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# Evaluation of morphological and anatomical characters for discrimination and verification of some *Medicago sativa* (L.) Cultivars

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**ABSTRACT**

*Medicago sativa* L. (alfalfa) is one of the most important legume forages in the world. The objective of this study was to characterize and discriminate among 15 alfalfa cultivars with a different geographical origin. Macro-morphological and anatomical characters as well as seed coat sculpture were investigated. Twenty five morphological characters were extracted directly from the fresh specimens. Transverse section in the main stem were carried out; stained and seventeen anatomical characters were examined by light microscope. Seed coat surface was investigated using Scanning Electron Microscope (SEM). Data obtained were coded and analysed using NTsys-Pc software (Version 2.02) and the resulted dendrogram is discussed. The results showed morphological and anatomical variation between the studied taxa. Vascular bundles ranged from 16 to 23. The Egyptian cultivar Nubaria has the lowest number of vessels (16) while the American Super supreme has the larger number (23). The seed coat ornamentation revealed five main surface patterns and suggests the presence of variations in anticlinal boundaries and periclinal walls that provide stable diagnostic characters for morphologically closely related taxa. The dendrogram showed that the Egyptian cultivar Nubaria was the most distant and clustered separately from all the other alfalfa cultivars which were grouped into two main clusters. Seed coat morphology and combination of other plant morphological and anatomical characters permitted identification and discrimination between the examined cultivars. Results obtained in this work could be considered for further breeding strategies and studies.

**Key words**: *Medicago sativa* L., Macro-morphology, Stem anatomy, Seed coat sculpture, SEM.

## INTRODUCTION

*Medicago sativa* L. (Alfalfa or Lucerne) is a perennial herbaceous legume, exists in many countries. Alfalfa originated from the Mediterranean basin and southwest Asia and was one of the first forage crops to be domesticated (Cook *et al*., 2005). It is cultivated in more than 80 countries in an area exceeding 35 million ha (Radovic *et al*., 2009). Alfalfa has good adaptability to different environmental conditions due to its variable genetic base (Radovic *et al*., 2009). Due to its high nutritional quality, alfalfa is one of the most important legume forages of the world. There are numerous cultivars of alfalfa have been improved over the last century, primarily through breeding for disease resistance and stress conditions such as winter hardiness, drought and salinity resistance (Lehman *et al*., 1992). This improvement process may have altered some of the traits of ancestral populations (Rumbaugh, 1991). Testing techniques are required to ensure that any cultivar released by plant breeders is not previously registered one. In the early part of cultivar improvement, problems were created by frequent changing of the names of cultivars. Protection of plant varieties and farmers right authority insists on characterization and registration of extant and new varieties as a part of national and botanical asset.

Plant morphological characters are the direct expression of the genetic construction of a genotype. Morphological characters as those of habit, flower, fruit, and seeds have been used in different systems of classification (Smith *et al*., 1991; Loumerem *et al*., 2007). According to the International Union for the Protection of New Varieties of Plants (UPOV 1988, 2005) the three basic criteria; distinctness, uniformity and stability (DUS) should be fulfilled for registration of a new cultivar. Knowledge of this variability should provide useful information concerning the potential value of these cultivars.

Anatomical parameters may play an important role in plant taxonomy (Metcalfe and Chalk, 1957; Teuber and Brick, 1988). However, they have been largely unexploited in taxonomic studies because of their restricted value for distinguishing species or taxa of infra specific rank. Anatomy can supply useful information for establishing interrelations between the species (Zoric *et al*., 2012); sometimes, it can also help in individual identification (Zoric *et al*., 2014).

Seeds tend to show less phonetic plasticity in comparison with other organs. The morphology of seed coat is usually stable and is little influenced by external environmental conditions (Fayed and Hassan, 2007).

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Therefore, seed characters can provide useful data in the delimitation and identification of taxa and has been found to useful taxonomic feature. The importance of ultra-structural pattern of the seed coat observed under the SEM has been well recognized as a reliable approach for assessing phonetic relationship and identification of taxa (Barthlott, 1981). Observations of many plant groups have shown that seed morphology and anatomic features are rather conservative, and hence taxonomically important (Zoric *et al*., 2012; Gabr, 2014; Heneidak and Abdel-Khalik, 2015).

The wide geographical distribution of Alfalfa populations across different environments will help in developing genotypes adapted to harsh environmental condition. Therefore, the present study is developed to discuss whether the plant morphology, stem anatomy and seed coat characters can provide an additional fundamental tool helping in assessing variability among local and exotic Alfalfa accessions. This will be helpful in directing conservation and breeding programs for the improvement of Alfalfa.

## MATERIALS AND METHODS

Fifteen cultivars collected from different geographical origin were used in the present study. These cultivars comprised seeds of two varieties obtained from the Forage Crop Department, Ministry of Agriculture, Giza, Egypt and thirteen exotic varieties kindly obtained from NAFA Agriculture Company, Saudi Arabia (Table 1). Some of the available cultivars have specific features of interest for breeding, such as characters for resistance to pests (SW 9720, Siri Nafa) or salinity (Super 10) and high productivity (Supreme forger, Nafa extra, Siriver).

Seeds of *Medicago sativa* L. were planted in botanical garden, Faculty of Science, Ain shams University. The macro-morphological characters of the studied cultivars were extracted directly from the fresh specimens. Data were recorded from ten plants from each cultivar and sample mean were calculated. Weight of 100 seed was obtained. Transverse section in the stem (third internode from the apex) was carried out, stained and examined by light microscope.

For SEM investigation, the seeds were fixed to specimen stub and to the specimen holder of SEM (FEI inspect S). Three samples of mature seeds were examined in each case. The detailed structure of the seed surface sculpture was observed far from the hilum. SEM photographs were taken using the computerized digital system of the microscope.

The descriptive terminologies of seed surface sculpture used here were that proposed by Barthlott (1981), Karam (1997) and Salimpour *et al*. (2007). In order to construct trees elucidating the relationships among the examined taxa, the coded data were analyzed using UPGMA (Sokal and Michener,1958) method based on a distance matrix. All analyses were performed with NTSYS-pc (Rohlf, 2000).

## RESULTS AND DISCUSSION

The cumulative macro-morphological characters of the taxa under investigation are shown in Table 2. All the accessions are herb showed an erect growth habit and most of them had medium internode length. Height of plants varied from cultivar to cultivar ranged from 42 to 80 cm. All accessions except the cultivar Nafa-extra have hairy petiole with length ranged from 7 to 40 mm. Terminal and lateral leaflet shape are grouped into four main types; obovate, ovate, rhomboid and elliptic. Leaf size is one of the morphological characters recommended for cultivar identification. Width/length ratio of terminal leaflet ranged from 0.19 to 0.53 while ranged from 0.25 to 0.43 in lateral leaflet. The Australian cultivar Siriver have the highest leaflet Width/length ratio. All terminal leaflet apex shape is mucronate except the cultivars Supreme forager and Perfect are hooked, while lateral leaflet apex shape is grouped into mucronate, notched and hooked. Nine accessions showed leafy stipule while six showed hairy stipule. Flower colour:

**Table 1:** List of 15 *Medicago sativa* L. cultivars used in the study and their origins

| Cultivar | Source |
|---|---|
| SW 9720 | Cal/West seeds Co. Genetics International Inc. California USA – |
| SW 9628 | Cal/West seeds Co. Genetics International Inc. California USA |
| Super Supreme | California USA (produced for ICS-Canahill Comp. Saudi Arabia) |
| Supreme Forager | California USA (produced for ICS-Canahill Comp. Saudi Arabia) |
| Cuf -101 | California USA |
| Grasis II | California USA |
| Super 10 | California USA |
| Magna 901 | Diary Land Comp. USA (produced for ICS-Canahill Comp. Saudi Arabia) |
| Perfect | Cal/West, California USA (produced for ICS-Canahill Comp. Saudi Arabia) |
| Siri Nafa | Produced for Nafa Agriculture Comp. Saudi Arabia by SEEDMARK-Australia |
| Siriver | Australia |
| Nafa extra | USA, Produced for Nafa Agriculture Comp. Saudi Arabia |
| Super-fast | Nafa Agriculture Comp. Saudi Arabia |
| Ismailia I | Egypt (Forage Crop Department, Ministry of Agriculture, Egypt) |
| Nubaria | Egypt (Forage Crop Department, Ministry of Agriculture, Egypt) |

**Table 2**: Macro-morphological characters of the studied *Medicago sativa* L. cultivars.

| | SW 9720 | SW 9628 | Super supreme | Supreme forager | cuf-101 | Grasis II | Super 10 | Magna 901 | Perfect | Siri Nafa | siriver | Nafa extra | Super fast | Ismailia I | Nubaria |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Herb | Herb | Herb | Herb | Herb | Herb | Herb | Herb | Herb | Herb | Herb | Herb | Herb | Herb | Herb |
| 2 | 68 | 55 | 70 | 58 | 80 | 42 | 47 | 63 | 54 | 60 | 49 | 73 | 45 | 68 | 51 |
| 3 | 3 | 1 | 4 | 2 | 5 | 3 | 1 | 2 | 2 | 2 | 4 | 2 | 2 | 3 | 1 |
| 4 | 13 | 11 | 20 | 14 | 27 | 16 | 12 | 13 | 11 | 12 | 16 | 15 | 11 | 13 | 13 |
| 5 | 6.5 | 4.5 | 4 | 3 | 5 | 3 | 5.5 | 6.5 | 5 | 6.5 | 3 | 6 | 5 | 6.5 | 6 |
| 6 | 2.5 | 1.5 | 2.5 | 2 | 3 | 1.5 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 2.5 | 2.5 |
| 7 | 7 | 18 | 23 | 12 | 7 | 16 | 16 | 12 | 12 | 18 | 16 | 30 | 40 | 12 | 18 |
| 8 | Hairy | Hairy | Hairy | Hairy | Hairy | Hairy | Hairy | Hairy | Hairy | Hairy | Hairy | Glabrous | Hairy | Hairy | Hairy |
| 9 | Rhomboid | Ovate | Ovate | Rhomboid | Obovate | Obovate | Rhomboid | Elliptic | Rhomboid | Rhomboid | Rhomboid | Obovate | Elliptic | Elliptic | Obovate |
| 10 | 26 | 19 | 16 | 20 | 15 | 13 | 17 | 26 | 18 | 21 | 19 | 22 | 20 | 19 | 28 |
| 11 | 7 | 8 | 8 | 6 | 5 | 3 | 6 | 5 | 5 | 6 | 10 | 9 | 5 | 6 | 9 |
| 12 | 0.27 | 0.42 | 0.50 | 0.30 | 0.33 | 0.23 | 0.35 | 0.19 | 0.28 | 0.29 | 0.53 | 0.41 | 0.25 | 0.32 | 0.32 |
| 13 | Elliptic | Ovate | Rhomboid | Rhomboid | Obovate | Obovate | Obovate | Elliptic | Rhomboid | Ovate | Obovate | Obovate | Obovate | Elliptic | Obovate |
| 14 | 22 | 18 | 14 | 15 | 12 | 11 | 15 | 23 | 19 | 21 | 14 | 27 | 15 | 18 | 24 |
| 15 | 6 | 6 | 6 | 5 | 4 | 3 | 4 | 8 | 6 | 6 | 6 | 9 | 5 | 5 | 6 |
| 16 | 0.27 | 0.33 | 0.42 | 0.33 | 0.33 | 0.27 | 0.27 | 0.34 | 0.32 | 0.29 | 0.43 | 0.33 | 0.33 | 0.28 | 0.25 |
| 17 | Mucronate | Mucronate | Mucronate | Hooked | Mucronate | Mucronate | Mucronate | Mucronate | Hooked | Mucronate | Mucronate | Mucronate | Mucronate | Mucronate | Mucronate |
| 18 | Mucronate | Mucronate | Mucronate | Hooked | Mucronate | Mucronate | Mucronate | Mucronate | Hooked | Mucronate | Mucronate | Notched | Mucronate | Mucronate | Mucronate |
| 19 | Entire/ serrate at apex | Entire/ serrate at apex | Entire /dentate at apex | Entire /serrate at apex | Entire/ serrate at apex | Entire/ serrate at apex | Entire / toothed | Entire / toothed | Entire | Entire/ serrate at apex | Entire/ serrate at apex | Entire/ serrate at margin | Entire | Entire | Entire/ser rate at apex |
| 20 | Leafy | Leafy | Leafy | Hairy | Hairy | Leafy | Hairy | Hairy | Leafy | Leafy | Leafy | Leafy | Hairy | Hairy | Leafy |
| 21 | 9 | 10 | 8 | 6 | 8 | 7 | 9 | 9 | 10 | 7 | 12 | 10 | 10 | 8 | 12 |
| 22 | 16 | 12 | 18 | 38 | 4 | 8 | 5 | 12 | 24 | 5 | 29 | 12 | 28 | 12 | 5 |
| 23 | Violet | Violet | Violet | Violet | Violet | Violet | Violet | Violet | Violet | Violet | Violet | Violet | Violet | Violet | Violet |
| 24 | 2 | 5 | 4 | 12 | 5 | 5 | 2 | 3 | 5 | 2 | 7 | 2 | 4 | 3 | 2 |
| 25 | 10 | 4 | 9 | 9 | 7 | 5 | 4 | 4 | 7 | 3 | 9 | 4 | 7 | 4 | 3 |

1: Growth habit; 2: Plant height (cm), 3: Stem number; 4: Number of nodes on main stem; 5: Internode length (mm); 6: Stem thickness (mm); 7: Petiole length (mm); 8: Petiole hairiness; 9: Terminal leaflet shape; 10: Terminal leaflet length (mm); 11: Terminal leaflet width (mm); 12: Terminal leaflet width/ length ratio, 13: lateral leaflet shape; 14: lateral leaflet length (mm); 15: Lateral leaflet width (mm); 16: Lateral leaflet width/ length ratio; 17: Terminal leaflet apex shape; 18: Lateral leaflet apex shape; 19: leaflet margin; 20: leaf stipules shape; 21: Stipules length (mm), 22: Peduncle length (mm); 23: Flower colour; 24; Number of flowers per inflorescence; 25: Flower length (mm).

violet. Number of flower per inflorescence ranged from 2 to12 in each, while flower length ranged from 3 to 10 mm (Table 2).

Morphological data suggest the presence of variation among Egyptian, American or Australian alfalfa cultivars used in this study (Table 2), but none of the characters provided data whereby it was possible to differentiate among all of the cultivars. These results are consistent with those of Dehghan-Shoar *et al*. (2005) who reported that none of the morphological characters were sufficient to identify or discriminate among four Iranian and two New Zealand Lucerne cultivars. Tucak *et al*. (2008) also reported that eight morphological characters were not sufficient to determine the differences among fourteen alfalfa cultivars with different geographical origin. There are several possible explanations for this i) the few number of morphological characters ii) the influence of environmental conditions that prevented recognition of considerable variation. Despite criticisms of using morphological traits to identify cultivars or study genetic variability, they have proven useful in many studies on Lucerne (Smith *et al.*, 1991; Abbasi *et al.*, 2003, 2007; loumerem *et al*., 2007; Basafa and Taherian, 2009; Al-Faifi, 2013). Abbasi *et al.* (2007) reported large phenotypic variation for central leaflet area, plant height and 1000 seed weight among alfalfa accessions of National Plant Gene Bank of Iran.

According to UPOV (2005) a cultivar of a plant species must be clearly distinguishable from any other cultivar by one or more characters, and the distinctive features of cultivar should be maintained during multiplication and commercialisation. From morphological view point, the criteria from stem and leaf macro-morphological alone are not sufficient for delimitation between the studied taxa. In this respect, stem anatomy and seed surface sculpture were examined to enhance the assessment of distinctness of Lucerne cultivars.

The use of anatomical characters for taxonomic purposes has proved very helpful for identification of plant and herbarium specimens (Metcalfe and Chalk, 1957). From the foregoing stem anatomical characters as cited in Table 3 and plate 1, it was observed that stem is semi-circular to angular in cross-section. Epidermis is composed of small cells with thin cuticle except the cultivars Supreme forager, Perfect, Nafa extra and Nubaria which have thick cuticle. Under epidermis, all cultivars, except cultivars super-fast, have a layer of collenchyma tissue. In a circle in central cylinder, 16 to 23 collateral vascular bundles, arranged. The Egyptian cultivar Nubaria has the lowest number of vessels (16) while the American cultivar Super supreme has the larger number (23). Xylem vessels are in radial rows. The reduction in the proportion of lignified tissues was observed in all studied taxa. Central stem portion has large, thin-walled parenchyma cells. Crystals are sometimes present in parenchyma cells of pith.

Jewett and Barnes (1994) reported that the genetic variation of the forage quality of Lucerne might be related to stem anatomy. Our analyses showed that the stem anatomy of the studied taxa has lower proportion of tissues composed of thick-walled cells and there is a reduction in lignified tissues. As collenchyma tissue is composed of cells with thick cell walls, it gives additional strength to the plants and provides mechanical support, resistance to disease, insects, temperature extremes and other biotic and abiotic stresses (Buxton and Redfearn, 1997). On the other hand, reduction of the lignified tissues in the stem cross-sections was highly correlated to the digestibility of Lucerne stem (Guines *et al*., 2003). Krstic *et al*. (2008) showed that a reduction in the fiber content, especially in stems, has been suggested as one of the most important objectives of *Trifolium* breeding programs for improved digestibility. This was recorded mostly in species that are used as forage.

The characteristics of seed morphology and seed coat sculpture are listed in Table 4. It appears that 100 seed weight ranged from 0.23-0.29 g. The Egyptian cultivar Ismailia I showed the highest 100 seed weights. Seed are compressed or not compressed. Seed colour: Brown, dark brown, yellow or yellow to brown. Seed shape: Globose, ovate, kidney, oblong. Seed texture: Smooth, wrinkled, granulates.

According to the epidermal cell patterns seen by SEM, seeds shows some variation between the studied taxa (Plate 2). Five types of seed surface sculpture are recognized; Reticulate/foveate, reticulate/ruminate, reticulate, colleculate, verrucate. Anticlinal wall: Depressed, elevated or leveled; surface sculpture varies between granulate, wrinkled, smooth, ruminate, rugose. Periclinal wall: Depressed, elevated or leveled; surface sculpture varies between granulate, wrinkled, smooth. Hilum: Sub-terminal, lateral; Level: depressed, leveled; Shape: Globose, ovate (Table 4).

Seed characters such as shape, seed sculpture, anticlinal boundaries and periclinal walls were proved to be of taxonomic importance. The seed coat sculpture have low phenotypic plasticity and are less affected by environmental condition. Many authors used SEM for micro-morphological characters of the seed coat as the taxonomic tool in discriminating plant species or cultivars (Gabr, 2014; Heneidak and Abdel-Khalik, 2015). Seeds of the studied cultivars showed morphological variations in seed coat ornamentation (Table 4 and Plate 2). This is in agreement with Husain *et al*. (1994) who showed that the seed samples of *Medicago sativa* collected from different localities in Pakistan exhibit smooth to papillate and sometimes rugose surface pattern.

**Table 3**: Micro-morphological characters of stem anatomy of the studied *Medicago sativa* L. cultivars.

| Char \ Taxa | SW 9720 | SW 9628 | Super supreme | Supreme forager | cuf-101 | Grasis II | Super 10 | Magna 901 | Perfect | Siri Nafa | siriver | Nafa extra | Super fast |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cross section | Semi-circular | Semi-circular | Angular | Angular | Angular | Angular | Semi-circular | Angular | Semi-circular | Semi-circular | Angular | Semi-circular | Semi-circular |
| Cuticle | Thin | Thin | Thin | Thick | Thin | Thin | Thin | Thin | Thick | Thin | Thin | Thick | Thin |
| Stem | Hollow | Solid | Hollow | Solid | Hollow | Solid | Solid | Solid | Solid | Solid | Solid | Solid | Solid |
| Periderm | Present | Absent | Present | Absent | Absent | Absent | Absent | Present | Absent | Absent | Present | Absent | Absent |
| Epidermis | Uni-serriate | Uni-serriate | Uni-serriate | Multi-serriate | Uni-serriate | Uni-serriate | Uni-serriate | Uni-serriate | Uni-serriate | Multi-serriate | Uni-serriate | Uni-serriate | Uni-serriate |
| Epidermal cell shape | Radial Papillose | Tang. | Papillose | Radial / Pap. | Tang. / Pap. | Radial | Radial | Radial | Radial / Pap. | Radial / Pap. | Tang. | Tang. / Pap. | Tang. / Pap. |
| Trichomes | Unicell. / Uni-serriate | Unicell. / Uni-serriate | Unicell. / Uni-serriate | Bicell. / uni-serriate | Absent | Bicell. / uni-serriate | Absent | Absent | Absent | Unicell. / Uni-serriate | Absent | Absent | Absent |
| Cortex thickness | Narrow | Narrow | Narrow | Narrow | Narrow | Narrow | Narrow | Narrow | Narrow | Narrow | Wide | Wide | Wide |
| Cortex type | Coll. + Par. + Chlor. | Coll. + Par. + Chlor. | Coll. + Par. | Coll. + Par. + Chlor. | Coll. + Par. | Coll. + Chlor. | Coll. + Par. + Chlor. | Coll. + Par. + Chlor. | Coll. + Chlor. | Coll. + Par. | Coll. + Par. + Chlor. | Coll. + Chlor. | Par. + Chlor. |
| Chlorenchyma if present | Comp. cylinder | Incomp. cylinder | | Comp. cylinder | | Comp. cylinder | Incomp. cylinder | Incomp. cylinder | Incomp. cylinder | | Comp. cylinder | Incomp. cylinder | Comp. cylinder |
| Crystals in cortex | Present | Absent | Absent | Present | Absent | Absent | Absent | Absent | Present | Absent | Absent | Absent | Absent |
| Idioplast | Present | Absent | Present | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Present | Absent |
| Vascular bundle | Distinct | Contiguous | Distinct | Distinct | Distinct | Distinct | Distinct | Distinct | Distinct | Distinct | Distinct | Distinct | Contiguous |
| No. of V.B | 21 | 22 | 23 | 22 | 21 | 21 | 19 | 21 | 20 | 19 | 18 | 22 | 18 |
| Pericyclic fibers | Absent | Present | Present | Present | Present | Absent | Present | Present | Present | Absent | Present | Present | Present |
| Pith | Wide | Wide | Wide | Wide | Wide | Wide | Wide | Wide | Wide | Wide | Narrow | Narrow | Narrow |
| Crystals in pith | Absent | Absent | Absent | Absent | Present | Absent | Present | Absent | Absent | Absent | Present | Absent | Absent |

Tang = Tangential; Pap= Papillose; Bicell= Bicellular; Unicell= Unicellular; Coll. = Collenchyma; Par. = Parenchyma; Chlor.= Chlorenchyma; V.B.= Vascular bundle; Comp= Complete; Incomp= Incomplete

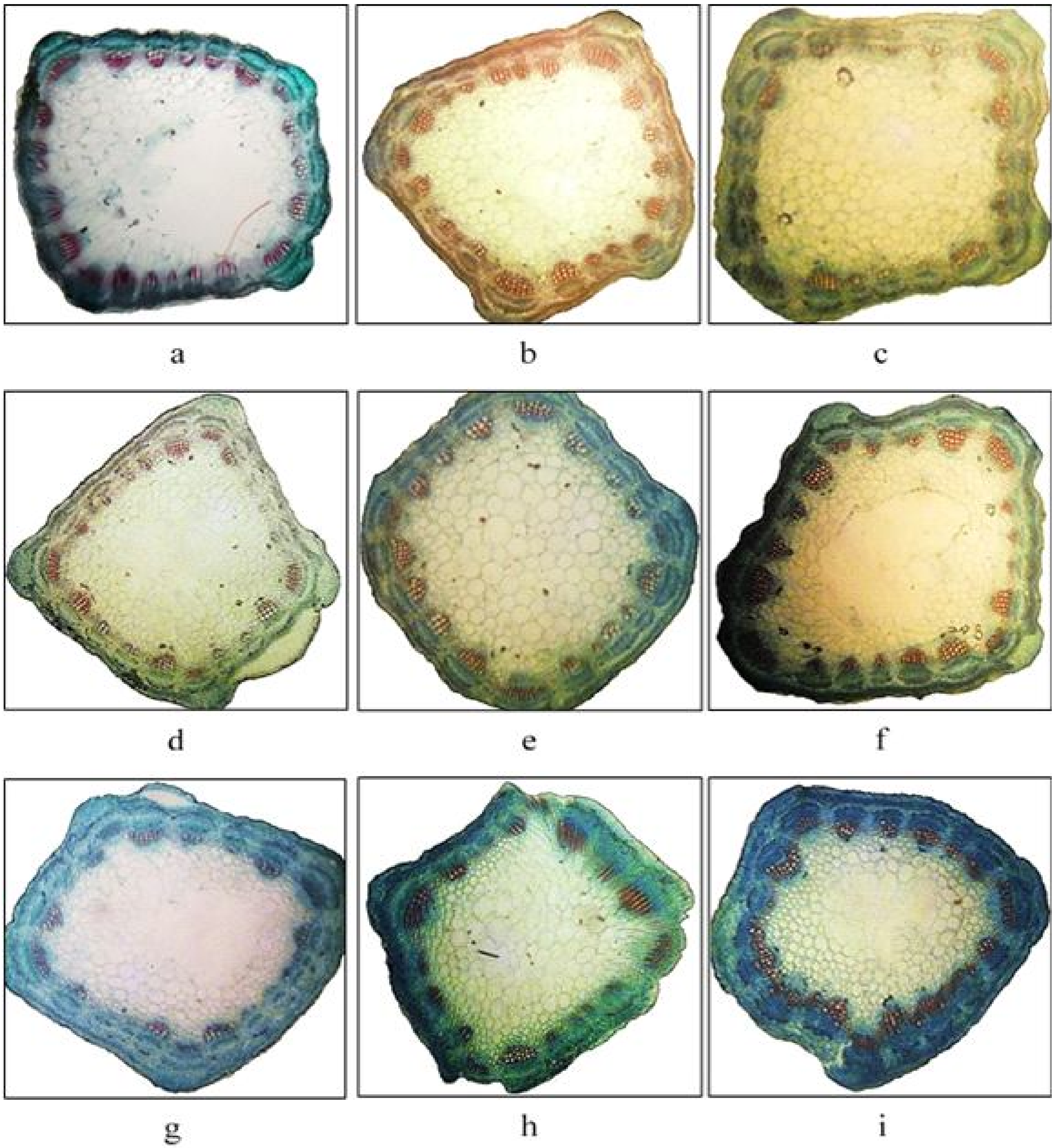


**Plate 1 (a-i)**: Light micrographs of stem cross-sections in some *Medicago sativa* L. cultivars. a= SW 9720, b= Supreme Forager, c= Cuf -101, d= Grasis II, e= Super 10, f=Magna 901, g= Perfect, h= Siriver, i= Super-fast

The dendrogram obtained from UPGMA cluster analysis based on macro- micro morphological and anatomical characters is shown in Figure 1. The dendrogram showed that the Egyptian cultivar Nubaria was the most distant and clustered separately from all the other alfalfa cultivars which were grouped into two main clusters. The first cluster included five American cultivars; SW9720, Grasis II, Super supreme, Culf 101 and Supreme forger. The second cluster included local accession Ismilia I as well as eight introduced American and Australian accessions in which the smallest distance was observed between the two American cultivars SW9628 and Perfect. The results were consistent with those of Touil *et al.* (2008) who found that Lucerne population did not cluster according to their geographical origins. The regrouping of some introduced cultivars with local accessions in the same cluster indicate similarities which may be the results of long cultivation history of local and some introduced cultivars in the same area. The clustering of these cultivars could be explained by common ancestry. On the other hand, Al-Faifi *et al.* (2013) found that local Saudi alfalfa accessions tend to group together in the same cluster or formed individual cluster.

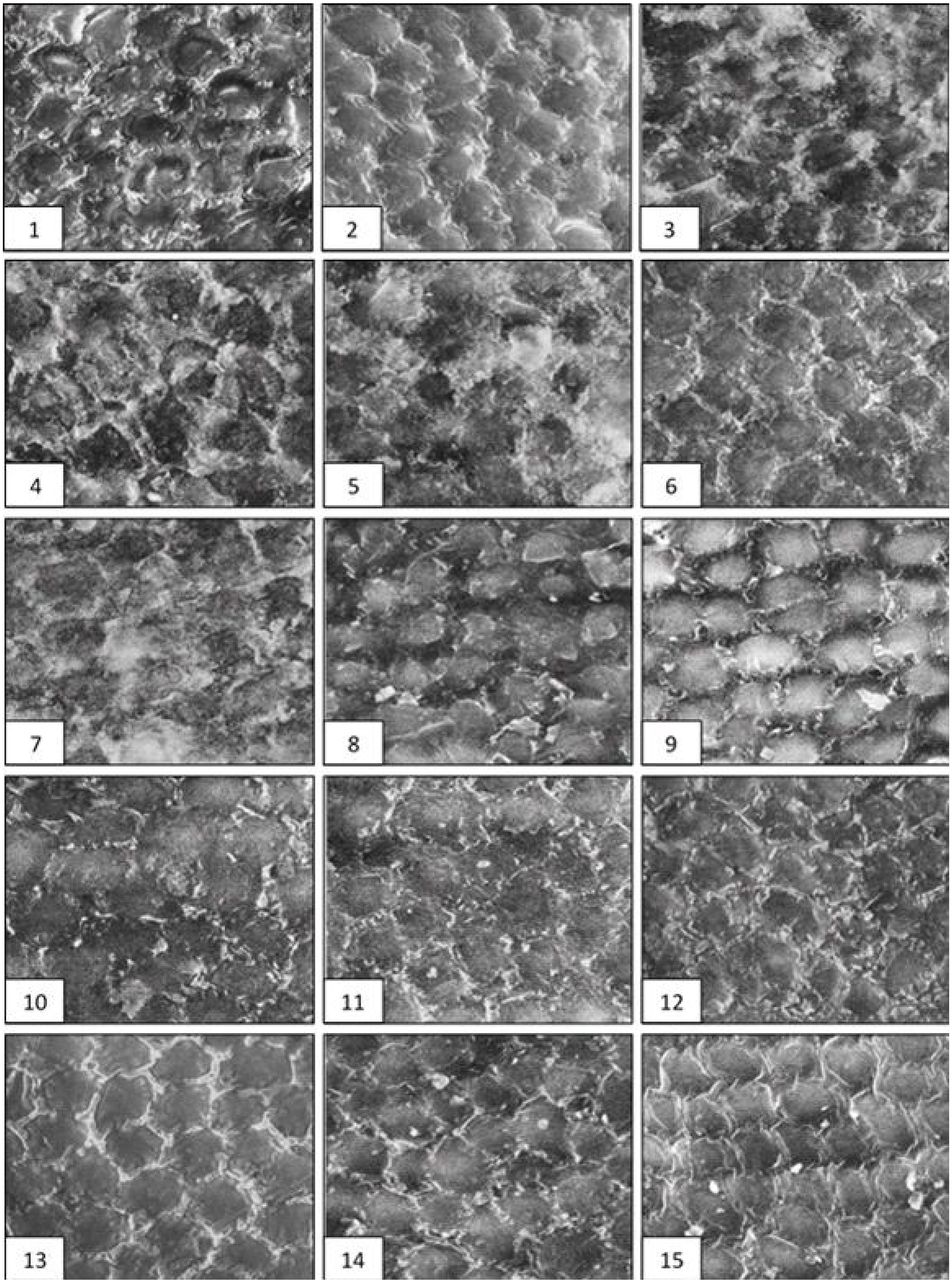


**Plate 2**: Scanning electron micrographs of *Medicago sativa* L. seed coat surface (*Medicago sativa* names from 1-15 as listed in Table 1)

**Table 4**: Seed micro-morphological characters of the studied *Medicago sativa* L. cultivars.

| Seed/ Character | | | Seed | | | | SEM | | | | | Hilum | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Compression | Shape | Texture | Seed colour | 100-seed weight (g) | Seed Sculpture | Anticlinal Wall | | | Periclinal Wall | | Position | level | Shape |
| | | | | | | | | Level | 2ry Sculpture or straight | undulate | Level Sculpture | 2ry | | |
| SW9720 | Compressed | Globose | Smooth | Brown | 0.27 | Reticulate / Foveate | Elevated | Granulate | Undulate | Depressed | Granulate | Sub-terminal | Depressed | Ovate |
| SW9628 | Not compressed | Ovate | Smooth | Brown | 0.26 | Colleculate | Depressed | Wrinkled | Straight | Elevated | Smooth | Lateral | Depressed | Ovate |
| Super supreme | Not compressed | Globose | Granulate | Brown | 0.27 | Reticulate / Foveate | Elevated | Granulate | Undulate | Depressed | Granulate | Lateral | Depressed | Globose |
| Supreme forager | Compressed | Ovate | Smooth | Brown | 0.23 | Reticulate / Foveate | Elevated | Granulate | Undulate | Depressed | Granulate | Lateral | Leveled | Globose |
| Cuf 101 | Not compressed | Oblong | Granulate | Brown | 0.27 | Reticulate / Foveate | Elevated | Granulate | Undulate | Depressed | Smooth | Lateral | Depressed | Globose |
| Grasis II | Compressed | Kidney | Smooth | Brown | 0.26 | Reticulate / Foveate | Elevated | Granulate | Undulate | Depressed | Granulate | Sub-terminal | Depressed | Ovate |
| Super 10 | Compressed | Kidney | Granulate | Dark/ brown | 0.26 | Reticulate / Ruminate | Elevated | Smooth | Straight | Depressed | Wrinkled | Lateral | Leveled | Globose |
| Magna 901 | Compressed | Oblong | Smooth | Brown | 0.24 | Verrucate | Depressed | Smooth | Undulate | Elevated | Granulate | Sub-terminal | Leveled | Ovate |
| Perfect | Not compressed | Ovate | Smooth | Brown | 0.27 | Colleculate | Depressed | Ruminate | Undulate | Elevated | Smooth | Lateral | Depressed | Ovate |
| Siri Nafa | Not compressed | Kidney | Smooth | yellow | 0.26 | Reticulate / Ruminate | Leveled | Wrinkled | Straight | Leveled | Smooth | Lateral | Depressed | Globose |
| Siriver | Not compressed | Ovate | Smooth | Yellow/ brown | 0.26 | Reticulate | Elevated | Wrinkled | Straight | Depressed | Smooth | Sub-terminal | Depressed | Ovate |
| Nafa extra | Not compressed | Globose | Wrinkled | yellow | 0.23 | Reticulate / Foveate | Elevated | Wrinkled | Straight | Depressed | Smooth | Lateral | Depressed | Globose |
| Super fast | Not compressed | Globose | Smooth | Brown | 0.27 | Reticulate | Elevated | Wrinkled | Straight | Depressed | Wrinkled | Sub-terminal | Depressed | Ovate |
| Ismailia I | Not compressed | Kidney | Smooth | yellow | 0.29 | Colleculate | Depressed | Wrinkled | Straight | Elevated | Smooth | Sub-terminal | Depressed | Globose |
| Nubaria | Compressed | Kidney | Smooth | yellow | 0.27 | Colleculate | Depressed | Rugose | Undulate | Elevated | Smooth | Lateral | Leveled | Globose |

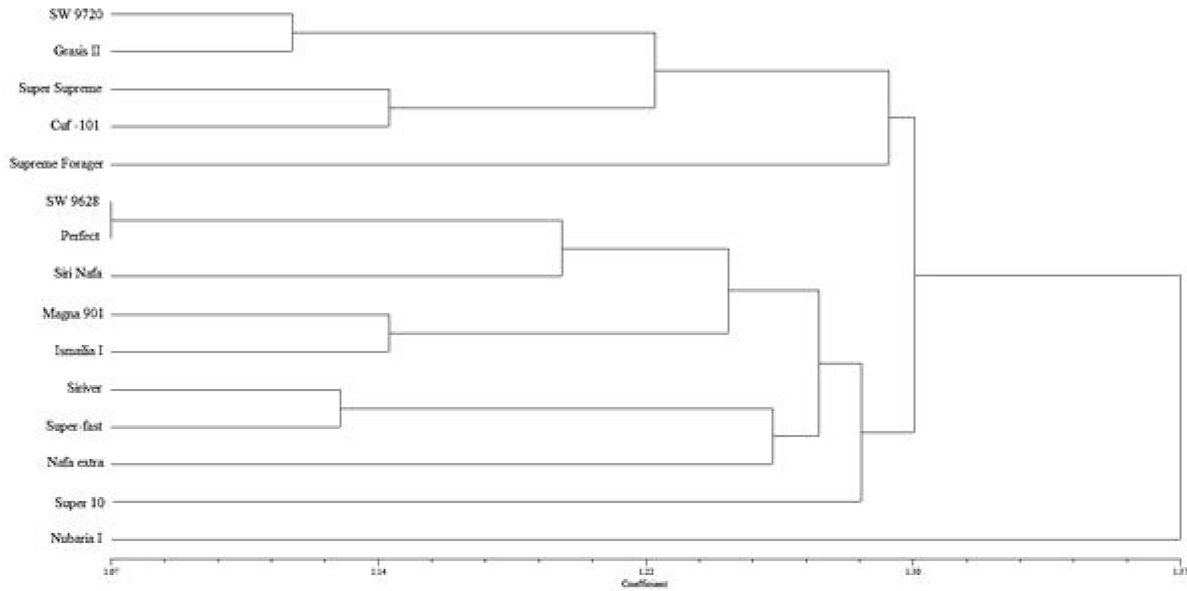


**Fig 1**: UPGMA tree illustrating the relationships among 15 *Medicago sativa* cultivars based on macro-morphological, anatomical characters and seed coat sculpture characters.

Bahar *et al*. (2006) found that alfalfa populations which were adapted to cold climates were grouped in one cluster and populations which belonged to the tropical area were placed in the cluster.

Conservation of Lucerne as forage crop is important worldwide, and in the Middle East in particular. Collection and characterization of genetic resources are required for the development of new cultivars. The apparent variation in plant morphology and seed-coat structure throw light regarding variability in these plants. The present study emphasizes the importance of seed characters for recognizing most of the studied taxa and supports the use of seed coat patterns as parameters for cultivar identification. The combined use of SEM and light microscopy has revealed numerous taxonomically useful characteristics.

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