ReseaRch

Domesticated pea (Pisum sativum L.) is a major food legume in temperate cropping systems across Europe, Asia, and North America and a traditional protein crop in the East African highlands (Zohary et al., 2012). Pea is also grown for fodder and as a source of green seeds for processing, as well as a vegetable crop (e.g., snap pea) (Davies, 1993; Warkentin et al., 2015). Thanks to its symbiosis with nitrogen-fixing bacteria and to its role as a break crop for pathogens and pests in cereal-dominated cropping systems, pea is important in temperate agroecological systems.

Genetic bottlenecks associated with domestication and breeding have eroded genetic diversity in many crops ( Tanksley and McCouch, 1997), thus making them vulnerable to stresses. Hence, a major objective of plant science is to identify useful alleles in crop wild relatives and reintroduce them into modern germplasm (Gur and Zamir, 2004). Moreover, comparative study of wild species and their domesticated counterparts offers an opportunity to understand the physiological and genomic consequences of domestication and thereby the genetic basis of crop adaptations.

Drought Response and Genetic Diversity in Pisum fulvum, a Wild Relative of Domesticated Pea

Erez Naim-Feil, Maya Toren, Grégoire Aubert, Mor Rubinstein, Ada Rosen, Ravit Eshed, Amir Sherman, Ron Ophir, Yehoshua Saranga, and Shahal Abbo*

ABSTRACT

Productivity of grain crops in semi-arid environments is often affected by drought, which is likely to increase due to predicted climate changes. Wild pea (Pisum fulvum Sibth. & Smith, Pf) accessions sampled across its ecological amplitude in Israel (350–850 mm annual precipitation) were used to assess the genetic diversity for drought responses. We hypothesized that native species evolving under Eastern Mediterranean climate carry adaptive traits to cope with drought stress. Accessions were classified according to single-nucleotide polymorphism variation pattern and habitat ecogeographic parameters. Significant differences were found between the accession groups, but grouping in both systems did not match. Subsequently, 52 Pf accessions and three domesticated pea (P. sativum L.) genotypes were evaluated during 2 yr under well-watered (~580 mm) and water-limited (~340 mm) treatments. Total dry matter, grain yield, harvest index, and average grain weight were higher in domesticated pea than wild Pf; however several Pf accessions exhibited lower drought susceptibility indices (i.e., greater stability across environments) than domesticated genotypes. Of special interest are a number of Pf genotypes in which low susceptibility to water stress was coupled with relatively high productivity. The sampling habitats of those low susceptibility–high productivity accessions are characterized by mild (400–530 mm) annual precipitation. Further sampling and evaluation of Pf from such locations may improve our understanding of pea drought adaptation and yield physiology.

DOMESTICATED pea (Pisum sativum L.) is a major food legume in temperate cropping systems across Europe, Asia, and North America and a traditional protein crop in the East African highlands (Zohary et al., 2012). Pea is also grown for fodder and as a source of green seeds for processing, as well as a vegetable crop (e.g., snap pea) (Davies, 1993; Warkentin et al., 2015). Thanks to its symbiosis with nitrogen-fixing bacteria and to its role as a break crop for pathogens and pests in cereal-dominated cropping systems, pea is important in temperate agroecological systems.

Genetic bottlenecks associated with domestication and breeding have eroded genetic diversity in many crops ( Tanksley and McCouch, 1997), thus making them vulnerable to stresses. Hence, a major objective of plant science is to identify useful alleles in crop wild relatives and reintroduce them into modern germplasm (Gur and Zamir, 2004). Moreover, comparative study of wild species and their domesticated counterparts offers an opportunity to understand the physiological and genomic consequences of domestication and thereby the genetic basis of crop adaptations.


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P. humile. East through the Trans-Caucasus towards Turkmenistan, Hungary, Bulgaria, and the Crimea, and from the Middle Eastern Mediterranean. Its distribution also extends to the Iberian Peninsula, affinities, as expressed in their natural distribution patterns (Ben-Ze’ev and Zohary, 1973) and phyloge-

According to Davis (1970), the genus Pisum consists of two species namely, wild P. fulvum Sibth. & Smith (Pf) and P. sativum. The latter species contains a complex of subspecific forms, including wild (subsp. elatus that includes var. pumilio, var. elatus, and var. brevipeduncula-
tum) and domesticated taxa (subsp. sativum that includes var. sativum, the garden pea, and var. arvense, the field pea). Ben-Ze’ev and Zohary (1973) studied the cytological affinities of intra- and interspecific hybrids within Pisum and concluded that it includes two biological species, Pf and P. sativum, the latter being an aggregate containing three intraspecific forms: P. sativum, P. elatus Steven ex M. Bieb., and P. humile Boiss. & Noe. Maxedt and Ambrose (2001) named three species: Pf, P. sativum with two sub-

Water deficiency is the main abiotic factor limiting crop productivity in many world regions (Boyer, 1982). Water availability for agriculture is becoming limited, and climate-change scenarios predict increased aridity in certain regions. Development of new cultivars with more efficient water use and greater drought resistance is considered a sustainable and economically viable approach (Condon et al., 2004). For pea production in dryland systems, the most common water shortage scenarios are either at the vegetative stage, mainly affecting establishment and survival, or during grain filling (terminal drought), which has a detrimental effect on grain yield and quality.

Since Pf is native to a wide range of environments, we hypothesize that it is likely to harbor a wide array of allelic variation in physiological adaptation loci. The ecological amplitude of Pf in Israel partly resembles that of wild emmer wheat, Triticum dicoccoides Schrank ex Schübl. (Peleg et al., 2005, 2008). Interestingly, in wild emmer, both the widest DNA allelic diversity and the greatest potential of drought adaptation were not found in populations native to the most arid habitats, but rather in regions of intermediate aridity, with wide seasonal rainfall fluctuations between years (Peleg et al., 2008). The similar distribution of wild emmer and Pf in Israel does not necessarily suggest a similar pattern of structured allelic diversity or adaptive phenotypes. The contrasting yield physiology and growth habit of wheat, a monocot with determinate growth habit, and pea, a dicot with indeterminate growth able to host N₂-fixing bacteria, may dictate different physiological-ecogeographic associations (Abbo et al., 2009). At present, however, no data are available on the phenotypic amplitude and water-stress-adaptive traits repertoire in wild cool-season grain legumes in general, and Pisum in particular. Moreover, we have no clue of any associations between geographic variables and either DNA allelic diversity or stress physiology attributes in wild pea, and this severely restricts our ability to offer effective conservation policies for natural ecosystems or to identify useful stocks for pea improvement. Accordingly, this study aimed at the following: (i) DNA marker (single-nucleotide polymorphism, SNP) characterization of a Pf germplasm collection spanning its ecological amplitude in Israel, (ii) phenotypic evaluation of selected Pf accessions under well-watered (WW) and water-limited (WL) regimes, and (iii) investigation of the associations between drought responses of Pf accessions, their ecogeographic origin, and their genetic profile.
**MATERIALS AND METHODS**

**Plant Materials**
A set of 135 wild Pf accessions spanning the documented ecological amplitude of the species in Israel and its surrounding region was assembled (Fig. 1). Seed increase of all accessions took place in a common garden in Rehovot, Israel, during winter 2012 to eliminate maternal effects.

**Ecogeographic Classification of Germplasm Sampling Sites**
Geography database included GPS reading of sampling sites (latitude, longitude, altitude above sea level) and distance to a major water body (i.e., the Mediterranean, Sea of Galilee). A set of meteorological parameters that reflect the seasonal growing and postgrowing conditions at each site, including the parameters of minimum and average temperature during January, maximum and average temperature during August, and average yearly precipitation, was retrieved from nearby meteorological stations. A combined ecogeographic database was then used to characterize the sampled habitats (Ben-David et al., 2010). Complete ecogeographic data were available for 82 (out of 135) sampling sites. According to these data, sampling sites were grouped into six clusters color coded in Fig. 1 (below) using the SPSS software package (IBM, 2012) for Ward’s hierarchical clustering method (Fig. 1).

Fifty-two Pf accessions were chosen to represent the ecogeographic amplitude of the entire collection for phenotypic evaluation. Forty-one accessions were selected according to...
their ecogeographic clustering, and 11 accessions (for which no adequate meteorological data were available and therefore were not included in the SPSS clustering) were selected based on geographical location. In addition, three domesticated pea genotypes (‘Alaska’, ‘Dunn’, and an Eritrean landrace) were used as check cultivars. Alaska is an extra-early-flowering field pea cultivar obtained from Dr. N.F. Weeden, Montana State University. Dunn is a field pea cultivar of Australian origin, presently grown in Israel for hay only. Eritrea is a single-seed descent line isolated from a seed sample obtained in 2003 from a field along the road from Mendefera to Barantu (14°54′ N, 38°41′ E, 1820 m asl).

**Genetic Grouping (SNP-Based Classification)**

Eighty-eight Pf accessions were subjected to SNP genotyping. A set of 96 SNP loci, polymorphic among *P. fulvum* accessions, were selected (Tayeh et al., 2015a, 2015b; Aluome et al., 2016). To assess the relationship between Pf accessions, we estimated the genetic distance as $D = [1 – the proportion of shared alleles (PSA)]$. The PSA was calculated as:

$$PSA = \sum_{i=1}^{L} \frac{PS_i}{2L}$$

where PS is the proportion of shared alleles for each locus, and $L$ is the total number of loci (Bowcock et al., 1994).

Hierarchical clustering was performed on a pairwise $D$ distance matrix, and the Ward agglomerative method (Odong et al., 2011) was applied. The confidence limits of the tree topology were calculated by applying bootstrap method (1000 resamplings of loci). To count the number of bipartitions fit to the tree, we used the “ape” R package (Paradis et al., 2004; Popescu et al., 2012) and presented the bootstrap values as percentages.

The subpopulation structure underlying the germplasm collection was estimated by running a simulation of STRUCTURE software 2.3.3 (Pritchard et al., 2000) with 5000 burn-in periods and 50,000 repetitions. The number of populations, $K$, was inferred by running the simulation of $K = 1$ to $K = 10$ (20 runs for each $K$) and using the likelihood method of $\Delta K$ (Evanno et al., 2005).

The fixation index $F_{ST}$ (Wright, 1950) was calculated as:

$$F_{ST} = (H_T – H_s)/H_T$$

where $F_{STR}$ is the genetic differentiation of a subpopulation due to genetic drift, $H_s$ is the weighted average of all subpopulations’ expected heterozygosity, and $H_T$ is the expected heterozygosity in the entire population (germplasm collection).

**Experimental Design and Growth Conditions**

Two experiments were performed during the 2014 and 2015 seasons at the Hebrew University experimental farm in Rehovot (31°54′ N, 34°47′ E; 54 m asl), a site with brown-red sandy soil (Rhodoxeralf) consisting of 76% sand, 16% clay, and 8% silt.

Seeds coats were partly removed to abrogate dormancy. Seeds were germinated in a peat:tuff:vermiculite (1:1:1) mixture. About 2 wk after germination (i.e., after second leaf expansion), the apical bud was removed to promote basal branching. This procedure helps in maintaining the plants more similar to their wild phenotype while growing inside a screen-house (see below). Three weeks after germination, seedlings were transplanted into two insect-protected (50 mesh) screen-houses, one for each treatment (described below). Under both treatments, each of the 52 Pf genotypes was planted in three different plots (repeats), and each of the three check varieties was planted in six different plots (repeats), using a randomized block design. Each plot (experimental unit) contained five plants (15 cm between plants). Plots were spaced 50 and 150 cm within and between rows, respectively. We did not aim to evaluate the wild accessions in a commercial field-like stand because, in nature, Pf always grow in patchy and rather sparse stands.

Drip irrigation was applied during winter and spring months (December–April) to mimic Eastern Mediterranean rainfall patterns. Two irrigation regimes, WW (control) and WL, were applied, and water quantities were offset weekly to complete portions with natural precipitation. The WW treatment was irrigated twice weekly, along the season with a total amount (rainfall + irrigation) equivalent to 554 mm during the 2014 season and 605 mm during the 2015 season. The WL irrigation treatment was irrigated twice weekly at the beginning of the growing season (vegetative stage) and once weekly when differential irrigation was applied (reproduction stages). The total irrigation amount of the WL treatment was equivalent to 299 mm during the 2014 season and 387 mm during the 2015 season. To estimate the seasonal water consumption by the plants, soil samples for gravimetric water content were taken at planting and during the harvest from 0 to 90 cm depth ($3 \times 30$ cm layers $\times 10$ replicates), according to the amount available to the plants during the growth period (residual from earlier season + rainfall + irrigation – end-of-season residual water content) and ignoring direct evaporation from bare soil. Ammonium nitrate (18%) fertigation was applied only during the 2014 season (as needed) at a rate of 38 kg pure nitrogen ha$^{-1}$.

Temperature in both screen-houses varied between minima of 5 to 26°C and 4 to 25°C and maxima of 14 to 43°C and 14 to 42°C in 2014 and 2015, respectively. During 2015, a polyethylene rain cover was spread over the WL treatment screen-house. As a result, the minimum weekly average temperature measurements recorded during the 2015 season were higher (by $1$–$2°C$) in comparison with the noncovered screen-house. We did not observe any growth abnormalities or deformations that could have been attributed to the temperatures. Ten weeks after planting, the rain cover was removed due to low rainfall probability. The 2014 growth season was relatively dry, and therefore no polyethylene cover was necessary. Hence, the temperatures were similar in both screen-houses used for the two treatments.

**Phenology and Physiology Characterization**

All variables were recorded individually for each experimental unit (a five-plant plot). Day of flowering onset was determined when open flowers were observed on two different plants in the plot and was used to calculate the number of days from sowing to flowering (DTF).

Samples for physiological measurements were collected in late March (1 mo after deferential irrigation was applied). For osmotic potential (OP) measurement, the youngest fully expanded leaves including petioles were sampled during morning hours, placed in plastic tubes with petioles dipped in distilled water, and kept 6 h in a dark cold box for full rehydration. Leaves were removed from the water, blotted with a paper towel, placed in a plastic test tube, frozen in liquid nitrogen, and stored at $-80°C$ until measurement. Tubes were then defrosted, pierced at their bottom, placed into
a larger clear tube, and centrifuged for 15 min at 10,000 rpm in a refrigerated centrifuge to extract leaf sap. The OP of the leaf sap was assessed using a vapor-pressure osmometer (model 5520; Wescor). Osmotic adjustment was calculated as the difference between OP in the individual WL plots and the genotypic mean in the WW treatment (Blum, 1989).

For total chlorophyll content (TC) and specific leaf weight (SLW), 10 4.5-mm-diam. discs were collected from young fully expanded leaves of each experimental unit using a hole puncher. Five discs were placed in a tube and stored at −80°C until measurement. Once defrosted, 1 mL acetone (80%) and two stainless steel beads (5 mm) were inserted into the tube for tissue extraction (Qiagen, TissueLyser 2). Tubes were placed for 1 h in a dark environment and centrifuged twice (90 s, 13,200 rpm) to obtain clear sap, which was then subjected to spectrophotometer (Genesios 10S Vis) absorbance measurements. Total chlorophyll content was calculated according to Arnon (1949) as TC = (20.2 × A645 + 8.02 × A663)/LA, where A645 and A663 are the absorbance at 645 and 663 nm, respectively, and LA is the leaf discs’ surface area. The remaining discs were oven dried (65°C for 72 h) and weighed (LW) to calculate SLW as LW/LA.

During the reproductive stage, physiologically mature pods were collected every 3 d to minimize the risk of pod shattering. At maturity, the aboveground material from each experimental unit was harvested, stored in sacks, and air dried for 10 wk. When dried, grains and vegetative matter were manually separated. Total grain yield (TGY) was weighed, whereas vegetative dry matter (VDM) was oven dried (65°C for 72 h) before weighing. Harvest index (HI) was calculated as a ratio between TGY and total dry matter (TDM). Grains from each experimental unit were counted to calculate average grain weight (AGW).

Drought susceptibility index (S) was calculated after Fischer and Maure (1978) as:

\[
S = [1 - \frac{Y_{(WL)}/Y_{(WW)}}{1 - X_{(WL)}/X_{(WW)}}]
\]

where \(Y_{(WL)}\) presents a single repeat performance of a certain genotype under WL treatment and \(Y_{(WW)}\) is the mean performance of the same genotype under WW treatments; \(X_{(WW)}\), and \(X_{(WL)}\) represent the mean of all genotypes under the respective treatments.

Statistical Analysis
Statistical analyses were conducted using JMP 12.2 package (SAS Institute, 2015). Distribution normality was assessed using the Shapiro–Wilk test for each histogram. Analyses of variance were performed for each measured variable, with genotype and irrigation considered as fixed effects and irrigation × genotype interaction, block, and block × irrigation interaction considered as random effects. To assess the potential effect of ecogeographic clustering, ANOVA was performed with cluster and irrigation as fixed effects. In cases of nonsignificant irrigation effects, the values of both WW and WL experimental units were used for ANOVA.

RESULTS
Phenotypic Diversity under Well-Watered and Water-Limited Conditions
Frequency distributions of the measured variables of the 52 Pf accessions and 3 domesticated \((P. sativum)\) genotypes are depicted in Fig. 2. In most cases, the histograms show normal distribution (marked with an asterisk in top left corner of each histogram). Almost as a rule, values recorded under WL conditions were lower relative to the WW treatment. Averaged across all genotypes, TGY showed a reduction of 70% between the WW and WL treatments for the first season and a 45% decrease for the second season (198 to 61 g plot−1 and 311 to 175 g plot−1, respectively; Fig. 2). Total dry matter showed a 65% reduction between treatments for the first season (372 to 132 g plot−1) and a 35% decrease for the second season (597 to 387 g plot−1, Fig. 2). A minor effect of irrigation in both seasons across all genotypes was observed for average HI (15% decrease in the first season and 12% reduction in the second season, Fig. 2) and for average grain weight (13% decrease in the first season and 1% increase in the second season, Fig. 2).

Under the WW regime, the productivity variables VDM, TGY, and TDM, showed wider ranges compared with the values recorded under the WL treatment. However, HI, OP, SLW, TC, and AGW had a similar range under both water regimes.

Susceptibility indices (S), reflecting stability of the measured variables across environments, were distributed normally in most cases (Fig. 2). Two (out of three) domesticated genotypes (Dunn and Eritrea, marked with arrows) showed higher productivity (VDM, TGY, TDM, HI, and AGW) compared with the performance of the wild Pf genotypes under both irrigation regimes, with intermediate positions in the respective susceptibility indices. Apparently, a considerable number of the Pf genotypes are less vulnerable (lower S values) to water limitations than the domesticated genotypes.

Regarding the year effect, it appears that the recorded biomass variables (VDM, TGY, and TDM), as well as TC, were higher during the 2015 growth season than in 2014. As for OP, HI, SLW, and DTF, similar ranges were recorded during both growing seasons. Average grain weight exhibited a consistent and rather stable range during both seasons and under the two irrigation treatments. The differences in productivity between years appeared to result from a longer growth season during 2015 and the rain cover that caused higher temperatures in the WL net-house during that year.

Analysis of variance for the entire dataset (52 genotypes × 2 water treatments × 2 yr) exposed significant year × treatment interactions for all variables (not presented), and analyses were therefore performed separately for each year (Supplemental Table S1). A significant genotypic effect was recorded for all measured variables. The irrigation treatment had a significant effect on all growth traits (VDM, TGY, TDM, and HI) that were also affected by significant genotype × irrigation interactions. The irrigation treatments did not have statistically significant
Fig. 2. Frequency distribution of 52 *Pisum fulvum* genotypes and three domesticated cultivars (Alaska [A], Dunn [D], and Eritrea [E], marked with arrows) under well-watered (WW) and water-limited (WL) treatments and in terms of susceptibility indices (S) in two growth seasons. (a) Days to flowering, (b) vegetative dry matter, (c) total grain yield, (d) total dry matter (TDM), (e) harvest index (HI), (f) average grain weight (AGW). Values along the vertical axes indicate number of genotypes. Normal distributions are marked with an asterisk in the top left corners.
effects on OP and SLW during both seasons and on TC in the first season. In both experiments, the block did not have significant effects on the measured variables. However, block × irrigation interaction effects were recorded for some traits (Supplemental Table S1).

**Ecogeographic Associations of Performance under Water Limitation**

Ecogeographic clustering for 41 (out of 52) tested Pf accessions (above) divided them into six clusters reflecting (among other characteristics) an aridity gradient—Cluster 3 (arid, average rainfall 401 mm), Cluster 2 (496 mm), Cluster 4 (592 mm), Cluster 1 (659 mm), and Clusters 6 and 5 (lush 748 and 826 mm, respectively). Analyses of variance performed for each growth season, with irrigation and eco-clusters assignment for each accession as class variables, showed significant cluster effects on DTF, HI, AGW, and OP in both years, SLW in 2014, and VDM and TDM in 2015. The irrigation effect was significant for most variables in both years, and no significant cluster × irrigation interaction was found (Supplemental Table S2).

Table 1 and Supplemental Table S3 show the multiple range tests for the growth and physiological variables between ecogeographic clusters under WW and WL.
treatments, as well as the respective susceptibility indices, during the two growth seasons. Although genotypic differences (between accessions) in production and physiological variables were apparent (Fig. 2, Supplemental Table S1), in many cases, no statistically significant differences were recorded between mean cluster values (Supplemental Table S3). For example, in 2014, no significant differences were observed between clusters in VDM, TDM, and TC over both examined water treatments (Supplemental Table S3).

Of special interest was the effect of irrigation treatment across clusters. A major decrease in TGY was observed in Cluster 5 accessions (81% at the first and 53% at the second year), whereas the lowest productivity decrease occurred during 2014 in Cluster 2 (66%) and during 2015 in Cluster 3 (38%), with similar reduction in Cluster 2 (41%). Moreover, in both years, higher TGY values were obtained by Cluster 2 members, whereas the lowest values were recorded for Cluster 5 members (Table 1).

Significant HI differences between clusters were recorded under both irrigation treatments during both seasons. Cluster 2 had the highest HI values under both treatments and both seasons, with low $S$ value in 2014 and an intermediate $S$ value in the 2015 season (Table 1). As expected from their origin in relatively lush environments,

<table>
<thead>
<tr>
<th>Cluster</th>
<th>WW</th>
<th>WL</th>
<th>TGY</th>
<th>HI</th>
<th>AGW</th>
<th>DTF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014</td>
<td></td>
<td>2015</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>167.9</td>
<td>52.0</td>
<td>307.7</td>
<td>46.9</td>
<td>58.2</td>
<td>76.5</td>
</tr>
<tr>
<td>2</td>
<td>195.7</td>
<td>66.0</td>
<td>315.4</td>
<td>48.2</td>
<td>59.5</td>
<td>76.4</td>
</tr>
<tr>
<td>3</td>
<td>187.8</td>
<td>53.5</td>
<td>266.8</td>
<td>44.5</td>
<td>54.5</td>
<td>77.4</td>
</tr>
<tr>
<td>4</td>
<td>180.8</td>
<td>42.6</td>
<td>303.8</td>
<td>41.5</td>
<td>56.1</td>
<td>81.4</td>
</tr>
<tr>
<td>5</td>
<td>182.6</td>
<td>34.1</td>
<td>311.2</td>
<td>34.6</td>
<td>48.4</td>
<td>84.2</td>
</tr>
<tr>
<td>6</td>
<td>151.9</td>
<td>41.4</td>
<td>314.2</td>
<td>15.6</td>
<td>52.8</td>
<td>82.3</td>
</tr>
</tbody>
</table>

* Significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probability level.

† Table 1 present data from the analysis of 41 genotypes (*Pisum fulvum* that are classified according to ecogeographic clusters). The numbers of genotypes for each cluster are the following: Cluster 1 (10), Cluster 2 (7), Cluster 3 (9), Cluster 4 (7), Cluster 5 (5), and Cluster 6 (3). In addition, both susceptibility index and the effect of treatment were examined for each cluster.

‡ Numbers preceded by different letters are statistically different ($P \leq 0.05$).

§ NS, not significant.

¶ Indicates parameters with nonsignificant treatment effect; therefore, presented data refers to overall average repeats (both treatments).
Cluster 5 and 6 accessions had the lowest HI values under both treatments and two seasons. Similarly, related to the collection sites’ average annual rainfall, accessions assigned to Clusters 1 and 2 were the first to flower (statistically significant only in 2014), whereas accessions belonging to Clusters 5 and 6 were last to flower (Table 1). Cluster 2 had the heaviest grains, whereas Cluster 5 had the lightest grains (Table 1). Cluster 1 had relatively heavy grains, albeit significantly different from Cluster 5 only in 2015 (Table 1).

**SNP Variation among P. fulvum Accessions**

The genotype calls of 88 Pf accessions were detected over 91 loci. Those loci were found to be inherited independently (linkage disequilibrium of $r^2 > 0.7$), polymorphic (polymorphic information content > 0.1), and had calls in >90% of the samples. The Pf accessions were assigned into two major genetically related groups (G1 and G2 in Fig. 3, upper panel); however, these SNP-based groups did not clearly correspond with the clusters identified on the basis of the ecogeographical parameters of the sampling sites. Group 1 holds a larger number of ecogeographically unclassified accessions (black color in Fig. 3a) relative to G2 (SNP groups). Another feature of the SNP grouping is the assignment of all three tested accessions from Cluster 6 to G2 (Fig. 3a). Estimates of $F_{ST}$ may provide indirect evidence of gene flow. Indeed, the $F_{ST}$ values are consistent with gene flow between the two major genetic groups (median $F_{ST}$ of 0.021) and between the identified ecogeographic groups—for example, between Clusters 1 + 2 and Clusters 5 + 6 with a median $F_{ST}$ of 0.024, or a median $F_{ST}$ of 0.008 for Cluster 2 and 5 + 6 as sub-populations. The gene flow is supported by the admixed structure depicted in the lower panel of Fig. 3.

Significant differences were observed between the two SNP groups (Fig. 3, upper panel) in several phenotypic variables. For example, G2 exhibited higher TGY and AGW values in 2014, with respective lower $S$ for TGY, TDM, and HI (Table 2).

**DISCUSSION**

**Phenotypic Response to Differential Water Availability**

The tested accessions showed wider phenotypic ranges for almost all measured variables under the WW treatment compared with those observed under the WL regime (Fig. 2). Apparently, certain accessions require a minimal soil moisture level to express their full growth potential, hence the narrow distribution under WL treatment. Note that some accessions (e.g., Pf126 [Mt. Grizim] in both seasons, Pf108 [Nahal Oren, north facing slope] in the
2015 season, and Pf67 [Machsiya] in the 2014 season) had only a limited growth response to the WW regime and, as a result, did not benefit from the ample irrigation. In this context, it is important to note that, during the 2015 growth season, it was necessary to install a rain cover over the WL screen-house. As a result, minimum temperatures in the WL screen-house were slightly higher (2°C) for 10 wk after planting. Consequently, early plant growth between January to mid-March 2015, and before limited irrigation was imposed, advanced faster in the covered WL screen-house compared with the WW screen-house, which experienced lower temperatures.

Average grain weight appeared as a relatively stable character (Fig. 2, Table 1). Apparently, rather than directly affecting grain weight, limiting water supply resulted in lower biomass production, which in turn resulted in smaller number of fertile pods. Grain size is a prime adaptive trait, especially in semi-arid regions. Large grains enable rapid seedling growth after the season’s onset, a period that in the Near East is characterized by an erratic rain pattern. Indeed, a number of accessions sampled from the drier sites have heavier grains (e.g., Pf84 [Burj El-Malich], Pf74 [Balad A-Sheikh]) compared with accessions sampled from the relatively high-rainfall sites (e.g., Pf35 [Adamit], Pf29 [Mt. Meron], Pf30 [Mt. Adir]).

From Table 1 and Supplemental Table S3, it appears that clusters with an intermediate to low performance range under WW usually had poor performance under the WL treatment (e.g., Clusters 1 and 3). However, the small differences in phenotypic performance between the two water treatments usually led to low $S$. It is tempting to assume that these genotypes have lower water requirements due to their compact or low-vigor growth habit and therefore did not experience severe water deficiency, as did more vigorous genotypes. Thus, an optimal genotype should combine high productivity with high stability (low susceptibility) across environments. Regarding productivity measures, the domesticated check Dunn was superior to all tested Pf accessions, whereas some Pf accessions presented higher TDM values compared with the Eritrean check. However, as apparent from Fig. 2, Dunn’s stability values were intermediate.

Although lower biomass of a single plant can be compensated in the field by increasing plant density, high drought susceptibility cannot be compensated. Therefore, medium to high productivity combined with low susceptibility, as presented by certain Pf accessions (e.g., Pf117 [Deir Abu Mashal], Pf73 [Migdal] and Pf97 [Kinneret]) (Fig. 4) may promote crop performance if introgressed into domesticated genotypes.

### Correlations between Adaptive Traits

Harvest index values were positively correlated with AGW and negatively correlated with DTF (Table 3). Accordingly, early-flowering accessions mostly presented high AGW. A strong correlation was apparent between
Fig. 4. Principle component analyses (PCA) for genotypic performance based on productivity, phenology, and susceptibility parameters under water-limited (WL) treatment regime in two growth seasons. Green vectors indicate promoting traits, and red vectors mark negative effects. Ecogeographic clusters are marked with colors as: 1 = blue, 2 = turquoise, 3 = green, 4 = yellow, 5 = red, 6 = pink, unclassified genotypes = black. Genetic (single-nucleotide polymorphism) groups are marked by symbols: 1 = triangle, 2 = square, unclassified genotypes = circle. AGW, average grain weight; DTF, days to flowering; HI, harvest index; S, susceptibility index; TDM, total dry matter; TGY, total grain yield.

Table 3. Correlation coefficient ($r$) indices measuring the association between performance (phenology and productivity parameters), physiological attributes, and their derivatives among 52 Pisum fulvum accessions. The upper value for each parameter refers to the 2014 season and the lower value to 2015.

<table>
<thead>
<tr>
<th></th>
<th>DTF WW</th>
<th>DTF WL</th>
<th>TGY WW</th>
<th>TGY WL</th>
<th>TDM WW</th>
<th>TDM WL</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-TGY</td>
<td></td>
<td></td>
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* Significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probability level.
† DTF, days to flowering; WW, well-watered; WL, water limited; TGY, total grain yield; TDM, total dry matter; S, susceptibility index for the respective parameters; HI, harvest index; AGW, average grain weight; OP, osmotic potential; OA, osmotic adjustment; SLW, specific leaf weight; TC, total chlorophyll content.
‡ NS, not significant.
S–VDM and S–TYG; namely, low susceptibility of vegetative production is associated with low susceptibility of grain yield. In addition, stable grain production (low S–TYG) was associated with early flowering, a key adaptive trait that facilitates terminal drought escape. For example, Pf86 and Pf88 that were sampled in relatively dry sites (ecotone between Mediterranean and Jordan Valley ecosystems) present very early flowering, relatively low TDM, and low susceptibility to water limitation. It is interesting to know whether this combination is a result of adaptive linkage blocks (Harlan et al., 1973).

Ecogeographic Association of the Measured Variables

Although ecogeographical Clusters 1, 3, and 4 presented an inconsistent pattern over the two seasons and two treatments with respect to production variables, it seems that, in general, Cluster 2 differed from Clusters 5 + 6, with Clusters 2 and 5 placed at opposing sides of the principal component analysis (PCA) plot (Fig. 5, Table 1, and Supplemental Table S3). The TGY of Cluster 2 was significantly higher compared with Cluster 5 in the 2014 WL treatment, with similar (though not significant) pattern in 2015. Clusters 2 and 5 also differed significantly in their HI (WL 2014 and WW 2015) and AGW (WW 2014, similar trend but not significant in WL). Accordingly, and as apparent from the PCA plot, Cluster 2 presents high production potential at both the WW and WL treatments, combined with relatively low susceptibility to water limitations (Fig. 5).

In agreement with the ANOVA results, the PCA plot shows that the production measures of Cluster 6 accessions and Cluster 5 are susceptible to WL conditions. Interestingly, Clusters 5 and 6 hold accessions sampled in the high-rainfall districts of Israel, mainly in the north. On the other hand, Cluster 2 accessions that are more productive and less susceptible to drought (above) were not sampled from a distinct geographic unit, but rather from sites with an intermediate average annual rainfall, subjected to strong yearly fluctuations. For example, accessions Pf17 (Horshim), Pf117 (Deir Abu-Mashal), and Pf99 (Dumeide) presented the highest production (TGY and TDM) and showed low S under WL conditions.

DNA Polymorphism Pattern

DNA diversity of the Pf germplasm collection was evaluated via three different approaches: a dendrogram of the genetic relationship based on the proportion of shared allele was constructed, the genetic population structure was inspected, and the fixation index of the two major groups indicated by the dendrogram (G1 and G2) was calculated. It seems that, across Israel, Pf can be considered in terms of an admixed (meta)population. This interpretation is supported by an $F_{ST}$ value close to zero (0.02), which can be seen as indirect evidence of gene flow across the sampled Israeli range of Pf. The finding that subpopulations were not observed is interesting because Pf is a self-pollinating species, as indicated by the high homozygosity of the analyzed accessions ($F_{ST}$ index median = 0.94). Indeed, it is expected for a self-pollinating species to rapidly differentiate (Zohary, 1999; Ballesteros-Mejia et al., 2016).

* Hordeum spontaneum* K. Koch (wild barley) is another important crop wild relative native to Israel. It is a self-pollinating annual that occurs across the Mediterranean district but also extends into steppe and desert habitats. Using phenotypic data and habitat characteristics, Hübner et al. (2013) have identified three genetic groups among Israeli *H. spontaneum* (i.e., desert, coast, Mediterranean). Using a set of SSR markers, these authors have shown that, despite the relatively short propagule dispersal range and its self-pollinating nature, a significant degree of gene flow occurs across Israeli *H. spontaneum* populations (Hübner et al., 2012, 2013). Interestingly, the $F_{ST}$ values determined for ecogeographic Pf Clusters 1 + 2 and 5 + 6 are in the same range as those reported for *H. spontaneum* across similar Levantine ecogeographic amplitude (Hübner et al., 2013). At present, it is unclear whether the similar degree of gene flow estimated by Hübner et al. (2013) and in the present study is the result of different or similar ecological and evolutionary forces. One possible explanation for our finding is that the current Pf distribution represents a relatively recent expansion (i.e., too young to create a spatial differentiation as reported for weedy rice [*Oryza sativa* L.] in Sri Lanka; He et al., 2014). Seed transfer in the gut of migrating herbivores (e.g., gazelles, deer [in the past], or livestock at present) is another possible explanation.

The observed ecotypic differentiation among the Israeli Pf (meta)population is corroborated by the significant differences of certain phenotypic variables between the two identified SNP-based genetic groups (Supplemental Table S4). We used Tayeh et al.’s (2015a, 2015b) SNP tests that were developed from expressed sequences, some of which may be associated with phenology or production determinants. The apparent association between SNP-based grouping and phenotypic performance may suggest that, despite the overall background of an admixed population, allelic changes at certain physiological adaptive loci are selected for at a higher rate than those at other genomic regions.

The Potential of *P. fulvum* Germplasm for Domesticated Pea Improvement

Pea geneticists and breeders have attempted to use wild Pf for cultivar improvement, mainly for introducing disease and pest resistance alleles (Clement et al., 2009; Jha et al., 2012; Kosterin, 2016). Most wild pea accessions in germplasm banks represent random sampling, rather than systematic collections. Consequently, the ability of pea geneticists to identify promising genotypes was limited. Hence, our wild Pf collection that spans an aridity gradient and a range of bedrock and soil types may facilitate a significant advance.
Fig. 5. Principle component analyses for ecogeographical clusters based on productivity parameters (a–d: total dry matter [TDM], total grain yield [TGY], harvest index [HI], average grain weight [AGW]) and their susceptibility indices (S, e–f) under two irrigation regimes in two growth seasons. Panels a and b present water-limited (WL) and well-watered (WW) treatments during 2014, respectively, whereas panels c and d present WL and WW treatments during 2015, respectively, and panels e and f present susceptibility indices during 2014 and 2015, respectively. Green vectors indicate promoting traits, and red vectors mark negative effects. Ecogeographic clusters are marked with colors as: 1 = blue, 2 = turquoise, 3 = green, 4 = yellow, 5 = red, and 6 = pink.
for improvement of domesticated pea against both biotic and abiotic stresses. We are unaware of a similar germplasm array from either *P. elatius* or *P. humile* that is available for research. Given the wide natural range of the former species (from the Crimea and Azerbaijan in the north through to Israel in the south), it should be possible to conduct a similar phenotypic evaluation and probably identify distinct eco-types. For example, a *P. elatius* accession sampled in Israel from the eastern Galilee flowered earlier when compared with *P. elatius* accessions sampled in Nahal Keziv, only 38 km away in a northwesterly direction, where higher rainfall and cooler average winter and spring temperatures prevail (Golani et al., 2016). It is therefore expected that other physiological and production traits would vary across the natural range of the species.

The last decade saw the development of genome-wide DNA marker systems for pea (Deulvot et al., 2010; Tyeh et al., 2015a, 2015b; Aloume et al., 2016). Recent advances in plant stress physiology and parallel efforts made in plant functional genomics enable association between physiological attributes, plant anatomy, specific gene action, and hormonal, developmental, and physiological networks (Bramley et al., 2013). However, to the best of our knowledge, studies associating classical stress-physiology (whole plant phenotypes) and DNA variability (or functional genomics) were not reported for wild *Pisum* sp. We intend to extend this study using deeper genomic DNA polymorphism screening, as well as further intrapopulation physiological characterization. To this end, we have recently sampled additional Pf accessions from three high- and three intermediate-rainfall habitats. These new Pf lines will enable us to also evaluate the within-population diversity using DNA markers and stress response variables.

**Supplemental Material Available**

Supplemental material for this article is available online.

**Acknowledgments**

This work was funded by Grant no. 837-0117 from the Chief Scientist of the Israeli Ministry of Agriculture and Rural Development. S. Abbo is the incumbent of the Jacob and Rachel Liss Chair in Agronomy; Y. Saranga is the incumbent of the Jacob and Yehuda Haim Gvati Chair in Agriculture. E. Naim-Feil was the recipient of the Tzion Cohen scholarship in 2014. The authors thank Vered Barak for OP measurement. We also thank the four anonymous reviewers for many useful comments.

**References**


