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Taxonomic Evaluation of *Spinacia oleracea* L. Accessions by Morphological and Anatomical Markers

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ABSTRACT

Spinacia oleracea L. is an edible crop and considered as super food due to high nutrient values. Due to these properties the pot-based project was designed to explore the most diverse accession of this species, 31 local *S. oleracea* accessions were evaluated taxonomically on the basis of morphological and anatomical markers. Seeds were sown in clay pots to evaluate different morphological characters. The germination period varied from 6 to 15 days while variation in number of leaves was observed to be from 12 to 68 leaves per plant. Moreover, abaxial and adaxial epidermal leaf analysis was examined under a light microscope to study the existing anatomical variations. Substantial variations were observed among quantitative characters including number of subsidiary cells (3-5 on both abaxial and adaxial sides), stomata number per unit area (6-21 on abaxial side; 8-18 on adaxial side) and stomatal index (13.9-28.6 on abaxial side; 14.8-25.5 on adaxial side). The shape of epidermal cells varied from tetragonal to pentagonal, hexagonal and irregular. The results concluded that leaf epidermal anatomical markers could be applied considerably in delimiting the species to solve the existing taxonomic problems among them. However, for more authentic results it would be preferred to use more taxonomic tools in integration with anatomy to study intraspecific variations.

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INTRODUCTION

Spinacia oleracea L. is an important food crop commonly known as Spinach or Palak. It belongs to the family Amaranthaceae. It is cultivated entirely all over the world covering almost 800,000 hectares and an annual herb (Hu *et al*., 2007). It bears simple, alternate leaves and yellow-green flowers with lumpy fruit clusters containing several seeds. It grows in subtropical, temperate regions and mostly in winter season (Rashid *et al*., 2014). Spinach has a rich number of antioxidants, great nutritional value when quickly boiled or steamed and also in fresh form. It is a wealthy source of vitamins K, C, E, A, manganese, magnesium, iron and folate (Metha *et al*., 2014). The eating of this plant has various positive effects on the health of humans by decreasing the risk of various degenerative diseases of aging (Singh *et al*., 2015).

Along with its high nutritional values a lot of its potential activities have been reported. It has antipyretic, hypoglycemic, anthelmintic, antioxidant, hepatoprotective, anticancerous, antidepressant, antimicrobial and virus inhibiting properties (Singh *et al*., 2015; Patil *et al*., 2009; Gupta *et al*., 2006; Verma *et al*., 2003). Different parts of this plant are useful in the treatment of blood and brain disorders, fevers, leucorrhoea, lumbago, bowel, sore-throat, joints' pain, thirst, sneezing, asthma, leprosy, biliousness, breast cancer and urinary calculi (Longnecker *et al*., 1997; Chopra *et al*., 1956).

Taxonomy is the backbone of all sciences, as it provides baseline data mostly for biological studies (Khan *et al*., 2014). Diversity at intraspecific level may get affected due to different environmental conditions, hence creating a lot of taxonomic confusions. The confusion can be resolved using different taxonomic markers. Studying phenotypic characters is the simplest one and most plant's taxonomic data which gathered is mostly dependent upon morphometry (Stace, 1991). But morphological markers are openly exposed to the environment leading towards changes in phenotypes of plant species. Therefore, the environmental influence is leading towards enhancement of little more taxonomic confusion (Gilani *et al*., 2002). Leaf epidermal anatomical markers like stomata and trichomes are quite important tools to describe different taxa in terms of phylogenetic and taxonomic consideration (Roale *et al*., 2009; Baranova, 1972; Jones, 1986). Leaf epidermal anatomy was first used by Kioug *et al*. (1998) for the taxonomic purpose. The features of leaf epidermis are now vastly used for resolving taxonomic confusion (Stace, 1991).

Lin and Tan (2015) have used leaf epidermal characters to distinguish *Allium* at interspecific levels. (Aly *et al*., 2023) has studied spinach leaf anatomical characters. Previous studies have also shown the use of anatomical markers in correlation with morphological markers (Yousaf *et al*., 2008). A lot of research has been done on anatomy of different plant species (Tripathi *et al*., 2023; Chapeta *et al*., 2023) but there are limited studies at intraspecific level of spinach plant species. Therefore, an attempt has been made to examine different accessions of spinach by leaf epidermal anatomical markers, in association with morphological markers.

MATERIAL AND METHODS

Taxonomically, variations among 31 accessions of spinach (*Spinacia oleracea* L.) were investigated that was based on their anatomical and morphological traits. Seeds of 31 Spinach accessions were gained from genetic resource (IABGR) and institute of Agri biotechnology gene bank, National Agriculture Research Center (NARC), Islamabad Pakistan. Each accession seeds were sown in clay pots. Morpho-anatomical characteristics were started on two months crop. The average environmental conditions were also recorded. The temperature ranged from 17 to 30 degrees Celsius. The relative humidity ranged from 56% to 72%. The spinach seeds were grown in loamy soil with a pH of neutral (7.0).

Morphological study

Following morphological markers were recorded on the basis of qualitative (i.e., seed type, leaf texture, stem anthocynin, petiole shape vegetative leaf shape, reproductive leaf shape, leaf edge, leaf color) and quantitative (i.e., germination period, germination rate, petiole length, plant height, leaf width, leaf length, number of leaves /plant) traits.

Anatomical study

Fresh leaves of spinach accessions were used for leaf epidermal study. The peel off method was used for this study. Both sides of leaves adaxial and abaxial epidermis were examined under light microscope. Leaf samples according to modified was prepared used by Yousaf *et al*. (2008). For this method Leaf tissue was softened by using lactic acid.

The epidermis from both sides (abaxial and adaxial side) of leaf was peeled off by spreading the leaf on slide followed by scratching it till the removal of chlorophyll. The labeled slide was then observed under a binocular light microscope (model: Meiji Techno). Diverse leaf epidermal characters were observed according to the descriptive terminology of Dilcher (1974), Charlton (1988) and Croxdale (1998). The characters included types and sizes of epidermal cells, subsidiary cells, guard cells and stomatal apertures. Digital camera fitted on light microscope used for microphotographs of mounted specimen. Anatomical characters were identified by using power at lower power plan was (10×/0.25, ∞/0.17, F=200, WD=7.3) and at high power plan (40×/0.65, ∞/0.17, F=200, WD=0.5). The identification and characterization of these micrographs were based on microscopic features, and different types of subsidiary cells and stomata were studied.

Data analysis

Data for all morphological and anatomical characters was recorded in Microsoft Excel. Qualitative characters were coded by following the method of Boratynski and Davis (1971). To find out intraspecific relationship data was calculated by cluster analysis and co-relation matrix. Unweighted Pair Group Method with Arithmetic Average (UPGMA) using computerized statistical software (Minitab 17.1) used for construction of dendrogram.

RESULTS AND DISCUSSION

Spinach (*Spinacia oleracea* L.) is an economically important green leafy vegetable with useful nutritional content (Ball, 2006), therefore obtaining a superior genotype of this crop is particularly profitable. For this purpose, it was necessary to explore the diversity of spinach cultivars and choose the best genotype among them. Thirty-one local accessions of spinach were examined on the basis of anatomical and morphological markers to solve their existing taxonomic confusion. All these accessions were separable from each other on the basis of some anatomical and morphological properties.

Morphological variation

In the present piece of work, different qualitative and quantitative characters were made as a base to explore morphological variations. Seed shape, seed color, stem color, leaf edge, leaf shape and petiole attitude were considered as qualitative characters while germination period, germination rate, leaf width, leaf length, stem length, plant height, number of leaves per plant, petiole length and leaf size were taken as quantitative characters. The morphological variation of spinach accessions is given in Table 1 and 2. Most of the variations were found in quantitative characters while little differences were observed in qualitative characters (Figure 1). Similar results were stated by Rashid *et al.* (2014). According to the results obtained, accessions were then analyzed by finding correlation and cluster procedure. Same analyzing procedures were used by Sneath and Sokal (1973) to study agro-morphological and seed quality characters of Mustard plant accessions.

Figure 1. Morphological Variation among Local Accessions of *Spinacia oleracea* L.

Table No. 1. Morphological Variations of 1-16 Accessions of *Spinacia oleracea* L.

Accession Number	Germination Period (Days)	Germination Rate (%)	Seed Type	Leaf Texture	Stem Anthocyanin	Petiole shape	Vegetative Leaf Shape	Reproductive Leaf Shape	Leaf Edge	Leaf Color	Leaf Length (cm)	Leaf Width (cm)	Plant Height (cm)	Petiole Length (cm)	Number of Leaves / Plant
10648	13	67	Smooth	Smooth	Low	Erect	Ovate	Smooth	Smooth	Yellow, green	9	4	15.2	6	32
10654	10	67	Smooth	Smooth	Very high	Erect	Ovate	Smooth	Rippled	Yellow, green	9	5	20.3	8	18
10661	7	67	Smooth	Smooth	Very low	Semi spared	Ovate	Smooth	Smooth	Yellow, green	6.3	4	17.7	7	13
18381	6	67	Prickly	Smooth	Very low	Erect	Pinnatified	Smooth	Lobed	Yellow, green	4	3	9.7	7	22
18638	14	67	Smooth	Smooth	Low	Semi spared	Ovate	Smooth	Smooth	Grey, green	4.4	2	6.4	9	37
18642	11	100	Smooth	Smooth	Very high	Erect	Broad elliptic	Smooth	Rippled	Grey, green	4.5	2.3	10	4	28
18643	12	100	Smooth	Smooth	Intermediate	Semi spared	Broad elliptic	Smooth	Smooth	Grey, green	6	2.8	12	6	17
18645	12	34	Smooth	Smooth	Very low	Erect	Ovate	Pointy	Rippled	Grey, green	9	6	20.3	8	12
18646	9	100	Smooth	Smooth	Intermediate	Erect	Ovate	Smooth	Rippled	Grey, green	7.5	6.7	29.2	11.5	15
18647	11	67	Smooth	Smooth	Intermediate	Semi spared	Ovate	Smooth	Rippled	Yellow, green	5	4.5	15.2	6	14
18650	12	100	Smooth	Smooth	Very low	Erect	Ovate	Smooth	Rippled	Yellow, green	6.7	3.2	27	10.5	14
18651	14	100	Smooth	Smooth	Low	Erect	Ovate	Smooth	Smooth	Yellow, green	8.3	5	24.6	9.7	16
18653	11	34	Smooth	Smooth	Very low	Erect	Elliptic	Smooth	Smooth	Yellow, green	8.7	6.6	47	18.5	30
18654	8	67	Smooth	Smooth	Intermediate	Semi spared	Ovate	Smooth	Smooth	Yellow, green	7.8	5.5	20.3	8	24
18655	9	100	Smooth	Smooth	Low	Erect	Broad elliptic	Smooth	Smooth	Yellow, green	8	6.5	20.3	8	37
19330	12	34	Smooth	Smooth	Intermediate	Erect	Elliptic	Ovate	Smooth	Grey, green	8.5	5.5	27	10.5	14

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Table No. 2. Morphological Variations of 17-31 Accessions of *Spinacia oleracea* L.

Accession Number	Germination Period (Days)	Germination Rate (%)	Seed Type	Leaf Texture	Stem Anthocyanin	Petiole shape	Vegetative Leaf Shape	Reproductive Leaf Shape	Leaf Edge	Leaf Color	Leaf Length (cm)	Leaf Width (cm)	Plant Height (cm)	Petiole Length (cm)	Number of Leaves / Plant
19333	15	67	Smooth	Smooth	Very high	Semi spared	Ovate	Smooth	Smooth	Yellow, green	7.3	4.3	18	7	26
19340	11	34	Smooth	Smooth	Intermediate	Erect	Ovate	Smooth	Smooth	Yellow, green	7.3	3.6	19	7.5	27
19343	14	34	Smooth	Smooth	Very low	Erect	Ovate	Smooth	Smooth	Yellow, green	12	7	25.4	10	57
19344	10	34	Smooth	Smooth	Intermediate	Erect	Ovate	Smooth	Smooth	Grey, green	11	8	22	8.5	43
19345	10	34	Smooth	Smooth	Intermediate	Erect	Ovate	Smooth	Smooth	Grey, green	7.5	5.5	15.2	6	68
19830	6	67	Smooth	Smooth	Intermediate	Erect	Elliptic	Smooth	Smooth	Grey, green	6.2	4	15.2	6	33
19837	8	67	Smooth	Smooth	Very low	Erect	Ovate	Smooth	Smooth	Yellow, green	6.5	4.5	19	7.5	25
30517	8	67	Smooth	Smooth	Low	Semi spared	Ovate	Smooth	Smooth	Yellow, green	10	6	24.1	9.5	26
30519	12	34	Smooth	Slight crinkled	Absent	Erect	Ovate	Smooth	Smooth	Yellow, green	10	7	25.4	10	25
30520	8	67	Smooth	Smooth	Low	Erect	Ovate	Smooth	Smooth	Grey, green	5.5	2.7	10.1	4	26
30532	6	67	Smooth	Smooth	High	Erect	Ovate	Smooth	Smooth	Grey, green	10.5	7.9	25.4	10	45
30533	12	67	Smooth	Smooth	Intermediate	Semi spared	Elliptic	Smooth	Smooth	Yellow, green	6.7	2.7	17.7	7	28
32122	8	100	Smooth	Smooth	Low	Semi spared	Ovate	Smooth	Smooth	Yellow, green	9.5	5.5	12.7	5	28
32618	7	100	Smooth	Smooth	Intermediate	Erect	Elliptic	Smooth	Smooth	Grey, green	7.5	4.5	20.3	5	17
34160	7	100	Smooth	Smooth	Very low	Semi spared	Ovate	Smooth	Smooth	Yellow, green	7	4.2	17.7	7	18

Cluster Analysis based on Morphological Characters

The cluster analysis highlights an extent of variation, which may be useful in future breeding programs (Sultana *et al*., 2006). Many authors utilized this method for morphological characterization of various species indicating their intraspecific relationship (Khadivi *et al*., 2018; Boampong *et al*., 2018). To find morphological intraspecific relationship between 31 local accessions of *Spinacia oleracea* were split into two main clusters. Similarly, Rashid *et al*. (2020) separated the east Asia spinach accessions into clusters to clear the morphological difference among them. At 48% genetic linkage distance group, cluster 1 was further divided into two sub clusters 1a and 1b. Based on morphological appearance 14 accessions were in cluster 1a having similar characters and 9 accessions were included in cluster 1b with no major difference. Cluster 2 showed difference than the cluster 1, at 52% genetic linkage distance cluster 2 was divided into two sub cluster 2a and 2b. 3 accessions were included in cluster 2a, and 5 accessions were included in 2b. These eight accessions showed major difference among all the thirty-one accessions (Figure 2).

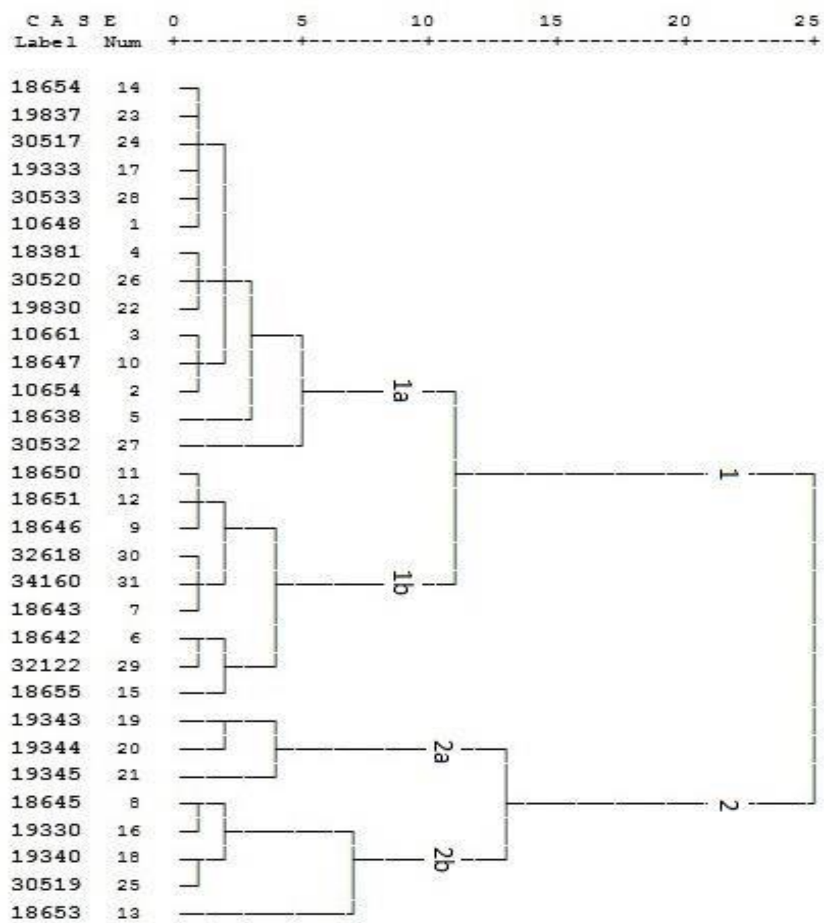

Figure 2. Dendrogram of Morphological Characters of Thirty-one Accessions of *Spinacia oleracea* L.

Correlation based on Morphological Characters

Correlation was calculated on the basis of morphological characters of accessions i.e., leaf texture, seed type, stem anthocyanin, petiole attitude, vegetative leaf shape, reproductive leaf shape, germination period, leaf length, leaf width, plant length, germination rate and petiole length (Table 3). Correlation ranged from 0.02 - 0.93 among the morphological characters. The value of correlation for plant length/ was 0.93. The value of correlation for leaf edge and petiole length was 0.02. Negative correlation for morphological characters ranged from -0.01 to -0.38. The value of correlation for leaf edge/number of leaves was -0.38. Minimum negative correlation for leaf edge/ number of leaves, plant numbers/germination rate and number of leaves/ germination rate was -0.01.

Table No. 3. Correlation among Morphological Characters of thirty-one Accessions of *Spinacia oleracea* L.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1															
2	-0.03	1														
3	0.37	0.21	1													
4	-0.13	-0.13	-0.09	1												
5	-0.1	0.58	-0.04	-0.13	1											
6	-0.05	-0.05	0.15	-0.17	-0.04	1										
7	-0.1	0.13	-0.08	-0.19	0.06	0.29	1									
8	0.16	0.16	0.48	0.17	-0.09	-0.29	-0.21	1								
9	0.13	-0.29	0.01	0.03	-0.18	0.18	0.07	0.08	1							
10	-0.25	-0.01	-0.14	0.25	0.25	-0.35	0.2	0.04	-0.22	1						
11	0.21	-0.33	0.03	-0.24	-0.42	0.14	-0.25	0.18	0.08	-0.36	1					
12	0.24	-0.21	0.06	-0.29	-0.31	0.15	-0.11	0.02	-0.1	-0.36	0.85	1				
13	0.14	-0.24	0.22	-0.32	-0.18	0.09	-0.01	0.24	0.1	-0.27	0.56	0.64	1			
14	-0.03	0.03	-0.16	-0.22	-0.02	-0.28	-0.39	-0.08	0.02	-0.39	0.32	0.29	-0.07	1		
15	-0.25	-0.01	-0.14	0.25	0.25	-0.35	0.2	0.04	-0.22	1	-0.36	-0.36	-0.27	-0.39	1	
16	0.14	-0.06	0.27	-0.26	-0.1	0.09	0.01	0.27	0.16	-0.32	0.46	0.57	0.94	-0.02	-0.32	1

Key (1-16): Leaf texture, Seed type, Stem anthocyanin, Petiole attitude, Vegetative leaf shape, Reproductive leaf shape, Leaf edge, Leaf color, Germination period, Plant number, Leaf length, Leaf width, Plant length, Leaf number, Germination rate, Petiole length.

Anatomical Variations

In taxonomy anatomical characters are basic tools to find out variations within species, genera or a family (Metcalf and Chalk, 1950). In taxonomic evaluation leaf epidermis anatomical characters establish to be an important tool (Yousaf et al., 2008; Gilani et al., 2002). Davis and Heywood (1963) also projected anatomical characters as useful and consistent constant within a tax on. Watson and Dallwitz (1992) had reported leaf epidermis abaxial side variation a consistent character in many of the grasses. Since the 19th century plant anatomy is a successful tool for characterizing species in taxonomy (Metcalfe and Chalk, 1950; Akhtar et al., 2022). From the anatomical results, it was observed that these accessions showed variations in different qualitative and quantitative characters. Major differences were perceived in size and shape of long cell, short cell, margin of epidermal cells, shape of epidermal cells, length and width of stomata and guard cells. In accession 30533 long cells were observed, while accession 18650 showed shortest cell as compared other accessions. Maximum length (Figure 3 and 4). Similar types of variations were reported by Younus et al. (2015) in the leaf epidermal anatomy of Euphorbiaceae members. According to Metcalf and chalk (1950), Van Greuning et al. (1984). For the identification of species leaf epidermal cell shape is a useful character Sonibare et al. (2005). In the present piece of work various shapes of epidermal cells were observed from irregular to tetragonal, pentagonal and hexagonal with mostly smooth or wavy margins. Ullah et al. (2011) observed rectangular, hexagonal, squarish, fusiform and irregular shapes of epidermal cells in Arthraxon prionodes. Polygonal shape of epidermal cells was observed in Citrus L. by Ogundare and S.A. Saheed (2012). The shape and margin of some long cells were observed to be smooth and hexagonal respectively on both adaxial and abaxial side of the leaf epidermis. While Raole et al. (2009) reported during epidermal studies of Rottboelia that cells were hexagonal and smooth type.

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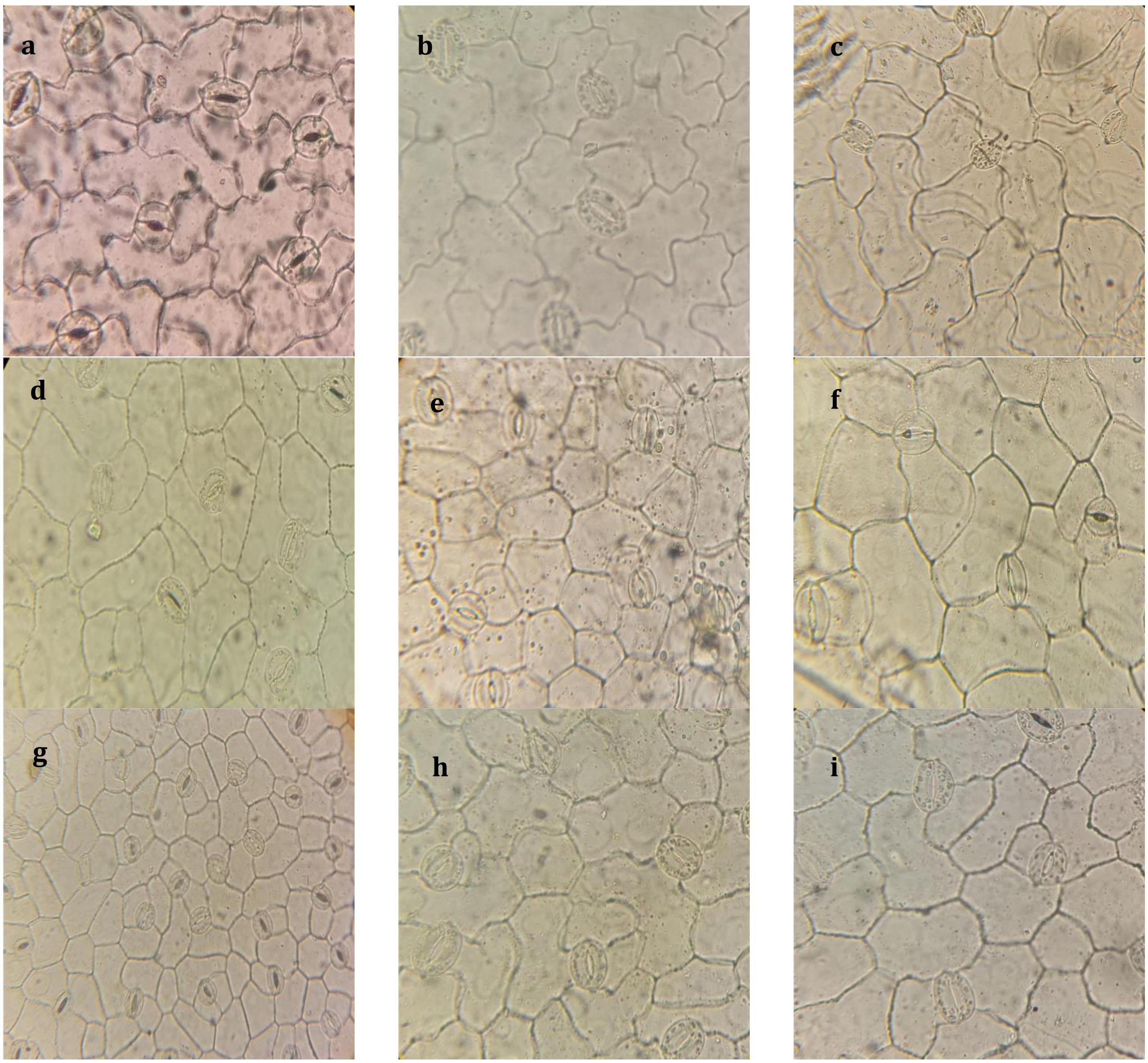

Figure 3. Leaf Epidermal Variation among Abaxial Sides of *Spinacia oleracea* L. Accessions; A. 18638. B. 18642, C. 18646, D. 30520, E. 30532, F. 32618, G. 18655, H. 19345. I. 30519.

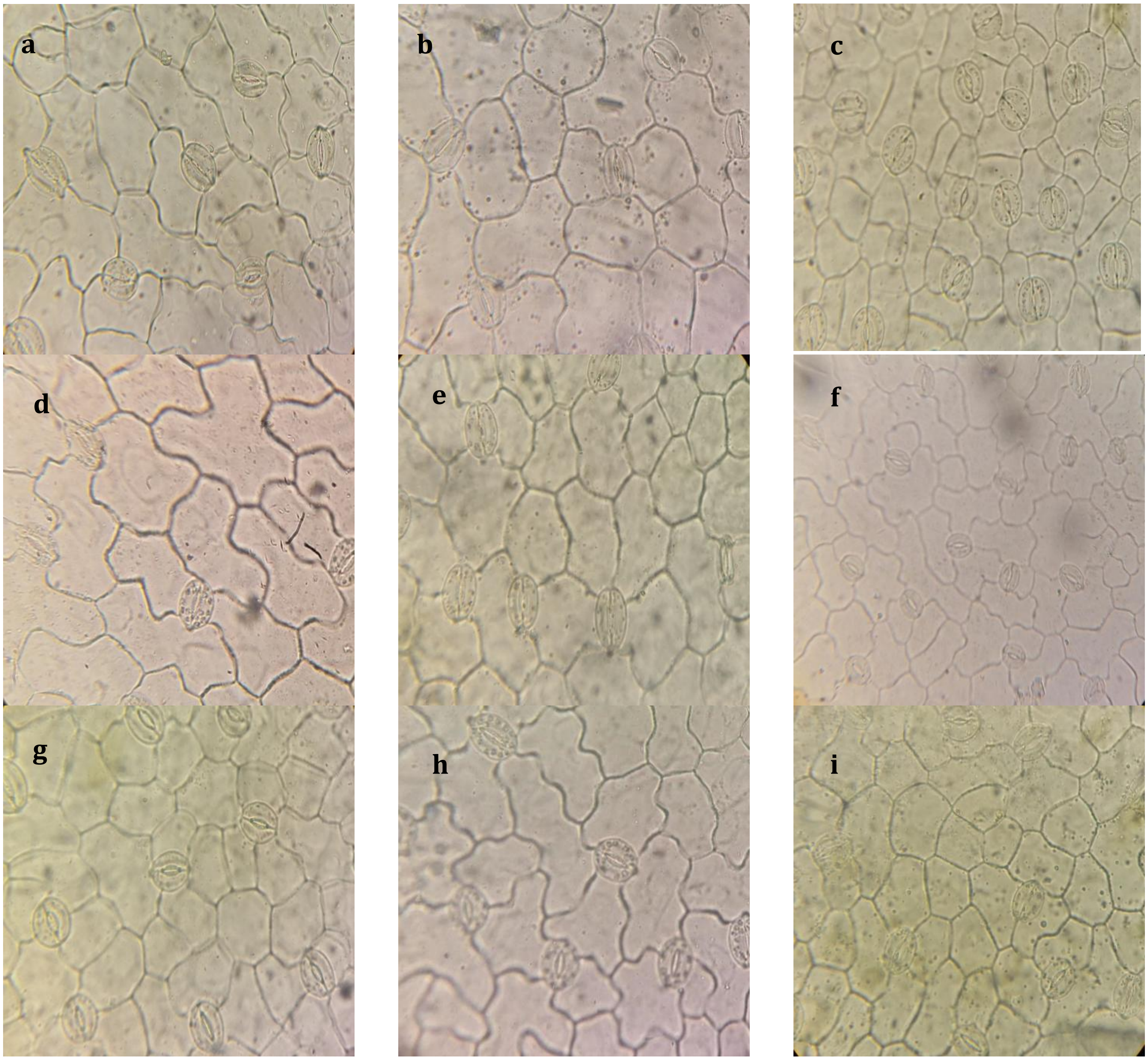

Figure 4. Leaf Epidermal Variation among Adaxial Sides of *Spinacia oleracea* L. Accessions A. 34160, B. 32618, C. 18645, D. 18646, E. 18651, F. 18655, G. 30517, H. 19345, I. 19830.

Maximum length of long cell was ranging from 263.5 – 274.2 µm in 30533 accessions while for short cell it was ranging from (29.8–32.5 µm) in 18650 accessions. Similarly, Ahmad *et al*. (2011) examined long cells in genus *Eragrostis* having maximum length ranging from 90-165µm. The margins of epidermal cells were smooth and wavy on both abaxial and adaxial surfaces. Sahreen *et al*. (2010) also reported smooth walls in some of the species of *Silene* on both of their abaxial and adaxial surfaces. But one of its species (*S. viscosa*) had an adaxial surface had smooth wall and on abaxial surface had wavy wall. In the present results, guard cells of kidney shape were observed on both sides (adaxial and abaxial) of the leaf epidermis. Stomatal type was found to be anomocytic on both sides. Toda *et al*. (2016) also examined kidney shaped guard cells in *Oryza sativa*. Furthermore, anomocytic type of stomata was observed in one of its members i.e., *Vicia faba*. The development and pattern of stomata are usually regulated by both genetic and environmental signals (Wang *et al*., 2007).

The Stomatal index was observed to be in the range of 13.95–28.57 in accession 19837 on abaxial side while 25.53–14.75 on the adaxial side in accession 18651. Raole (2009) reported similar results during the anatomical studies of *Andropogon pilosus*, a member of Poaceae family. Hameed *et al.* (2008), in their studies of members of Polygonaceae family, reported that in adaxial epidermis of *Rumex hastatus* had highest stomatal index (24.78 µm) and lowest in *Rumex austral* was (11.25 µm). While in abaxial epidermis stomatal index was lowest (17.99 µm) in *Rumex dentatus* and highest (48.52 µm) in *Rumex hastatus*.

Leaf epidermal attributes of all accessions were quite different in width and length of short and long cells along with width and length of guard cells, width, and length of the stomata. Similar findings were reported by Raole (2009) for epidermal studies of *Desmostachya* species, that width and stomatal cavity and length of short, long guard cells were diverse in their lengths and widths. Ferris *et al*. (2002) also observed variations by analyzing stomatal index, stomatal density, co-efficient of variance and epidermal cells per unit area.

There was no variation found in the type of stomata on both adaxial and abaxial side of leaf epidermis in all accessions (Figure 3 and 4). Anomocytic stomata were present on both sides of the leaf. Klimko (2008) reported that stomata present in all species of the genus *Dracaena* were also anomocytic. Order of Polygonales and Centrospermae had anomocytic type pf stomata studied by Metcalfe and Chalk (1950). Padmini and Rao (1995) reported in mostly dicot species irrespective of the specific family or order had anomocytic type of stomata except the family Amaranthaceae had diverse type of stomata like paracytic, anisocytic, anomocytic and diacytic (Padmini and Rao, 1995).

Similarly, *Cleome viscosa* is also distinguished due to anomocytic type of stomata (Perveen *et al*., 2007). The findings of the present study were also in agreement to those of Hameed *et al*. (2008) where *Rumex dentatus* leaf epidermis on both abaxial and adaxial epidermis had anomocytic type of stomata. Length of stomatal pore was observed to be ranging from 20.6 µm–47.35 µm on adaxial side, while on abaxial side, it was ranging from 16.55–64.55. Similar findings were stated by Hameed *et al*. (2008) reported upper epidermis stomatal pore length to be 24 µm in *Polygonum plebejum* and 14 µm in *Rumex australe*. Length of stomatal cavity ranged from 39.7–44.1 µm on abaxial side in accession 18651 and 60.8–68.3 µm on adaxial side in accession 32122. On the other hand, width of stomatal cavity ranged from 8.2–19.1 µm on adaxial side in accession 19330 while 23.7–24.6 µm on abaxial side in accession 18655. Fanourakis *et al*. (2014) also found such type of quantitative values during the study of leaf epidermis sides.

Length of short cell ranged from 101.7–109.9 µm on abaxial side while that on adaxial side ranged from 53.1–91.9 µm in accession 34160. Width of short cell ranged from 41.2–74.8 µm in 34160 accessions. Rehman *et al*. (2015) described similar results in the leaf epidermal studies of *Artocarpus integrifolia.* Guard cells average length ranged from 73.65 µm-35.85 µm on abaxial side in accession 19333. Average width of guard cell was ranged from 26.85-11.2 µm on abaxial side while 39.7-9.65 µm on adaxial side in 32618 accessions. Hameed *et al*. (2008) also studied leaf anatomy of *Rumex hastatus* and *Rumex austral,* the adaxial side leaf epidermis average length of the guard cells showed variation. In abaxial epidermis guard cells average width was lowest in *Rumex austral* and was highest in *Polygonum plebe*. Chaudhary & Imran (1997) described variations among the percentage of opening and closing of stomata, type of stomata, guard cells and size of stomatal pore. Anatomical characters were analyzed by cluster analysis and correlation matrix to explore the existing similarities and differences among accessions. Similar analyses were performed by Rehman *et al.* (2015) and Younas *et al*. (2015) examined the taxonomic variation of species of family Moraceae and Euphorbiaceae respectively. Moreover, accession 19345 showed best results among all these accessions.

Correlation based on anatomical characters

Correlation of 31 spinach accessions was calculated on the basis of different anatomical characters i.e., width and length of epidermal cells (short and long cells), guard cells, shape of stomata length and width of stomata, epidermal cells, stomata per unit area, stomatal index, shape of epidermal cells and margin of epidermal cells. Correlation for abaxial side ranged from 0.01-0.85 (Table 4). The maximum value of correlation for length of short cell/ length of guard cell was 0.85. The minimum value of correlation for width of stomata/ number of subsidiary cells was .01. Negative correlation for this character ranged from -0.05 to-0.12. The maximum negative value for width of stomata/ stomatal index, epidermal cells per unit area/shape of epidermal cells was-0.12. The minimum negative value for stomatal index/ length of guard cell was -0.05. Correlation for adaxial side ranged from 0.04-0.62 (Table 5). Maximum co-relation for stomata per unit area/epidermal cells per unit area was 0.62. The minimum co-relation for length of stomata/stomatal index, number of subsidiary cells/lengths of guard cells, number of subsidiary cells/lengths of short cells was 0.05. Negative correlation ranged from -0.07 to -0.38. The value of correlation for stomatal index/epidermal cells per unit area, number of subsidiary cells/widths of short cells, length of stomata/margin of epidermal cells was -0.38. The value of correlation for number of subsidiary cells/shapes of epidermal cells was -0.07.

Table No. 4. Correlation among anatomical characters of abaxial side of thirty-one accessions of *Spinacia oleracea*. L.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1													
2	0.68	1												
3	-0.33	-0.12	1											
4	-0.19	0.02	0.4	1										
5	-0.04	0.05	0.19	0.25	1									
6	-0.21	-0.18	0.75	-0.25	-0.02	1								
7	0.36	0.64	-0.27	-0.06	-0.17	-0.28	1							
8	0.59	0.3	-0.3	-0.09	-0.17	-0.27	0.41	1						
9	0.04	0.21	-0.27	-0.23	-0.12	-0.15	0.53	-0.02	1					
10	0.08	0.27	-0.28	-0.06	-0.1	-0.28	0.54	0.05	0.86	1				
11	0.27	0.24	-0.33	-0.15	-0.05	-0.26	0.21	0.09	0.59	0.6	1			
12	-0.16	-0.19	0.04	0.29	0.28	-0.12	-0.17	-0.09	0.16	0.27	0.48	1		
13	0.04	0.07	0.1	-0.12	-0.34	0.29	-0.01	0.24	-0.3	-0.2	-0.05	-0.01	1	
14	0.24	0.04	-0.07	-0.15	0.36	0.01	-0.02	-0.03	0.03	-0.02	0.05	-0.02	-0.37	1

Key:(1-14): Stomata length, Stomata width, Stomata number, Stomatal index, Subsidiary cell number, Epidermal cell number, Long cell length, Long cell width, Short cell width, Guard cell length, Guard cell width, Epidermal cell shape, Epidermal cell margin.

Table No 5. Correlation among anatomical characters of adaxial side of thirty-one Accessions of *Spinacia oleracea* L.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1													
2	0.61	1												
3	0.23	0.17	1											
4	0.05	-0.09	0.46	1										
5	-0.11	0.04	-0.19	-0.02	1									
6	0.17	0.24	0.63	-0.39	-0.14	1								
7	0.41	0.17	-0.14	-0.1	-0.19	-0.06	1							
8	0.34	0.06	0.2	-0.18	0.06	0.33	0.46	1						
9	0.48	0.39	0.14	-0.18	-0.39	0.28	0.38	0.32	1					
10	0.58	0.42	0.17	-0.21	0.05	0.34	0.09	0.32	0.53	1				
11	0.35	0.27	0.25	-0.13	-0.1	0.36	0.4	0.57	0.56	0.34	1			
12	0.06	-0.14	0.09	-0.24	-0.12	0.32	0.22	0.28	0.2	0.05	0.49	1		
13	-0.08	-0.12	0.23	0.15	-0.08	0.09	-0.11	0.24	0.09	-0.06	0.17	0.01	1	
14	-0.39	-0.24	0.01	0.2	0.07	-0.17	-0.22	-0.3	-0.15	-0.14	-0.29	0.18	-0.19	1

Cluster Analysis based on Anatomical Characters

Cluster analysis constructed on anatomical characters of leaf epidermal on abaxial side shown that 31 local accessions of *Spinacia oleracea* L. were divided into two main clusters 1 and 2 with polygenetic distance 100%. At 40% genetic linkage distance group cluster 1 was further divided into two sub clusters 1a and 1b. Out of 25 accessions, 13 accessions were included in cluster 1a and 12 accessions were included in cluster 1b. At 68 % genetic linkage cluster two was divided into two further sub clusters 2a and 2b. 5 accessions were included in sub cluster 2a while accession 10661 was the only accession included in 2b sub cluster (Figure 5). Cluster analysis constructed on anatomical characters of leaf epidermal on adaxial side shown that 31 local accessions of *Spinacia oleracea* L. were divided into two main clusters 1 and 2 with polygenetic distance 100%. At 60% genetic linkage distance group cluster 1 was further divided into two sub clusters 1a and 1b. A total of 15 accessions were included in 1b and 14 accessions were included in 1a. Accession numbers 18645 and 18647 were included in cluster 2 (Figure 6).

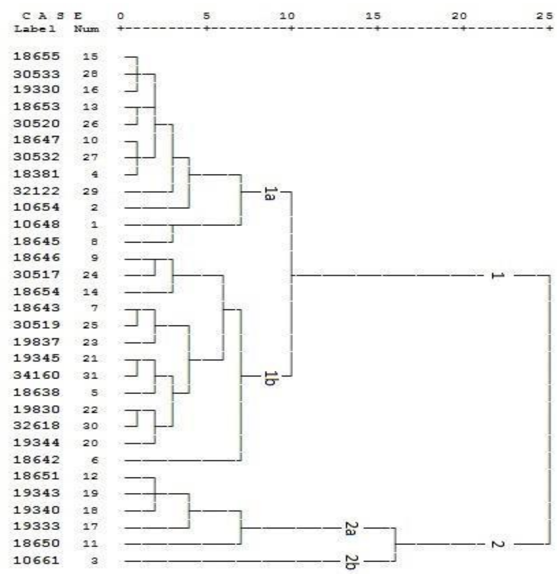

Figure 5. Dendrogram of Anatomical Characters from Abaxial Side of Thirty-one Accessions of *Spinacia oleracea* L.

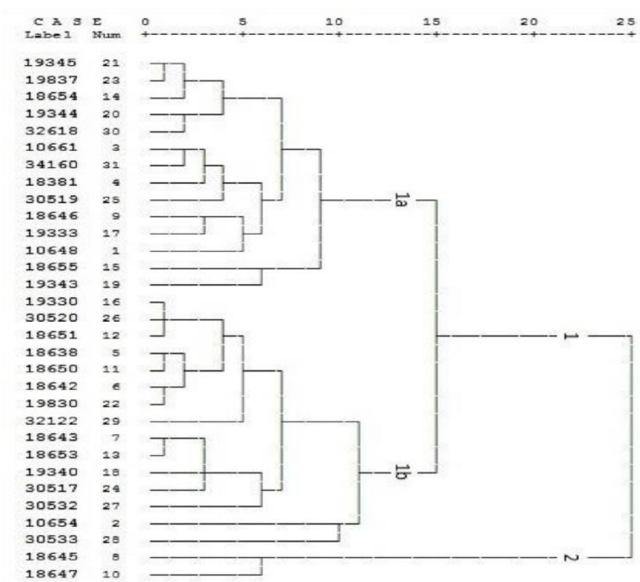

Figure 6: Dendrogram of Anatomical Characters from Adaxial Side of Thirty-one Accessions of *Spinacia oleracea* L.

CONCLUSION

The taxonomic study of thirty-one local spinach accessions showed both morphological and anatomical differences to some extent. Quantitative characters showed more variation, while qualitative characters showed more similarity. Moreover, features on both epidermises were not so much different with reference to each other. Main significant differences were found among epidermal cells shapes, guard cells and sizes of stomata, and epidermal cells. These intra-specific variations have broadened the spectrum of available characters for systematic purposes. It was inferred that foliar epidermal characters are of substantial taxonomic value that could be used for authentication, correct identification and classification of closely related germplasms. Additional taxonomic tools like palynological, cytological and biochemical tools must also be studied in integration with anatomical tools to study minor intraspecific variability among accessions. Furthermore, a breeder may employ these different traits to develop novel varieties with desired characteristics such as disease resistance, higher antioxidant levels, and good crop quality and quantity.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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