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Characterization of *Pisum sativum* Mediterranean accessions by qualitative traits.

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Abstract

Nowadays, pea field is getting more attention because of its nutritional values. The objective of this paper is to characterize and identify relationships among 21 Mediterranean pea accessions. Fifteen accessions of *Pisum sativum* subsp. elatius and six accessions of *Pisum sativum* subsp. *arvense* were provided by ICARDA (International Centre for Agricultural Research in Dry Areas). Twenty qualitative traits were selected according to the guidelines of the International Union for the Protection of New Varieties of Plants (UPOV). We measured eight vegetative qualitative traits such as presence or absence of leaflets, type of plant axis development and presence or absence of anthocyanin coloration. We measured twelve floral qualitative traits such as standard color, pod color and curvature of the pod. In order to study the clustering of our accessions, we used Past4.03 with the test UPGMA (unweighted pair group method with arithmetic mean). The biometric and descriptive analysis we carried out reveals the presence of traits that discriminate accessions. Among these traits, we can cite C15 (vegetative qualitative trait), C23, C31 and C35 (floral qualitative traits). C15 discriminates the accessions TUR10, TUR8, SYR1 and SYR2. C23 distinguishes TUR6, TUR11 and AL2 accessions from other accessions. C31 is a discriminating trait for TUR13 accession. The type of starch grain (C35) discriminates the TUR14 accession. Cluster analysis led to establishing dendrogram allowing a grouping of accessions according to their geographical affiliation. In fact, it is possible to discriminate significantly the accessions TUR10, SYR1 and SYR2 from the accessions TUR12, TUR13, GR1 and TUN which could be an incentive for further improvement and better exploitation.

1. INTRODUCTION

Legumes are the third largest family of angiosperms belonging to Fabaceae/ Leguminosae (Gepts et al. 2005; Gepts et al. 2005). *Pisum sativum* are the main leguminous food and the second largest in the world after common beans Phaseolus vulgaris L. The growing demand for protein rich in raw materials for animal and/or human feed has led to greater interest in this crop as a source of protein (Kharrat and Maatougui, 2019 ; Sai Kachout et al. 2021). Their culinary benefits on human health as well as their benefits on animal nutrition are well recognized (Kharrat and Maatougui, 2019).

In fact, pea seeds are rich in protein (23 to 25%), slowly digesting starch (50%), soluble sugars (5%), fiber, minerals and vitamins (Janko et al. 2017; Sai Kachout et al. 2021). Protein peas (harvest of mature seeds), derived from box peas, were selected for their high production of high protein and starch seeds and low in anti-trypsic compounds (Santos et al. 2019). Green pod and dry seed of *Pisum sativum* L are also essential sources of protein and vitamins used for human and animal food. An increase in unit area yield and pod quality could be achieved by growing new cultivars with high pod yields and / or by improving cultivation practices (Rashwan and El-Shaieny, 2016).

Pisum sativum L. is an economically important crop as it contributes to the development of low input farming systems by fixing atmospheric nitrogen and it serves as a disruption crop which further minimizes the need for external inputs (Alizadeh et al. 2016). It is suitable for head rotation, increases soil fertility and erosion control (Srarfi Ben Ayed et al. 2017; Coyne et al. 2020). Furthermore, field peas rank third in world production among food legumes (Ghafoor and Arshad, 2008).

Peas are subdivided into different species, subspecies and groups, according to the size of the plant (tall, half-dwarf, dwarf), the type of foliage (afila, leaf), the coloration of the flowers (white, purple, etc.) and the morphology of the fruits and seeds. Boyeldieu (2003) reported that there are three main species of the genus Pisum or subspecies of *Pisum sativum*. The first is P. sativum elatius, representing wild peas. The second is *Pisum arvense* L., encompassing field peas or fodder peas, this species is characterized by its colorful flowers and small, stipules with red collar, leaves with 2-4 leaflets and small seeds with colored teguments. The third main species, *P. sativum* L. or *P. sativum* hortense Asch and Graebn is garden pea. The protein cultivars were selected from this species by hybridization between *P. sativum* and *P. arvense*. In addition, Cousin (1992) classified pea varieties into different groups: Feed peas, Market peas, Edible peas, Casserie peas, Canned peas and Protein peas (Srarfi Ben Ayed et al. 2017).

Aware of the agronomic and economic interests that could be presented by the cultivation of this species in Tunisia, rehabilitation programmes have been suggested to intensify production in appropriate regions (humid wet and sub-humid wet bioclimatic stages), the research and exploitation of varieties adapted to certain biotic and abiotic stresses and the improvement of the organoleptic and nutritional qualities of the seed (Mohamed et al. 2019) Improved legume production can create local value-added processing opportunities, boost domestic demand and provide off-farm employment, income sources and enriched food for smallholder and resource-poor farmers (Getachew, 2019; Kebede, 2020). Therefore, the selection of new varieties of peas becomes a priority in Tunisia to restore this crop to its true place in regional crop rotations as a welding plant and as a plant that improves the physico-

Table 1. List of traits recorded on several accessions of the field pea following the International Union for the Protection of New Varieties of Plants (UPOV, 2009) recommendations for *Pisum sativum*.

Vegetative qualitative traits		**Code**
1	Presence or absence of leaflets	C3
2	Type of plant axis development	C4
3	Presence or absence of anthocyanin coloration on stem and foliage	C5
4	Presence or absence of anthocyanin coloration on stipules	C6
5	Leaflet teeth	C8
6	Size of leaflets	C11
7	Maculation on the stipules	C12
8	Stipule size	C15
Floral qualitative traits		**Code**
9	Standard color	C20
10	Wing color	C21
11	Pod color	C23
12	Curvature of the pod	C26
13	Shape of the distal part on the pod	C27
14	Seed shape	C30
15	Seed color	C31
16	Color of hilum on seed	C32
17	Cotyledons color	C33
18	Intensity of wrinkles on cotyledons	C34
19	Type of starch grains	C35
20	Presence or absence of purple or pink spots on the seed coat	C38

chemical qualities of the land. Selection objectives must meet both productivity and nutritional requirements.

This study was carried out with the aim of exploiting the genetic diversity of twenty-one accessions of *Pisum sativum* from a collection provided by ICARDA, in order to determine the diversity between genotypes in terms of morphological traits and to detect the level of genetic diversity between the genotypes studied. The most interesting material will be used as breeding material for future crossings as part of the pea enhancement program (*Pisum sativum*).

2. MATERIALS AND METHODS

2.1. Plant material

The study covered 21 accessions of the genus *Pisum sativum*, from different Mediterranean countries, provided by ICARDA (International Centre for agricultural research in dry areas). We have 15 *Pisum sativum* subsp eliatus: one from Greece, two from Syria, 11 from Turkey and one from Morocco. We have six *Pisum sativum* var. *arvense*: three from Turkey, two from Algeria and one from Tunisia. Tunisian variety is called Yamama supplied by INRAT and listed in the J.O.R.T. (2009).

2.2. Experimental design and trial management

The experimental design is completely randomized based on 30 plants initially for each accession. The trial was conducted on a plot at the Faculty of Science of Tunis in plastic pots. Before planting, hand weeding was carried out to remove the roots of the weeds and to clean the soil of the parasites (larvae or insects) thus minimizing the risk of competition or disease transmission. In several species of the legume family, the seed is covered with an impermeable layer that protects the surface of the seed and prevents water from entering the embryo. For germination to occur, this impermeable layer must be broken, shredded or cracked (Vallée et al. 1999). For this reason, we performed a scarification of the seed before planting. Daily maintenance and monitoring of the plot was carried out from planting to harvesting.

2.3. Measured qualitative traits

In order to study the diversity of our 21 accessions, we have chosen 20 qualitative traits from UPOV (2009) described in Table 1 and Fig.1.

2.3.1. Vegetative qualitative traits

Presence or absence of leaflets (C3): a leaflet is a leaf-like part of a compound leaf. Though it resembles an entire leaf, a leaflet is not borne on a main plant stem or branch, as a leaf is, but rather on a petiole or a branch of the leaf.

Type of plant axis development (C4): plagiotropic axis or for all the accessions. Similar vegetative metamers may behave slightly differently becoming either a vertical or horizontal axis. A vertical axis, which is also called an orthotropic axis, has an essentially radial symmetry. The horizontal, or plagiotropic axis, is normally dorsiventrally flattened but can arise in other ways (Ingrouille, 1992).

Presence or absence of anthocyanin coloration on stem and foliage (code C5): on young plants or new twigs, when the production of chlorophyll has not yet begun and the plant is

Fig. 1. Vegetative and floral qualitative traits. **a** : Anthocyanin coloration on stem ; **b** : Anthocyanin coloration on leaflet ; **c:** Maculation on stipules ; **d:** Anthocyanin coloration on stipules ; **e**: Intensity of wrinkles on cotyledons ; **f**: Light color of the hilum on the seed ; **g**: Dark color of the hilum on the seed ; **h**: Standard colors ; **i**: Purple on seed tegument.

therefore unprotected against ultraviolet, the production of anthocyanin increases. As soon as chlorophyll production begins, anthocyanin production is reduced. The level of anthocyanins produced depends on the type of plant, substrate, light and temperature. It has also been found that red coloring makes it possible to camouflage against herbivores that are unable to see the red wavelengths. In addition, anthocyanin synthesis often coincides with the synthesis of phenolic compounds unpleasant to taste. Anthocyanin coloration should be considered present if there is anthocyanin in one or more of the following: seed, foliage, stem, axilla, flower or pod.

Presence or absence of anthocyanin coloration on stipules (code C6): stipules have sometimes red spots, characteristic of certain varieties, especially in feed peas.

Leaflet teeth (code C8): it can be absent (0), weak (1), medium (2), strong (3) or very strong (4).

Leaflet size (code C11): It can be very small (0), small (1), medium (2) or large (3).

Maculation on stipules (code C12): maculations are white spots that are dotted all over the surface of the stipules. The examination should be performed on the main rod only. The presence of maculas on a forepart of the main stem means that maculas are present. Care must be taken to ensure that, at the lower nodes, senescence has not begun on the foliage prior to observation. The plant must have at least eight nodes because in some varieties the macules do not express at the lower nodes (UPOV 2009).

Stipule size (code C15): the stipule size can be either very small (0), small (1), medium (2), large (3), or very large (4).

2.3.2. Floral qualitative traits

Standard colors (C20): five standard colors were observed (pale pink, bluish white, white, pink and purple).

Wing colors (C21): four wing colors were observed (dark purple, purple, pink and white).

Pod colors (code C23): color is either yellow (0) or green (1).

Curvature of the pod (code C26): curvature is either absent or very weak (0), weak (1) or medium (2).

Shape of the distal part on the pod (code C27): shape of the distal part on the pod is measured qualitatively. The shape is either pointed (1) or truncated (2). Observations should be made on several nodes of each plant when the pods are fully developed but prior to senescence (UPOV 2009).

Seed shape (code C30): the seed shape can be:

- ellipsoid (0): seeds with zero or very low compression on the root and/or distal surfaces.
- cylindrical (1): seeds compressed on root and distal surfaces. Square to rectangular or with rounded sides in the longitudinal section.
- Rhomboid (2): seeds compressed irregularly on the root and distal surfaces but also irregularly on abaxial surfaces.
- irregular (3): seeds compressed irregularly; they do not have any of the above forms. All these descriptions are drawn according to the principles of UPOV (2009).

Seed color(C31): three different colors were observed (green , brownish green and yellow).

Color of the hilum on the seed (code C32): it is an observation with the naked eye of the color of the hilum on the seed. Color may be the same or lighter than in teguments (0) or much darker than in teguments (1).

Cotyledon color (code C33): this is an eye observation of the cotyledon color. The color is green (0), yellow (1) or orange (2).

Intensity of wrinkles on cotyledons (code C34): The intensity of wrinkles on cotyledons is absent or low(1), medium(2) or high(3).

Type of starch grains (code C35):

1) After removal of the integuments, fine tissue fragments shall be extracted from a cotyledon and placed on a microscope slide. A water droplet is added to the extracted tissue and another microscope slide placed above it. The water and fabric mixture is then slightly flattened between the two blades. Excessive pressure causes grain fragmentation; too low pressure makes the object holder insufficiently thin for examination (UPOV, 2009).

2) A microscope with X16 eyepieces and X10 or X40 lenses is most appropriate. For the examination of compound grains, the objectives at the highest magnification must be used (UPOV 2009).

3) Single beans are similar in shape to wheat or coffee beans and often present as a full-length suture line (UPOV 2009).

4) Compound grains have an irregular star shape and appear to consist of several segments. Their centre may appear cruciform. In varieties with a high degree of sweetness, compound starch grains are very small and few in number (UPOV 2009).

Presence or absence of purple or pink blotches on seed tegument (code C38): two types of blotches on seed tegument were observed.

2.4. Cluster analysis

The cluster analysis was performed using Past 4.03 using unweighted pair-group method with arithmetic averages (UPGMA) based on Euclidean distances as similarity measures. UPGMA allows us to group accessions based on qualitative traits.

3. RESULTS AND DISCUSSION

Qualitative biometric and descriptive analysis carried out revealed the presence of discriminatory characteristics with regard to accessions. These traits include those that discriminate the most accessions.

3.1. Vegetative qualitative traits

The development of axis (C4) is plagiotropic for all the studied accessions. For anthocyanin trait (C5), no discrimination was observed between the accessions and they all show pigment spots either on the stem or on the leaves but no anthocyanin coloration was observed at the level of the flowers. Accessions TUR2, TUR 9, TUR 13, TUR 14, AL1, AL2, and MAR1 don't show anthocyanin coloration on stipules (C6). However, the other accessions show a nectar spots in the central part of the stipules. No variation was observed for the trait presence or absence of leaflets (C3) since all accessions have leaves. However, the trait size of the leaflets (C11) brings together the accessions in four different groups. A group with a very small size of leaflets formed by TUR8, SYR1 and SYR2. A group with small leaflets including TUR1, TUR3, TUR4, TUR5, TUR6, TUR7, TUR9, TUR10, TUR11 and TUR14. A group formed by TUR2, TUR13, AL1, AL2, MAR1 and TUN. These accessions have a medium leaflets size. In addition, a group that includes accessions GR1 and TUR12 with a large size of leaflets (Fig. 2.).

Accessions can be classified into five different groups: (1) the TUR10, SYR1, SYR2 group with a very small stipules; (2) The group comprising TUR4, TUR5, TUR6, TUR7, TUR9, TUR11 and TUR14 which represent a small stipules size; (3) A group formed by TUR1, TUR2, TUR3, AL1, AL2 and MAR1 with medium stipules size; (4) A group of accessions GR1, TUR13 and TUN with large stipules size; (5) A last group formed by TUR12 characterized by a very large stipules size (Fig. 3.). For the trait maculation on stipules (C12) accessions can be classified into five different groups. Accessions with a very loose maculation on the stipules TUR9, TUR10, TUR12, TUR14, SYR1 and SYR2. Accessions with loose maculation are TUR1 and TUR13. Accessions with medium maculation are GR1, TUR3, TUR4, AL1, AL2, MAR1 and TUN. Accessions with a dense maculation on the stipules are TUR6 and TUR11 and accessions with a very dense maculation on the stipules are TUR2, TUR5 and TUR7.

3.2. Floral qualitative traits

Only thirteen of the first 21 accessions

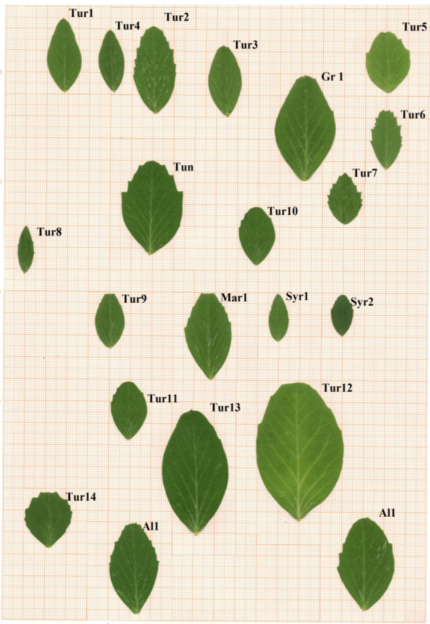

Fig. 2. Leaflets size of the studied accessions.

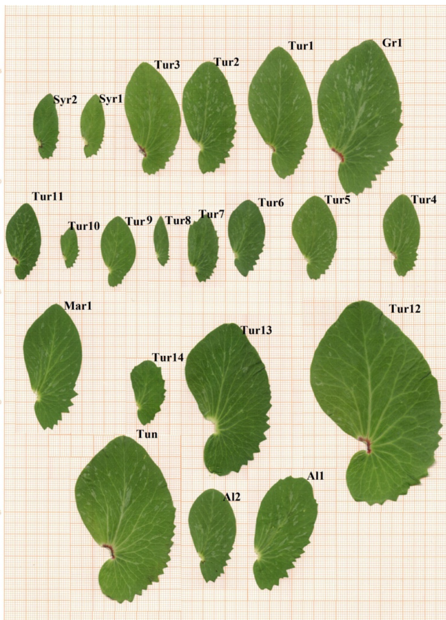

Fig. 3. Stipule size of the studied accessions.

concerned the floral stage, taking into account the decrease in the number from the vegetative to the floral stage, which leads us to treat the stages separately. The accessions at this stage concerned: GR1, TUR1, TUR2, TUR3, TUR4, TUR5, TUR6, TUR11, TUR12, TUR13, AL1, AL2 and TUN. For standard color (C20) Accessions can be classified into five different groups. A first group of accessions with a pale pink color of the standard includes GR1, TUR1, TUR6, TUR11, AL1 and AL2. A second group characterized by a blue white standard comprises the following accessions: TUR1, TUR12 and TUN. A third group is formed by TUR4 and TUR13. These accessions are characterized by the white color of the standard. A fourth group formed by TUR5 which presents a pink color of the standard. The last group is formed by TUR3. This accession has a purple color of the standard. Wing color trait (C21) differs from one accession to another. Four colors are highlighted. Reddish purple: this color is observed in the following accessions: GR1, TUR2, TUR5 and TUR6. Violet: this color is noted in TUR1, TUR3, TUR12, AL1 and TUN. Pink: this color applies to the following accessions TUR4, TUR11 and AL2. White: this color is observed only in TUR13 accession.

Pod color trait (C23) may be green or yellow. This character makes it possible to distinguish the accessions TUR6, TUR11 and AL2 from the other accessions which have a yellow color of the pod. The accessions with a green color of the pod are GR1, TUR1, TUR2, TUR3, TUR4, TUR5, TUR12, TUR13, AL1 and TUN.

This trait curvature of the pod (C26) allows us to classify accessions into three groups: (1) the accessions with absence or very low curvature of the pod and which are: TUR1, TUR3, TUR5, TUR6, TUR11, TUR13, AL1, and AL2; (2) the accessions with a low curvature of the pod: TUR2, TUR4 and TUN; (3) Accessions with a medium curvature of the pod: GR1 and TUR12.

The trait shape of the distal part on the pod (C27) subdivides accessions into two groups: accessions having a truncated shape of the distal part of the pod (GR1, TUR2, TUR3, TUR4, TUR11, TUR12, AL1,AL2 et TUN) and accessions TUR1, TUR5, TUR6 and TUR13 having a pointed shape of the distal part of the pod.

Examination and observation of the seeds harvested from the accessions studied show that seed shape trait (C30) they can be grouped into three groups: ellipsoid seeds (TUR1, TUR3, TUR6 and TUR13); cylindrical seeds (TUR2, TUR4, TUR5, TUR11, AL1, AL2 and TUN); seeds of irregular shape (GR1 and TUR12).

However, according to seed color trait (C31), we distinguish three different seed colors. C31 is a qualitative trait discriminating for TUR13 accession. Indeed, this accession has a seed color (yellow) that differs from other accessions. Green seeds are characteristic of the following accessions: GR1, TUR1, TUR2, TUR3, TUR4, TUR6, AL1, AL2 and TUN accessions. Brown green seeds are observed in TUR5, TUR11 and TUR12.

The observation of the color of the hilum on the seed (C32) for the 13 accessions studied makes it possible to partition them in two groups: accessions with the same hilum color or much lighter than integument are GR1, TUR5, TUR12, TUR13, AL1, AL2, and TUN; accessions having a darker color of the hilum than the integument and are TUR1, TUR2, TUR3, TUR4, TUR6 and TUR11.

The character color of cotyledons (C33) is characterized by the discrimination of TUR13 accession, which presents an orange color of the cotyledons. The remaining accessions have a yellow cotyledon color.

The intensity of wrinkles observed on the cotyledons (C34) of the different accessions may be absent or weak, medium or strong. The accessions that are characterized by absence or low intensity on wrinkles are TUR4, TUR13 and TUN. The accessions with an average intensity of wrinkles on the cotyledons are TUR1, TUR2, TUR3, TUR5, TUR6, TUR11, AL1 and AL2. Accessions with high wrinkle intensity are GR1 and TUR12.

The type of starch grain C35 which is a qualitative trait makes it possible to discriminate TUR14 accession which presents a compound starch grain unlike other accessions characterized by a single type starch grain.

The presence or absence of purple or pink spots on seed coat (C38) trait breaks up accessions into two different groups: (1) a first group formed by accessions GR1, TUR4, TUR5, TUR11 and TUR12 which have purple spots on the seed coat; (2) A second group that gathers the rest of the accessions and does not have purple or pink spots on the seed coat.

In conclusion, the biometric and descriptive analysis that we carried out reveals the presence of traits that allow discriminating accessions. Among these traits, we can cite C15 that makes it possible to distinguish between the accessions TUR10, TUR8, SYR1 and SYR2 which differ very significantly from the accessions TUR12, TUR13, GR1 and TUN. C23 makes it possible to distinguish TUR6, TUR11 and AL2 accessions from other accessions which have a yellow color of the pod. C31 is a qualitative discriminating characteristic for TUR13 accession. Indeed, this accession has a seed color (yellow) which differs from other accessions. The type of starch grain (C35) which is a qualitative characteristic makes it possible to discriminate the TUR14 accession which has a compound starch grain unlike other accessions characterized by a starch grain of simple type.

Turkey, one from Tunisia and one from Greece. B2 included 6 Turkish accessions that have small leaflets and small stipules. B3 consisted of 3 accessions from the adjacent countries Algeria and Morocco and that are characterized by similar vegetative traits: do not show anthocyanin coloration on stipules medium leaflets size, medium stipules size and medium maculation on the stipules. The accessions TUR1, branched also in group B rather independently

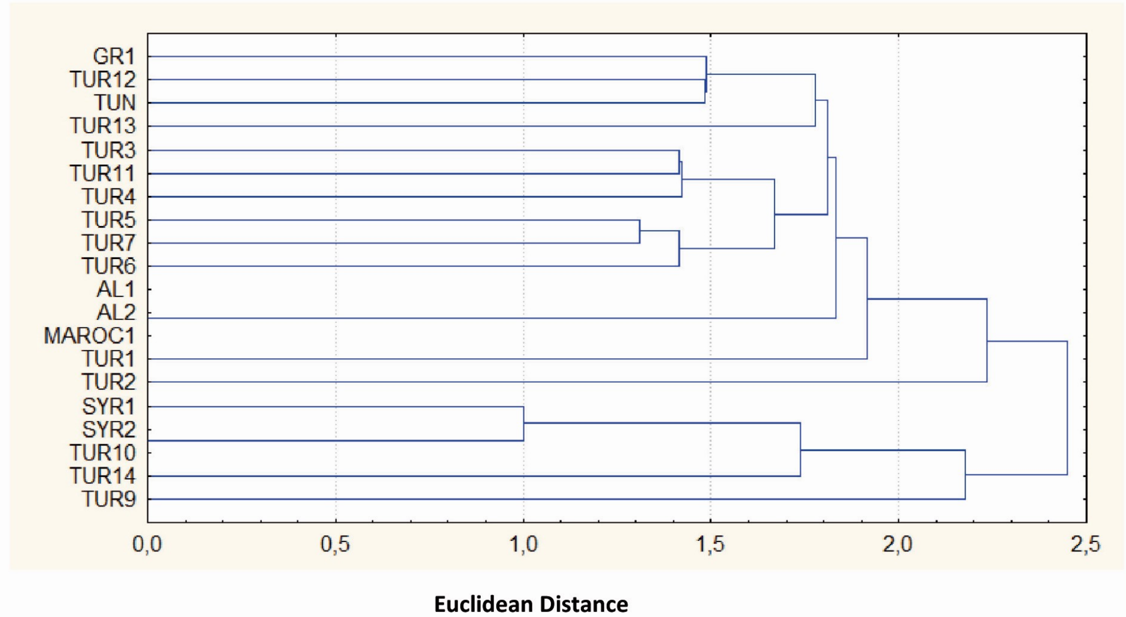

Fig. 4. Dendrogram of hierarchical clustering of *Pisum sativum* accessions based on 8 vegetative qualitative traits.

3.3. Cluster analysis of vegetative qualitative traits

TUR8 accession has showed a low number of accessions at vegetative state less than 10. For thus, it was not considered for the study of the dendrogram. The dendrogram generated based on eight vegetative qualitative traits for the remained 20 accessions on vegetative data set (Fig. 4) showed two main groups: group A including 5 accessions and group B including 15 accessions. Group A consisted of the two Syrian accessions (SYR1 and SYR 2) and 3 Turkish accessions (TUR9, TUR10 and TUR14). Accessions in this cluster are characterized by very small leaflets, very small stipules and a very loose maculation on the stipules for SYR 2 and TUR10 showed the same vegetative traits. Group B, including 15 accessions and subdivided in 3 main subgroups. B1 comprised 4 accessions that showed large leaflets and large stipules. Two of them (TUR12 and TUR13) originate from from the other accessions which is characterized also by small leaflets, small stipules and a very loose maculation on the stipules.

Dendrogram revealed confirm a distinction between the accessions related to the discrimination found among qualitative traits. In fact, it is possible to discriminate significantly the accessions TUR10, SYR1 and SYR2 from the accessions TUR12, TUR13, GR1 and TUN. The qualitative traits studied confirmed that TUR13 and TUR14 diverged from the other accessions.

3.4. Cluster analysis of floral qualitative traits

The dendrogram generated by the 12 floral qualitative traits for the 13 accessions studied with a number of accessions more than 25 showed the cluster represented by Fig. 5. We observed two main groups. Group A including the first one grouped (GR1, TUR2, TUR6, TUR4, TUR11, AL1, and AL2) with low curvature of the pod. Group B including (TUR1, TUR3, TUN and

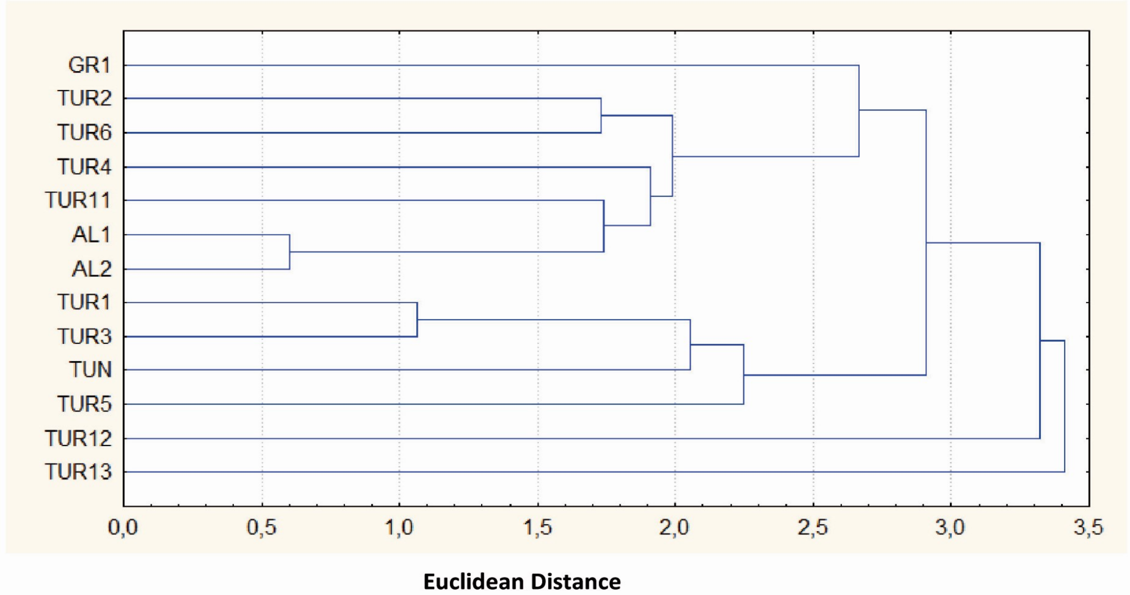

Fig. 5. Dendrogram of hierarchical clustering of *Pisum sativum* accessions based on 12 floral qualitative traits.

TUR5). The accessions TUR12, and TUR13 branched rather independently from the other accessions characterized TUR12, and accession). C15 trait makes possible to distinguish between TUR12, TUR13 from the other accessions. However, C31 discriminate TUR13 accession.

4. CONCLUSION

In the scenario of the present subject, we have reasoned that the qualitative traits were useful to assess the phenotypic diversity in *Pisum sativum* accessions. The results revealed considerable diversity within *Pisum sativum* accessions. Our work could be incentive for their improvement and exploitation in our agriculture. The most interesting material will be used for future crosses are TUR12 which is close to TUN with a significant vegetative mass and anthocyanin central coloration spot on stipules. Indeed, the lack of research on the genetic and adaptive potential of pea varieties to biotic and abiotic stress has contributed significantly to the decline of their culture (Halila, 2003). For this, precise labelling of plant material agronomic, biochemical, physiological and genetic characteristics must be established to create varieties adapted to our environment.

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