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Morphological and agronomic characterization of Turkish *Vaccaria hispanica* (Mill.) Rauschert populations

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Abstract: *Vaccaria hispanica* (Mill) Rauschert, also known as cow cockle, is an important candidate for industrial crop species that has yet to be fully domesticated. Therefore, characterization of germplasms is crucial to initiate and select for economic use. The study reports cultivation, characterization, and seed production of 66 wild *V. hispanica* genotypes, collected throughout Turkey that represent the available genetic pool. The study was carried out in an unheated greenhouse in Antalya, under Mediterranean climate from December to early June. The populations were evaluated for 21 morphological and 6 agronomic traits. The highest coefficient of variation (CV) for morphological traits was detected for the length of the smallest leaf (84.76%), the number of main branches on stem (57.5%), degree of petals imbricate (46.5%), and the flower number of the top five dichasium (42.27%). Among the agronomic traits, the number of seeds per capsule (27.88%), seed yield per plant (24.34%), and total yield per plant (23.74%) showed the highest three CVs. The minimum-maximum values of the seed yield per plant, total yield per plant, 1000 seed weight, and number of seeds per capsule were determined as 54.4–186 g, 173.3–616 g, 4.5–7.9 g and 3.3–15.3, respectively. The results revealed the potential cultivation of *V. hispanica* under greenhouse and Mediterranean climate conditions. In addition, various plant characteristics enabled us to select different phenotypes and variants for future breeding studies for both industrial and ornamental plant use.

Key words: Breeding, cow cockle, domestication, industrial crop, seed yield, TSW

1. Introduction

The global nutraceutical market is growing rapidly. Its value was about $15–20 billion (U.S.) (Ferrie et al., 2005) in 2005 and global market expected to grow to $722 billion by 2027 (Grand View Research, 2021). The increasing interest, need, and demand for natural medicinal and aromatic plants is the driving force of this rapid growth. This enormous economic potential motivates efforts to increase plant-derived metabolite production and develop new varieties with higher content.

Among the natural plants, one of the species that has attracted particular attention recently is *Vaccaria hispanica* (Mill) Rauschert, also known as cow cockle. It is a diploid plant species from the Caryophyllaceae family with a somatic chromosome number of 2n = 30 (Löve, 1942; Kocyigit and Alp, 2018). Although *V. hispanica* is a member of the monotypic genus *Vaccaria*, it has many synonyms such as *V. grandiflora* Jaub. & Spach, *V. segetalis* (Neck) Garke, *V. vulgaris* Host. (Zhou et al., 2016), *V. oxydonta* Boiss., *V. pyramidata* Medik., *Saponaria ampicimus* Miller, *S. hispanica* Miller, *S. oxydonta* (Boiss.) Boiss., and *S. vaccaria* L. (Memon et al., 2013). It has been widely distributed in Europe and Asia (Sang et al., 2003), and introduced to North America (Duddu et al., 2015a). *V. hispanica* is an annual herbaceous plant up to 70 cm tall, has erect stem, lanceolate-ovate sessile glabrous leaves (Memon et al., 2013), two styles, perfect flowers with five petals in spreading dichasial panicles (Cullen, 1967), seeds similar in shape and size to canola (Mazza et al., 1992), and top root system (Fletcher, 1897; Duddu et al., 2015a). The inflorescence is widely branched and many-flowered (Cullen, 1967). Flowers are mostly pink in color and bloom in April–July. According to Torzewski et al. (2018), *V. hispanica* is a thermophilic plant that thrives in calcium-rich soils.

The seeds of *V. hispanica*, also known as semen Vaccariae or Wang-bu-liu-xing, are used in Chinese medicine for nearly 2000 years (Zhou et al., 2016). However, *V. hispanica* has until recently been underutilized natural plant in the world, except for a small number of ethnobotanical uses (Cakilcioglu and Turkoglu, 2010; Ishtiaq et al., 2021) and a limited ornamental use (Sang et al., 2003). It is mostly

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known as a common annual weed in many countries (Holm et al., 1997). Even, *V. hispanica* was reported by Alex (1968) as an important invasive weed species for crop fields at a density exceeding 300 plants m^{-2} in Western Canada. For this reason, this species has been treated as a weed for a long time and has been intensively struggled all over the world. It has been reported that over time, the frequency and distribution of *V. hispanica* in Canada has decreased drastically (Thomas et al., 2007; Harker et al., 2009), thus reducing the effect of this species as a weed (Duddu et al., 2015a). In addition, although one of *V. hispanica*'s centers of origin is the continent of Europe, according to Torzewski et al. (2018), it has been withdrawing from all of Europe in recent years. The main reasons for this worldwide decrease were stated as the intensification of agriculture, improvement of seed cleaning methods, the change in the physical and chemical properties of the soils, and particularly the weed management practices such as herbicides and zero tillage (Kiraly et al., 2006; Harker et al., 2009; Torzewski et al., 2018). However, *V. hispanica* is almost considered a gold mine today due to its valuable ingredients. The high seed content of *V. hispanica* particularly in terms of unique small starch grains and high saponin ingredient and its favorable agronomic properties such as early maturity (90–100 days), medium stature, less nutrient-demand, similarity to canola seeds, ease of seed threshing, and suitability for cultivation with canola mechanization (Mazza et al., 1992; Watson et al., 2007; Balsevich, 2008) made it an important candidate as a new alternative medicinal-industrial crop for Canada lately (Willenborg and Johnson, 2013). It is estimated that an agronomically superior bred through doubled-haploid *V. hispanica* cultivar could have a return of $10 millions in a 100-ha area (Oelck, 2008). The most valued ingredients of *V. hispanica* are usually collected in their seeds. About 85% of the seeds have valuable compounds that are segetalin-type cyclic peptides (0.3%–1%), quillaja-type saponins (2%–4%), antioxidant phenolics (0.4%–1%), ribosome-inactivating proteins (11%–14%), low amount of oil (3%–4%), and high amount of small-sized (0.5–1.6 μm) starch grains (60%–65%) (Balsevich, 2008). These compounds have the potential to be used in the medicinal, nutraceutical, vaccine (as adjuvant), herbal, food, feed (as additive), veterinary, and cosmetics industries (Balsevich, 2008). Given its economic potential and importance as a medicinal-industrial plant, the production of valuable breeding lines or hybrid varieties of *V. hispanica* would be extremely important goal.

Consequently, the development of conservation strategies and the management of the existing *V. hispanica* genetic resources for both sustainable cultivation and domestication and breeding studies are indispensable for the true evaluation of these sources. Thus, it is necessary to characterize the available germplasms. This is particularly important for capturing intraspecies variations in *V. hispanica* that belongs to a monotypic genus. Domestication and breeding studies in *V. hispanica* are limited both in scope and in geography. The domestication studies in Canada involved characterization of the *V. hispanica* germplasms for morphological (Goering et al., 1966; Mazza et al., 1992; Duddu et al., 2015a), seed composition (Mazza et al., 1992; Balsevich, 2008), seed dormancy and germination ecology (Duddu and Shirtliffe, 2014), seed persistance (Duddu et al., 2015b), agronomic (Goering et al., 1966; Willenborg and Dosdall, 2011; Willenborg and Johnson, 2013; Duddu et al., 2015a), and phytochemical properties (Mazza et al., 1992; Biliaderis et al., 1993). As for Turkey, excluding flora/vegetation based topics, studies to promote economic potential of *V. hispanica* are lacking. Recently, we have carried out a comprehensive characterization study in order to initiate *V. hispanica* breeding, primarily for the pharmaceutical industry, and possibly for the ornamental plant sector. The project aimed to characterize Turkish *V. hispanica* germplasm, 66 populations, representing the whole geographic locations of Turkey. The genotypes were characterized at DNA level (SSR and SRAP molecular markers), germination data, morphological, and agronomic traits, as well as haploid induction performance (through isolated microspore culture) data; and the multiplied seeds were analyzed for physical (size, diameter, 1000 seed weight (TSW), bulk density) as well as chemical (starch, protein, saponin, cyclopeptide, phenolics, fat and fatty acid composition) structure. Seed analysis and haploid results were reported by Cam et al. (2018) and Ari et al. (2022), respectively, while germination and molecular studies will be reported soon. In this work, we present the morphological and agronomic characterization of 66 wild Turkish *V. hispanica* genotypes.

2. Materials and methods

2.1. Plant material

As previously stated by Cam et al. (2018), the seeds of 66 wild *V. hispanica* genotypes were collected in the summer of 2013 according to Turkey grid system (Davis, 1965–1985), representing all regions of Turkey (Figure 1). The plant samples were collected with the permission of the General Directorate of Agricultural Research under the Ministry of Agriculture and Forestry. During the field surveys carried out in 27 squares, attention was paid to leave a distance of at least 100 km among the locations and to collect at least two genotypes from each square.

Plants multiplied from the seeds were used as the source of morphological observations, while the produced seeds were used for agronomic characterization in this study.

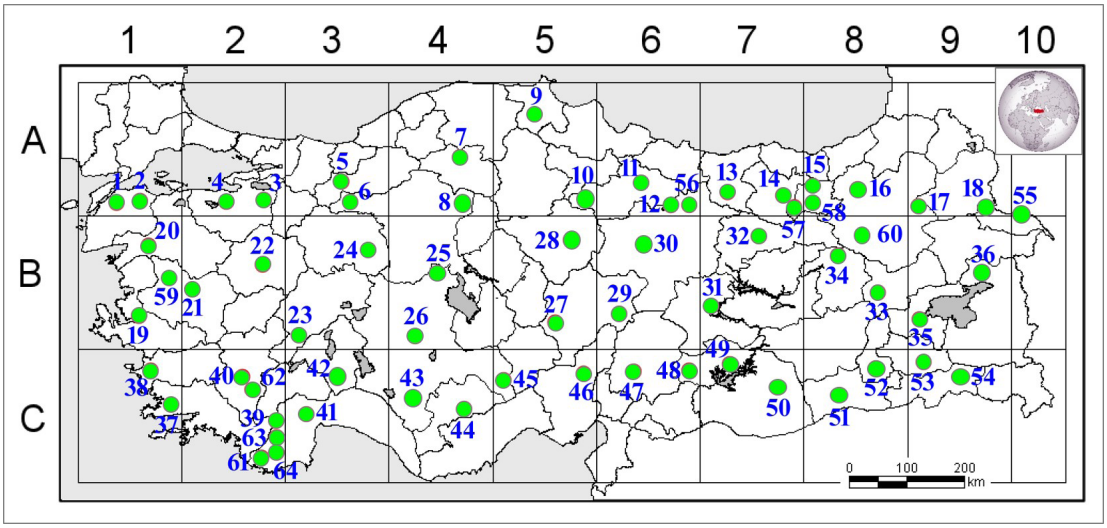

Figure 1. Seed collection points of *Vaccaria hispanica* genotypes according to Turkey grid system (Davis, 1965-1985).

The seeds collected from wild were sown in containers with a mixture of 65% peat, 25% perlite, and 10% vermiculite in December 2013. One thousand three hundred and twenty seedlings representing 66 genotypes, 20 seedlings for each, were transplanted to the solarized soil in an unheated greenhouse at Akdeniz University (36°53′54″ N latitude; 30°38′19″ E longitude), Antalya, Turkey, with a Mediterranean climate at the end of January 2014. The recorded minimum, average, and maximum air temperatures of the greenhouse were 5.2, 19.4, 41.8 °C, respectively, from December 2013 to early June 2014. Seedlings were planted double rows in ground beds at intervals of 100 cm × 80 cm and 60 cm crosswise. Plants were regularly irrigated twice a week, and also regularly fertilized by drip irrigation with NPK (18-18-18 or 16-8-22 w/w), monopotassium phosphate, and microelements. Fertilization was stopped after the first seed capsule was formed. In addition, protective acaricide and insecticide applications were made with a back pump.

2.2. Morphological traits

In the study, a total of 21 morphological features were examined on three randomly selected plants in each of 66 genotypes. These are; 1. plant height (*ph*) (cm), 2. stem base diameter (*sbd*) (mm), 3. length of the largest leaf at stem base (*lll*) (cm), 4. length of the smallest leaf at the top (*lsl*) (cm), 5. number of days until flowering (*nduf*) (day), 6. number of main branches on stem (*nmbos*) (n), 7. flower (/flower bud) number of top five dichasium (*fntfd*) (n), 8. flower (/flower bud) number of last alar (*fnla*) (n), 9. width of calyx (*woc*) (mm), 10. length of calyx (*loc*) (mm), 11. length of pedicel (*lop*) (cm), 12. petal length (with claw) (*pl*) (cm), 13. width of petal lamina (*wpl*) (mm), 14. catalog number of petal color (*cnpc*), 15. degree of petals imbricate (*dopi*), 16. degree of petal emarginate (*dope*), 17. degree of teeth of petal margin (*dotpm*), 18. degree of petal vein (*depv*), 19. presence of leaf pruinose (*polp*), 20. presence of leaf involute (*poli*), 21. shape of calyx teeth (*soct*).

The first 13 of these features can be characterized as quantitative and the next eight as qualitative. Quantitative features were measured in the specified units, while qualitative features were evaluated according to the visual rating scale observations stated below. Determination of the 14th trait was made on a 0–3 visual rating scale (0, 1, 2, and 3) (0: none, 1: No.65-A, 2: No.65-B, and 3: No.65-C) using the Royal Horticultural Society's Color Charts (RHSCC, Fan 2). A 0–4 visual rating scale (0: none, 1: very little, 2: little, 3: moderate, and 4: excess) was used to evaluate the 15th, 16th, 17th, and 18th traits, while the absent/present rating (–/+) (0/1) was used to assess the 19th and 20th traits. Also, the narrow/wide (0/1) assessment was used for the 21st trait.

2.3. Agronomic traits

V. hispanica genotypes were harvested in early June 2014. The criterion for harvest maturity was that the capsules began to open at the tip and the inner septal membrane was visible from above. Since the plants were harvested when they were quite dry, the aboveground parts of the plants tended to break off immediately from their roots, and therefore only the aboveground parts of the plants were collected during harvest. Twenty plants of each genotype were collected in this way and weighed after compacting into a large sack. Then, the sacks were kept in the greenhouse for about 2 months until the plants were completely dried. After the sacks were weighed again, the dry plants in each sack were passed through the sieve of a seed cleaning machine used for the canola harvest and the seeds were cleaned. Finally, the amount of seeds obtained from 20 plants in a sack for each genotype was weighed in

bulk. Then, six agronomic traits were examined using the seeds harvested from 66 *V. hispanica* genotypes. These are as follows:

1. Total yield (biomass yield) per plant (single plant weight) (ty/p) (g plant^{-1}): It is the average weight (total of stem and seed weights) of a single plant calculated after drying all the aboveground parts of 20 plants except the roots.

2. Seed yield per plant (sy/p) (g plant^{-1}): It is the yield per plant calculated as mean seeds obtained from 20 plants.

3. Harvest index (hi): It was calculated as the ratio of seed yield per plant to total biomass yield of a plant (stem + seed).

4. Seed number per capsule (sn/c) (n): The average of the number of seeds in 12 capsules, three capsules randomly selected from four randomly selected plants from each genotype.

5. TSW (tsw = 1000 seed weight) (g): The weight of 50 randomly selected seeds in three replicates was recorded with a precision balance and proportionally transformed into TSW according to Lara-Fioreze et al. (2013) (Cam et al., 2018).

6. Seed diameter (sd) (mm): The width and length (shortest and longest diameter) of 20 randomly selected seeds were measured with a digital caliper in three replications and averaged according to Razavi and Milani (2006) (Cam et al., 2018).

2.4. Statistical analysis

For morphological and agronomical traits, statistical analyses were performed using the R suite (R Core Team, 2020). The simple statistical parameters consisting of minimum, maximum, and mean values, also standard deviation and coefficient of variation (CV) were calculated using the inbuilt functions of the program. The relation between traits was determined by bivariate correlation analysis using the Pearson correlation coefficient method ($p < 0.01$) (R package: *Hmisc* and *corrplot*). Principal component analysis (PCA) was performed using ape package of the suite. Cluster analysis was performed by the k-means clustering method, using the embedded function in the program. The distance between each cluster was determined by calculating the Euclidean distance between cluster centers.

3. Results

In the study, 66 wild Turkish *V. hispanica* genotypes were characterized in terms of 27 traits, 21 morphological and 6 agronomic. Genotypes did not show variation in the last 4 of 21 morphological traits mentioned in the method section, and therefore these traits were not subjected to statistical analysis. Degree of petal vein was on the 3rd scale (moderate) of the 0–4 visual rating scale in all genotypes except for only one genotype. The remaining genotype was the 8th genotype, which was probably apomictic since it formed flower buds but could produce seeds without blooming, and this observation could not be made on its petals as the buds were always closed. The most observable feature in the flower buds of the 8th genotype is that only the tips of the light-colored petals, whose color cannot be fully distinguished from the closed flower buds, are visible. On the basis of absent/present assessment, leaves had a floury appearance (leaf pruinose) in all 66 genotypes, but none had leaf curl (leaf involute). In addition, according to the narrow/wide evaluation, the shape of the calyx teeth was wide in all genotypes, except for the 8th genotype, where this observation could not be made clearly due to the unopened flower buds.

As for a total of 23 traits, including morphological (17) and agronomic (6) traits for which variation exists, the analysis for basic statistical parameters presented the high variation among the genotypes. The CV of 10 out of 23 traits was found to be above 20%. Morphological traits (7 of 17, 41%) and agronomic traits (3 of 6, 50%) showed similar levels of variance (Table 1).

As seen from Table 1, the highest three CV were found among morphological traits. The length of the smallest leaf had the highest CV of 84.8%, while the variation was low in the length of the largest leaf at stem base (16% CV). It was followed by the number of main branches on stem (57.5%) and degree of petals imbricate (46.6%). The length of the smallest leaf was measured between 0.27 cm and 2.50 cm. The number of dichasium was alternating from 0 to 5, while the degree of petals imbricate was from 0 to 3.7.

Among the agronomic traits, the highest three CVs were determined for the number of seeds per capsule, seed yield per plant and total yield per plant, which were 27.9%, 24.3%, and 23.7%, respectively. The seed number per capsule varied between 3.3 and 15.3. The seed yield per plant had a minimum of 54.4 g and a maximum of 186 g, while the total yield per plant value ranged between 173.3 g and 616 g (Table 1).

All four measured morphological features related to flowering and petal showed a high degree of variation, more than 20% CV. The flower number of the top five dichasium ranged from 7 to 57, the catalog number of petal color from 1 to 2.67, degree of teeth of petal margin from 1 to 3.33, and the number of days the plants needed before flowering from 23 days to 87 days (Table 1).

Creating a selection formula using the relationships between traits is a method frequently applied in plant breeding. As an example, by studying such relations amongst the traits, breeders can make an early selection for yield (Aman et al., 2020). These relationships also useful to improve existing breeding programs (e.g., improvement for one trait influences another; indirect selection) and to identify hard-to-detect traits. Therefore, the correlation analysis is a powerful tool to unearth bivariate relationships.

Table 1. Descriptive statistics for the morphological and agronomic characters of 66 wild Turkish *Vaccaria hispanica* genotypes.

No.	Full name and unit of character	Abbr.	CV (%)	Min	Max	Mean	SD	Genotypes with Min and max values	
Morphological characters								**With min value**	**With max value**
1	Plant height (cm)	ph	11.33	77.00	138.00	107.79	12.21	57	17
2	Stem base diameter (mm)	sbd	16.03	13.00	28.00	19.46	3.12	31	34
3	Length of the largest leaf at stem base (cm)	lll	16.00	8.73	19.60	13.13	2.10	28	2
4	Length of the smallest leaf at the top (cm)	lsl	84.76	0.27	2.50	0.54	0.46	57	2
5	# of days until flowering (day)	nduf	20.89	23.00	87.00	64.15	13.40	61	17
6	# of main branches on stem (n)	nmbos	57.52	0.00	5.00	2.64	1.52	19, 21, 24-25, 60-62, 64, 66	40, 43, 53-54
7	Flower (/flower bud) # of the top five dichasium (n)	fntfd	42.27	7.00	57.00	23.75	10.04	21	1
8	Flower (/flower bud) # of the last alar (n)	fnla	14.88	2.33	3.67	2.63	0.39	5, 12-13, 15, 18-19, 21, 23, 25, 27, 29-32, 35, 37, 40, 44-45, 47-49, 51, 53, 57-59, 61, 63-64, 66	8, 10-11, 17, 34, 55
9	Width of calyx (mm)	woc	17.81	3.50	8.50	5.64	1.00	22	56
10	Length of calyx (mm)	loc	8.82	11.50	18.00	13.55	1.20	32	56
11	Length of pedicel (cm)	lop	14.34	2.00	4.73	3.50	0.50	58	48
12	Petal length (with claw) (cm)	pl	6.03	1.90	2.70	2.32	0.14	13	31, 33
13	Width of petal lamina (mm)	wpl	14.67	3.00	8.00	5.65	0.83	13	57, 65
14	Catalog number of petal color	cnpc	29.18	1.00	2.67	1.30	0.38	1-7, 9, 11, 13, 15-16, 19-21, 23-24, 26-28, 30, 38-39, 41-43, 46, 49, 51-52, 54-55, 58-59	60
15	Degree of petals imbricate (0–4 visual rating scale)	dopi	46.55	0.00	3.67	2.02	0.94	46	16, 31, 53
16	Degree of petal emarginate (0–4 visual rating scale)	dope	16.35	1.00	3.67	2.84	0.46	32, 49	47
17	Degree of teeth of petal margin (0–4 visual rating scale)	dotpm	21.61	1.00	3.33	2.60	0.56	13, 28	2-3
Agronomic characters								**With min value**	**With max value**
1	Total yield per plant (g plant^{-1})	ty/p	23.74	173.25	616.00	401.80	95.38	63	5
2	Seed yield per plant (g plant^{-1})	sy/p	24.34	54.35	186.02	117.42	28.58	63	5
3	Harvest index	hi	12.86	0.19	0.45	0.29	0.04	6	46
4	Seed number per capsule (n)	sn/c	27.88	3.33	15.33	8.88	2.48	14	51
5	1000 seed weight (g)	tsw	13.81	4.55	7.92	6.09	0.84	27	9
6	Seed diameter (mm)	sd	3.19	1.92	2.24	2.07	0.07	54	1

Forty-eight of the bivariate relationships were found to be significant ($p < 0.05$) for morphological traits (Figure 2A and Supplemental Table 1). The strongest positive correlation was between length of the smallest leaf and flower number of the top five dichasium. Also, stem base diameter showed a positive correlation with number of

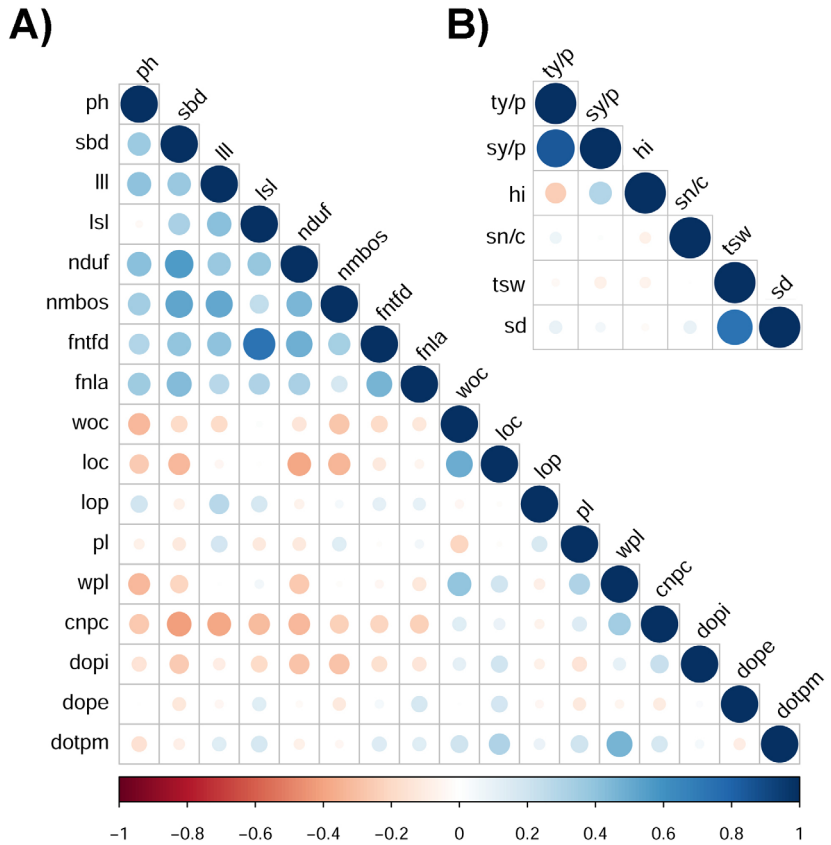

Figure 2. Bivariate correlation of the traits among the 66 wild Turkish *Vaccaria hispanica* genotypes. A: Heat map for morphological traits. B: Heat map for agronomic traits. Blue indicates a positive relationship, while red indicates a negative relationship. Size of the dot represents the degree of the correlation. For the correlation and statistical significance, (*p*) values for each bivariate relationship please refer to supplementary information (Supplemental Tables 1 and 2).

days until flowering and number of main branches on stem. However, it showed a strong negative correlation with catalog number of petal color. The latter also showed a strong negative correlation with length of the largest leaf at stem base.

For agronomic traits, four correlations were found to be significant ($p < 0.05$). Total yield per plant was positively correlated with seed yield per plant, as can be expected. Both traits were also positively correlated with the harvest index, TSW and seed diameter (Figure 2B and Supplemental Table 2).

A multivariate analysis technique, the principal component analysis (PCA), was applied to the datasets (i.e. separately for morphological and agronomic) to reveal the patterns and to represent the data in the best way with the minimum loss. The eigenvalues and the percentage of variance values are presented in Table 2. For the morphological traits dataset, the first five PCA had eigenvalues higher than 1, while for the agronomic traits dataset, only the first three PCA had eigenvalues higher than 1. Thus, according to Kaiser's criterion (Kaiser, 1958), the morphological traits can be condensed into five factors, which account for 62.5% of the total variance. On the other hand, 79.7% of the variance present among the agronomic traits can be explained by three factors.

In Figure 3, contributions of the morphological and agronomic traits to the first two principal components are presented. Several traits contributed both positively and negatively to the first two principal components of the morphological traits PCA. Among these traits, length of the smallest leaf, length of the largest leaf at stem base, and flower number of the top five dichasium contributed positively to the first two principal components, while width of calyx, length of calyx, and width of petal lamina contributed negatively. Among the agronomic traits, total yield per plant and seed yield per plant contributed positively to the first component, as expected (Figure 2B). Similarly, TSW and seed diameter contributed positively to the second principal component.

The genotypes were then further analyzed and grouped using k-means algorithm on the biplot of the first two principal components. The optimum number of clusters

Table 2. PCA analysis of *Vaccaria hispanica* genotypes. Analysis was done separately for morphological and agronomic datasets. PoV: percentage of variation, CPoV: cumulative percentage of variation.

For morphological dataset				**For agronomic dataset**			
PCA component	**Eigenvalue**	**PoV**	**CPoV**	**PCA component**	**Eigenvalue**	**PoV**	**CPoV**
PCA 1	4.398	25.873	25.873	PCA 1	1.863	31.062	31.062
PCA 2	2.212	13.010	38.883	PCA 2	1.750	29.173	60.235
PCA 3	1.676	9.861	48.744	PCA 3	1.168	19.474	79.710
PCA 4	1.309	7.701	56.444	-	-	-	-
PCA 5	1.038	6.105	62.550	-	-	-	-

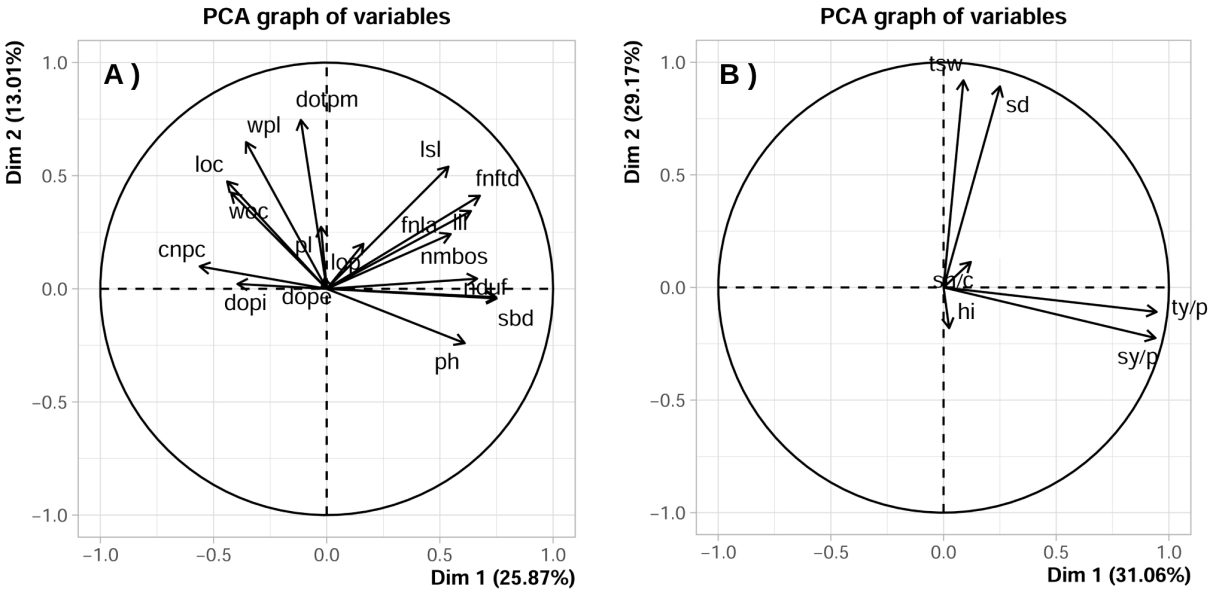

Figure 3. Contributions of morphological (A) and agronomic (B) traits for the first two principal component of each PCA. Each trait is represented by a vector and its magnitude is the measure of its effect (positive or negative).

was found using the elbow method on total within cluster sum of squares of plot of the clusters. Three optimum cluster numbers were found for morphological traits and two for agricultural traits (Figure 4).

Results of PCA and k-means were used to plot different populations among the genotypes (Figures 5 and 6). Morphological variations existed among genotypes grown under the similar conditions. The genotypes in proximity were similar in terms of traits affecting the first two principal components. For morphological traits, three clusters were formed each containing 11, 16, and 38 genotypes, respectively (Figure 5). First and second principal components explained 25.9% and 13% of the total variations, respectively. The cluster 1 (Figure 5, cluster in red) was characterized by several morphological traits (stem base diameter, number of days until flowering, flower number of the top five dichasium, length of the largest leaf at stem base, plant height) highlighted in Figure 2A. The cluster 3 in green in Figure 5 was associated with color, calyx, and petal characteristics. Because the traits were expressed when grown under similar conditions, selection can be made based on morphological characteristics of genotypes.

For agronomical traits, two clusters were found to be the optimum number of clusters. The 31 genotypes with high TSW and seed diameter were placed into cluster 2 (Figure 6, cluster in green), while the 35 genotypes were placed into cluster 1 (in red). The cluster 1 was characterized by low single plant weight and seed yield. Apparently, TSW, seed diameter, single plant weight and seed yield carried high enough variation to separate the genotypes into distinct groups. Therefore, for breeding purpose for a given agronomic trait, either selection can be made directly for a genotype or different genotypes can be crossed to improve the trait of interest.

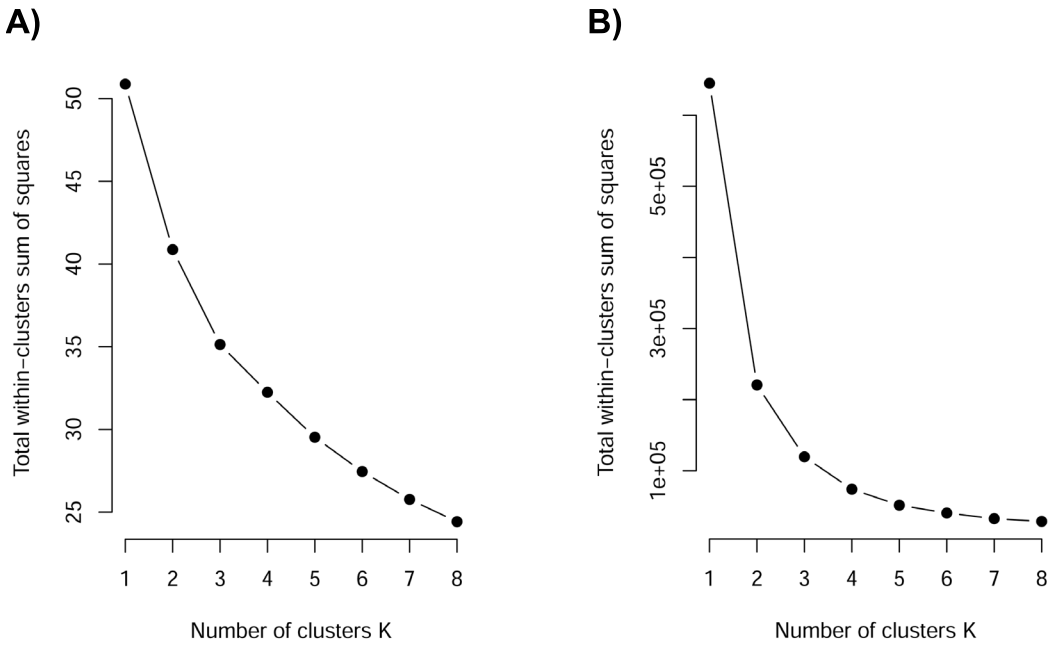

Figure 4. Total within cluster sum of squares of clusters for morphological (A) and agronomic traits (B) determined in *Vaccaria hispanica* genotypes. Cluster size were sampled from 1 to 8.

In addition to all these analyzes, preselections were made among the plants that have the potential to be used in future breeding studies for both industrial and ornamental purposes. Besides the agronomically important genotypes, 159 individuals with ornamental potential were selected based on their plant height, plant growth form and particularly flower structure criteria consisting of size, shape, arrangement and color of petals, and the seeds of these individuals were preserved.

Apart from the abovementioned individuals, during the greenhouse observations, especially in the 57/1st phenotype selected from the 57th genotype, it was observed that the flower and seed size were larger than the general view of *V. hispanica* flowers and seeds. Additionally, a difference was detected in the seed surface and pollen structure in the electron microscope and it was determined that the vein structure of the cotyledon leaves was quite different from other plants in germination studies. Further, although genetic variations were found in plant productions made from the selfed seeds of other genotypes, phenotypic homogeneity was observed in each of the 1st and 2nd generation individuals of the 57/1st phenotype. Because the study presents various data sets, not all the details of individual selections are mentioned here, but views of some selected plants or variants with different flower characteristics are given in Figure 7, which shows *V. hispanica* plant cultivation and some of the morphological characteristics and variants determined in the study.

4. Discussion

Plant domestication is the process of adapting wild plants to artificial conditions, and thereby transforming wild forms into cultivated or crop plants through evolution and selection. The morphological differences, related to the survival and adaptation of the plants, play a key role in selection by early humans at the beginning of domestication (Fuller, 2007; Duddu et al., 2015a). According to Vavilov (1926), a significant number of Old World crops have initially entered the crop community through the domestication of their weedy forms. Recently, *V. hispanica* as a weed species created an interest for domestication especially in Canada because of its valuable and profitable seed composition and attractive agronomic characteristics (Duddu et al., 2015a). But, the available agronomic information regarding the cultivation of *V. hispanica* as a crop is very limited and the domestication status of *V. hispanica* is still unknown exactly. However, adaptation of the natural germplasms to cultivation is the main initial criterion (Duddu et al., 2015a). In the present study, plant growth for preadaptation, characterization, and seed production studies of 66 wild *V. hispanica* genotypes (Figures 7A–7C) collected from different regions of Turkey were carried out under Mediterranean climate and greenhouse culture conditions for the first time. Gathering as much information as possible about the morphological and agronomic characteristics of the genotypes is extremely important for future breeding studies of *V. hispanica* for industrial crop or ornamental

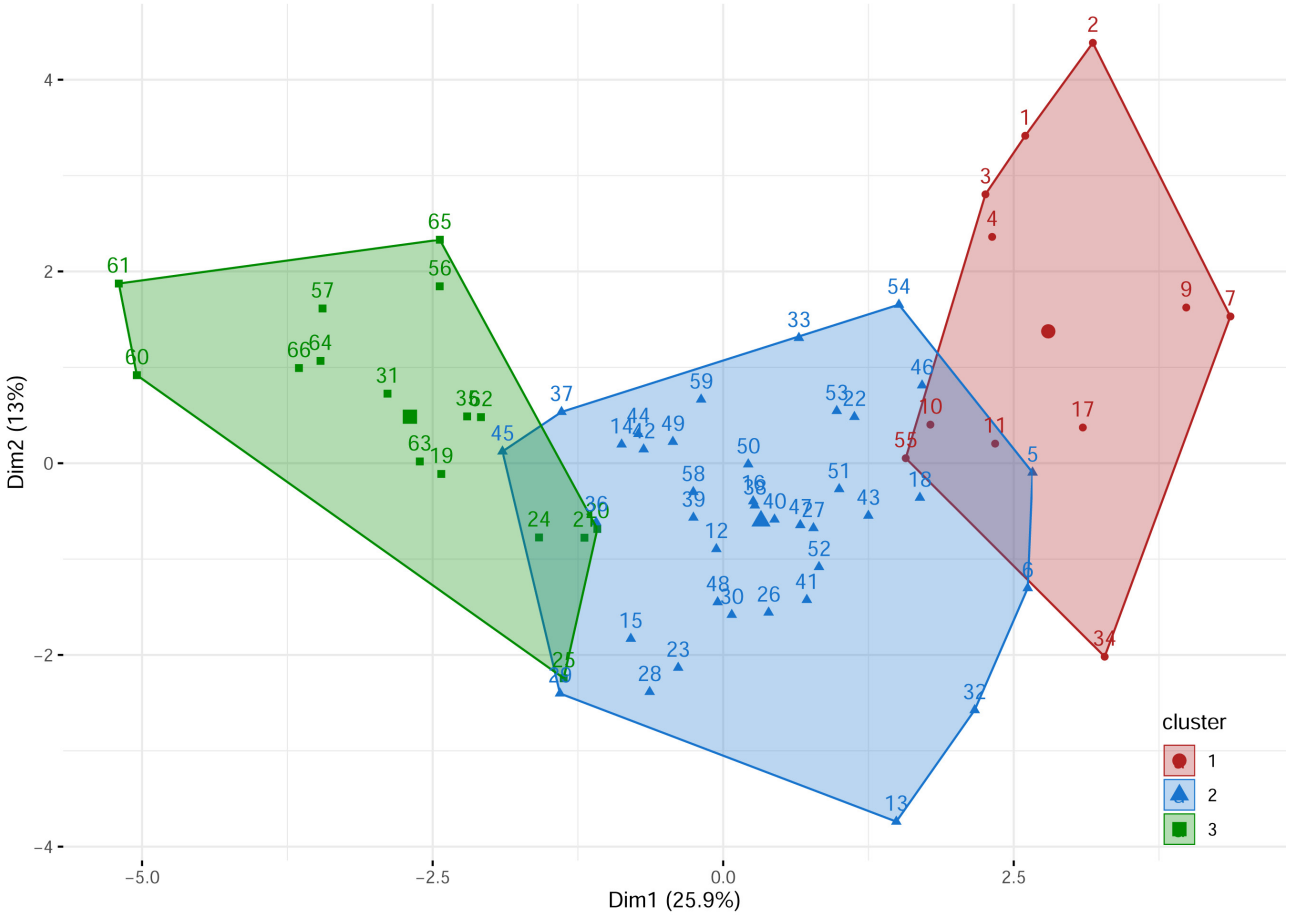

Figure 5. Cluster plots of morphological traits in 66 Turkish *Vaccaria hispanica* genotypes.

plant sector. In order to better recognize these genotypes, they were screened for 21 morphological and 6 agronomic traits.

A total of 1320 seedlings representing 66 genotypes were grown in a greenhouse, with a 0.75 m^2 $plant^{-1}$ density. The highest total aboveground biomass yield per plant and seed yield per plant were 616 g (equal to 821 g m^{-2}) and 186 g (equal to 248 g m^{-2}) (in the 5th genotype), respectively, while the maximum TSW was 7.9 g (in the 9th genotype). The trait of seed yield per plant was positively correlated with the harvest index, TSW and seed diameter. Meanwhile, the highest harvest index value was 0.45 (in the 46th genotype) and the maximum seed diameter was 2.24 mm (in the 1st genotype). As for the recent Canadian studies performed under field fertilized soil conditions, in a comprehensive adaptation study, Duddu et al. (2015a) compared 15 *V. hispanica* populations (six cultivated and nine wild) from different parts of the world for 12 agronomic and morphological traits. One of the cultivated population (referred to as Turkey) originated from a single germplasm accession from Turkey with code PI 304488 from USDA-ARS. Cluster analysis based on the agronomic and morphological similarities of the 15 populations revealed three main population groups. The second cluster including the cultivars Pink Beauty, Turkey, PB-87, Scott and UMan-89 was characterized by taller plants (approximately 58 cm) with large seeds (approximately 7.7 g) and high seed yield (approximately 2400 kg ha^{-1}). The possible reason for the high agronomic potential in the populations of this cluster has been attributed to their preadaptation to domestication. In the study of Duddu et al. (2015a), the populations were planted in 2 × 6 m plots and the seeds were sown at a target plant density of 300 plants m^{-2}, with a row spacing of 20 cm. Among the 15 populations, Turkey was the best performing population in terms of seed yield (about 2500 kg ha^{-1}, equal to approximately 250 g m^{-2} from 171 plants m^{-2}) and almost TSW (approximately 7.8 g). In other Canadian study conducted earlier than Duddu et al. (2015a), but under similar field conditions, Willenborg and Dosdall (2011) used 12 of previous 15 *V. hispanica* genotypes, including Turkey, and reported that seed yield and seed weight varied significantly among genotypes. Seed yields of the 12 genotypes, which were grown to achieve a desired



Figure 6. Cluster plots of agronomic traits in 66 Turkish *Vaccaria hispanica* genotypes.

stand density of 200 plants m^{-2}, ranged between 1328 kg ha^{-1} and 6500 kg ha^{-1}, while TSW was between 3.7 g and 8.8 g. The seed yield and TSW of Turkish genotype was 5215 kg ha^{-1} (equal to 521 g m^{-2}) and 8.5 g, respectively. As seen, the compared agronomic values of Canadian studies and our study were obtained from quite different working conditions in terms of the differences of genotypes, growth season, field/greenhouse, seeding/seedling, plant density, fertilization and climate. Meanwhile, it is worth noting that different geographic origins with varying environmental conditions experienced by the mother plants as well as genotype x environment interaction may have an impact on these results, as reported for seed yield in winter canola (Erdogdu and Esendal, 2021) and disease resistance in wheat (Chattopadhyay et al., 2022). The genotypic effect due to geographical origin was also previously reported for also seed germination in *V. hispanica* by Duddu and Shirtliffe (2014). Anyway, at least, the growth of *V. hispanica* plants in soil conditions of a greenhouse appears to be the first in our study and its results might give new ideas and bring new insights for *V. hispanica* plant production in different parts of the world.

Among the morphological traits, one of the most important characteristics that determine the form of the plant is the number of main branches on the stem. This trait had the second highest CV (57%) among all traits including also agronomic ones. While four genotypes with the highest mean of branching (5) were determined, the number of genotypes that did not show branching (i.e. developed only on the main stem) was eight (Table 1, Figures 7D and 7E). Duddu et al. (2015a) reported the number of branches per plant as 8-13 in the 15 *V. hispanica* populations and 8 in Turkey population. The reason for the high difference between Duddu et al.'s (2015a) and our study might be that only the main branches were counted in our study. Although not effective alone, lots of branching can increase the seed yield of the plant. In this study, the genotypes with more branches, in direct proportion to the height of the genotypes, had higher seed yields per plant, as expected. Also, excessive branching is an advantage for compact plant selection in ornamental plant breeding.

Although *V. hispanica* is less nutrient-demanding than other crops (Watson et al., 2007), Balsevich (2008) notified that it responds strongly to nitrogen fertilizer. The plant

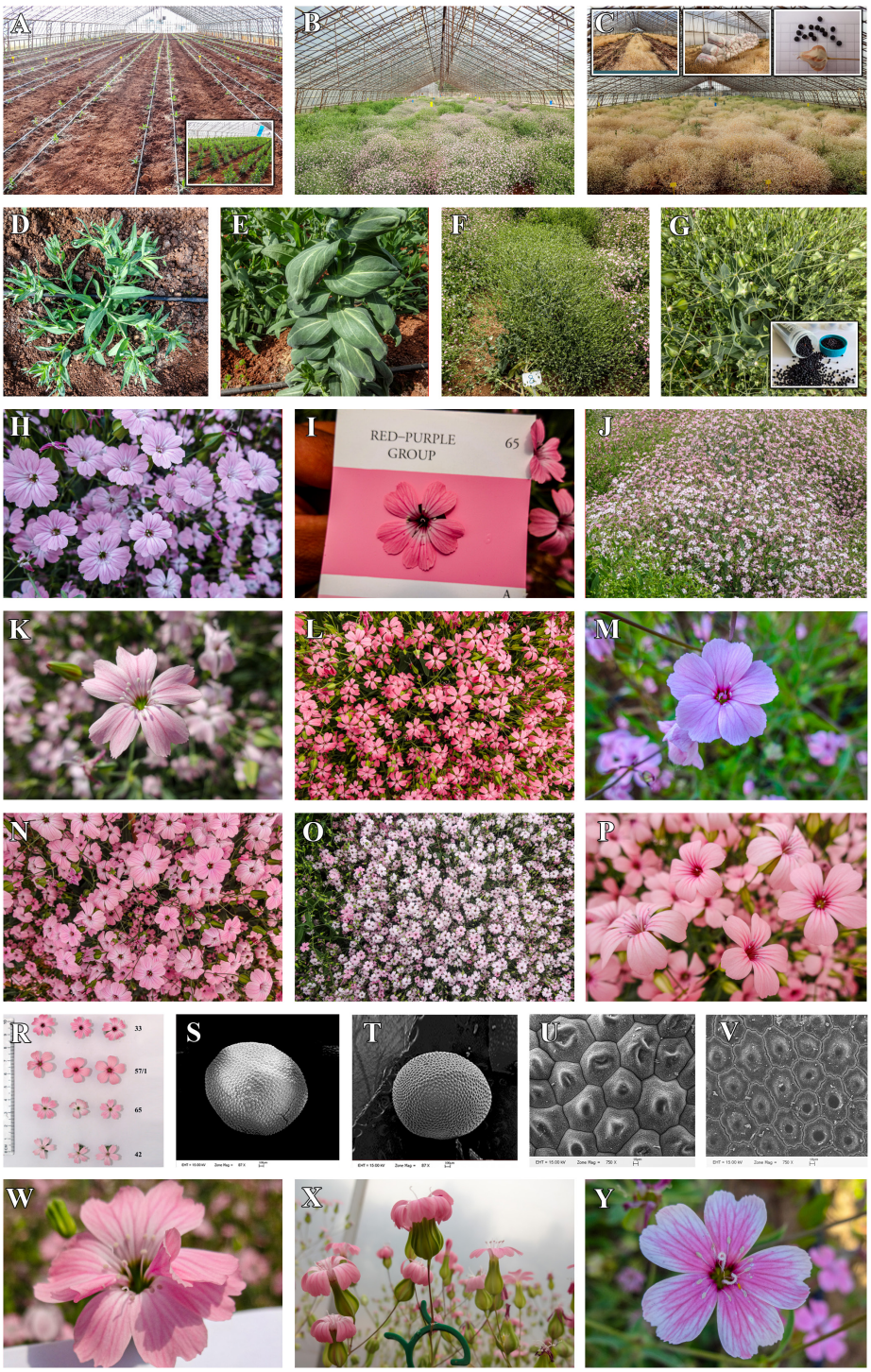

Figure 7. Plant cultivation of 66 Turkish *Vaccaria hispanica* genotypes in an unheated greenhouse in Antalya under Mediterranean climate and some of the morphological characteristics and variants determined in the study. **(A)** Newly planted *V. hispanica* seedlings; **(B)** genotypes in flowering and; **(C)** harvest period; **(D-E)** plants of the 40th and 21st genotypes with 4-5 **(D)** and 0 **(E)** main branches on stem, respectively; **(F-G)** probable apomictic 8th genotype in flowering **(F)** and harvest **(G)** period; **(H)** the earliest (35th) genotype with different petal tips and soft pink colored flowers; **(I)** determination of catalog number of flowers; **(J)** a plant of the 45th genotype with white flowers; **(K)** a plant of the 47th genotype with high degree of emarginated petals; **(L)** a plant of the 7th genotype with bigger flowers, dense inflorescence and compact plant growth; **(M)** a plant of the 53rd genotype with wider petal structure and high imbricated petals; **(N)** a plant of the 17th genotype with dense inflorescence, compact plant growth and light pink flower; **(O)** a plant of the 52nd genotype with bigger flowers, dense inflorescence and compact plant growth**; (P)** a phenotype (57/1) of the 57th genotype with different flower structure and bigger seeds; **(R)** comparison of flower size and morphology of 57/1st phenotype with some other genotypes; **(S)** comparison of seed size of 57/1st phenotype with; **(T)** that of 57th genotype under scanning electron microscope (SEM) (87×); **(U)** comparison of seed surface of 57/1st phenotype with; **(V)** that of 57th genotype under SEM (750×); **(W)** a variant with multipetal structure from the 31st genotype; **(X)** a variant with multi-petal and multiovary structure from the 27th genotype; **(Y)** a variant with chimeric petal color from the 46th genotype.

height of *V. hispanica* under natural conditions in Turkey was reported as 15–60 cm by Cullen (1967), while the heights of the plants varied between 77 cm and 138 cm in the present study performed under greenhouse fertilization conditions, which confirmed Balsevich (2008). In Duddu et al.'s (2015a) study, Turkey population also came to the fore with regard to morphological characters. It was the second tallest population (60 cm) after Mongolia (almost 65 cm). Additionaly, in the study of Willenborg and Dosdall (2011), the plant height of the highest genotype (Florist Rose) was 70.7 cm, while the Turkish genotype was 63.3 cm. As can be expected, the height growth of *V. hispanica* plants grown in greenhouse conditions is much higher than their counterparts in field conditions, despite being fertilized in both conditions.

As a morphological characteristic that supports and decorates compact plant growth, the number of flowers on the plant might be an important criterion for both seed yield and ornamental plant selection. A high variation (42.2% CV) ranging between 7 (the 21st genotype) and 57 (the 1st genotype) was determined in the flower number of the top five dichasium among the genotypes.

During the greenhouse observations, an unusual variation was encountered in one of the 66 genotypes. One of the genotypes with the highest flower/flower bud number on the last alar region is the 8th genotype (Table 1). However, it is a probable apomictic. Although flower buds formed in the 8th genotype, these buds did not open, but their ovaries were swollen and finally formed seed capsules (Figures 7F and 7G). When the capsules were examined, it was determined that this genotype formed healthy, plump seeds like other normal flowering genotypes. Its average total and seed yields per plant were 313.5 g plant^{-1} and 82.5 g plant^{-1}, with 0.26 harvest index. The average seed number per capsule, TSW, and seed diameter was 11.1, 6.3 g, and 2.1 mm, respectively. The values of these first two traits in the 8th genotype were higher than the average of 66 genotypes. According to Oelck (2008), *V. hispanica* is a self-pollinating species with a rate of more than 80%. However, as far as it is known, the existence of apomictic feature in this species has never been mentioned in the literature until now. More interestingly, during our previously completed isolated microspore culture study on these 66 genotypes, we were able to obtain microspore-derived embryos from this putative apomictic genotype with an average embryo yield of 17 embryos 100 buds^{-1} (Ari et al., 2022). Reproductive assurance is a main advantage of apomictic hermaphroditic plants and apomictic plants have the advantage over changing climatic conditions or fluctuating pollinator frequencies (Hörandl, 2008). Therefore, the 8th genotype, which is likely to have apomictic characteristic but needs to be confirmed by further research, can be an important genetic resource for future breeding studies.

One of the most important criteria determining the cultivation of a species in different regions and climates is the number of days that plants need before flowering. It indicates whether a genotype is early or late. In this study, it was noticed that some genotypes were early even at the seedling stage. For example, seedlings germinated from the seeds of the 35th and 36th genotypes formed flower buds while still in the nursery conditions and showed a tendency to bloom rapidly. In this case, only the seedlings of these two genotypes were pruned from the tops. Otherwise, they would complete their generation earlier. This situation probably affected their flowering after transplanting to the soil and their equal comparison with others regarding the number of days until flowering. The number of days until flowering ranged from 23 days to 87 days among the 66 *V. hispanica* genotypes in soil conditions. The earliest genotype was the 61st genotype (flowered on the 23rd day after planting), while the latest genotype was the 17th genotype (on the 87th day). Meanwhile, the 36th (on the 41st day) and 35th (on the 43rd day) (Figure 7H) genotypes were determined as the 6th and 8th earliest genotypes, respectively, although they were pruned previously. In our culture conditions in the unheated glass greenhouse in Antalya, under Mediterranean climate, and in the culture period that includes the combination of winter and spring months, the average number of days until flowering of 65 genotypes, excluding the 8th genotype, was 64.15.

A high variation was found in observations of the petal color of *V. hispanica* genotypes (Figure 7I), which usually have light pink flowers, with a CV of 29.18%. Petal color is one of the most important morphological characteristics used in breeding studies. Unlike previous reports, variations with light or dark baby pink color, medium or dark pink color, and even white color (Figure 7J), which can be an important advantage in breeding, were encountered among 66 *V. hispanica* genotypes. In addition, petal length, width of petal lamina, degree of petal emarginate (Figure 7K), degree of teeth of petal margin, and degree of petals imbricate (Figures 7L and 7M) are eligible characteristics to be used in ornamental plant breeding. The last treat is especially important for the development of multi-petal varieties. It was determined that this treat has the 3rd highest CV (46.55%) in the study. Additionally, the variation in the number of flowers of the plants facilitated the selection of plants with abundant inflorescence (Figures 7N and 7O) among the genotypes for the high ornamental plant potential. Since genotypes were still heterogeneous, it was usual to encounter different variations within a genotype. However, a phenotype called 57/1st (Figures 7P–7V) and some variants (Figures 7W–7Y), found to differ significantly in terms of petal size, shape and color, were preserved due to their potential to be evaluated in ornamental plant breeding. Petal length of

V. hispanica was specified by Cullen (1967) in the range of 14–16 mm. In this study, the petal length of 65 genotypes was determined between 19 mm and 27 mm including claw, with the average of 23.2 mm. On the other hand, Duddu et al. (2015a) reported the average flower diameter of 15 genotypes to be 1.3 cm. The two highest flower diameters were measured in two ornamental cultivars, Florist Rose (20 mm) and White Beauty (17 mm), while the flower diameter of the Turkey population was about 12.5 mm (Duddu et al., 2015a). Considering that the flower diameter observation is almost the sum of the two petal lengths, the flower size of Turkish *V. hispanica* genotypes in the present study appears to be larger. As two additional floral morphometrics, the calyx width and length of *V. hispanica* were reported by Jürgens (2006) to be 7.5 and 13.5 mm, respectively. In this study, calyx width (17.8% CV) varied between 3.5 mm and 8.5 mm, while calyx length (8.8% CV) was determined between 11.5 mm and 18 mm. Although *V. hispanica* has a spectacular beauty, it has not received the value it deserves as an ornamental plant. The fact that the number of commercial cultivars known in this species is negligible indicates that ornamental plant breeding studies are extremely limited. It is hoped that the results reported in this study will be useful for future studies.

As a result, in characterization studies performed on 66 Turkish *V. hispanica* genotypes, it was determined that there is a wide variation between genotypes in terms of morphological and agronomic characteristics. These genotypes formed the starting individuals of an important and qualified gene pool for future agronomic, phytochemical and ornamental plant breeding studies.

Acknowledgement
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References

Alex JF (1968). Competition between linseed flax and *Saponaria vaccaria*. Canadian Journal of Plant Science 48 (2): 139-147.

Aman J, Bantte K, Alamerew S, Sbhatu DB (2020). Correlation and path coefficient analysis of yield and yield components of quality protein maize (*Zea mays* L.) hybrids at Jimma, Western Ethiopia. International Journal of Agronomy https://doi.org/10.1155/2020/9651537.

Ari E, Bedir H, Deniz IG, Genc I, Seguí-Simarro JM (2022). Evaluation of the androgenic competence of 66 wild Turkish *Vaccaria hispanica* (Mill.) Rauschert genotypes through microspore culture. Plant Cell, Tissue and Organ Culture 148: 209-214. https://doi.org/10.1007/s11240-021-02169-1

Balsevich JJ (2008). Prairie carnation (*Saponaria vaccaria*)–A potential new industrial/medicinal crop for the Prairies. In: Fuelling the farm, SSCA annual conference, February 2008; Regina, Saskatchewan, Canada. pp. 46-50.

Biliaderis CG, Mazza G, Przybylski R (1993). Composition and physico-chemical properties of starch from cow cockle (*Saponaria vaccaria* L.) Seeds. Starch-Stärke 45 (4): 121-127.

Cakilcioglu U, Turkoglu I (2010). An ethnobotanical survey of medicinal plants in Sivrice (Elazığ-Turkey). Journal of Ethnopharmacology 132 (1): 165-175. https://doi.org/10.1016/j.jep.2010.08.017

Cam IB, Balci-Torun F, Topuz A, Ari E, Deniz IG et al. (2018). Physical and chemical properties of cow cockle seeds (*Vaccaria hispanica* (Mill.) Rauschert) genetic resources of Turkey. Industrial Crops and Products 126: 190-200. https://doi.org/10.1016/j.indcrop.2018.10.022

Chattopadhyay N, Mandal R, Roy A, Bhattacharya PM, Chowdhury AK (2022). Assessment of wheat genotypes based on genotype-by-environment interaction for durable resistance to spot blotch disease in hot spot. Cereal Research Communications 50 (1): 95-102.

Cullen J (1967). *Vaccaria*. In: Davis PH (editor). Flora of Turkey and the East Aegean Islands. Edinburg, UK: Edinburgh University Press, Vol: 2, pp. 177-179.

Davis PH (1965-1985). Flora of Turkey and the East Aegean Islands. Edinburg, UK: Edinburgh University Press. Vol. 1-9.

Duddu HS., Shirtliffe SJ (2014). Variation of seed dormancy and germination ecology of cowcockle (*Vaccaria hispanica*). Weed Science 62 (3): 483-492. https://doi.org/10.1614/WS-D-13-00125.1

Duddu HS, Shirtliffe SJ, Willenborg CJ (2015a). Evolutionary adaptation in cow cockle *Vaccaria hispanica*: a potential alternative crop. Genetic Resources and Crop Evolution 62 (6): 879-891. https://doi.org/10.1007/s10722-014-0198-y

Duddu HS, Johnson EN, Blackshaw RE, Shirtliffe SJ (2015b). Evaluation of seed persistence in cow cockle. Crop Science 55 (2): 899-909. https://doi.org/10.2135/cropsci2014.04.0334

Erdogdu Y, Esendal E (2021). Multi-environment trial analysis by parametric and non-parametric stability parameters for seed yield in winter rapeseed (Brassica napus L.) genotypes. Turkish Journal of Field Crops 26 (1): 71-78.

Ferrie AMR, Bethune T, Kernan Z (2005). An overview of preliminary studies on the development of doubled haploid protocols for nutraceutical species. Acta Physiologiae Plantarum 27 (4): 735-741. https://doi.org/10.1007/s11738-005-0078-3

Fletcher J (1897). Weeds (Bulletin 28). Canada Department of Agriculture, Central Experimental Farm.

Fuller DQ (2007). Contrasting patterns in crop domestication and domestication rates: recent archaeobotanical insights from the Old World. Annals of Botany 100 (5): 903-924. https://doi.org/10.1093/aob/mcm048

Goering KJ, Eslick RF, Watson CA, Keng J (1966). Utilization and agronomic studies of cow cockle (*Saponaria vaccaria*). Economic Botany 20 (4): 429-433.

Grand View Research (2021). Nutraceuticals Market Growth & Trends [online]. Website https://www.grandviewresearch.com/press-release/global-nutraceuticals-market

Harker KN, Blackshaw RE, Beckie HJ, O'Donovan JT (2009). Weed invasions in western Canada cropping systems. In: Management of Invasive Weeds (Series: Invading nature – Springer Series in Invasion Ecology), Dordrecht, Netherland: Springer, Vol: 5, pp. 151-166.

Holm L, Doll J, Holm E, Pancho JV, Herberger JP (1997). World weeds: natural histories and distribution. John Wiley & Sons.

Hörandl E (2008). Evolutionary implications of self-compatibility and reproductive fitness in the apomictic *Ranunculus auricomus* polyploid complex (Ranunculaceae). International Journal of Plant Sciences 169 (9): 1219-1228. https://doi.org/10.1086/591980

Ishtiaq M, Maqbool M, Ajaib M, Ahmed M, Hussain I et al. (2021). Ethnomedicinal and folklore inventory of wild plants used by rural communities of valley Samahni, District Bhimber Azad Jammu and Kashmir, Pakistan. Plos one 16 (1): e0243151. https://doi.org/10.1371/journal.pone.0243151

Jürgens A (2006). Comparative floral morphometrics in day-flowering, night-flowering and self-pollinated Caryophylloideae (*Agrostemma, Dianthus, Saponaria, Silene*, and *Vaccaria*). Plant Systematics and Evolution 257 (3): 233-250. https://doi.org/10.1007/s00606-005-0379-4

Kaiser HF (1958). The varimax criterion for analytic rotation in factor analysis. Psychometrika, 23 (3): 187-200.

Kiraly G, Pinke G, Mesterhazy A (2006). Changes in the distribution of certain arable weed species in West Hungary-different responses to the modification of agricultural management methods. Journal of Plant Diseases and Protection Special Issue 20: 557-566.

Koçyiğit M, Şevket A (2018). Seed Morphology, Leaf Anatomy and Karyotype Analysis of the medicinal and ornamental plant; *Vaccaria hispanica* (Miller) Rauschert. Yuzuncu Yıl University Journal of Agricultural Sciences 28 (1): 10-18. https://doi.org/10.29133/yyutbd.306727

Lara-Fioreze ACC, Tomaz CA, Fioreze SL, Pilon C, Zanotto MD (2013). Genetic diversity among progenies of Crambe abyssinica Hochst for seed traits. Industrial Crops and Products 50: 771-775. https://doi.org/10.1016/j.indcrop.2013.07.039

Löve D (1942). Some contributions to the cytology of Silenoideae. Svensk Botanisk Tidskrift 36: 262-270.

Mazza G, Biliaderis CG, Przybylski R, Oomah BD (1992). Compositional and morphological characteristics of cow cockle (*Saponaria vaccaria*) seed, a potential alternative crop. Journal of Agricultural and Food Chemistry 40 (9): 1520-1523. https://doi.org/10.1021/jf00021a009

Memon RA, Bhatti GR, Khalid S, Mallah A, Ahmed S (2013). Illustrated weed flora of wheat crop of Khairpur District, Sindh. Pakistan Journal of Botany 45 (1): 39-47.

Oelck MM (2008). The new SAPONIN business - Pink(s) to the prairies. In: The Agricultural Biotechnology International Conference (ABIC), 24-27 August 2008 [Oral presentation]; Cork, Ireland (https://www.slideshare.net/MOelck/saponin-at-abic-2008-cork-ireland)

Razavi SM, Milani E (2006). Some physical properties of the watermelon seeds. African Journal of Agricultural Research 1 (3): 065-069.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria [online]. Website https://www.R-project.org/.

Sang S, Lao A, Chen Z, Uzawa J, Fujimoto Y (2003). Chemistry and bioactivity of the seeds of Vaccaria segetalis. In: Oriental Foods and Herbs, American Chemical Society Symposium Series 859: 279-291. https://doi.org/10.1021/bk-2003-0859.ch021

Thomas AG, Leeson JY (2007). Tracking long-term changes in the arable weed flora of Canada. In: Clements D, Darbyshire S (editors.), Invasive Plants: Inventories, Strategies and Action. Topics in Canadian Weed Science 5: 43-69.

Torzewski K, Kazienko A, Drapiewski A, Sienkiewicz T (2018). *Vaccaria hispanica* (Caryophyllaceae) rediscovered in Poland. Steciana 22 (4): 129–132. https://doi.org/10.12657/steciana.022.015

Vavilov NI (1926). Studies on the origin of cultivated plants. Institut de Botanique Applique´e et dAAme´lioration des Plantes, Leningrad, p.248.

Watson PR, Oelck M, Johnson E, Kharbanda P, Yang J et al. (2007). Prairie Carnation®: a new crop for western Canada. In: Soils and Crops Workshop, March 2007 [Poster presentation]; University of Saskatchewan, Canada (http://hdl.handle.net/10388/9389).

Willenborg C, Dosdall L (2011). First report of redbacked cutworm damage to cow cockle [*Vaccaria hispanica* (Mill.) Rauschert], a potential new crop for western Canada. Canadian Journal of Plant Science 91 (2): 425-428. https://doi.org/10.4141/CJPS10143

Willenborg CJ, Johnson EN (2013). Influence of seeding date and seeding rate on cow cockle, a new medicinal and industrial crop. Industrial Crops and Products 49: 554-560. https://doi.org/10.1016/j.indcrop.2013.05.028

Zhou G, Tang L, Wang T, Zhou X, Kou Z et al. (2016). Phytochemistry and pharmacological activities of *Vaccaria hispanica* (Miller) Rauschert: a review. Phytochemistry Reviews 15 (5): 813-827. https://doi.org/10.1007/s11101-015-9425-1

Supplemental Table 1. Pearson's correlation coefficients for different morphological traits among wild *Vaccaria hispanica* genotypes.

	ph	**sbd**	**lll**	**lsl**	**nduf**	**nmbos**	**fnftd**	**fnla**	**woc**	**loc**	**lop**	**pl**	**wpl**	**cnpc**	**dopi**	**dope**	**dotpm**
ph	1																
sbd	0.369**	1															
lll	0.400***	0.379**	1														
lsl	–0.030	0.322**	0.412***	1													
nduf	0.411***	0.567***	0.373**	0.380**	1												
nmbos	0.349**	0.528***	0.510***	0.244	0.455***	1											
fnftd	0.290*	0.393**	0.401***	0.731***	0.488***	0.337**	1										
fnla	0.353**	0.433***	0.274*	0.305*	0.325**	0.178	0.463***	1									
woc	–0.329**	–0.184	–0.182	0.020	–0.138	–0.279*	–0.188	–0.128	1								
loc	–0.254*	–0.324**	–0.047	–0.010	–0.382**	–0.337**	–0.116	–0.054	0.499***	1							
lop	0.196	–0.077	0.271*	0.177	–0.064	0.040	0.119	0.106	–0.049	–0.014	1						
pl	–0.073	–0.116	0.184	–0.114	–0.120	0.136	–0.013	0.051	–0.215	–0.015	0.164	1					
wpl	–0.327**	–0.219	0.004	0.052	–0.267*	–0.018	–0.047	–0.127	0.395**	0.194	–0.081	0.300*	1				
cnpc	–0.268*	–0.416***	–0.388**	–0.311*	–0.321**	–0.239	–0.217	–0.236	0.134	0.089	–0.061	0.146	0.348**	1			
dopi	–0.148	–0.254*	–0.098	–0.184	–0.282*	–0.284*	–0.165	–0.136	0.114	0.192	–0.071	–0.146	0.108	0.227	1		
dope	–0.003	–0.122	–0.047	0.130	–0.029	–0.113	0.049	0.175	0.003	0.184	–0.043	–0.111	–0.050	–0.102	0.024	1	
dotpm	–0.159	–0.083	0.136	0.180	–0.076	–0.041	0.148	0.13	0.208	0.309*	0.083	0.208	0.466***	0.177	0.049	–0.091	1

For explanation of the measured character symbols, please see Table 1.
*, **, ***: Correlation is significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Supplemental Table 2. Pearson's correlation coefficients for different agronomic traits among 6 wild *Vaccaria hispanica* genotypes.

	ty/p	**sy/p**	**hi**	**sn/c**	**tsw**	**sd**
ty.p	1					
sy.p	0.843***	1				
hi	–0.247*	0.294*	1			
sn.c	0.076	0.012	–0.073	1		
tsw	–0.033	–0.080	–0.067	–0.002	1	
sd	0.095	0.054	–0.029	0.094	0.736***	1

For explanation of the measured character symbols, please see Table 1.
*, **, ***: Correlation is significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.