# ORIGINAL ARTICLE

# Pod, seed traits and cytotaxonomic studies of some *Vicia narbonensis* L. accessions (*Fabaceae*)

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**Abstract** This study aimed to characterize eight accessions of *Vicia narbonensis* L. originated from different Mediterranean countries. The cytology of these species is rarely known despite the fact of its great socio-economical and ecological interest in these arid and semi-arid zones. This work aimed mainly to characterize the karyotype, morphological pod and seed traits of the species. Karyotypes of all accessions were similar to a diploid number of $2n = 2x = 14$. All the accessions have submetacentric chromosomes with a secondary constriction attached to the long arm of pair VII close to the centromere. Variation in chromosome size was observed; it ranged from 5.86 μm to 7.62 μm. Indices of karyotype asymmetry were calculated as the total form percentage (TF%) and symmetric indices (Syi) which ranged from 33.75% to 35.42% and from 51.01% to 54.85%, respectively. The predominance of submetacentric chromosomes indicated that the karyotype is symmetrical and can be considered as primitive. However, the analysis of quantitative parameters measured on pods and seeds showed a significant variation between accessions. A relationship between centromeric index and the pod beak length was found. Estimation of phenotypic diversity using the Shannon diversity index ($H'$) showed that the length, the seed color and the number of seeds per pod are the most polymorphic traits with respectively, $H' = 0.92$, 0.80 and 0.83. Cluster analysis of karyological, pod and seed traits showed four groups of accessions. This clustering is partially due to the geographical origin of the studied accessions. The variation in chromosome size, pod

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## 1. Introduction

*Vicia narbonensis* L. is one of the species that have been domesticated in the early centuries of agriculture (Bryant and Hughes, 2011). It has its origin in North–West Asia but then it has been extended to the Mediterranean Basin. Since the 90s the crop growing of *V. narbonensis* has become more popular in Australia, and still is used as a multi-purpose legume for grain, fodder and green manure in areas of low to medium rainfalls (Dafwa, 2005). Narbon vetch is a promising forage legume with a good resistance to drought, cold, and aphids. The green parts are used as fodder, grazed or cut for hay. As a nitrogen fixing legume, Narbon vetch is used increasingly as green manure as well as trap crop for parasitic weed *Orobanche crenata* (Kroschel, 2001).

Narbon vetch (*V. narbonensis* L.) is an annual legume with thick, angular and erect pubescent stems that can grow without support (Ecocrop, 2013; Bryant and Hughes, 2011). The root system is well developed; the fruit is a dehiscent pod containing several smooth seeds, often called Narbon beans that are 5.5–6 mm wide and brown in color (Ecocrop, 2013; Bryant and Hughes, 2011; Hyppa, 2000).

*V. narbonensis* is a diploid plant with $2n = 2x = 14$ chromosomes (El-Bok et al., 2014). The total chromosome length was considerably long. The chromosomes are cytologically uniform but can be distinguished by minor variations in chromosome length. All accessions posses' satellites (SAT chromosome), a secondary constriction, connected to the long arm near centromeric region.

Pod and seed morphology traits are good descriptors for evaluating genetic resources (IPGRI, 1998; USDA, 2001). In *V. narbonensis*, the size of the pods and seeds are often important to distinguish between the botanical varieties (Schäfer, 1973), cited in Bennett and Maxted (1997) as well as characters like hilum color, funiculus persistence, shape of the pod and teething leaflets (Hassen, 2000). Karyological information is also useful for identification and analysis of taxonomical position of species. Furthermore, the correspondence of morphological and karyological characters is rather rare. In fact, this study's aims are to assess pod and seed morphological variation among *V. narbonensis* accessions originated from different geographical regions, to correlate morphological data with karyological one and for a better description of the accessions and their possible use in breeding programs.

## 2. Materials and methods

### 2.1. Plant materials

Seeds of eight accessions of *V. narbonensis* L. from different Mediterranean origin are used in this study. The name, origin and botanical variety of these accessions are mentioned in Table 1. Seeds of six accessions were provided by ICARDA while seeds of other accessions were collected locally and preserved at INRAT forage and animal production laboratory.

### 2.2. Methods

#### 2.2.1. Karyological analysis

Seeds were germinated at room temperature. Root tips from germinated seeds were excised between 10 and 11 h (period of active cell division) and pretreated in 0.1% colchicine for 2 h at room temperature. After pretreatment, the root tips were fixed in freshly prepared Ethanol–Chloroform–Acetic Acid solution (6v:3v:1v) for at least 24 h at 4–5 °C. Roots were hydrolyzed in 1 N HCl for 15 min at 60 °C then stained with Feulgen reagent. The meristematic root part was squashed in 45% acetic water with acetic orcein and observed at 1000× magnification using optical microscope (type Hund, H 600 WILO-PRAX PL, Germany). A digital camera with 12 MP was used for chromosomes photography. Chromosomal nomenclature was carried out according to Levan et al. (1964). Measurements such as long ($l$) and short ($s$) arm lengths were made for each cell. Calculations were made to determine the total chromosome length, arm ratio ($l/s$), centromeric index ($s/l$) and total haploid chromosome length ($\sum$TCL). The ratio between the sum of the lengths of the short arms of individual chromosome and the total length of the complement in percentage (TF%), the ratio between the average length of the short arms and the average length of the long arms (Syi) and the classification of Stebbins (1971) reported by Zuo and Yuan (2011) were used to perform the analysis. The classification of Stebbins (1971) is based on relative frequency of chromosomes with a long arm ratio greater than 2 and on the ratio between the lengths of the longest and the shortest chromosome in the complement. Karyograms and idiograms were constructed by arranging the chromosomes in homologous pairs by order of their length.

#### 2.2.2. Morphological analysis of pods and seeds

The morphological study of the pods of accessions was carried out on a sample of thirty pods per accession repeated three times which leads totally to the result of 90 pods per accession.

**Table 1** Names of *V. narbonensis* accessions used in this study, their origin and botanical varieties.

| Entry N | Name | Origin | Botanical variety |
|---------|------|--------|-------------------|
| 1 | Sel 2376 | Lebanon | *aegyptiaca* |
| 7 | Sel 2387 | Lebanon | *narbonensis* |
| P1 | P1 | Tunisia | *aegyptiaca* |
| 545 | 545 | Tunisia | *narbonenis* |
| 8 | Sel 2392 | Lebanon | *narbonensis* |
| 9 | Sel 2393 | Morocco | *aegyptiaca* |
| 10 | Sel 2466 | Lebanon | *aegyptiaca* |
| 14 | Sel 2561 | Turkey | *narbonensis* |

*Abbreviations:* 1, accession 1; 7, accession 7; P1, accession P1; 545, accession 545; 8, accession 8; 9, accession 9; 10, accession 10; 14, accession 14.
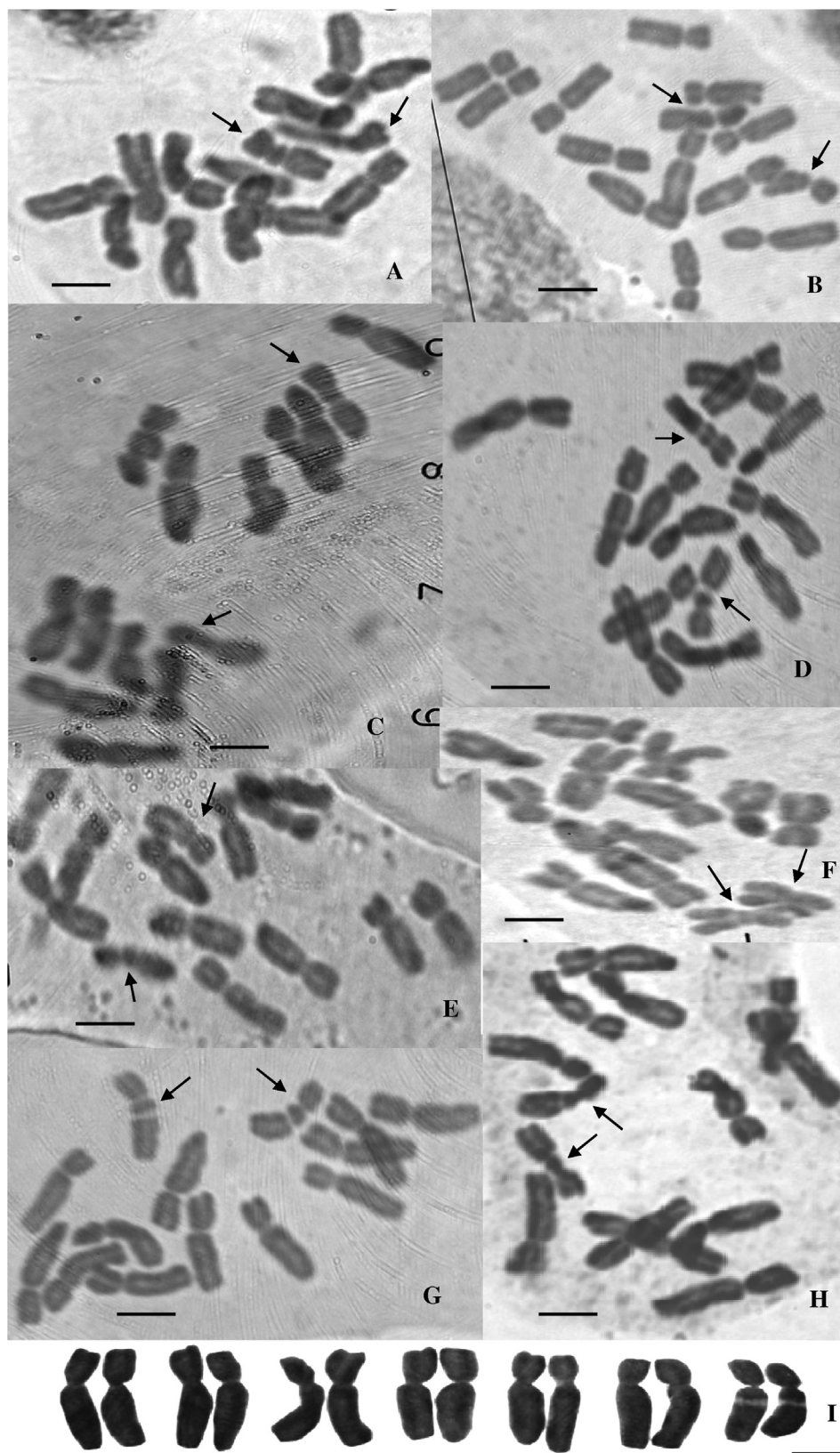
**Figure 1**    Mitotic metaphase chromosomes of the studied *V. narbonensis* L. accessions: A = accession 1; B = accession 7; C = accession P1; D = accession 545; E = accession 8; F = accession 9; G = accession 10; H = accession 14; (Scale bar = 5 μm; the arrows indicate the chromosomes with secondary constriction) and I = karyotype of accession 10. (Scale bar = 5 μm.)

**Table 2** Karyotype asymmetry of the studied accessions of *Vicia narbonensis* L.

| Accession | TF% | SYi index | Stebbins categories[*] |
|---|---|---|---|
| Acc. 1 | 33.80 | 51.12 | A2 |
| Acc. 7 | 33.86 | 51.12 | A2 |
| Acc. P1 | 34.02 | 51.64 | A2 |
| Acc. 545 | 33.75 | 51.01 | A3 |
| Acc. 8 | 35.42 | 54.85 | A2 |
| Acc. 9 | 35.37 | 54.61 | A2 |
| Acc. 10 | 34.51 | 52.70 | A2 |
| Acc. 14 | 34.69 | 53.07 | A1 |

*Abbreviations:* Acc. 1, accession 1; Acc. 7, accession 7; Acc. P1, accession P1; Acc. 545, accession 545; Acc. 8, accession 8; Acc. 9, accession 9; Acc. 10, accession 10; Acc. 14, accession 14; TF%, ratio between the sum of the lengths of the short arms of individual chromosomes and the total length of the complement; SYi index, ratio between the average length of the short arms and the average length of the long arms.

[*] Classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971).

Seven quantitative parameters were measured including: length and width of the pod (mm), beak length (mm), number of seeds per pod, pod weight (g) and pod length/width ratio. The morphological study of the seeds was carried out on a sample of 20 seeds per accession and repetition. The following traits were measured: seed length and width (mm) and 100 seed weight (g). These analyses did not include accession 545 due to the lack of sufficient pods. Seed color was estimated on seeds of 30 pods taken randomly from each accession and repetition. There were five classes of color determined, 0: dark green, 1: light green; 2: light brown, 3: brown, 4: dark brown.

### 2.2.3. Statistical analysis

Data were expressed as mean ± standard deviation (SD). Karyotype and pod morphological parameters were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test when the *F*-value was significant. *P*-value less than 0.05 was considered significant.

In classifying the studied accessions, cluster analysis using SAS software (SAS, 2009) was performed and then a dendrogram was constructed. To determine the phenotypic diversity, the Shannon index diversity (*H'*) was used (Al-Nashash et al., 2007). Generally, this index can be applied for qualitative traits and quantitative ones through calculation of frequencies of phenotypic classes for each character.

## 3. Results and discussion

### 3.1. Karyological traits

The diploid chromosome number of *V. narbonensis* L. was found to be 14 in all the studied accessions and this result agrees with the previous counts of Schäfer (1973), Yamamoto (1973), Raina et al. (1989), Cremonini et al. (1998), Perrino and Pignone (1981), El-Bakatoushi and Ashour (2009) and Lamine (2009). Fig. 1 illustrates the mitotic metaphase chromosomes of the studied accessions and the karyogram of one of them. All the studied accessions have



**Figure 2** Morphology of pod types of the seven studied *V. narbonensis* L. accessions: A = accession 1; B = accession 7; C = accession P1; D = accession 545; E = accession 8; F = accession 9; G = accession 10. (Scale bar = 1 cm.)

the same karyotype formula (7sm). This agrees with that of Raina et al. (1989) who found that all chromosomes are sub-metacentrics (7sm). According to Navrátilová et al. (2003) and El-Bakatoushi and Ashour (2009), the existence of a single karyotype formula for different accessions of *V. narbonensis* L. may be due to the closest geographical origin and/or the similarity of altitudes from their original sites. However, Perrino and Pignone (1981) have mentioned two karyotypes in their study differing in chromosome rearrangement: karyotype with chromosomes all metacentric (7m) and karyotype with chromosomes all submetacentric (7sm). Yamamoto (1973) revealed four pairs of sub-metacentric chromosomes and three pairs of sub telocentric chromosomes (4sm + 3st).

Analysis of variance (ANOVA) showed that total haploid length, total chromosome length, chromosome length and total form TF% were significantly different among the studied *V. narbonensis* L. accessions ($P < 0.0001$) (Tables 2 and 3). In fact, the length of the total haploid complement (THCL) is considerably high (49.02 μ) compared to what was found by Yamamoto (1973) and was 39.15 μ for Australian accessions of *V. narbonensis* and what was also found by Cremonini et al. (1998) and was 27.14 μm for an Italian collection of *V. narbonensis* L. It varied from 41.02 μ for accession 9 to 53.35 μ for acc.10. The highest MCL was found in accession 10 (7.62μ) while the lowest MCL was found in accession 9 (5.86 μm). Karyotype formula was similar to all accessions. It is composed of seven metacentric chromosomes (7sm) one with secondary constriction.

All the studied accessions have karyotypes of sub metacentric chromosomes with a secondary constriction on the long arm of the shortest chromosome pair (Fig. 1). These results agree with those of Raina et al. (1989) but differ from those obtained by Perrino and Pignone (1981), Yamamoto (1973), Navrátilová et al. (2003), Kamel (1999) and El-Bakatoushi and Ashour (2009). In Reference to Sinha and Roy (1979), the presence in our material of only one pair with a secondary constriction revealed that the studied accessions did not undergo much of hybridization and they might have retained some of their primitive characters.

Our finding showed that the degree of karyotype asymmetry as indicated by TF% values ranged from 33.75% in accession

**Table 3** Karyological data of the studied accessions of *V. narbonensis* L.

| Accession/traits | THCL[*] | MCL[*] | Range | MCI[*] | KF |
|---|---|---|---|---|---|
| 1 | 47.37[ab] | 6.78[d] | 5.46–7.95 | 33.95[ab] | 6sm + 1sm$_{cs}$ |
| 7 | 51.56[ab] | 7.37[b] | 6.10–8.91 | 33.77[ab] | 6sm + 1sm$_{cs}$ |
| P1 | 51.56[ab] | 7.37[b] | 6.27–8.66 | 34.11[ab] | 6sm + 1sm$_{cs}$ |
| 545 | 52.15[ab] | 7.45[b] | 6.21–8.60 | 33.63[b] | 6sm + 1sm$_{cs}$ |
| 8 | 44.63[bc] | 6.38[e] | 5.33–7.56 | 35.32[a] | 6sm + 1sm$_{cs}$ |
| 9 | 41.02[c] | 5.86[f] | 4.55–6.93 | 35.36[a] | 6sm + 1sm$_{cs}$ |
| 10 | 53.35[a] | 7.62[a] | 6.14–8.93 | 34.54[ab] | 6sm + 1sm$_{cs}$ |
| 14 | 50.51[ab] | 7.22[c] | 6.02–8.53 | 34.67[ab] | 6sm + 1sm$_{cs}$ |

*Abbreviations:* 1, accession 1; 7, accession 7; P1, accession P1; 545, accession 545; 8, accession 8; 9, accession 9; 10, accession 10; 14, accession 14; THCL, total haploid chromosome length; MCL, Mean chromosome length; Range, range of chromosome length; MCI, mean centromeric index; KF, karyotype formula.
[*] Means with similar letters in a column are not significantly different at 5% level.

**Table 4** Comparison between accessions for pod and seed traits according to Duncan test at 5% and relative diversity index ($H'$).

| Traits/accessions | Acc.1 | Acc. 7 | Acc. P1 | Acc.8 | Acc.9 | Acc.10 | Acc.14 | $H'$ |
|---|---|---|---|---|---|---|---|---|
| Pod length (mm) | 56.95ab | 55.23b | 56.62b | 47.25c | 55.44b | 58.87a | 57.13ab | 0.75 |
| Pod width (mm) | 11.07a | 10.49c | 10.73bc | 8.81d | 11ab | 10.68bc | 10.60c | 0.68 |
| Seed number | 4.61bc | 4.38c | 4.33c | 5.02a | 4.43bc | 4.76ab | 4.53bc | 0.83 |
| Pod beak length (mm) | 5.49a | 4.78b | 3.99cd | 3.87d | 3.73d | 4.08cd | 4.31c | 0.75 |
| Pod weight (g) | 1.36a | 1.05cd | 1.11cd | 0.75e | 1.16bc | 1.26ab | 1.03d | 0.70 |
| PL/PW ratio | 5.17cd | 5.28bc | 5.29bc | 5.42ab | 5.06d | 5.52a | 5.41ab | – |
| Seed length (mm) | 7.4a | 7.3ab | 7bc | 5.8d | 7bc | 6.8c | 7bc | 0.80 |
| Seed width (g) | 5.7abc | 5.8a | 5.7ab | 4.5d | 5.6abc | 5.4c | 5.5bc | 0.92 |
| 100 SW (g) | 22.82a | 18.92b | 21.16ab | 13.82c | 19.52b | 20.01ab | 18.37b | 0.76 |

*Abbreviations:* Acc. 1, accession 1; Acc. 7, accession 7; Acc. P1, accession P1; Acc. 8, accession 8; Acc. 9, accession 9; Acc. 10, accession 10; Acc. 14, accession 14; PL/PW ratio, pod length/pod width; 100 SW, 100 seed weight; $H'$, relative diversity index.

545 to 35.42 in accession 8. The degree of symmetry as indicated by the SYi index varied from 51.01% in accession 545 to 54.85% in accession 8 (Table 2).

According to Stebbins (1971) classification, all the studied accessions fall in the A2 categories except accession 14 and accession 545 which fall in A1 and A3 category, respectively (Table 2). These results agree with those cited in Cremonini et al. (1998) who mentioned the following values for *V. narbonensis*: 56.05 for SYindex, 34.57 for TF% and A2 for the Stebbins category. As revealed by Zuo and Yuan (2011), a symmetrical karyotype is characterized by the predominance of m and sm chromosomes of the same size approximately. According to Kamel (1999), symmetrical karyotype and longer chromosomes are considered more
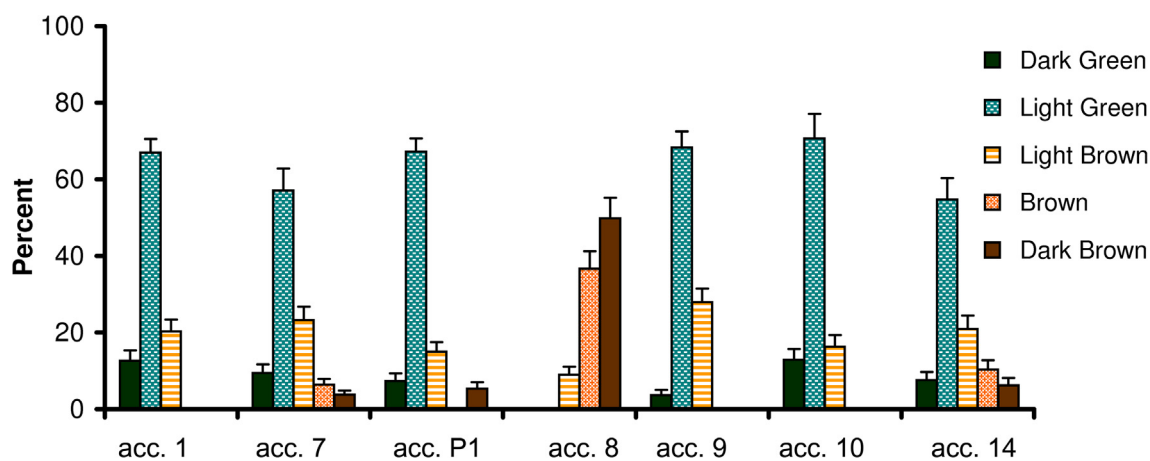


**Figure 3** Variation within and between the seven studied accessions of *V. narbonensis* L. for seed color. *Abbreviations:* Acc. 1, accession 1; Acc. 7, accession 7; Acc. P1, accession P1; Acc. 8, accession 8; Acc. 9, accession 9; Acc. 10, accession 10; Acc. 14, accession 14. Data are plotted as mean (±SD) percentage of seed color for each accession.

primitive than asymmetrical chromosomes and shorter ones. In addition, it is considered that median centromeres with chromosome arms of same length are more primitive than chromosomes with arms of different length.

The MCI value varied between 33.63% and 35.36% (Table 3). This result agrees with that observed by Raina et al. (1989), Cremonini et al. (1998) and Ashour et al. (2005). According to El-Bakatoushi and Ashour (2009) and Navrátilová et al. (2003), the similarity between accessions for the karyotype formula could be due to the similarity of their geographic origin and/or to the similarity of altitude of their origin sites.

### 3.2. Pod and seed characteristics

#### 3.2.1. Quantitative traits
Analysis of variance of pod and seed traits revealed significant differences among accessions ($P < 0.01$) for all measured traits (Table 4).
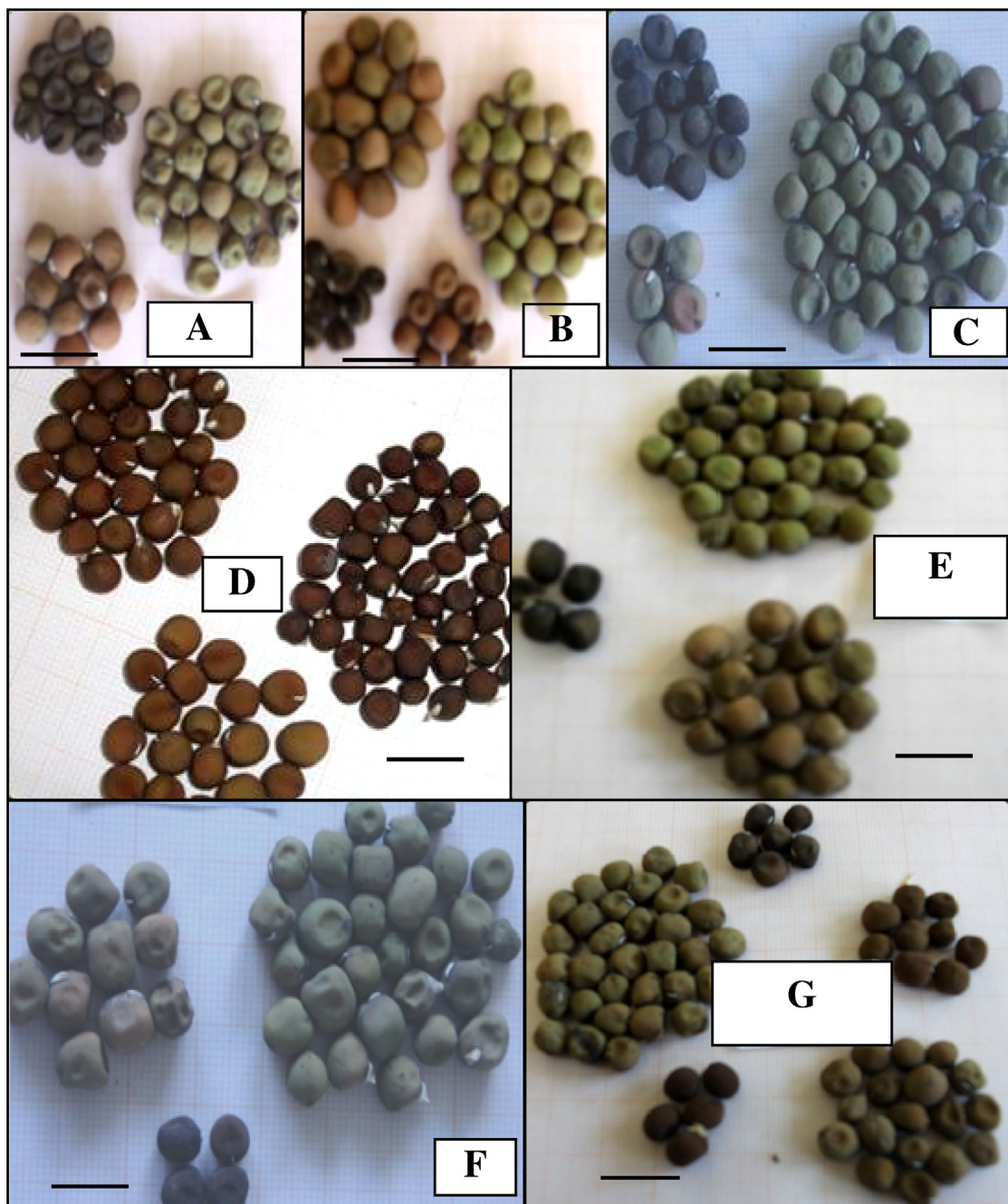


**Figure 4** Variation in seed color of the seven studied *V. narbonensis* L. accessions: A = accession 1; B = accession 7; C = accession P1; D = accession 545; E = accession 8; F = accession 9; G = accession 10. (Scale bar = 1 cm.)

The average pod length ranges from 47.25 mm in accession 8 to 58.87 mm in accession 10 while the average width of the pod ranges from 8.81 mm in accession 8 to 11.07 mm in accession 1.

These parameters were measured by (Berger et al., 2002) for the variety *narbonensis* and the observed values are 44 ± 0.1 mm for the average length of the pod and 9 ± 0.03 mm for the average width of the pod which approximates accession 8.

Concerning the other accessions, the observed values are close to those found by the same author in the variety *aegyptiaca* which are 54 ± 0.2 mm for the length and 11 ± 0.05 mm for the width of the pod. Pod beak length varied between 3.73 for accession 9 and 5.49 for accession 1 (Fig. 2).

### 3.2.2. Qualitative traits

Seed color is the only qualitative parameter measured and evaluated according to five types of classes. A significant variation was observed between and within accessions for this parameter (Fig. 3).

There is a predominance of the light green color in all accessions except in accession 8 in which the dark brown color dominates (Fig. 3). Accession 7 and accession 14 have seeds with 5 colors while accession 1, accession 9 and accession 10 have seeds with 3 colors and accession P1 has seeds with 4 colors (Figs. 3 and 4). A previous study on color traits in common vetch (*Vicia sativa* L.) has shown a variation in seed color which was explained by pleiotropism (Chowdhury et al., 2004).

### 3.2.3. Diversity index (H′) for the pod and seed traits

To estimate the phenotypic variation of the measured parameters, the relative diversity index (H′) was calculated for each morphological parameter from the frequencies calculated for each phenotypic class on pod and seed traits (Table 4). Morphological description of the accessions showed significant phenotypic diversity for the following characters: seed length and color (H′ = 0.92 and 0.80, respectively) and seed number per pod (H′ = 0.83).

In fact, seed length is highly polymorphic showing three phenotypic classes namely: small seeds, large seeds and medium seeds. The number of seeds per pod is highly polymorphic with the prevalence of the class with 5 seeds per pod. Seed color also showed a significant polymorphism characterized by the predominance of light green color (55.01% of seeds) compared to the other colors (7.7% of seeds with dark green color, 18.9% of seeds with light brown color, 7.63% of seeds with brown color and 9.32% of seeds with dark brown color).

### 3.3. Correlations between pod and seed morphology and karyology

Significant correlations were found: centromeric index and pod beak length ($r = -0.76^*$. $n = 7$); pod length is correlated with pod weight ($r = 0.84^{**}$), seed length ($r = 0.79^*$), seed width ($r = 0.82^*$) and 100 seed weight ($r = 0.84^{**}$); seed number is correlated with seed width and length ($r = -0.82$; $r = -0.90$) and 100 seed weight ($r = -0.62$). Pod weight is correlated with seed length and width ($r = 0.75$. $r = 0.72$, respectively) and 100 seed weight ($r = 0.93$). These correlations showed that accessions with long beak pods have low centromeric index. According to Yamamoto (1973), there is no clear relationship between morphological parameters and karyotype in the genus *Vicia*. Also, the higher the seed number the smaller they are.

### 3.4. Cluster analysis

Cluster analysis of all traits showed three groups of accessions (Fig. 5): accession 8 and accession 9 from two separate groups while the remaining accessions form one group with two sub groups (accession 1 as a first sub group and accession 7, accession 14, accession P1 and accession 10 as the second subgroup). Accession 8 is the more distant one. It has the shortest mean chromosome length (MCL), the lowest mean centromeric index (MCI) and the smallest seeds and pods. It is also the only accession which has dark brown seed color. Accession 9 forms an out group; it has the shortest pod beak. Both accessions (accession 8 and accession 9) have different geographical origin and belong to different botanical varieties. Accession 1 had the largest pods and seeds and the longest pod
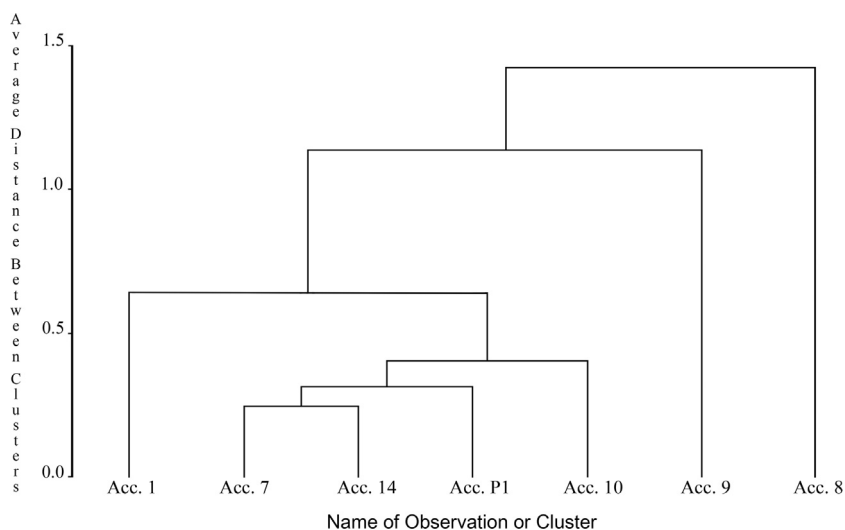


**Figure 5** Dendrogram of *Vicia narbonensis* accessions according to cluster analysis based on karyological and pod and seed morphological traits. *Abbreviations:* Acc. 1, accession 1; Acc. 7, accession 7; Acc. 14, accession 14; Acc. P1, accession P1; Acc. 10, accession 10; Acc. 9, accession 9; Acc. 8, accession 8.

beak. Accession 7 and accession 14 are similar for all traits but different in origins. Accession P1 and accession 10 are quite similar botanically, different in geographical origin and attached to the previous ones.

## 4. Conclusion

All accessions of *V. narbonensis* L. revealed to be diploid ($2n = 2x = 14$). The karyotype consists of 7 pairs of submetacentric chromosomes, the shortest one with a secondary constriction on the long arm. These features argue for a symmetrical and primitive karyotype. However, a variation in chromosome length was observed among the accessions. The similarity of the geographical origins of the studied accessions may explain the existence of a common karyotype formula. For breeding purposes and with reference to the results got we have to take into account in hybridization programs.

### Conflict of interest

The authors declare that there are no conflicts of interest.

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