186	P-20	tcaatgacgtgttgatcgagga	ccatgctttgcaccgaaagtaa	62
PSMPSAD237	P-21	agatcatttggtgtcatcagtg	tgtttaatacaacgtgctcctc	62
PSMSAA476	P-22	tagttttgaactttggccgtat	cacaccctaatctaggctatcc	60
X51594	P-23	caaccagccattatacacaaaca	ggcaataaagcaaaagcaga	60
PEACPLHPPS	P-24	gtggctgatcctgtcaacaa	caacaaccaagagcaaagaaaa	58
PSMPSAA456	P-25	tgtagaagcataagagcgggtg	tgcaacgctcttgttgatgatt	60
PEAPHTAP	P-26	ggattggattggatgatga	tggagcccttagtccacaac	60
PSCAB66	P-27	cacacgataagagcatctgc	gcttgagttgcttgccagcc	55
X78581	P-28	ctgctatgctatgtttcacatc	ctttgcttgcaacttagtaacag	60
AF004843	P-29	ccatttctggttatgaaaccg	ctgttcctcattttcagtggg	54
PSP4OSG	P-30	caaccagccattatacacaaaca	ggcaataaagcaaaagcaga	58
AA430902	P-31	ctggaattcttgcggtttaac	cgttttggttacgatcgagcat	54
PSAJ223318	P-32	cagtggtgacagcagggccaag	cctacatggtgtacgtagacac	58

and divided by 1.29. The morphological characteristics of 48 pea landraces were determined on ten randomly selected plants from each plot.

2.4. SSR primers

In this study, 32 microsatellite primer pairs were selected from previous studies according to high polymorphism for pea germplasm (Cupic et al., 2009; Nasiri et al., 2009; Bouhadida et al., 2013). The information of primers is shown in Table 2.

2.5. Genomic DNA isolations and SSR analysis

Young leaves from ten randomly chosen field-grown plants were combined per landrace for genomic DNA isolation and SSR analysis. Genomic DNA was isolated from leaf samples using the modified CTAB (cetyltrimethylammonium bromide) method described by Rogers and Bendich (1985). DNA quality and quantity were analyzed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA).

PCR amplifications were conducted in total volumes of 25 µL comprising 1 µL of genomic DNA, 5 pmol of each forward and reverse primer, and 5 µL of 5X C Taq Master Mix (Promega Corporation, USA). The PCR products were handled using a Bio-Rad Thermal Cycler. Amplifications were performed with the following profile: 95 °C initial denaturation for 5 min, followed by 35 cycles of 30 s at 95 °C, annealing at 54–62 °C for 30 s, and 30 s at 72 °C. PCR products on 2.5% agarose gels stained with ethidium bromide (EtBr) in Tris-borate-EDTA (TBE) buffer were analyzed under UV light. To determine the size of each amplified product a DNA ladder of 100 bp (Promega Corporation, USA) was used. The gel was viewed using a Bio-Rad gel documentation machine. The gel picture was analyzed using Bio-Rad Image Lab software for the band size.

2.6. Statistical analyses

Traits were tested using the Kolmogorov–Smirnov test. One-way ANOVA of the data was performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) to determine differences among the geographic areas. The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

DNA marker data were processed with NTSYS version 2.1 software. The phylogenetic dendrogram was obtained by using the unweighted pair group method on arithmetic averages (UPGMA).

3. Results

3.1. The evaluation of morphological, yield, and quality features

All the qualitatively measured traits that revealed polymorphisms are presented in Table 3. Our results indicated that the landraces collected from high altitudes were distinguished morphologically.

The averages of three years of yield and quality characterizations of the accessions are presented in Figure 1. Time to harvest varied between 133 and 183 days without a significant difference. The average of plant heights varied between 55.6 and 178.8 cm. The T8, O5, and O6 landraces showed higher values than the cultivars in terms of plant height ($P < 0.05$). The average hay weight per plant varied between 9.58 and 39.42 g/plant in dry matter. The T8 landrace showed higher hay weight values than cultivars ($P < 0.05$). The average hay crude protein contents varied between 15.01% and 20.14%. The highest (but not significantly so) hay crude protein values were seen in the T8 landrace in terms of hay crude protein content. The average relative feed values varied between 137.45 and 252.12. The O1, O8, and T8 landraces showed higher relative feed values compared to the commercial cultivars ($P < 0.05$), which have prime features according to Horrocks and Vallentine (1999).

Ten clusters were obtained on the basis of the phylogenetic dendrogram with three years of morphological, quality, and yield data (Figure 2). In the dendrogram the cultivars were clustered together in one group. The maximum distance was observed between O1 collected from the lowest altitude and O13 collected from the highest altitude.

3.2. SSR analyses

Forty-eight landraces and 3 cultivars were successfully discriminated using 32 SSR markers, showing the high discriminating power of the set of markers used. In the study, 127 alleles were detected (Table 4). The number of alleles per primer ranged from 2 to 7, with an average of 3.97. All primers were determined as polymorphic, and the average polymorphism information content (PIC) value was 0.632, ranging from 0.175 for primer P-30 to 0.892 for primer P-28. The alleles were detected in a wide range (90–662 bp).

An UPGMA dendrogram was formed that clearly revealed the genetic relationship between landraces and cultivars tested. In the dendrogram, landraces and cultivars were clustered in 5 groups (Figure 3).

A joint analysis of molecular markers compared to morphological markers showed a low but positive significant correlation ($r = 0.356$).

4. Discussion

Using a combination of morphological traits and molecular markers has been shown to lead to more reliable conclusions in assessments of genetic diversity (Nikoumanesh et al., 2011; Zhou et al., 2015). As seen in Table 3, all the qualitatively measured traits, which are important for identification and characterization of pea species, indicated a high range of variation among the 48 landraces evaluated. Morphological traits are often known to be influenced by

Table 3. Morphological evaluation of the accessions.

Landrace	ss	cc	ac	a	st	fc	scc	dh
O1	Ellipsoid	Yellow	Absent	Simple	Intense	Pink	Brownish	Absent
O2	Ellipsoid	Yellow	Present	Compound	Intense	Pink	Brownish	Absent
O3	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Absent
O4	Irregular	Yellow	Present	Compound	Faint	Pink	Brownish	Absent
O5	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Absent
O6	Ellipsoid	Green	Absent	Compound	Intense	Pink	Green	Absent
O7	Ellipsoid	Yellow	Present	Compound	Intense	Pink	Green	Absent
O8	Ellipsoid	Yellow	Present	Simple	Faint	Pink	Brownish	Absent
O9	Ellipsoid	Green	Absent	Compound	Absent	Pink	Brownish	Absent
O10	Ellipsoid	Yellow	Present	Compound	Intense	Pink	Brownish	Absent
O11	Ellipsoid	Green	Absent	Compound	Intense	Pink	Brownish	Absent
O12	Round	Yellow	Absent	Compound	Intense	White	Green	Absent
O13	Rhomboid	Green	Absent	Compound	Absent	Reddish	Brownish	Present
O14	Round	Orange	Absent	Compound	Faint	Pink	Reddish	Present
G1	Ellipsoid	Yellow	Present	Compound	Intense	Pink	Brownish	Absent
G2	Ellipsoid	Yellow	Absent	Compound	Absent	Pink	Green	Absent
G3	Ellipsoid	Yellow	Absent	Compound	Faint	Pink	Brownish	Absent
G4	Ellipsoid	Green	Absent	Simple	Intense	Pink	Brownish	Absent
G5	Ellipsoid	Yellow	Absent	Compound	Faint	Reddish	Brownish	Absent
G6	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Absent
G7	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Absent
G8	Ellipsoid	Green	Absent	Compound	Faint	White	Brownish	Absent
G9	Ellipsoid	Yellow	Present	Compound	Absent	Pink	Brownish	Present
G10	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Absent
G11	Rhomboid	Orange	Absent	Simple	Absent	Reddish	Reddish	Present
G12	Irregular	Yellow	Absent	Compound	Faint	Pink	Green	Absent
T1	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Absent
T2	Ellipsoid	Yellow	Absent	Compound	Faint	White	Green	Absent
T3	Ellipsoid	Green	Absent	Compound	Intense	Pink	Cream	Absent
T4	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Cream	Absent
T5	Irregular	Yellow	Present	Compound	Intense	Pink	Brownish	Absent
T6	Rhomboid	Yellow	Absent	Compound	Absent	Pink	Brownish	Absent
T7	Irregular	Yellow	Absent	Compound	Faint	Pink	Brownish	Absent
T8	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Present
T9	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Present
T10	Irregular	Green	Absent	Simple	Faint	Pink	Brownish	Absent
R1	Rhomboid	Yellow	Absent	Compound	Absent	Pink	Brownish	Absent
R2	Ellipsoid	Yellow	Absent	Compound	Faint	White	Green	Absent
R3	Ellipsoid	Yellow	Absent	Compound	Faint	Pink	Green	Absent
R4	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Absent
R5	Ellipsoid	Yellow	Absent	Compound	Intense	White	Green	Absent
R6	Rhomboid	Orange	Absent	Simple	Faint	Pink	Green	Present
R7	Ellipsoid	Yellow	Absent	Compound	Intense	Pink	Brownish	Absent
R8	Irregular	Yellow	Present	Compound	Intense	Pink	Green	Present
A1	Ellipsoid	Yellow	Absent	Compound	Absent	Pink	Reddish	Absent
A2	Ellipsoid	Yellow	Present	Simple	Faint	Pink	Brownish	Absent
A3	Irregular	Green	Absent	Compound	Intense	Pink	Reddish	Absent
A4	Round	Yellow	Present	Compound	Intense	Reddish	Reddish	Present

ss: Seed shape, cc: cotyledon color, ac: anthocyanin coloration, a: auricle, st: spots on testa, fc: flower color, scc: seed coat color, dh: dark hilum.

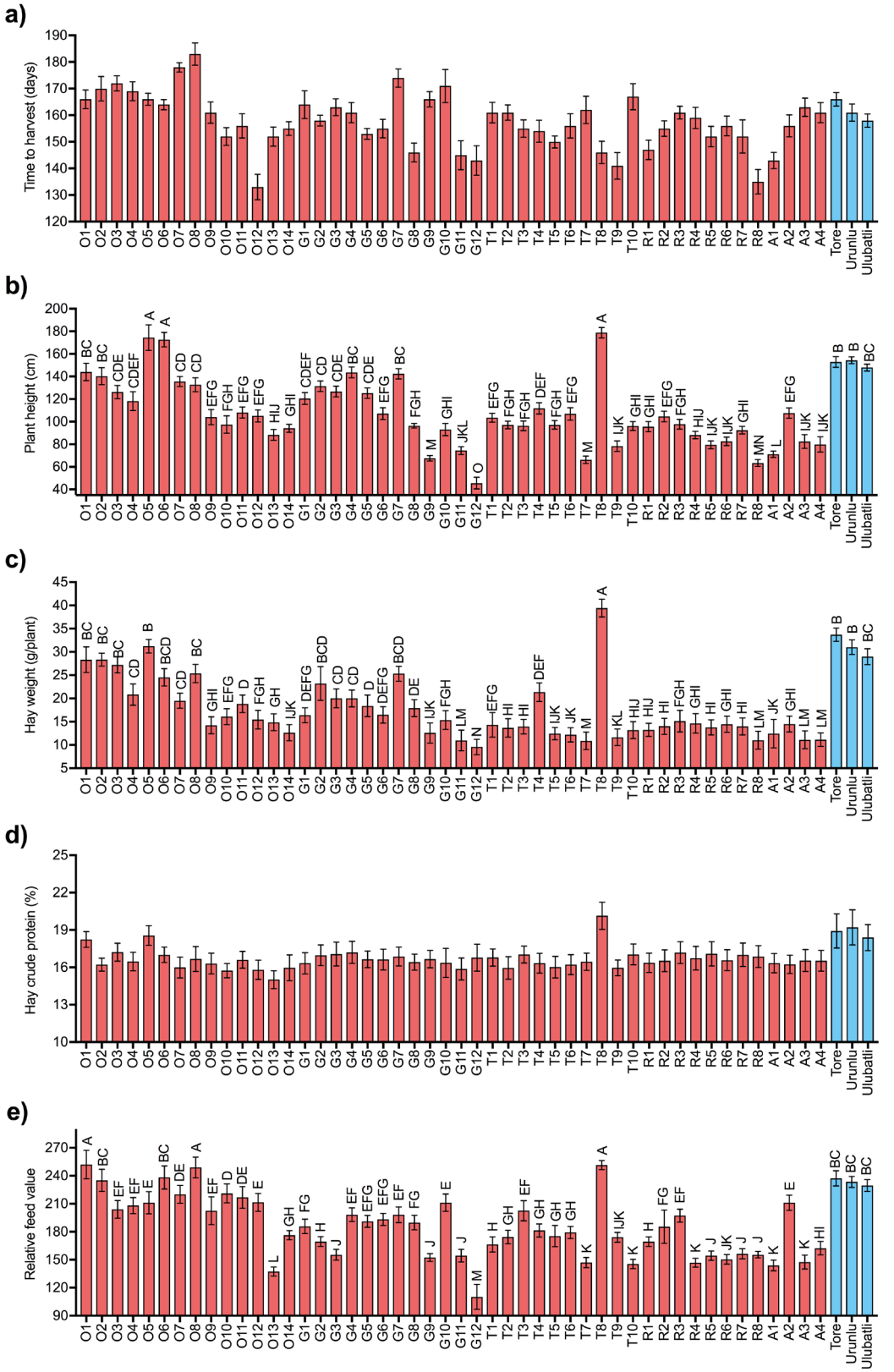

Figure 1. Average of three years of yield and quality traits (mean ± SD) and significance of the accessions: a) time to harvest, b) plant height, c) hay weight, d) hay crude protein, e) relative feed value. The bars colored with light blue represent the control cultivars. The values indicated are not significantly different ($P < 0.05$).

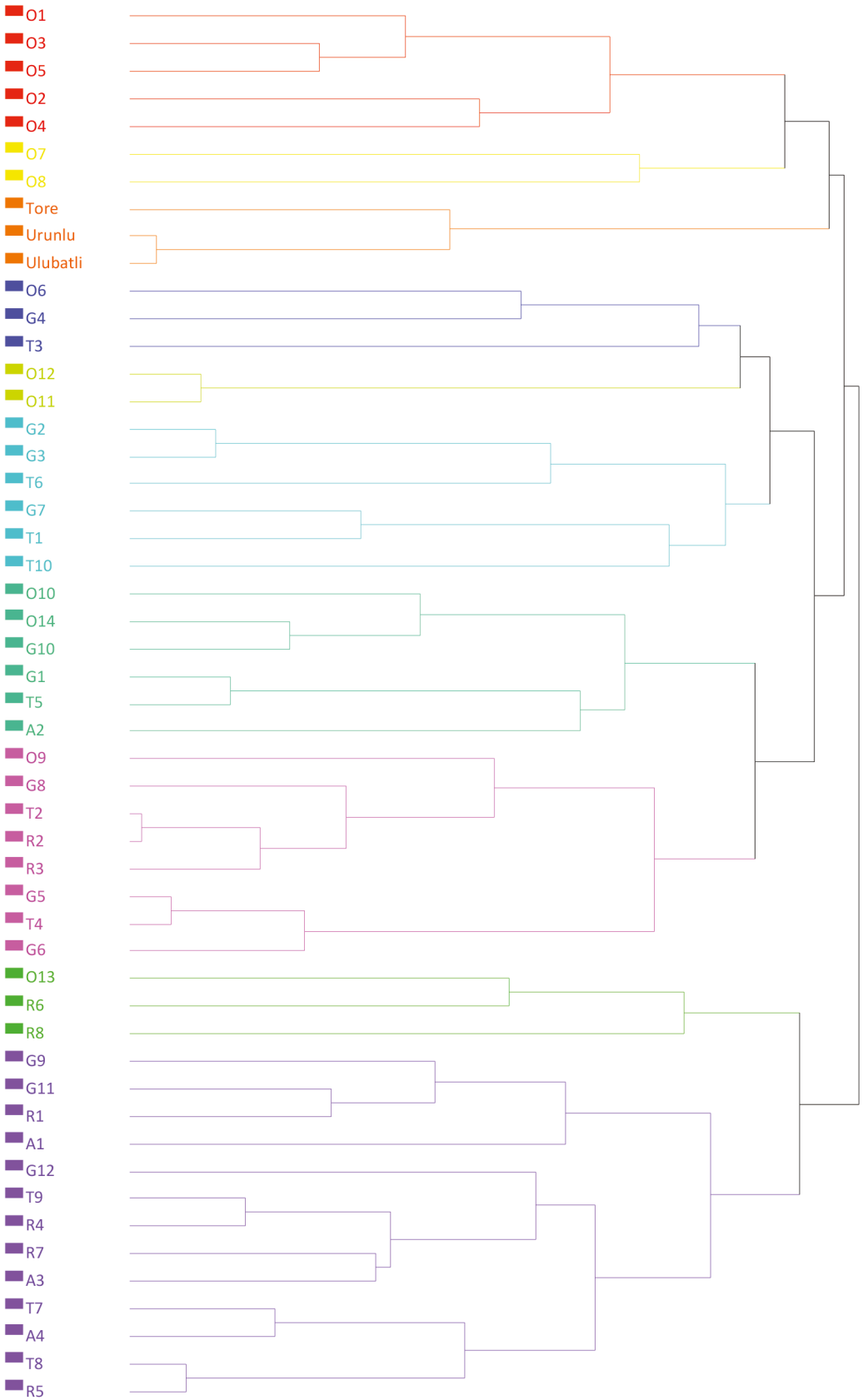

Figure 2. The phylogenetic dendrogram of a tree based on the morphological, quality, and yield data achieved by Ward method.

Table 4. The values of the primers evaluated in the study.

Primer code	Allele range (bp)	Allele numbers	PIC values
P-01	170–251	7	0.840
P-02	257–538	6	0.817
P-03	330–389	3	0.651
P-04	340–391	4	0.763
P-05	122–173	3	0.610
P-06	178–206	3	0.397
P-07	323–442	5	0.642
P-08	136–167	4	0.702
P-09	161–189	4	0.612
P-10	364–389	5	0.709
P-11	388–435	4	0.682
P-12	226–284	5	0.691
P-13	277–286	4	0.591
P-14	282–300	4	0.660
P-15	236–350	5	0.594
P-16	200–275	5	0.603
P-17	216–246	4	0.703
P-18	327–406	3	0.470
P-19	591–662	3	0.668
P-20	270–332	3	0.489
P-21	234–374	5	0.791
P-22	186–348	5	0.793
P-23	223–351	5	0.873
P-24	371–448	3	0.626
P-25	90–105	3	0.577
P-26	146–154	3	0.494
P-27	404–441	3	0.634
P-28	221–367	5	0.892
P-29	226–239	3	0.591
P-30	285–301	2	0.175
P-31	308–346	3	0.557
P-32	287–298	3	0.337
Average	90–662	3.97	0.632

environmental factors, but the importance of these traits cannot be underestimated for analyzing the diversity of a crop species as they are the primary constituents of overall diversity. It is suggested that use of morphological traits is unavoidable for distinctness, uniformity, and stability (Roldán-Ruiz et al., 2001; Cupic et al., 2009). Researchers have utilized morphological variability in combination with molecular markers to precisely estimate characteristic diversity for drawing inferences in many crops, including pea (Smýkal et al., 2008a; Sharma et al., 2010; Rana et al., 2017). In this study, the T8 landrace was determined as a promising landrace in terms of hay weight, plant height, hay crude protein, and relative feed value for yield and quality compared to commercial cultivars. All these traits are important objectives of overall improvement. The improvement of forage legumes has a final economic

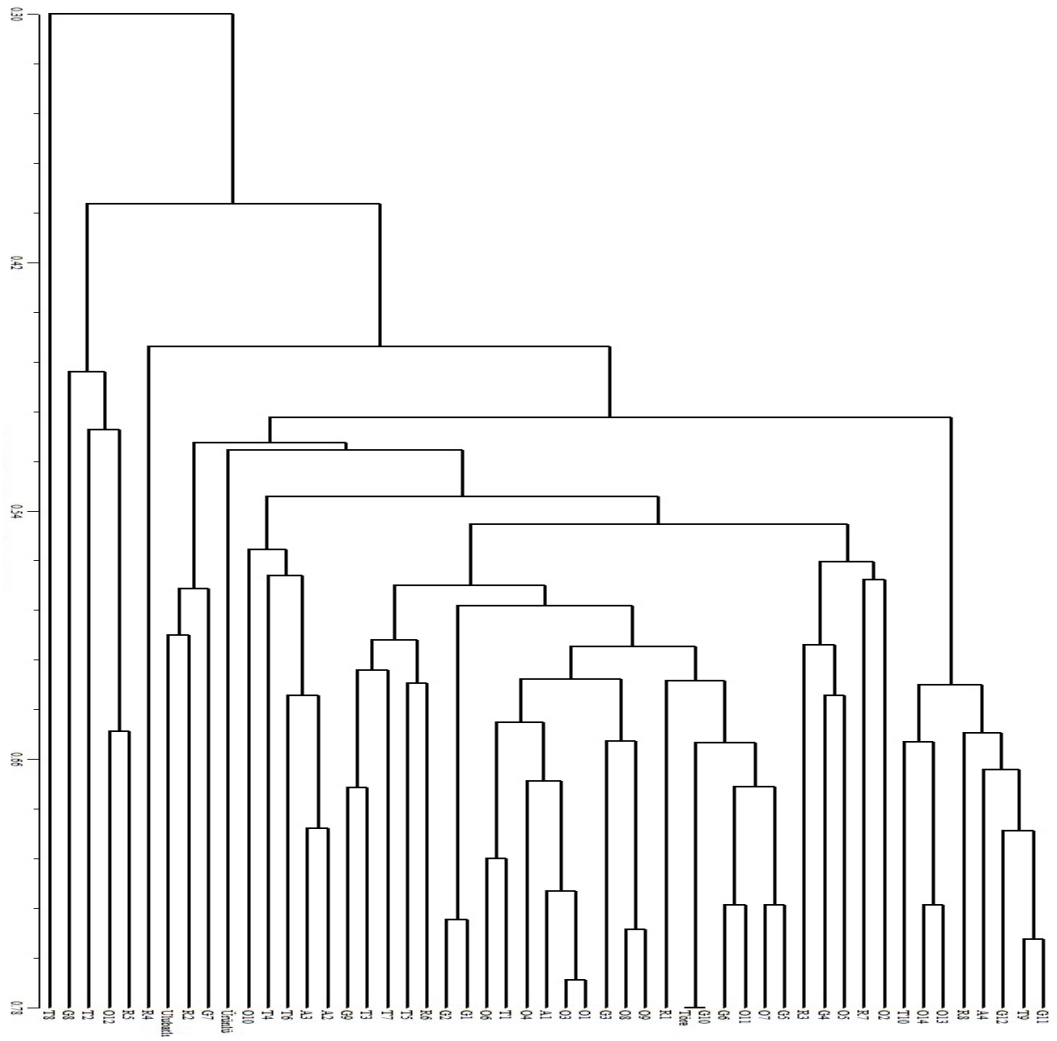

Figure 3. The phylogenetic dendrogram of the landraces based on the genetic similarity matrix data, achieved by unweighted pair group method of arithmetic averages (UPGMA) cluster analysis.

objective of maximizing the weight gain per animal and per area. In this regard, these traits are fundamental as criteria for forage legume selection to achieve economically viable use (Phelan et al., 2015; Simeão et al., 2017).

The morphological cluster analysis was effective for classifying the cultivars and landraces (Figure 2). The clustering of the landraces based on three years of morphological, quality, and yield data was partially explained by the collection altitudes. Results of this study were in agreement with the findings of Merkouropoulos et al. (2017).

Differences in altitudes did not significantly impact the hay crude protein and time to harvest values of the landraces, whereas altitude was found to be an important factor affecting the other yield and quality traits of the landraces (Figure 4). The landraces having 0–400 and 400–800 m altitudes showed the highest plant heights ($P < 0.05$). The landraces having 400–800 m altitude yielded the highest hay weight and relative feed value ($P < 0.05$). The differentiation among landraces has been reported and was attributed to their mating systems, gene flow, genetic drift, long-term evolutionary history (Hogbin and Peakall, 1999), habitat differentiation and management (Peter-Schmid et al., 2008; Merkouropoulos et al., 2017), and altitude differentiation (Acar et al., 2016).

Although the research area where the landraces were collected in the current study does not cover a wide region, our results revealed that either similar or higher diversity results were obtained compared to studies that collected landraces from wider regions. In terms of PIC and allele numbers, the same results were obtained by Sarikamis et

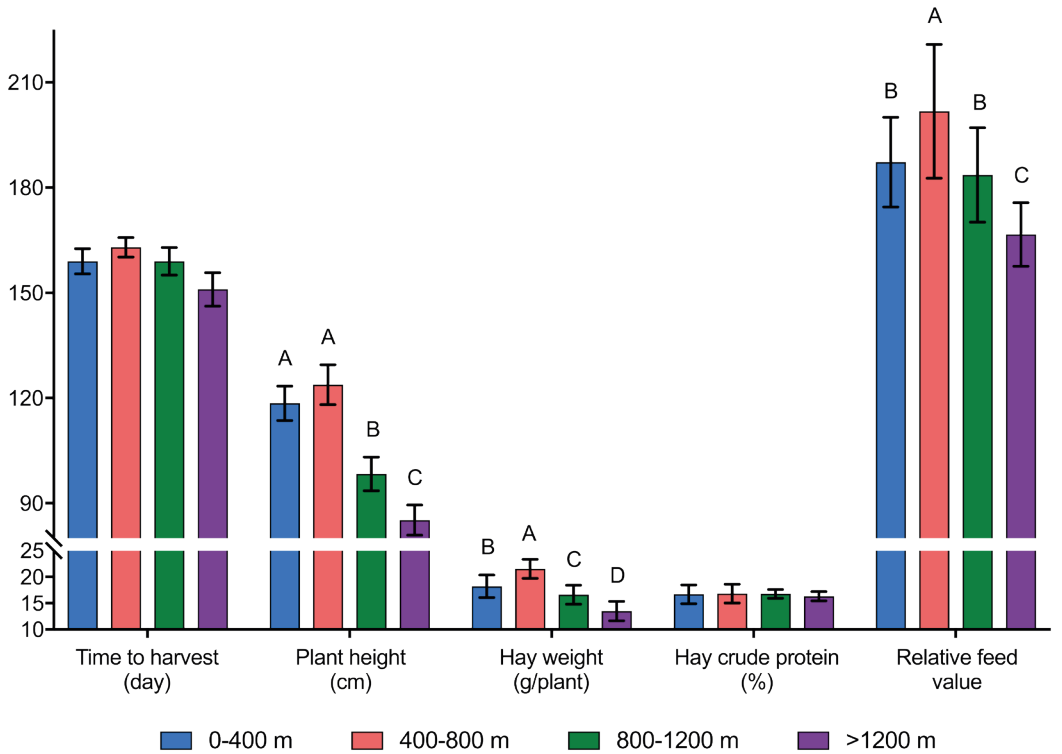

Figure 4. Yield and quality traits (mean ± SD) and significance of *P. sativum* var. *arvense* landraces according to four altitudes. The values indicated are not significantly different ($P < 0.05$).

al. (2010), who analyzed landraces collected from a wider area. This further emphasizes that the region where we collected our samples has a diverse set of landraces.

Moreover, the average PIC and allele values obtained in this research were higher than in the studies conducted by Handerson et al. (2014) and Jain et al. (2014) and were similar to studies done by Cupic et al. (2009), Cieslarova et al. (2012), Ahmad et al. (2015), Baloch et al. (2015), Prakash et al. (2016), Nisar et al. (2017), Wu et al. (2017), and Uysal et al. (2018). On the other hand, lower average PIC and allele values were observed in this study compared to those performed by Smýkal et al. (2008b) and Rana et al. (2017).

In the dendrogram (Figure 3), the first and third groups had only one landrace (T8 and R4, respectively), demonstrating that these landraces are genetically different from all others tested. All white flower landraces, except R2, were clustered in the second group, suggesting that, genetically, G8, T2, O12, and R5 are closely associated. This means that white flowers could have a main role for genetic diversity in selection. Most of the landraces were included in the fourth group, in which the commercial cultivars were also included. This means that, genetically, the commercial cultivars are more closely associated with landraces included in this group compared to others included in groups 1, 2, 3, and 5. The promising landraces (O1, O5, O6) in terms of yield and quality were clustered in the fourth group. The fifth group had eight landraces (T10, O14, O13, R8, A4, G12, T9, G11). The common feature of these landraces was that they were all collected from high altitude regions. This suggests that, similar to flower color, altitude could play a main role for genetic diversity. This finding is in agreement with Turpeinen et al. (2003) and Shakhatreh et al. (2016). In terms of flower color, similar results were also observed by Bouhadida et al. (2013). Overall, the dendrogram shows that the landraces collected in the Eastern Black Sea Region of Turkey are impressively different in genetic diversity. The observed differences in diversity among pea populations suggest differences in demographic history. The wide diversity observed in this region could be due to selection of unconsciously appropriate alleles by farmers with better adaptation to local climatic conditions. In pea, as in other organisms, selection appears to be a major differentiating and orienting force of regional evolutionary change, maintaining genetic polymorphisms under conditions of environmental heterogeneity and stress (Hübner et al., 2009; Shakhatreh et al., 2016). In the present study, except for two samples, no marker heterozygosity was detected, suggesting that these landraces were highly homozygous. This was expected because pea is a self-pollinating species. Moreover, in this study, the landraces have been used for

long years and were not exchanged with farmers from other regions, which prevents heterogeneity that could possibly occur over the years. The other reason could be that the region has differences in altitudes and climatically, which tend to improve the level of diversity.

In the present study, a joint analysis of molecular markers compared to morphological markers showed a low but positive significant correlation (r = 0.356). This indicates that SSR genetic distance tended to reflect morphological distance. Similar results with positive correlations were also reported in pea (Smýkal et al. 2008a, 2008b; Cupic et al., 2009; Handerson et al., 2014).

The collected landraces in this study will likely harbor additional genetic variation. Our data further suggest that pea landraces are surprisingly often unique. Thus, continuous efforts to sample plant genetic resources from farmers would result in more variation, possible to be exploited in breeding (Hagenblad et al., 2014). Gathering of crop biodiversity is often associated with landraces cultivated in areas with nonindustrialized agriculture. This study shows the importance of inventorying local landraces.

In conclusion, in this study, in addition to high diversity of morphological, yield, and quality features, the genetic variability among landraces was high enough to propose that genetic diversity of the landraces is sufficient for creation of new favorable gene combinations. We assessed 32 SSR markers that showed significant variability across 48 forage pea landraces. This suggests a potential use for these markers in association studies. The information revealed in cluster analysis may be useful in a breeding program.

The landraces collected from high altitudes morphologically and genetically distinguished themselves from those collected from lower altitudes. This indicates regional diversity. It means that diversity is structured at different altitudes and further suggests that the similarities and differences in their morphological features are dependent on environmental factors. Therefore, the distinctiveness of the high altitude plants should be included for selection programs of *P. sativum* var. *arvense*.

T8 was promising for forage in terms of having the highest forage value and high allelic diversity. This shows that locally adapted landraces may have better performances.

Acknowledgment

This research was supported by the Ordu University Scientific Research Projects Coordination Unit with project number TF-1324. The data were taken from the PhD thesis of Gürkan Demirkol.

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