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Full Length Research Paper

Genetic assessment of the genus *Pisum* L. based on sequence specific amplification polymorphism data

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The present study was conducted to analyze genetic diversity among 56 accessions of *Pisum sativum, Pisum elatius, Pisum pumilio, Pisum fulvum* and *Podandrogyne brevipedunculata* from Syria (42) and a set of 16 reference accessions from Israel (3), Ethiopia (2), Greece (2), Italy (2), Turkey (3), Tunisia (1) and Georgia (1). The study was based on the analysis of multiple data sets generated using sequence specific amplification polymorphism (SSAP) and associated morphological and ecogeographic data. The diversity analysis based on SSAP markers resulted in 83 scorable fragments in *P. sativum, P. elatius* (63)*, P. fulvum* (62), *P. pumilio* (53), *P. brevipedunculata* (31) and *P. abyssinicum* (29). Genetic variability was measured as Nei's gene diversity and maximum polymorphism was found in *P. elatius* (33.87%) and *P. fulvum* (32.74%) whereas minimum diversity was observed in *P. brevipedunculata* (12.48%). Pair wise band sharing among *Pisum* species was also calculated, significant band sharing was observed between *P. fulvum* and *P. elatius*, *P. elatius* and *P. sativum* and *P. pumilio* and *P. elatius*. The relationship among species as revealed by SSAP data could not significantly be correlated with those based on the agro-morphologic characters, suggesting that the two inferences are independent and estimates of genetic relations differently among *Pisum* taxa. The ecogeographic data associated with *Pisum* species (latitude, longitude) were also plotted using Arc View GIS 3.2 in order to show the distribution of species in Syria.

Key words: *Pisum*, sequence specific amplification polymorphism (SSAP), genetic assessment, neighbor joining, ecogeographic, amplification, polymorphism.

INTRODUCTION

Pisum is the fourth imperative food legume crop in the world based on its sowing area and production (Bezdicek et al., 2003). The center of origin of *Pisum* is in southwestern Asia (India, Pakistan, Union of Soviet Socialist Republics and Afghanistan) since 10000 years ago and then spread to the temperate zones of Europe (Cousin, 1997). However, wild pea species like *Pisum fulvum*, *pisum formosum* and now *Vavilovia*. *Pisum humile* found in Middle East imply that Northwest Asia is the origin of pea and from there it is scattered to West, North and East of Asia (Zohary and Hopf, 2002; Oelke et al., 2003; Kosterin and Bogdanova, 2008). In nature, cultivated peas have maximum diversity while minimum was observed in wild varieties (Ghafoor et al., 2001). Among wide diversity of *Pisum* species *Pisum sativum* showed reproductive isolation from *P. fulvum* which then overcrowded in the Anterior Asia (Baranger et al., 2004). *P. sativum* with *Pentarrhinum abyssinicum* from South Arabia and Ethiopia are also distinguished on the basis of chromosome rearrangements as well as some morphological traits (Ghafoor et al., 2005). Recent studies of the genus *Pisum* have focused on relationships among the cultivated garden pea*, P. sativum* (Mohammad et al., 2009) and wild annual species *P. fulvum* an eastern Mediterranean plant (Bisby et al., 2007), *P. humile* most often found in open vegetation

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near eastern steppe (Bezdicek et al., 2003) and *P. elatius* an Omni Mediterranean plant (Brkic et al., 2004) based on morphological characteristics.

Pea (*P. sativum*) with genome size of 4400 Mbps is an annual growing plant and well thought-out as one of the most important legumes for human, animal and environment. It is widely spread due to its many uses as fresh green peas, dry peas, tender green pods, green foliage and leaves, in the canning and freezing industry, ripened seed (Duke, 1981; Kay, 1981; Davies et al., 1985).

Much of the research on field pea improvement has been focused on disease resistance (as with many crop species) and molecular markers associated with many characters have been identified. Other work on pea has focused on using molecular markers to study the genetic factors controlling quantitative trait loci (QTLs) and creating linkage maps (Weeden et al., 1998). AFLP (Amplified fragment length polymorphism) and SSAP have a high multiplex ratio (Lu et al., 1996; Ellis et al., 1998) and offer a distinct advantage when genome coverage is a major issue. For an extensively inbreeding system such as pea dominance of the markers is not such an important consideration and it has been shown that SSAP markers produced by TEs are more informative than AFLP and RFLP (Ellis et al., 1998). SSAP reveals insertion site polymorphism and sequence variation in the flanking DNA which have employed in current study.

The present study is to examine the relationship among wild and cultivated species of *Pisum* using morphological characters, ecogeographic and molecular data for selected *Pisum* taxa. The potential of each dataset (molecular, morphological and ecogeographical) will help in discrimination of pea taxa studied and to evaluate the similarities and differences among wild and cultivated peas of Syria.

MATERIALS AND METHODS

The molecular, morphological and ecogeographic data pertaining to fifty six *Pisum* accessions from Syria (42), Israel (3), Ethiopia (2), Greece (2), Italy (2), Turkey (3), Tunisia (1) and Georgia (1) were analysed. Among these forty *Pisum* accessions were collected from Syria (Mumtaz et al., 2006) while remaining sixteen accessions were obtained with the courtesy of Mike Ambrose of John Innes Centre. The latter accessions are referred to as JI lines. These datasets was analyzed *in silico* to assess the pattern of diversity. DNA extraction and SSAP was carried out as described in Ellis et al., (1998). The data was recorded as presence (1) or absence (0) of band scored on a particular locus. Altogether 135 loci were scored. The data were used in revealing basic statistics like the total number of bands, polymorphic, monomorphic and unique bands. Pair wise band sharing was calculated among *P.* taxa. The data was subjected to cluster analysis as follows: A similarity coefficient matrix was generated using the Nei and Li coefficient. The cluster analysis was performed using Unweighted Pair Group Method (UPGMA) in NTSYS pc2.20 (Rohlf, 2006).

Morphological data was used as an input file for NTSYSpc2.20 software in order to calculate the similarity coefficient matrix. Cluster analysis was performed by using UPGMA in NTSYSpc 2.20 (Rohlf, 2006). A similar method was used to perform cluster analysis using qualitative characters. Ecogeographic data of 56 *Pisum* accessions was used to evaluate the diversity among species. Following parameters were selected to assess the diversity among *Pisum* species, that is, Country, Province, habitat, altitude, latitude, longitude, soil texture, parent rock, topography, aspects, slope, rainfall, plant association and pH. The coordinates associated with each of the herbarium specimen was used to plot distribution maps using ArcView GIS 3.2 (ESRI 1999). Similarly, the pH, altitude and rainfall data associated with each of the taxon was assessed to study the ecological and geographic associations.

RESULTS

Binary data obtained for six pea taxa: *P. sativum, P. elatus, P. pumilio, P. fulvum, Podandrogyne brevipedunculata and P. abyssinicum* was subjected to analysis using unweighted pair group method with arithmetic means (UPGMA). The analysis revealed a clustering pattern which corresponded well with the morphological identification with some exceptions. The genetic similarity level among accessions ranged from 0.77 to 0.98 with an average of 0.87 (Figure 1). Altogether, six clusters designated as A, B, C, D, E and F and have been identified at a similarity level 0.75. The total number of bands exhibited by various taxa in the genus *Pisum* (Figure 2) suggested three taxa: *P. sativum* (83), *P. elatius* (63) and *P. fulvum* (62), to be most diverse (Table 1). The level of genetic diversity in *P. sativum* is probably due to the breeding practices. Similarly *P. elatius and P. fulvum* are wild species and relatively more diversity depicting these to be ancient lineages. This is further endorsed by the percentage unique bands observed in the species.

Morphology data (both qualitative and quantitative) obtained for *P. sativum, P. elatius, P. pumilio, P. fulvum, P. brevipedunculata* and *P. abyssinicum* were subjected to cluster analysis. The UPGMA cluster analysis based on genetic similarity showed five groups when assessed at 0.28 (33%) similarity level (Figure 3). The genetic similarity for all accessions ranged from 0.28 to 0.80 with an average of 0.45. A subset of morphological data comprising of qualitative characters only were assessed using coefficient of simple matching and UPGMA. At 30% similarity criteria, two major clusters were revealed largely dissecting all accessions into the Syrian accessions and those obtained as reference lines from John Innes center. However, at a stricter criterion of 60%, the clustering pattern showed correspondence with the morphologically identified taxa. Cluster analysis placed accessions in to seven major groups (Figure 4). The genetic similarity for all accessions ranged from 0.56 to 0.92 with an average of 0.73.

Plants of *P. fulvum* were found growing in moderately moist conditions, with good seed production via aerial stems. *P. fulvum* showed allopatrism with *P. sativum* on the basis of field terraces and *P. pumilio* of woodland etc.

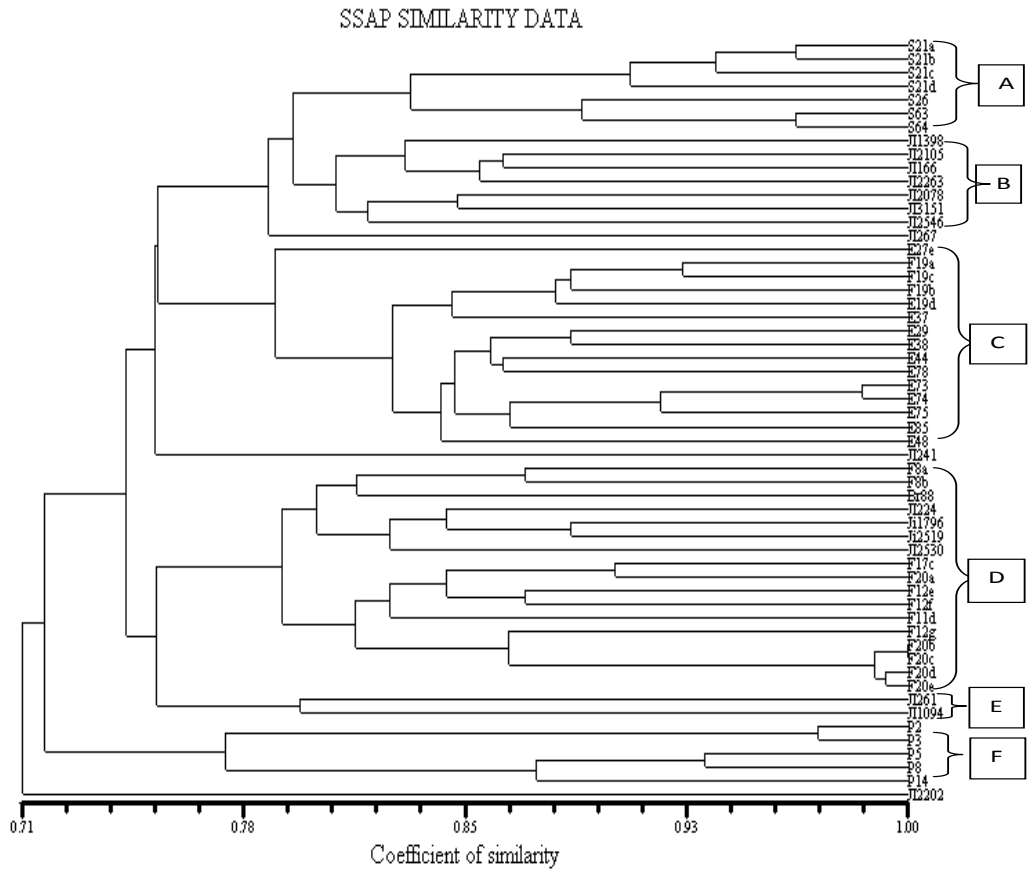


Figure 1. Cluster analysis of *Pisum* accessions based on SSAP at 75% similarity. Group A (*P. sativum* accessions, Syrian. Group B JI1398 *(P. elatius,* Greece), JI2546(*P. fulvum*, Georgia), JI2105 (*P. sativum*, Italy), JI166 (*P. sativum* Ethiopia), JI2263 (*P. sativum* Tunisia), JI2078 *P. elatius,* Italy), JI3151 (*P. sativum*, Turkey), Group C (Syrian *P. fulvum* and *P. elatius),* Group D (Syrian *P. fulvum* accessions and JI2519, 2530 (Syrian *P. fulvum*), JI1796 (*P. fulvum*, Israel), JI261(*P. elatius,* Turkey), JI1094 (*P. fulvum*, Greece), Group E JI261 (*P. elatius*), JI1094 (*P. fulvum*) and Group F (Syrian *P. pumilio* accessions).

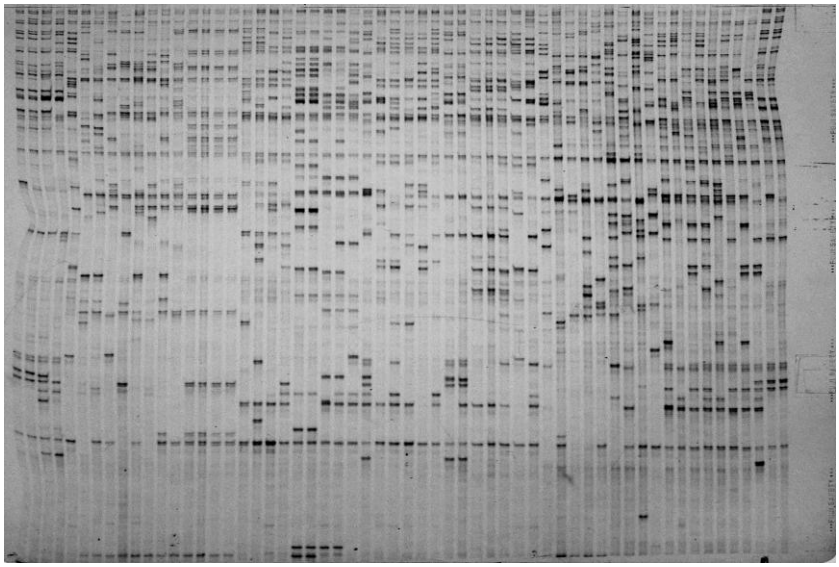


Figure 2. Electrogram of SSAP for 56 accessions of *Genus Pisum.*

Table 1. Comparative genomic diversity among *Pisum* species as assessed by number of polymorphic and unique bands.

S/N	Accessions	Total bands	Polymorphic bands (%)	Monomorphic bands (%)	Unique bands (%)
1	*P. sativum*	83	(29) 21.17	(54) 40	2.8
2	*P. elatius*	63	(45) 33.87	(18) 13.33	9.0
3	*P. fulvum*	62	(44) 32.74	(18) 13.33	12.5
4	*P. pumilio*	53	(31) 22.22	(22) 16.48	3.2
5	*P. brevipedunculata*	31	(17) 12.48	(14) 10.37	3.5
6	*P. abyssinicum*	29	(20) 14.72	(9) 6.66	8.20

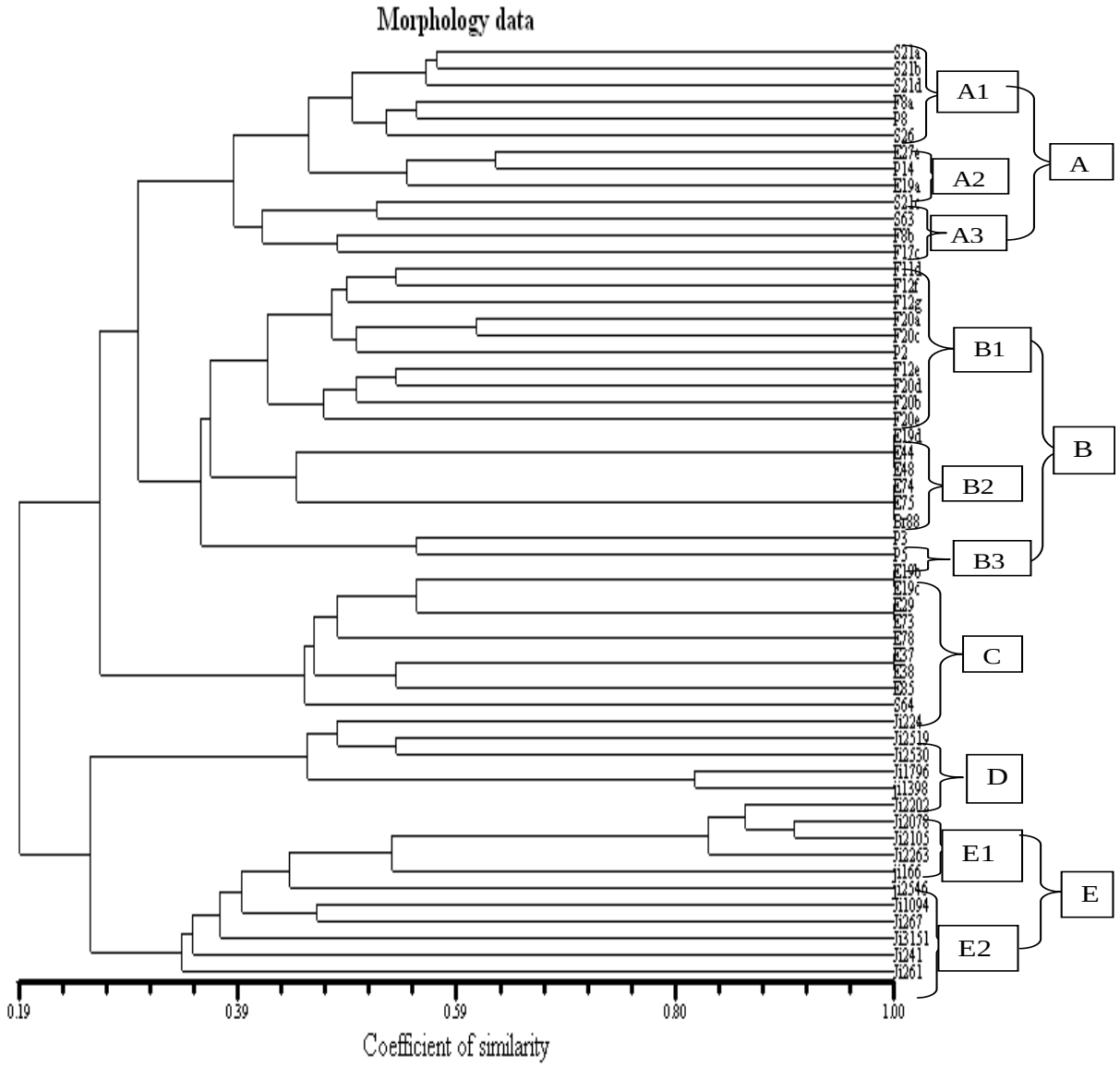


Figure 3. Clustering pattern of *Pisum* accessions based on Morphological data. The clustering is depicted at 33% similarity. Group A (Syrian *P. sativum* (S21a, b, c, d, S63, S26), Syrian *P. fulvum* (F8a, b), Syrian *P. elatius* (27e). Group B, Syrian *P. fulvum* (F20a, b, c, d, e, F12f, g, F11c, d), *P. pumilio* (P2, P3, P5) *P. elatius* (E19d, E44, E48, E74,E75), Group C (Syrian *P. elatius* E19b, c, 29, 73, 78, 37, 38, 85), Syrian *P. sativum* (S64) and Group D JI224 (*P. sativum*), JI2519, JI2530 (*P. fulvum*), JI1796 (*P. fulvum* Israel), JI1398 (*P. elatius*) and Group E (JI2202 (*P. abyssinicum*), JI2078 (*P. elatius*), JI2105 (*P. sativum* Italy), JI2263 (*P. sativum* Tunisia), JI166 (*P. sativum* Ethiopia), JI2546 (*P. fulvumGeorgia*), JI1094 (*P. fulvum,* Greece), JI267 (*P. elatius* Turkey), JI3151 (*P. sativum* Turkey), JI241 (*P. pumilio*, Israel), JI261 (*P. elatius*, Turkey).

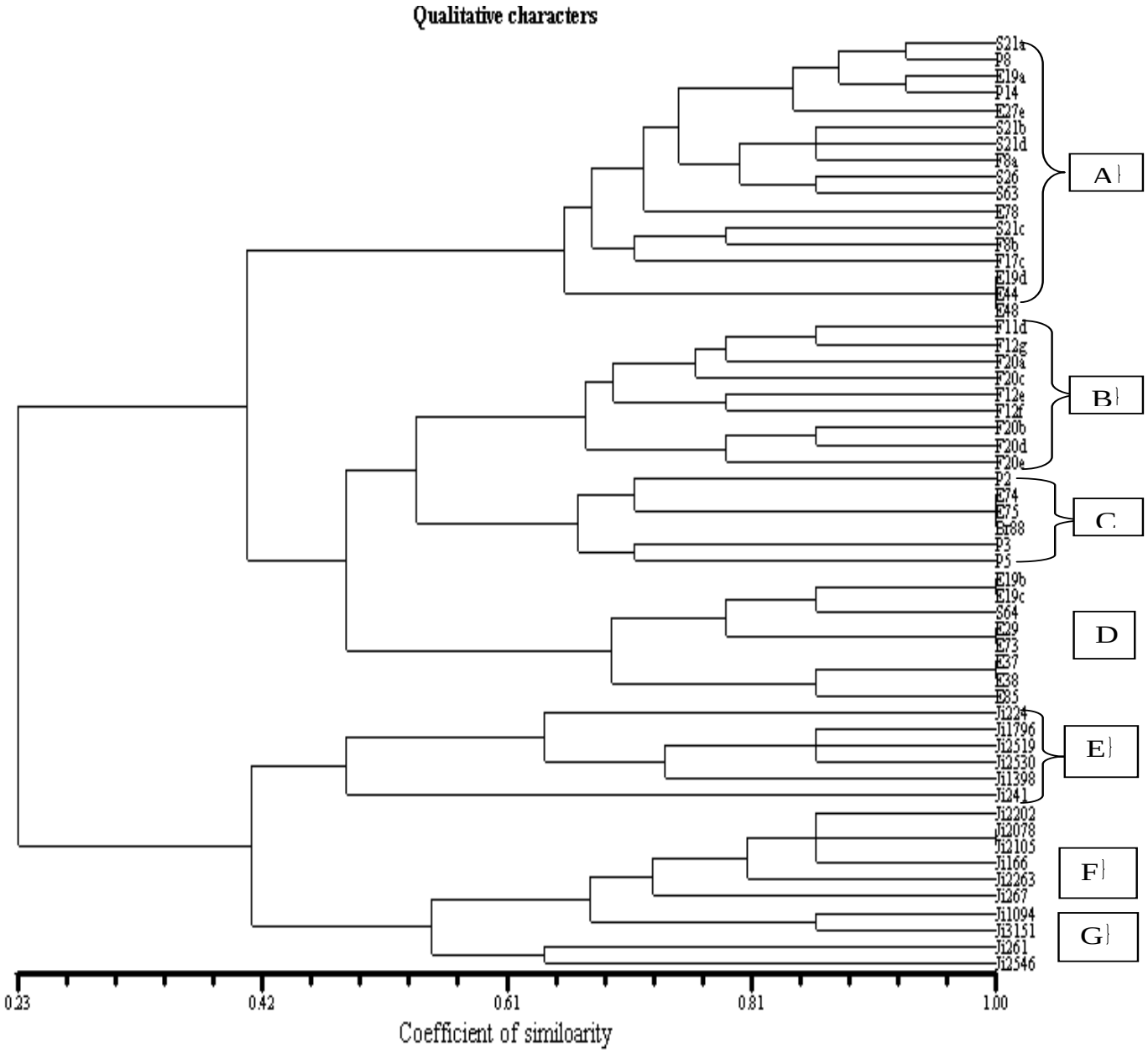


Figure 4. Cluster analysis of *Pisum* accessions based on Qualitative characters at 60% similarity. Group A Syrian *P. sativum* (S21a, b, c, d, S26, 63), Syrian *P. elatius* (19a, d, E19a, E27e, E78, E44, 48), Syrian *P. fulvum* (F8a, b, F17c), *P. pumilio* (P8, 14) Group B Syrian *P. fulvum* (F11d, 12g, F20a, b, c, d, e) Group C Syrian *P. pumilio* (P2, P3, P5), *P. elatius* (E74, E75) and *P. brevipedunculata* (Br88). Group D *P. elatius* (E19b, c, 29, 73, 37, 38, 85) and Syrian *P. sativum* (S64) and Group E JI224 (*P. sativum*), JI2519, JI2530 (*P. fulvum*), JI1796 (*P. fulvum* Israel), JI1398 (*P. elatius*) and Group F (JI2202 *P. abyssinicum*), JI2078 (*P. elatius*), JI2105 (*P. sativum* Italy), JI2263 (*P. sativum* Tunisia), JI166 (*P. sativum* Ethiopia), JI2546 (*P. fulvum Georgia*), JI1094 (*P. fulvum,* Greece), JI267 (*P. elatius* Turkey), JI3151 (*P. sativum* Turkey), JI241 (*P. pumilio*, Israel), JI261 (*P. elatius*, Turkey).

(Table 2). *P. fulvum* mostly occurred on hillside terraces where no other *Pisum* was found. This shows its ability to sustain itself under drier climatic conditions. *P. elatius* was frequently found in maquis (moist and well drained) habitats. These were found growing in small, scattered clumps of 5 to 10 individuals especially at Al Latakia province and showed affinity with moist and well drained conditions especially at coastal sites (Table 2). In Figure 5, we observed that *P. fulvum* showed wide range of occurrence, that is, it has been recorded at an altitude as high as (1000 m) and as low as (300 m). *P. elatius* and *P. sativum* found at high altitude (1000 m) only. In case of rainfall, *P. sativum* and *P. pumilio* showed maximum occurrence at (800 to 1000 mm) while *P. elatius* and

Table 2. Ecogeographical data associated with *Pisum* taxa collected from Syria.

S/N	Taxon	Provinces	Rainfall (mm)	Habitat	Altitude (m)	Parent rock	Soil texture
1	*P. fulvum*	Al Latakia Homs Hama Aleppo Qunaitra	350-1000	Woodland, road side field terraces, Hill side, and gorge etc.	320-1141	Basalt Limestone schist	Clay Clay with stony texture Sandy loam
2	*P. elatius*	Al Latakia	1000	Slope facing sea abandoned path, castle ruins, road side and field terraces	40-555	Basalt Limestone schist	Clay
3	*P. pumilio*	As Sweida Qunaitra	350-600	Woodland, field terrace and grassland	920-1400	Basalt Limestone	Clay
4	*Brevipedunculata*	Aleppo	350	Church ruins	509	Basalt/ Limestone	Clay with stony texture
5	*P. sativum*	Al Latakia Hama	0-1000	Field terraces, road side and field terraces	799-970	Schist Limestone	Clay Classic brown clay

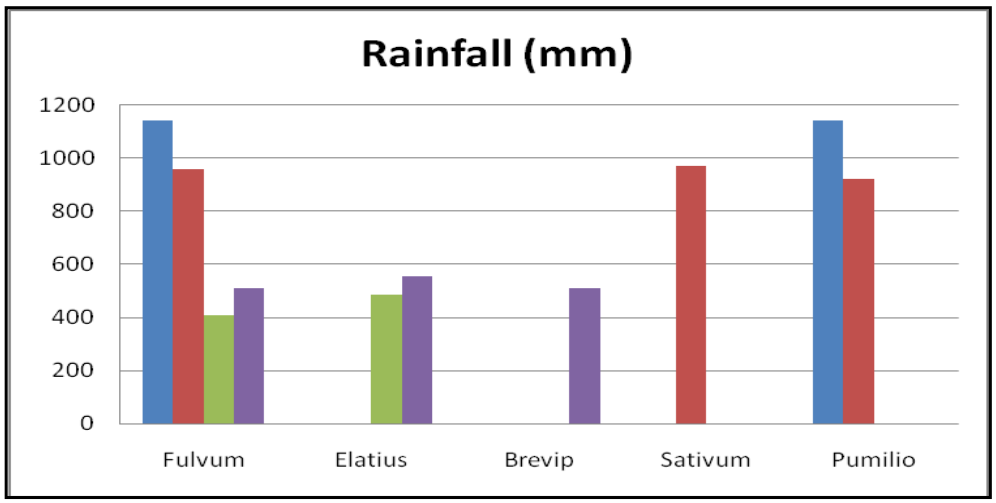
P. brevipedunculata have been observed at a lower rainfall (400 to 500 mm) area. Furthermore, *P. elatius*, *P. sativum* and *P. pumilio* occurred at pH range (6 to 7) while *P. fulvum* was found at a wider pH range, that is, 6 to 8 (Figure 5).

DISCUSSION

The present study demonstrated the potential of SSAP technique to study genetic polymorphism. The analysis is appropriate to study individual DNA and genetic diversity as utilized here for fifty six individuals of *Pisum* species. The data revealed clustering of morphologically identical accessions both at strict (0.98) and relaxed (0.75) levels of similarity. This has been the case for *P. fulvum* (F20a, c and d) and *P. elatius* (E73 and E74). Based on these observations we suggest that both *P. elatius* and *P. fulvum* lineages are genetically more diverse. Based on the analysis of morphology, ecology, cytogenetics and hybrid performance Ben Ze'ev and Zohary (1973) concluded that *P. fulvum* is a fully divergent species, whereas *P. pumilio*, *P. elatius* and *P. sativum* form a single species complex comprised of two main races, weedy forms (*elatius* and *pumilio*) and cultivated derivatives (*P. sativum*).

In contrast, *P. fulvum* and *P. elatius* showed highest proportion of polymorphic and unique bands, suggesting that both are more diverse among *Pisum* species. The cluster analysis based on SSAP data generally depicted similar pattern of similarity as mentioned in the above studies. *P. pumilio*, *P. elatius* and *P. sativum* form a monophyletic group while *P. fulvum* in each case clearly the most distinct of pea taxa (Polans et al., 1996). *P. abyssinicum* has low number of polymorphic, species specific and total number of bands from the *Pisum* gene pool which corroborate previous findings (Vershinin et al., 2003).

On morphological basis, the most ancient divergence event within the genus had separated *P. fulvum*, next divergence separated *P. abyssinicum*, while the remaining lineage of *P. sativum* irradiated to provide a variety of forms. *P. abyssinicum* is morphologically much closer to *P. sativum,* so that some authors consider them conspecific (Makasheva, 1979), but it was



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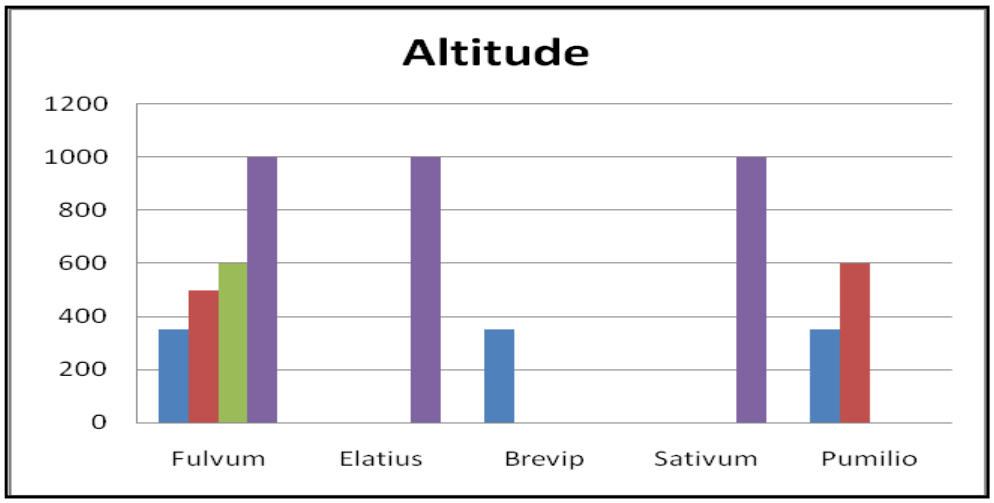

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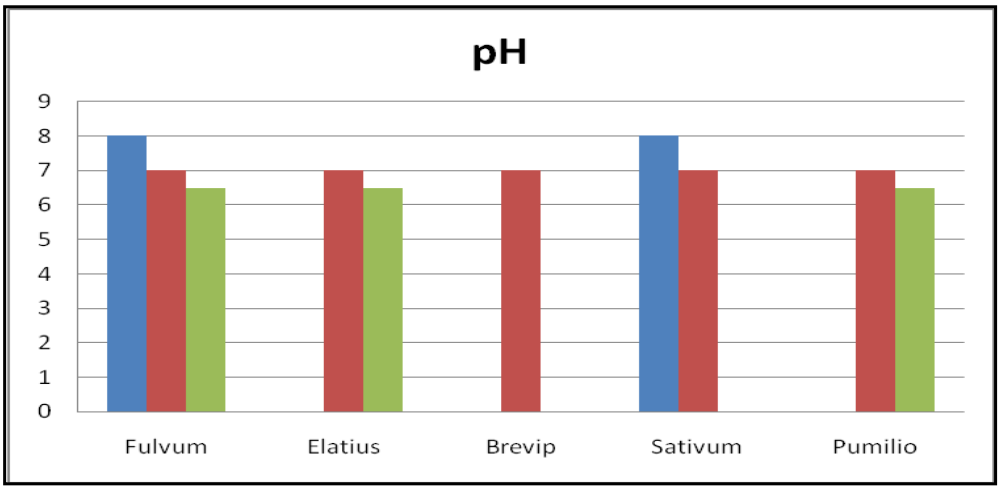

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Figure 5. Relative frequency of occurrence of different *Pisum* taxa in Syria (a) Color bars represent different values: Blue 300, Red 500, Green 600 and Purple 1000, (b) Color bars represent different values: Blue 1100, Red 900, Green 400 and Purple 500, (c) Color bars represent different values: Blue 8, Red 7 and Green 6.5.

domesticated independently of *P. sativum* (Govorov, 1937; Ellis et al., 1998). However *P. fulvum* and *P. sativum* showed only three markers in common which suggests the isolation of *P. sativum* from *P. fulvum* and *P. abyssinicum.* The relationships among the *Pisum* species, as revealed by molecular markers (SSAP), were not significantly correlated with those based on the morphological characters suggesting that the two systems give different estimates of genetic relations among the species.

Ecogeographic data suggested that *P. fulvum* has been found widespread throughout western Syria and sympatric to all other *Pisum* taxa collected. Occurrences of *P. fulvum* in both Mediterranean (coastal) as well as in continental (in land) climate zones indicated its adaptability to both sets of conditions (Bukvic et al., 2007). Colonies of *P. elatius* were frequently found in shady areas, such as historical ruins with restricted public and animal access. *P. pumilio* was collected only from south western provinces, As Sweida and Qunaitra (Fallon et al., 2006). Plants were found thriving in low rainfall (350 to 400 mm/yr) pastures with occasional scrub like thickets of *Quercus ilex*. The terrain of these sites was plain, with well drained terra rosa soil derived from basaltic or limestone parent rock, with an average altitude of 1200 m. A rare taxon *P. brevipedunculata* was found only at one site, among the ruins of an old church called “Saint Simeon” in northern Syria (Ghafoor et al., 2002). The area is climatically continental, with low rainfall (350 to 600 mm/yr) and limestone pavement with occasional canyons. Ecologically, it prefers a sunnier and drier habitat, similar to that of *P. pumilio* (Fowler et al., 2006). Data from the present expedition and previous reports (Davis, 1970) revealed its limited occurrence and allopatric distribution as compared to *P. elatius* and *P. pumilio.* These results may have arisen because the diversity at the molecular level, which is a priori neutral, may not reflect the diversity at the morphological or physiological level as described by Sardana et al. (2007).

Conclusions

The extensive genetic variability commonly associated with pea as the classical Mendelian organism is the basis for its multiplicity of forms. Systematists have often interpreted this variation by collectively recognizing many distinct pea species in a variety of different relationships. The present investigation revealed that *P. fulvum* and *P. elatius* are more diverse among pea taxa whereas *P. sativum,* a cultivated species was found less diverse. On the other hand, *P. abyssinicum* is a cultivated species from Ethiopia least diversity. Among wild taxa *P. elatius* showed closer relationship with *P. pumilio*, *P. fulvum* and *P. brevipedunculata*. Similarly, *P. elatius* was closely associated with *P. sativum*. In addition to this, the *P. sativum* was also found closely related to *P. pumilio* but not similarity with *P. fulvum* and *P. abyssinicum*. Among three methods, molecular and ecogeographical datasets were found useful for the assessment of variability within *P. taxa* especially the wild and cultivated taxa. Both data were more helpful and demonstrated that all cultivated taxa were well separated from the wild *P. taxa*. Furthermore, the morphological data were inconsistent with molecular and ecogeographic data suggesting that the molecular and ecogeographic datasets are more reliable.

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