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Investigation of the Structural Characteristics of Seed Surfaces of Some Soybean Genotypes by Using Scanning Electron Microscopy (SEM)

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HIGHLIGHTS

- One of the most important legume plants in the Fabaceae family, soybeans are essential for both human and animal nutrition.
- The characterization of different genotypes and varieties of soybeans is too important for plant breeding and SEM seed surface examinations are a low-cost and effective technique for characterizing of them.

Abstract

Soybean (*Glycine max*) is an annual plant in the Fabaceae family, native to East Asia. The plant is grown for its edible beans. Soybean plays a crucial role in East Asian cuisine and the animal feed industry as it is one of the plants with the highest protein yield per cultivated area and is rich in nutritional value. Since soybean cultivars are morphologically similar, molecular and genetic markers are mostly used to determine different varieties and lineages. It is extremely important to prefer more effective and practical approaches for faster and cheaper characterization of agricultural products. In this respect, SEM analyses, which allow seed surface characterization of not only soya varieties but also all grains are foreseen to be of critical importance as a candidate method and approach. SEM technique provides high-resolution images of the surfaces and allows detailed examination of the microstructure of the materials. This study used Scanning Electron Microscopy (SEM) to determine seed surface characteristics such as surface roughness, reticulation, tubercles, and raised and grooved surface decorations in 12 soybean cultivars. As a result of the study, soybean genotypes were divided into 3 types according to their micro-morphological characteristics and their similarities and differences were revealed. In conclusion, it is thought that such a classification based on surface traits could be a potential method to help identify and compare seeds, especially in the identification of hybrid plants. In addition, valuable data can be obtained with this method during the use of wild forms of cultivated plants in breeding programs.

Keywords: *Glycine max*, seed surface ornamentation, Türkiye.

1. Introduction

Soybean, an important legume plant from the Fabaceae family, is used in both human and animal nutrition (Kuromori et al. 2022). First identified in China and referred to as the 'miracle plant,' soybean was cultivated approximately 5000 years ago and later spread to the United States (USA) and Europe (Zhao and Gai 2004).

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The cultivated form of soybean, *Glycine max*, was first domesticated from the wild species *Glycine soja* in China, and from there it spread to Japan, South Korea, and North Korea (Boerma and Specht 2004). Although soybean is one of the most important cultivated crops in the worldwide today, it was not well known outside of the Far East until about 100 years ago. In our country, soybean was first cultivated as a primary crop in the Black Sea region during World War I, and as a secondary crop in the Mediterranean and Aegean regions between 1968 and 1970 (İşler and Coşkan 2009). According to the 2021 data from the Food and Agriculture Organization (FAO) of the United Nations, the global cultivation area of soybean is 126.95 million hectares, with a production quantity of 353.46 million tons, and an average yield value of 2.78 tons per hectare. The leading countries in terms of cultivation area are Brazil (37.1 million hectares), the USA (33.3 million hectares), Argentina (16.7 million hectares), India (12.1 million hectares), China (9.8 million hectares), Paraguay (3.6 million hectares), and Canada (2 million hectares) (FAO 2021). In Türkiye, soybean production amounted to 182.000 tons in an area of 43.891 hectares in 2021, with an average yield of 4.15 tons per hectare (TURKSTAT 2021). Adana, Mersin, Samsun, Osmaniye, and Kahramanmaraş provinces constitute 97% of the soybean cultivation area in Türkiye. In soybean production, Adana province ranks first with 62.8%, followed by Mersin with 16.8%, Kahramanmaraş with 7.1%, Osmaniye with 5.8%, and Samsun with 3.4%. Soybean ranks first among oilseed crops imported into our country. Türkiye is largely dependent on imports for its soybean needs, and in 2020, 3.040.452 tons of soybean were imported, with an expenditure of 1.2 billion dollars. Our self-sufficiency rate in domestically produced soybean is 4.9% (FAO 2021).

Soybean seeds contain on average 18-24 % oil, 36-40 % protein, 26 % carbohydrates, and 8 % mineral matter, making it an important food raw material for essential nutrients such as protein, fat, and milk (Arıoğlu 2007). Soybean ranks first among oil crops in terms of cultivation area and production quantity worldwide. Although numerous studies have been conducted on the nutritional value and content of soybean due to its consumption as food (Omoni and Aluko, 2005; Szostak et al., 2020; Dukariya et al., 2020; Carrera et al., 2021; Kumar et al.,2023), genotype characterization and determination of seed surface properties have not received sufficient attention. The importance of seed morphology in determining evolutionary patterns and species identification as well as phylogenetic relationships is supported by many features provided by seed micromorphology (Johnson et al. 2004; Attar et al. 2007). Ultrastructural features of the seed, especially the seed surface and cell shape, are considered important discriminators at intra- and interspecific levels (Kubitzki et al. 2013). In plant breeding, morphological markers which are based on visually accessible characteristics including flower colour, seed form, growth behaviours, and pigmentation are used to identify genetic variation (Govindaraj et al., 2015). In crop production, seed morphology (macro and micro) can be particularly important in breeding and identification of species/hybrid lines. In this context, Scanning Electron Microscopy (SEM) appears to be a powerful tool for obtaining high-resolution images of objects. With this technique, three-dimensional images are obtained by correlating surface features with depth. Seed characters revealed by scanning electron microscopy or SEM are widely used to address various issues primarily related to external characteristics, systematics and evolutionary relationships among species (Segarra and Mateu 2001). To our knowledge, there is no SEM study showing the surface ornamentation of soybean seeds, but there are studies on species belonging to the same family, and these studies clearly show how important seed surface morphology is in distinguishing genotypes (Zoric et al. 2010; Güneş 2013; Erkul et al. 2015; Rashid et al. 2018; Waheed et al. 2021; Kashyap et al. 2021; Rashid et al. 2021).

This study aims to visualize seed surface ornamentation in 12 soybean cultivars and varieties using the SEM technique. It will thus reveal similarities or differences between them using various features such as surface roughness and reticulation and determine the importance of these features in genotype selection.

2.Materials and Methods

Soybean seeds were provided by Prof. Dr. S. Ahmet BAĞCI. A total of 12 cultivars were examined for macro/micro-morphological variations. After cleaning the seeds, macromorphological measurements were made under a binocular microscope. The seeds were evaluated in terms of macromorphological characteristics (testa colour, hilum colour and shape), and the obtained data and measurements are given in Table 1. At least ten soybean seeds were used for each cultivar and the best one was selected. Then seeds were dehydrated, carbon dioxide-critical-point dried, and mounted on stubs with double-sided adhesive tape for SEM inspection. Using a JEOL JSM-6060 model SEM in low vacuum mode, the seeds were coated with gold at a thickness of around 20 nm to observe their surface features (Candan et al. 2009; 2016). SEM images were captured at 86× magnification for the overall view of the seeds, 10K× for the overall view of the seed surfaces, and 30K× for up-close views of the microstructural characteristics of the seeds for all genotypes. Structural nomenclature for seed surfaces was performed following the International Seed Morphology Association (ISMA) seed surface feature comparison chart (2022).

3.Results and Discussion

In this study, the seeds of soybean cultivars were examined macro- and micro morphologically. The seed macro morphological characteristics and seed surface patterns are given in Table 1, Table 2, and Figure 1. In the macromorphological measurements made in this study, seed length/width ratios (L/W ratio) were found to be very close to each other. The smallest seeds belonged to the Mersoy genotype (L/W ratio: 1.07) and the largest seeds belonged to Gapsoy, Lider and Mona genotypes (L/W ratio: 1.17). All seeds are yellow in colour, spherical flat type and medium-sized. The importance of seed morphology in determining phylogenetic relationships as well as evolutionary patterns and species identification is supported by a number of features provided by seed micromorphology (Johnson et al. 2004; Attar et al. 2007). It has been discovered that seed morphology, as well as the composition and morphology of the seed coat, are valuable taxonomic traits (Algan and Büyükkartal 2000; Segarra and Mateu 2001; Bobrov et al. 2004, Hassan et al. 2005). Many species or genera can be identified with great assistance from seed features (Juan et al. 2000; Moro et al. 2001; Segarra and Mateu 2001). Morphological characteristics, such as ornamentation on the testa and seed shape, are frequently employed to identify between species and variations (Aniszewski et al. 2001). Compared to other organs, fruits and seeds often exhibit lower levels of phenotypic plasticity (Bonilla-Barbosa et al. 2000). Seed characteristics are often unaffected by environmental conditions and commonly reflect genetic differences. Angiosperm taxa exhibit significant variation in seed morphology, with rather constant seed structure within small taxonomic groupings (Esau 1977; Hassan et al. 2005).

Table 1. Detailed information about soybean seeds macromorphological characteristics

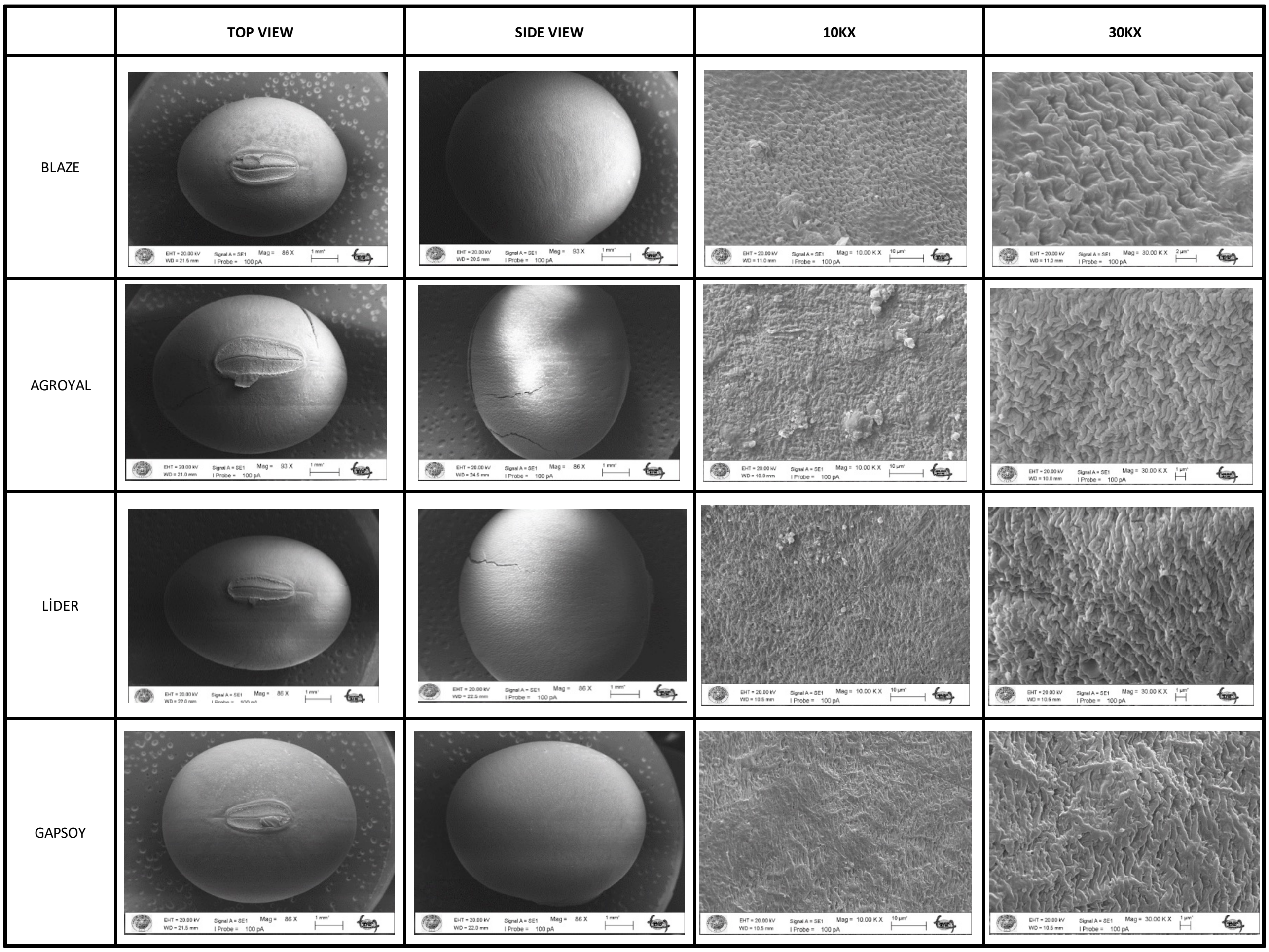
Soybean Cultivar	L (mm)	W (mm)	L/W ratio	Seed coat (testa) color	Hilum color	Seed shape
BLAZE	6.8	6.3	1.08	Yellow	Black	Spherical flattened
AGROYAL	6.4	5.5	1.16	Yellow	Black	Spherical flattened
LİDER	6.9	5.9	1.17	Yellow	Black	Spherical flattened
GAPSOY	6.9	5.9	1.17	Yellow	Dark Brown	Spherical flattened
ATLAS 3616	6.5	5.7	1.14	Yellow	Imperfect black	Spherical flattened
MAY 6301	6.9	6	1.15	Yellow	Dark Brown	Spherical flattened
MONA	6.9	5.9	1.17	Yellow	Black	Spherical flattened
ALPEREN	7.3	6.3	1.16	Yellow	Dark Brown	Spherical flattened
AVON	6.6	5.8	1.14	Yellow	Dark Brown	Spherical flattened
MERSOY	6.5	6.1	1.07	Yellow	Dark Brown	Spherical flattened
UMUT	6.6	6.1	1.08	Yellow	Dark Brown	Spherical flattened
PINAR	6.3	5.6	1.13	Yellow	Black	Spherical flattened

SEM technique was used to reveal the micro morphological similarities and differences between seed surfaces. Detailed information about the structure of the seed surface of these genotypes is provided in Table 2. In this study, seed similarity levels were evaluated as Type A, Type B and Type C based on surface characteristics. Firstly, when evaluated according to seed surface characteristics, Blaze seed belongs to Type A and was identified as having the highest level of distinctiveness with unique surface characteristics. It is distinguished from other seeds by its stippled/pitted surface roughness, wavy ridged reticulation, warty tubercles and decorative veins. Agroyal, Lider, Gapsoy, Atlas, May 6301, Mona and Alperen seeds were grouped in Type B since their surface characteristics were very similar. Looking at the seed surfaces, the stippled surface roughness, wavy ridged reticulation, warty tubercles and vein decoration are consistent among these seeds. The other seeds Avon, Mersoy, Umut, and Pınar have unique surface characteristics different from Type A and B seeds and are classified as Type C. Scurfy surface roughness, ridged reticulation, irregular tubercles and irregular dorsal ornamentation (ridges) are characteristic features of this group (Figure 1).

As a result, it was determined that morphological characteristics such as seed size, general shape and seed colour were not distinguishing characteristics in the studied varieties, but micromorphological characteristics such as seed surface ornamentation could be distinguishing characteristics for soybean genotypes.

Table 2. Seed surface characteristics of the studied soybean cultivars.

Cultivar name	Type	Surface Roughness	Reticulation	Tubercles	Raised and Grooved Surface Decoration
BLAZE	A	Stippled/Pitted	Wavy ridged	Warty	Veins
AGROYAL	B	Stippled	Wavy ridged	Warty	Veins
LİDER	B	Stippled	Wavy ridged	Warty	Veins
GAPSOY	B	Stippled	Wavy ridged	Warty	Veins
ATLAS 3616	B	Stippled	Wavy ridged	Warty	Veins
MAY 6301	B	Stippled	Wavy ridged	Warty	Veins
MONA	B	Stippled	Wavy ridged	Warty	Veins
ALPEREN	B	Stippled	Wavy ridged	Warty	Veins
AVON	C	Scurfy	Ridged	Irregular	Irregular ridges
MERSOY	C	Scurfy	Ridged	Irregular	Irregular ridges
UMUT	C	Scurfy	Ridged	Irregular	Irregular ridges
PINAR	C	Scurfy	Ridged	Irregular	Irregular ridges



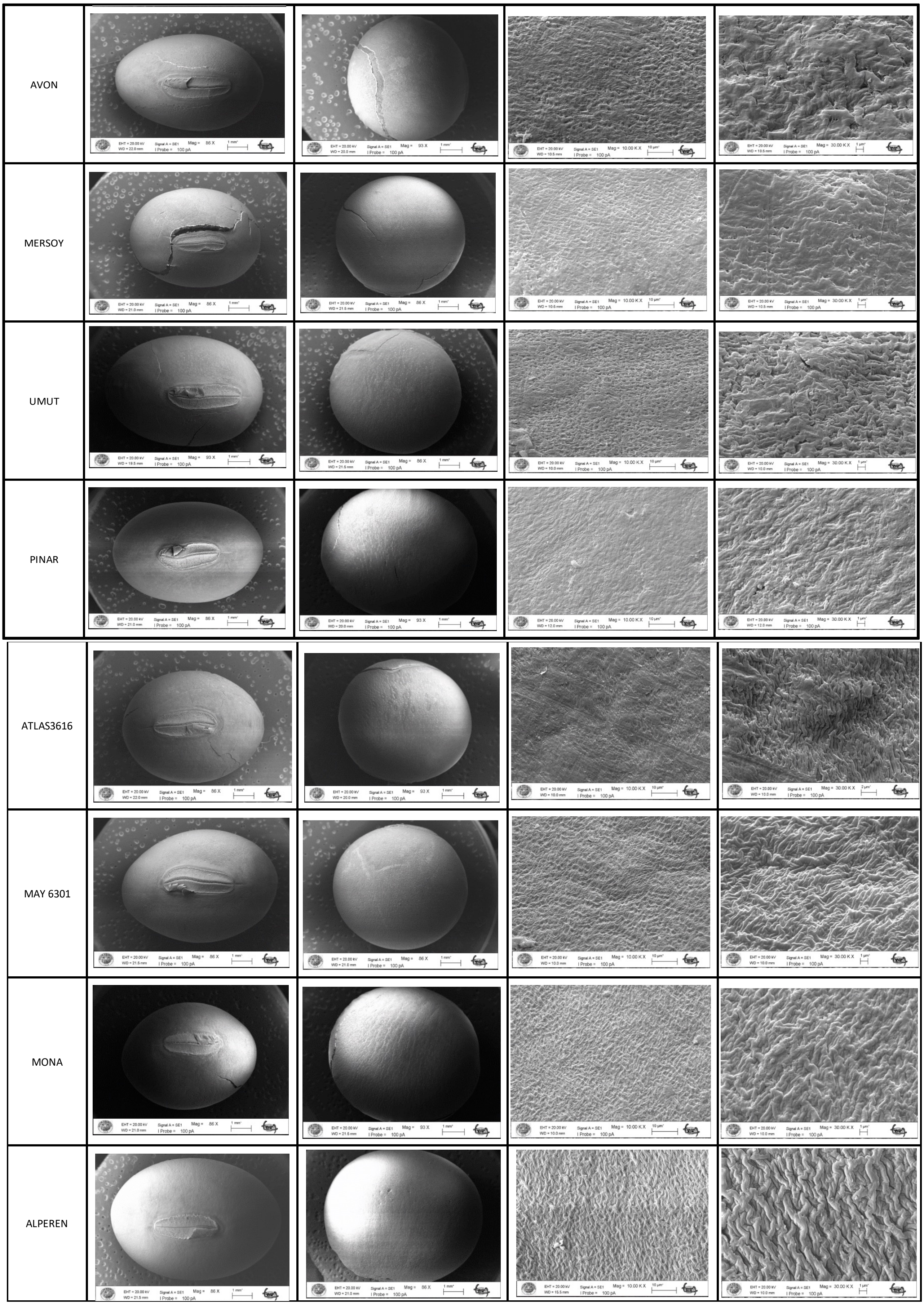


Figure 1. Scanning electron microscope photographs (SEM) of the investigated soybean seeds.

According to our literature review, there is only one study related to SEM analysis of soybean seeds, but in the study, instead of separating the cultivars, two soybean cultivars were evaluated with SEM technique in terms of water permeability and it was reported that the water permeability of the seed decreased due to small cuticle cracks on the seed surface (Chavan et al. 2021). However, there are similar studies on the determination of seed surface properties in species belonging to the *Fabaceae* (*Leguminosae*) family. Zohary and Heller (1984) carried out initial investigations on the seed surfaces of 24 *Trifolium* species. They identified five types of seed coat patterns and reported that the seed surface was smooth, rough, tuberculated, wrinkled or pitted. Algan and Büyükkartal (2000) described the ultrastructure of the seed coat of *Trifolium pratense* and reported that the seed coat was composed of elongated macroclerids. Slattery et al. (1982) analyzed the color of the seed coat of *Trifolium subterraneum* and its relationship with phenolic content and permeability and reported that seeds were oval and spherical in shape and the seed coat was smooth, striated and wrinkled. The study by Zoric et al. (2010) aimed to describe and compare the external seed morphological characteristics of 38 *Trifolium* species and to evaluate their possible use for taxonomic evaluations. As a result of the study, it was emphasized that more studies should be carried out on more characters to facilitate identification within these groups due to small differences in seed macro and micro morphological characteristics. Rashid et al. (2018) examined the micro-morphological and ultrastructural characteristics of seeds for 12 species within the Vicieae tribe in Pakistan to study taxonomic traits that can be useful in differentiating between species at the generic or infra-generic level. Their findings indicated that seed micromorphology was a highly helpful criterion for separating different species within the Vicieae tribe. To assess the taxonomical significance of macro/micro-morphological seed characteristics, Rashid et al. (2021) used SEM to examine the seed morphology of 12 species from 5 genera of tribes Astragaleae and Trifolieae (*Leguminosae*; *Papilionoideae*). They stated that an SEM investigation had identified important and remarkable seed morphological characteristics in several *Astragaleae* and *Trifolieae* tribe members. The seeds' size, shape and ornamentation are noted as important characteristics for identifying the species under study. Their results also demonstrated how latent morphological affinities between species can be found through SEM in seed morphology. Waheed et al. (2021) employed the scanning electron microscopy technique to compare the micromorphological characters of seeds in the identification of 12 Fabaceae species from Sanghar district of Pakistan and they reported that the SEM studies revealed significant characters of seed surfaces that contain enough information about seed to be recognized as determination of the species and genus levels in the subfamilies *Papilionoideae*, *Caesalpinioideae* and *Mimosoideae*.

4. Conclusions

In conclusion, SEM surface analysis is an inexpensive and effective method for the characterization of soybean genotypes. At the same time, the SEM method can be considered as a potential method for following and determining the characteristics of hybrid plants. In addition, this method has the potential to provide valuable data during the use of wild forms of cultivated plants in breeding programs. The data obtained will provide valuable information for agricultural research and practical applications and will lead to a better understanding of genetic diversity in soybeans.

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