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Anatomical and micromorphological characteristics of the seed coat of field pea (*Pisum sativum* L.) genotypes in relation to cracks and damage of seeds

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Abstract: In this paper, we present the morphological characteristics of the seed and micromorphological, anatomical and chemical characteristics of the seed coat of pea (*Pisum sativum* L.) genotypes, Jezero, Javor and NS Junior. Our aim was to investigate whether these genotypes can be differentiated based on seed coat morphoanatomical characteristics, depending on the harvest treatment. The observations and measurements of seed coat cross-sections were performed using light microscopy. The seed coat surface was observed using SEM. A tuberculate seed coat surface characterized all examined pea genotypes, and the average diameter of the tubercle was about 12 μm. Statistical and laboratory analyses revealed that major damage was the most frequent defect type as the result of mechanized harvest in all the examined genotypes. Genotype NS Junior had the shortest seed length (6.1 mm). Micromorphological analysis revealed that the seed surface was tuberculate in all genotypes. The genotype Jezero had the highest number of tubercle ribs (11.0) and a significantly higher proportion of parenchyma tissue (50.6%), while NS Junior was characterized by the greatest share of macrosclereids (49.8%). The highest number of osteosclereids (832/mm2) was counted in genotype Javor. In addition, genotype NS Junior stands out due to the highest percentage of crude fiber (62.75 g/100g) in the seed coat. There was a marked difference among the studied genotypes with regard to the seed coat morphoanatomical characteristics, which is confirmed by the results of multivariate discriminant analysis (MDA). These results suggested that the morphological, micromorphological and anatomical characteristics of the seed might have an impact on the seed coat damage level at harvest.

Key words: anatomy; seed micromorphology; seed coat

INTRODUCTION

The seed coat protects the embryo from mechanical injuries and facilitates regulation of the exchange of gases between the embryo and the environment, as well as imbibition. Different seed regions are characterized by varying seed coat thickness and absorb water and other substances differently. Seed coat permeability is also associated with its porosity and color, which affect seed viability and potential, as well as resistance to storage and fungal infections [1,2]. Consequently, the seed coat is a major modulator of embryonic response to ecological environmental conditions. Moreover, its characteristics are closely related to the temporal and spatial dispersion of seed germination in many plants. Time of germination is one of the key parameters in both natural and agroecosystems and is a major factor in yield determination [3]. The germination process is affected by numerous environmental factors, such as humidity, temperature, light and chemical factors. Come and Semadeni [4] concluded that seed coat permeability, and therefore seed germination, is affected by the seed coat color and the concentration of phenolic compounds. According to Bewley et al. [5], germination is the process of embryo growth related to the seed coat characteristics. The seed coat affects the seed growth rate and thus its final size [6]. The seed response to certain environmental conditions could be better understood by analyzing the characteristics

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of the seed coat. Rapid growth of cotyledons, which is often not aligned with the growth of the seed coat, is one of the most common causes of seed damage. This misalignment causes cracks, even during seed maturation, and thus reduces seed quality [7]. In an earlier study of Gillikin and Graham [8] it was found that seed coat susceptibility to mechanical damage is closely associated with its lignin content as a high content of the lignin polymer in the seed coat contributes to its increased strength and impermeability. However, increased lignin concentration in soybean seed coat is negatively correlated with seed coat permeability and positively correlated with resistance to mechanical damage [9-12]. Understanding seed coat characteristics is of fundamental importance for the development and improvement of seed production and handling, and could eventually lead to greater seed quality, thereby increasing agricultural production system efficiency. Seed coat features and functions have been the subject of many studies, each reflecting a specific interest, depending on the issues affecting particular species [1,13-17]. Karagić et al. [18] suggested that the occurrence of seed coat damage in different pea genotypes developed at the Institute of Field and Vegetable Crops in Novi Sad, depended on harvest treatment and genotype. The highest percentage of seeds with damaged seed coats occurred in the genotype with the largest seeds. The damage affects not only the physical seed quality but also seed germination.

The aim of this study was to determine if significant genotypic variability in morphological and micromorphological seed parameters, as well as anatomical seed coat parameters, exists in the seeds of different pea genotypes, and if it could be related to the types of seed coat damage observed following manual and mechanized pea harvesting.

MATERIALS AND METHODS

Plant material and environment data

In this study, we analyzed three pea genotypes: NS Junior, Javor and Jezero (Table 1). Plants were grown in field conditions at the experimental field of the Institute of Field and Vegetable Crops in Novi Sad during the 2008 and 2009 vegetation season. The area is characterized by a continental semi-arid to semi-humid climate with an annual precipitation of 617 mm and average annual temperature of 11.0°C. The experiment was conducted on loam soil type (pH 7.0, organic matter content 2.82%, N-NO_3 10.7 ppm, P_2O_5 and K_2O 30.8 ppm-26.6 ppm). At the stage of physiological seed maturity, two harvest treatments were applied: manual harvest (H) and mechanized, using the harvester Wintersteiger Nursery Master Expert at 800 r/min (M).

Table 1. Basic information of the analyzed genotypes.

Characteristics	Jezero	Javor	NS Junior
Seed color	light cream	light cream	cream
Seed shape	irregularly rounded	rounded	rounded
TSW (g)	270-290	280-350	130-170
Protein (%)	20	25	22

Morphological and micromorphological analysis

Fifty randomly selected seeds per genotype, treatment and year were selected. Morphological parameters (seed length and width (mm)) were measured using digital calipers. The seed index was calculated as the seed length to width ratio. Using a Leica MZ16 stereomicroscope, seed coat damage on the dorsal, ventral and lateral sides of the extrahilar zone was observed and recorded. Based on size and shape, incidences of damage were divided into three groups: (i) major damage (MD), (ii) large cracks (LC), and (iii) small cracks (SC) (Fig. 1 A-C). Changes in the seed coat color >2 mm with clear contours were marked as "major damage", cracks in the seed coat from 1-2 mm in length were marked as "large cracks", and cracks <1 mm were denoted as "small cracks".

Anatomical analysis

Ten randomly selected seeds per genotype and treatment were used for anatomical analysis. Parts of the seed coat located laterally from the hilum were separated and sections were created using a Leica CM 1850 cryostat at a temperature of -25°C. Partly macerated seed coat, obtained by heating in 30% KOH for 15 min, was used for the determination of the number of the osteosclereids/mm^2, osteosclereid size and the number of macrosclereids/mm^2 on the seed coat surface. Light microscopy was used for the analysis and measurement of seed coat cross-sections, using the Im-

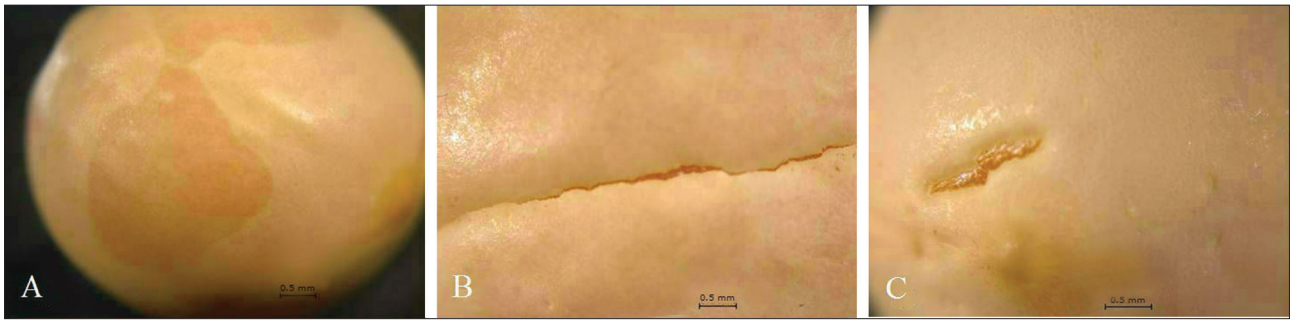

Fig. 1. Macroscopic observation of the seeds revealed the presence of damage on the seed coat of all three genotypes, irrespective of harvest type. Differences were recorded in both damage and crack distribution and size. Types of seed coat damage: **A** – Major damage; **B** – Large cracks; **C** – Small cracks.

age Analyzing System Motic 2000. Anatomical analysis included seed coat thickness (μm), macrosclereid layer thickness (μm), osteosclereid layer thickness (μm) and osteosclereid width (apical, medial and basal part, μm). Based on the measurements, the percentage share of certain tissues was calculated. The seed coat surface microstructure was analyzed using a scanning electron microscope (SEM). The seeds were coated with gold-palladium alloy (85:15) in the JEOL JEE 4B vacuum evaporator and observed under JEOL JS MT-35 SEM.

Chemical analysis

Chemical analysis of the seed coat included determination of the concentrations of pectin, pectic acid and propectin, as well as crude seed coat fibers. For this analysis, 2 g of whole seed coat per genotype were used. Dry seed coat was easily separated from the endosperm by hand. Fractions of pectin were determined colorimetrically by the carbazole method, while crude fibers were designated as crude cellulose by the Kirchner-Ganak method [19,20] as described below.

Fractions of pectic substances

Pectic substances were first precipitated as follows: 0.7-1 g of sample (seed coat) was transferred to a centrifuge cuvette that was filled with 95% ethanol solution (heated up to 75°C) to a volume of 40 mL. The mixture was mixed for 10 min in a water bath at 85°C. The volume was then filled to 50 mL. The cuvette was centrifuged at 3000 rpm for 15 min and the supernatant was rejected. The sediment was treated with 50 mL of 63% ethanol and mixed in a water bath at 85°C for 10 min. After 10 min, the cuvette was centrifuged at 3000 rpm for 15 min. The supernatant was discarded and the sediment used for fractional dissolution of pectic substances.

Extraction of pectin

Five mL of distilled water were added to the sediment obtained in the previous step and the content was mixed. A cuvette was filled up to 40 mL with distilled water and mixed for 10 min. The cuvette was centrifuged at 3000 rpm for 15 min. The supernatant was decanted into a 100-mL flask. Extraction with water was repeated and the supernatant was added to the same flask. Five mL of 1mol/L sodium hydroxide was added to the flask and the flask was filled with distilled water. The sediment was used for the next step.

Extraction of pectic acid

The sediment was treated with 5 mL of 0.75% ammonium oxalate solution and the content was mixed. The cuvette was filled up to 40 mL with the same solution and mixed for 10 min. The cuvette was centrifuged at 3000 rpm for 15 min. The supernatant was decanted to a 100-mL flask and the procedure was repeated once. Supernatant was again added to the flask. Five mL of 1 mol/L sodium hydroxide was added to the flask, and flask was filled with 0.75% ammonium oxalate solution.

Extraction of protopectin

The remaining sediment from the previous step was transferred to a 100-mL flask using distilled water. Five mL of 1 mol/L of sodium-hydroxide was added

Table 2. Number and position of damage types of pea genotypes of seed coat submitted to two harvest treatments (mean value±standard error, CV).

	Jezero		Javor		NS Junior	
Seed position and damage type	**H**	**M**	**H**	**M**	**H**	**M**
Ventral MD	0.33±0.21^{b} (155)	1.00±0.45^{ab} (110)	0.33±0.21^{b} (155)	2.00±0.89^{a} (110)	0^{b}	0.33±0.21^{b} (155)
LC	1.00±0.37^{a} (90.0)	0.50±0.22^{ab} (110)	0.83±0.31^{ab} (90.0)	0.83±0.40^{ab} (118)	0^{b}	0.33±0.33^{ab} (245)
SC	0^{b}	2.00±0.52^{a} (63.0)	1.17±0.65^{ab} (137)	0^{b}	1.33±0.71^{ab} (131)	0.50±0.34^{b} (167)
Dorsal MD	0.50±0.34^{c} (168)	0.67±0.33^{bc} (123)	3.33±1.02^{ab} (75.0)	4.83±1.70^{a} (86.0)	0.67±0.71^{bc} (131)	1.00±0.52^{bc} (127)
LC	0,33±0.21^{a} (155)	0.67±0.33^{a} (123)	0.50±0.22^{a} (110)	0.50±0.34^{a} (168)	0.33±0.21^{a} (155)	1.00±0.52^{a} (126)
SC	0^{a}	0.83±0.83^{a} (245)	0.50±0.22^{a} (110)	0^{a}	0^{a}	0^{a}
Lateral-right MD	8.33±0.99^{a} (29.0)	4.83±0.87^{bc} (44.0)	5.67±0.49^{bc} (21.0)	7.00±1.03^{ab} (36.0)	4.00±0.37^{c} (22.0)	2.33±0.67^{d} (70.0)
LC	0.17±0.17^{a} (245)	0.50±0.22^{a} (110)	0.67±0.33^{a} (123)	0.83±0.40^{a} (118)	0^{a}	0.33±0.33^{a} (245)
SC	0.33±0.21^{bc} (155)	0.83±0.31^{b} (90.0)	1.67±0.33^{a} (73.0)	0.17±0.17^{bc} (245)	0^{c}	0^{c}
Lateral-left MD	6.17±0.65^{ab} (26.0)	6.17±1.01^{ab} (40.0)	4.00±0.63^{bc} (39.0)	7.00±1.06^{a} (37.0)	4.67±0.61^{abc} (32.0)	2.83±0.79^{c} (69.0)
LC	0.50±0.34^{b} (167)	1.50±0.56^{a} (92.0)	0.33±0.33^{b} (245)	0^{b}	0.17±0.17^{b} (245)	0.17±0.17^{b} (245)
SC	0.17±0.17^{b} (245)	1.67±0.61^{a} (90.0)	0.33±0.33^{b} (245)	0^{b}	0^{b}	0.33±0.21^{b} (155)
Summation of seed coat damage, irrespective of location						
Summation of all MD	15.33±1.67^{ab} (27.0)	12.67±1.58^{b} (31.0)	13.33±1.30^{b} (36.0)	20.83±3.57^{a} (42.0)	9.33±1.28^{bc} (48.0)	6.50±1.80^{c} (48.0)
Summation of all LC	2.00±0.52^{ab} (63.0)	3.17±0.87^{a} (68.0)	2.33±0.84^{ab} (89.0)	2.17±0.31^{ab} (35.0)	0.50±0.3^{b} (167)	1.64±1.17^{ab} (173)
Summation of all SC	0.50±0.34^{b} (168)	5.33±1.17^{a} (54.0)	3.67±1.28^{a} (86.0)	0.17±0.17^{b} (245)	1.33±0.71^{b} (131)	0.83±0.31^{b} (90.0)

H – manual harvest; M – mechanized harvest;
MD – major damage; LC – large cracks; SC – small cracks
*Duncan's test values marked with the same letter in the same row are not significantly different (the level of significance $p \leq 0.05$)

to the flask. The flask was filled with distilled water and shaken for 15 min. The content was filtered and used for colorimetric determination.

Colorimetric determination of pectic substances

One mL of each extract was pipetted into two cuvettes. To one cuvette, 0.5 mL of 0.1% alcoholic carbazole solution was added. To the second cuvette (blank probe) 0.5 mL of purified alcohol was added. Six mL of concentrated sulfuric acid was added to both cuvettes. The cuvettes were heated in a water bath (85°C) for 5 min. Absorbance was measured at 525 nm. Standard solutions of galacturonic acid were prepared the same way for calibration. The content of pectic substances fractions was expressed as equivalents of galacturonic acid.

Crude fibers

The sample (0.2 g) was transferred to a flask and 45 mL of 80% acetic acid and 4.5 mL of nitric acid were added. The mixture was heated with a reversible condenser for 25 min. Hot liquid was filtered through a dried and weighed glass filter, and dried to a constant mass at 105°C. Crude fibers were expressed as g/100 g of sample.

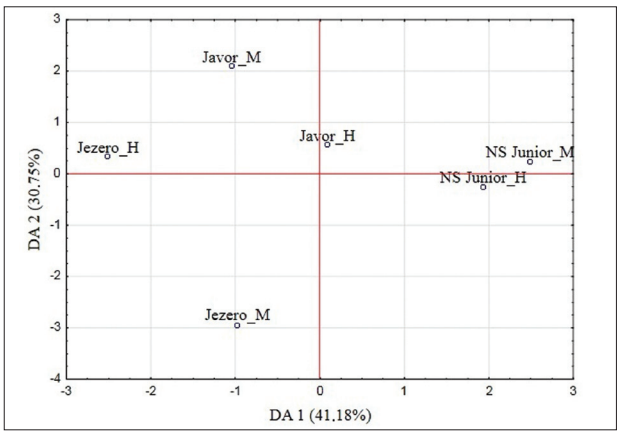

Fig. 2. Position of centroids in the space delimited by the first and second discriminant axis based on the seed damage observed in the studied pea genotypes induced by different harvesting methods. The figure shows that the first discriminant axis separated genotype NS Junior (M and H) from the two other analyzed genotypes according to characters such as the number of ventral large cracks (LC), dorsal major damage (MD), and lateral-right major damage (MD) and large cracks (LC). With regard to the second discriminant axis, genotypes Jezero (M) and Javor (M) are separated by the number of ventral small cracks (SC), dorsal major damage (MD) and lateral-left small cracks (SC).

Statistical analysis

The obtained results were statistically processed using Statistica for Windows version 10 (2011). To determine whether differences among the studied parameters of the analyzed genotypes were statistically significant, one-way ANOVA was applied, followed by Duncan's post hoc test for a significance level of $p \leq 0.05$ [21]. To test the hypothesis that the analyzed sample comprised morphologically distinct groups, discriminant component analysis (DCA) was applied.

RESULTS

Macroscopic observation of the seeds revealed the presence of damage on the seed coat of all three genotypes, irrespective of the harvest type. Differences were recorded in both damage and crack distribution and size (Fig. 1, Table 2). The lowest number of defects in the form of cracks (large and small) for all genotypes was present on the dorsal side of seeds. Most of the major damage was found on the lateral side of seeds, with significant genotypic differences. Major damage was the most frequent defect type in all genotypes, regardless of the part of the seed coat. Mechanical harvesting resulted in the significantly greater prevalence of major damage in the genotype Javor (20.83), compared to Jezero (12.67) and NS Junior (6.50) (Table 2). The incidence of all types of seed coat damage was very variable in all examined genotypes and treatments, resulting in extremely high values of coefficient of variation (Table 2).

The DCA results showed that the separation of seeds harvested using different harvesting methods, based on the analyzed components, was not possible in genotype NS Junior (Table 3). The first discri-

Table 3. Discriminant component analysis of quantitative characters of seed damage in the studied pea genotypes exposed to different harvest treatments.

Characters	DA1	DA2	DA3	DA4	DA5
Ventral MD	-0.366490	-0.330853	-0.121451	**-0.772762**	0.112087
Ventral LC	**-0.886703**	0.165114	-0.163730	-0.008218	0.621692
Ventral SC	0.248688	**-0.899204**	0.199249	-0.051278	-0.490549
Dorsal MD	1.071796	**0.755525**	-0.238062	0.057304	-0.477355
Dorsal LC	0.501853	0.262627	0.0900389	0.220678	0.178743
Dorsal SC	0.109926	0.239825	-0.329818	0.0557882	-0.181457
Lateral-right MD	**-0.793075**	-0.156805	0.292301	0.346183	-0.103325
Lateral-right LC	**-0.854772**	0.092772	-0.496653	-0.583273	0.397328
Lateral-right SC	-0.303753	0.019545	**-0.885828**	0.314931	-0.070177
Lateral-left MD	-0.698292	0.231077	0.261839	-0.186059	-0.378460
Lateral-left LC	-0.057481	-0.426514	0.415474	0.102720	-0.137497
Lateral-left SC	-0.176092	**-0.782445**	-0.349544	-0.257242	0.405004
Characteristic value	3.658456	2.731880	1.545171	0.683226	0.265984
The cumulative percentage	0.411770	0.719250	0.893164	0.970063	1.000000

MD – major damage; LC – large cracks; SC – small cracks

minant axis, which contributed 41.18% to the total discrimination, clearly separated genotype NS Junior (M and H) from the two other analyzed genotypes. Specific quantitative characters that contributed the most to discrimination among the genotypes along the first axis were the number of ventral LC, dorsal MD and lateral-right MD and LC. The second discriminant axis, which contributed 30.75% to the total discrimination, clearly separated the Jezero (M) and Javor (M) genotypes. The second discriminant axis was defined by the number of ventral SC, dorsal MD and lateral-left SC. On the third axis, with a much lower contribution to the total discrimination, the number of lateral-right MC stood out, whereas the number of ventral MD was allocated to the fourth discriminant axis (Fig. 2, Table 3).

The three examined genotypes significantly differed in seed length (Table 4). The seeds of genotype NS Junior were characterized by spherical shape and a significantly shorter length (6.1 mm). A low variability in the seed morphological characters was indicated by the low values of the coefficients of variation.

Micromorphological analysis revealed that the seed surface was tuberculate in all genotypes (Fig. 3 A, B, C). Evenly and densely distributed tubercles, measured laterally from the hilum, were on average 13.5 μm in diameter. Each tubercle had 6 to 15 ribs, depending on its location. Statistically significant differences among the genotypes were observed in the number of tubercle ribs, and the genotype Jezero was characterized by the highest values (11.0) compared to the other two genotypes (Table 4)

A significantly thinner seed coat was noted in NS Junior (127 μm), while that measured in genotypes Javor and Jezero did not differ significantly (153 μm and 161 μm, respectively). In all analyzed genotypes, the macrosclereids were arranged in a single layer, except in the region of the hilum where two layers were present (Fig. 4 A, B). Statistically significant differences among genotypes in percentage share of macrosclereids were recorded (Table 4). NS Junior was had the greatest share of macrosclereids (49.8%) in the total seed coat thickness. Genotype Jezero had the smallest share of macrosclereids (35.1%) in the total seed coat thickness, as well as the greatest number of macrosclereids per mm^2 (4737) of the seed coat surface.

Table 4. Micromorphological and anatomical characteristics of the seed coat of the three investigated pea genotypes (mean value±standard error; coefficient of variation CV%).

Variable	Jezero	Javor	NS Junior
Seed length (mm)	7.0±0.1^{a*} (11.0)	6.9±0.08^{a} (9.0)	6.1±0.06^{b} (8.0)
Seed width (mm)	6.4±0.09^{a} (12.0)	6.0±0.06^{a} (9.0)	6.1±0.08^{a} (11.0)
Seed index	1.11± 0.02^{a} (16.0)	1.16±0.02^{a} (14.0)	1.00±0.01^{b} (12.0)
Number of tubercular ribs	11.0±0.42^{a} (19.0)	7.9±0.2^{b} (13.0)	8.4±0.2^{b} (14.0)
Tubercle diameter (μm)	14.4±0.24^{a} (9.0)	13.0±0.3^{b} (12.0)	14.0±0.42^{a} (15.0)
Number of macrosclereids/mm^2	4737±85^{a} (4.0)	4356±173^{b} (9.0)	4505±211^{ab} (11.0)
Number of osteosclereids/mm^2	697±44.2^{b} (20.0)	832±25.7^{a} (10.0)	699±33.2^{b} (15.0)
Osteosclereid diameter (μm)			
apical	19.4±1.2^{c} (52.0)	27.8±1.2^{a} (32.0)	23.3±0.8^{b} (29.0)
medial	12.0±0.9^{c} (57.0)	16.1±0.7^{a} (33.0)	13.4±0.6^{b} (31.0)
basal	33.2±0.9^{ab} (19.0)	34.6±0.7^{a} (15.0)	32.1±0.7^{b} (16.0)
seed coat thickness (μm)	161±3.5^{a} (17.0)	153±3.4^{a} (17.0)	127±2.9^{b} (18.0)
% of macrosclereid layer	35.1±0.8^{c} (18.0)	47.2±0.9^{b} (15.0)	49.8±0.8^{a} (13.0)
% of osteosclereid layer	14.2±0.7^{a} (38.0)	11.1±0.4^{ab} (31.0)	10.8±0.5^{b} (35.0)
% of parenchyma layer	50.6±1^{a} (16.0)	41.6±1^{b} (19.0)	39.4±0.9^{b} (18.0)

* Duncan's test values marked with the same letter were not significantly different (the level of significance $p \leq 0.05$).

A significant difference among genotypes was found with respect to the number and dimensions of the individual osteosclereid cells. Genotype Javor stood out due to a significantly high number of osteosclereids per seed coat unit area (832/mm^2) and their large dimensions (Table 4). The osteosclereid size and osteosclereid layer thickness were very variable, as evidenced by the high values of the coefficients of variation. The seed coat parenchyma was comprised of the greatest number of layers of densely packed cells of irregular shape (Fig. 4 C). A significantly higher proportion of parenchyma tissue was observed in genotype Jezero (50.6%) relative to NS Junior (39.4%) and Javor (41.6%), which could not be distinguished based on this character (Table 4).

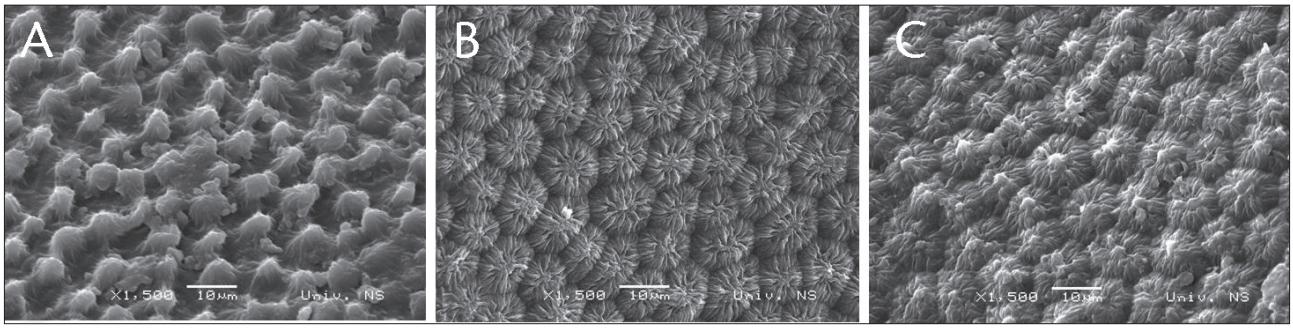

Fig. 3. Scanning electron micrographs of the seed coat surface. **A** – NS Junior; **B** – Jezero; **C** – Javor. It has been found that the seed surface was tuberculate in all genotypes. Each tubercle had 6 to 15 ribs, depending on its location.

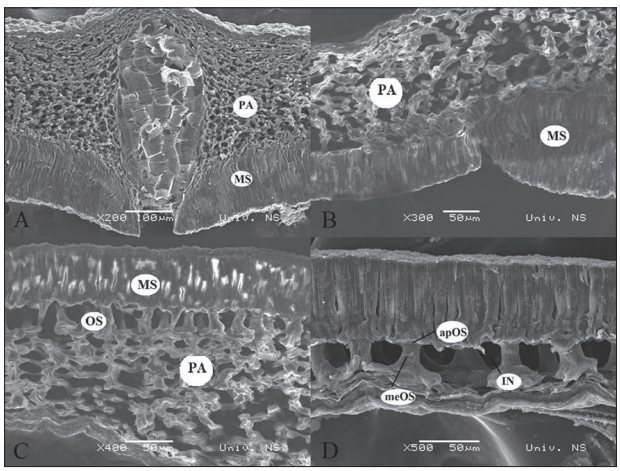

Fig. 4. Scanning electron micrographs of the seed coat cross-sections. **A**, **B** – Cross-section of seed coat in the hilar region. **C** – Multilayered parenchyma (MS -macrosclereids; PA - parenchyma; OS – osteosclereids); (D) APOS – apical part of osteosclereids; MEOS – medial part of osteosclereids; IN – intercellular.

Discriminant analysis of the main components revealed that the studied genotypes could be significantly differentiated based on morphological and anatomical seed characteristics (Fig. 5, Table 5). The characters that contributed most to discrimination among the genotypes and defined the first two discriminant axes were seed length and width, seed index, seed coat thickness and percentage of macrosclereid layer thickness. Moreover, the first discriminant axis was identified by the number of tubercle ribs and the number of osteosclereids/mm^2 and contributed to the separation by a remarkable 66.1%. Based on the characters of the first discriminant axis, genotype Jezero was separated from the other two genotypes. The second discriminant axis, in addition to the aforementioned characters, was also defined by tubercle diameter, number of macrosclereids/mm^2, as well as percentage of osteosclereid layer and parenchyma thickness. Genotypes Javor and NS Junior were separated by the characters located on the second discriminant axis.

The seed coat chemical analysis of pectin, pectic acid and protopectin (g/100 g) revealed no significant differences in the seed coat chemical structure of the three studied genotypes. The highest content of crude fiber (62.75 g/100 g) was found in the genotype NS Junior (Table 6).

DISCUSSION

The results of discriminant analysis pointed to genotypic specificity in the micromorphological and histological characteristics of pea seed coat, based on which the three studied genotypes could be clearly separated into distinct groups. The effect of mechanical damage to the seed coat due to different harvest treatments (manual/mechanized harvest at 500, 650 and 800 r/min) on the most important parameters of seed quality (germination energy, germination, development of atypical plants and seed weight), was investigated by Karagić et al. [18]. The authors reported that the proportion of damaged pea seeds was significantly different, depending on the genotype. This finding was subsequently confirmed by the examination of surface damage of pea seeds, observed by stereomicroscope, which was visible in all genotypes, irrespective of the harvesting method. NS Junior, the genotype with the thinnest seed coat, exhibited the lowest susceptibility to damage. In Javor and Jezero, a greater impact of harvest treatment to seed coat damage was recorded. The greatest number of defects in the form of small or large cracks was observed in the genotype Javor.

Table 5. Discriminant component analysis of quantitative characters of seed coat in the studied pea genotypes and loadings on five discriminant axes.

Characters	DA 1	DA 2	DA 3	DA 4	DA 5
Seed length	**2.68823**	**-5.05342**	-5.63018	-1.39525	0.10507
Seed width	**-2.34711**	**4.94331**	7.05949	1.92991	-0.44066
Seed index	**-3.85819**	**5.58482**	8.30703	1.89082	-0.60213
Number of tubercle ribs	**0.90703**	0.57363	-0.30137	0.46729	-0.35816
Tubercle diameter	0.38421	**-1.04764**	0.14464	-0.48230	0.07083
Number of macrosclereids	1.65998	**-4.01559**	2.22053	-1.41274	0.48401
Number of osteosclereids	-0.85229	-0.36995	-0.91480	0.20924	-0.53194
Diameter of apical part of osteosclereids	-0.28672	0.35092	0.36236	-0.42093	0.46361
Diameter of medial part of osteosclereids	0.15645	-0.21240	-0.18134	-0.27999	0.01505
Diameter of basal part of osteosclereids	0.23074	-0.40025	-0.38480	0.00207	-0.11331
Seed coats thickness (μm)	**-3.55385**	**4.05078**	-2.36436	4.19504	1.81602
% of macrosclereid layers	**-3.35776**	**4.83868**	-1.92181	2.17791	-0.77032
% of osteosclereid layers	-0.32532	**-2.18958**	1.45593	3.09653	4.91780
% of parenchyma layers	0.45931	**3.34400**	-1.61770	-3.37342	-5.28303
Characteristic value	11.09635	4.10217	0.80750	0.50959	0.26299
Cumulative percentage	0.66134	0.90583	0.95395	0.98433	1.00000

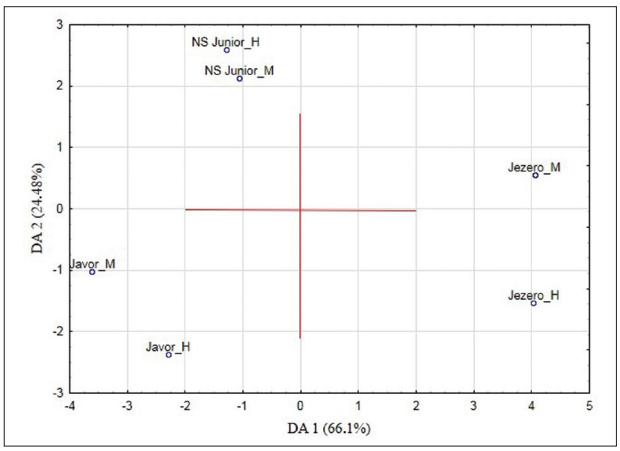

Fig. 5. Position of centroids in the space delimited by the first and second discriminant axis based on the seed coat characters of the studied pea genotypes. Characters located on the first discriminant axis separated the genotype Jezero from the other two genotypes, while characters that defined the second discriminant axis separated the genotypes Javor and NS Junior.

According to Karagić et al. [18], the genotype NS Junior is characterized by the lowest seed weight. These results are consistent with those reported in other studies, where seeds with damaged seed coats had a significantly higher length and width, as well as seed surface, compared to seeds with intact seed coats [22,23]. In the case of large-grained genotypes, damage was reported to occur on seed coats even before harvest, during the period of seed filling [24].

It is believed that seed coat damage in the case of plants with large seeds (as is the case with Javor) is a result of the loss of seed coat elasticity, arising due to the earlier maturation of the seed coats, i.e., uneven development of the cotyledons and the seed coat [25]. During the 2008/2009 growing season, when the material for this study was sampled, seed viability of the same pea genotypes was examined. By applying different tests, Karagić et al. [18] concluded that Javor had the lowest germination percentage, which was the highest in Jezero, in all modes of testing. These results are in concordance with those obtained by our morphological analysis of seed coat damage. The highest degree of seed coat damage was found in Javor, irrespective of the harvesting method.

Table 6. Content of certain chemical components in the seed coat of the studied pea genotypes (g/100g).

Variable	Jezero	Javor	NS Junior
Pectin	0.30	0.31	0.28
Pectic acid	0.31	0.38	0.33
Protopectin	0.86	1.05	0.94
Crude fibers	56.42	55.34	62.75

The anatomical features of macrosclereids and the presence of cuticle and wax on the surface of these cells, affect the ornamentation of seed coats, as well as the imbibition process [2,17,26,27]. In NS Junior, the highest percentage of macrosclereid thickness and maximum percentage share of crude fiber in the seed coat were recorded. This can be correlated with the smallest number of defects recorded in the same geno-

type. This assumption was confirmed by the results of the discriminant and correspondent analysis.

The subepidermal cell layer is comprises of osteosclereids, with clearly visible intercellular spaces, which are involved in the exchange of gases between the seed and the external environment [28,29]. The hypodermis development varies with the genotype. The presence of the highest number of the largest osteosclereids/mm^2 and of the smallest number of macrosclereids/mm^2 in Javor could be related to seed coat damage in this genotype. Wolf and Baker [30] reported that irregular cracks in soybean seed coats led to the separation of epidermal and hypodermal cells, thus revealing the parenchyma tissue located beneath. Although parenchyma cells are not associated with the seed imbibition process, in clover seed coats, elevated callose content in this layer was observed. This phenomenon may be associated with lower impermeability [31]. Results reported by Duke et al. [22] indicated that seed coat damage directly affects the cotyledon tissue located beneath. These results were also confirmed by SEM of soybean coat, which revealed differences in the cotyledon texture in places with and without damage. Examination of mature seeds also revealed that the damage to the surface layers mainly affected seed coat, cuticle, macrosclereid and osteosclereid cells [32]. The macrosclereid cuticle is considered the key determinant of seed coat permeability [33].

Mechanical damage to pea seeds is determined by the genotypic variability of histological characteristics of seed coats that directly affect seed quality parameters. The position of the damage on the seed coats could be linked to the seed coat structural and physiological characteristics. These results suggested that the morphological, micromorphological and anatomical characteristics of the seed might have an impact on the seed coat damage level at harvest.

In addition to the basic knowledge about seed coat structure, tracking changes that occur in the chemical composition of macrosclereids and osteosclereids during seed filling would be important for understanding the occurrence of damage on the coats of mature seeds.

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