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Seed microstructure and genetic variation of characters in selected grass-pea mutants (*Lathyrus sativus* L.)

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A b s t r a c t. Significant differences in a mutant form of Polish grass-pea obtained from parental cultivars Derek and Krab by chemomutagenesis were shown. Agricultural properties of plants (plant height, number of branches, number of pods per plant and their length and width, as well as number and weight of seeds per pod and maturity), geometrical features of mature seeds, and their microstructure were investigated. It was shown that the range of traits for the analysed features of mutants exceeded the variation observed for the original cultivars – Krab and Derek. This is especially visible for such yield structure traits as pod number per plant, seed number and weight per pod. From the agricultural point of view, very promising are mutants with reduced plant height and biomass, decreased number of lateral branches, improved lodging resistance and earliness. Analyses of technological usability factors (harvesting, drying, storage) involved assays of seed coat thickness and cotyledone cell microstructure. The obtained results are of key importance to the selection of the most promising material for further genetic trials and breeding practice.

K e y w o r d s: chemomutagenesis, *Lathyrus sativus*, plant properties, seed microstructure

INTRODUCTION

Grass-pea *Lathyrus sativus* L. is a protein-rich pulse, easily grown on marginal land under adverse environmental conditions, and has been cultivated in the Balkans as early as around 8000 BC (Lambein and Yu-Haey Kuo, 1997), and in South Asia and Ethiopia for over 2,500 years. In archaeological excavations in Turkey and Iraq, *Lathyrus species* were found as collected or cultivated (Kislev, 1986). It is a popular drought-tolerant crop for food and feed uses in drought prone areas of Africa and Asia. Its ability to provide an economic yield under diverse conditions has made it a popular crop in subsistence farming in many developing countries; it also offers a great potential for use in marginal rainfall areas (Abd El Moniem *et al*., 2000). The grass-pea is mainly cultivated in India, Bangladesh, China, Nepal and Pakistan, and locally in Europe (Spain, France, Italy, Bulgaria, Ukraine, and Poland).

Grass-pea plays a marginal role in Polish agriculture, however, since the 17th century it has been locally cultivated in the South-East Poland (Milczak *et al*., 2001). The cultivated landraces of grass-pea are very well adapted to Polish environmental conditions and serve as an ideal pro-ecological plant. Under our conditions, grass-pea displays high resistance to low temperatures in early spring, excellent resistance to drought (Rybiński and Pokora, 2002), fungal diseases, attacks of insects, and tolerance to salinity and soil types. As a result of continued experiments and selection, the most promising landraces designated as Der and Kra were released as new cultivars – Derek and Krab. Although these cultivars with improved agronomical traits indicate a great breeding progress and contribute to its cultivation in Poland, broader introduction of grass-pea elsewhere in Poland is still limited. This is mostly due to the very narrow gene pool as well as the necessity of improving such traits as indeterminate growth, too long ripening time, susceptibility to lodging, and production of high biomass with many lateral branches.

Grass-pea has good yield potential and very tasty seeds with high content of protein (up to 30%) and lysine. Although the seeds of *Lathyrus sativus* are tasty and

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protein-rich, over-consumption can cause an upper-neurone disease known as neurolathyrism, an irreversible paralysis of the lower limbs (Abd El Moniem *et al*., 2000). Seeds of such legumes as *Lathyrus ssp*. are characterized by high content of anti-nutrients (Hanbury *et al*., 1999; 2000) which hinder free nutritional utilization in monogastric animals and humans (Grela *et al*., 2001). The most frequently occurring anti-nutritional substances in grass-pea include a neurotoxin identified in 1964 as β-N-oxalyl-L-α, β-diaminopropionic acid or β-ODAP (Rao *et al*., 1964). Efforts to develop safer cultivars with lower levels of toxins or their complete removal are one of the most important goals in grass-pea improvement. Classic breeding and genetic engineering can be used to improve agricultural traits and reduce levels of anti-nutrients. Additionally, mutation breeding can be a valuable supplement to create a broad genetic variability that may be utilized by a plant breeder in development of cultivars for specific purposes or with specific adaptability (Campbell *et al*., 1994).

The goal of the presented paper was to demonstrate how mutagen agents influence plant habit, maturity, branching as well as yielding parameters – pod size, seed size and number and weight of seeds per pod and plant. It was also interesting to identify the effect of mutation on the seed structure of a microscopic level. It was expected that some of the mutants obtained can constitute interesting material for further investigation and become also valuable food and/or fodder.

MATERIAL AND METHODS

Plant material

The list of released original varieties in Poland, among many legume cultivars, includes two varieties of grass-pea (*Lathyrus sativus* L.) – Derek and Krab. The seeds of both cultivars constituted the initial material for mutation induction. For this purpose two chemomutagens – N-nitroso-N-methylurea (MNU) and sodium azide (NaN_3) – were used. The M_1 progeny, similar to that obtained by Nerkar (1976) and Singh and Chaturvedi (1987), in comparison to M_2 provided the information on mutagenic sensitivity, effectiveness and efficiency of successful selection of mutants in subsequent generation. In M_2 , the treated and control materials were screened for mutation frequency on the basis of chlorophyll and morphological mutations. In M_3, the selected forms were sown in plots to confirm whether the changed genotypes were stable mutants, compared to the initial material. Twenty of them, characterized by wide genetic variability of traits, were chosen for further analyses in M_4 and M_5.

Field tests

The seeds of mutants and both initial cultivars were sown in a randomized block design with 3 replications at the experimental field of the Institute in Cerekwica, with a spacing of 50 x 25 cm. Data were recorded for plant height (PH), first legume height (FLH), number of branches per plant (NBP), number of pods/plant (NPP), number of fertile pods/plant (NFPP), number of sterile pods/plant (NSPP), pod length (PL), pod width (PW), seeds number/ pod per main stem (S/P), seeds weight/pod per main stem (SW/P), seeds number/plant (SNP), seeds weight/plant (SWP), days to maturity (DM) of 20 mutants tested – 17 originated from cultivar Krab and 3 from Derek.

Scanning electron microscopy

For analysis of microscope pictures, two mutants of cv. Krab (K 29 and K 63) and two of cv. Derek (D 4 and D 13) were chosen. Microscope analyses were performed according to Frias *et al*., (1998). Preparations were mounted by sliver adhesive to aluminium specimen holders, and coated with carbon and gold in vacuum evaporator JEE 400. Following coating, the preparations were viewed in a JSM 5200 scanning electron microscope, operated at accelerating voltage of 10 keV.

Mass and geometrical properties

Mass and geometrical properties were determined by weighing the seeds on an electronic balance and measuring their two dimensions: diameter and thickness.

RESULTS AND DISCUSSION

The population arose from natural variation and sexual recombination. Contemporary plant breeding is based on creating variation, selection, evaluation and multiplication of desired genotypes (Ahloowalia and Małuszyński, 2001; Ahloowalia *et al*., 2004). One of the elements of variation creation is spontaneous mutation which, unfortunately, occurs with low frequency and is difficult to recognize. To create a new variation, the use of chemical and physical mutagens is an interesting method which has become an established technology. In this way many induced mutants have been released as cultivars (Małuszyński *et al*., 1995).

The results presented in Table 1 indicate that the chemomutagens used increased the range of obtained variation of traits in mutants as compared to control. The plant height for cultivar Krab ranged from 104.2 to 111.5 cm, and that of the mutants from 89.9 to 129.4 cm. The selection of mutants with plant height reduction is particularly important in connection with high susceptibility of grass-pea to lodging. Using gamma rays and EMS (Waghmare and Mehra, 2000), the induced polygenic variability of plant height in M_2 and M_3 reached 26-120 and 37-118 cm, respectively. A wide variation was noticed for traits affecting yielding ability. It was particularly true for pod number per plant, number and weight of seeds per pod and plant. The number of pods/plant ranged from 56.2 to 69.8 for cv. Krab and from 37.1 to 95.4 in their mutants. In M_2 and M_3 the variability of this trait

T a b l e 1. Range of the induced variability of traits ($X_{min.} – X_{max.}$) in mutants and their initial cultivars – Krab and Derek

Traits	Cultivars and mutants			
	KRAB	Mutants	DEREK	Mutants
PH* (cm)	104.2–111.5	89.9–129.4	110.1–119.1	92.4–93.8
FLH (cm)	23.8–26.2	19.3–41.9	18.5–21.4	21.5–27.0
NBP	8.9–11.6	8.9–16.2	18.2–20.4	8.70–9.60
NPP	56.2–69.8	37.1–95.4	118.2–132.4	64.5–89.0
NFPP	47.2–58.8	36.0–89.0	116.8–129.8	54.3–70.6
NSPP	10.2–12.4	0.0–17.8	1.6–3.8	5.9–18.4
PL (cm)	3.52–3.70	2.89–3.80	3.54–3.70	3.27–3.55
PW (cm)	1.25–1.29	1.14–1.32	1.10–1.18	1.17–1.22
S/P	3.34–3.52	2.32–4.14	3.84–4.00	2.88–3.85
SW/P (g)	0.36–0.42	0.20–0.46	0.24–0.28	0.19–0.31
SNP	168.2–185.4	96.3–250.6	385.4-418.2	142.2–227.2
SWP (g)	18.4–20.2	10.3–32.0	24.4–26.6	9.2–17.3
DM	124–128	116–130	124–126	119–122

*PH – plant height, FLH – first legume height, NBP – number of branches per plant, NPP – number of pods/plant, NFPP – number of fertile pods/plant, NSPP – number of sterile pods/plant , PL – pod length, PW – pod width, S/P – seeds number /pod per main stem, SW/P – seeds weight/pod per main stem, SNP– seeds number/plant, SWP – seeds weight/plant, DM – days to maturity.

accounted for 6-560 and 5-394, respectively (Waghmare and Mehra, 2000). Under Polish environmental conditions, mutants with a shorter vegetation period are particularly desirable. The range of variability indicates the possibility of selecting mutants which ripen 8 days earlier as compared to the initial cultivar Krab.

The results compiled in Table 1 confirm high effectiveness of mutagens in inducing wide variability of grass-pea traits, which was also observed by other investigators (Nerkar, 1972; 1976; Singh and Chaturvedi, 1987; Waghmare and Mehra, 2000; Rybiński 2001). According to the results of induced polygenic variation of traits obtained for Andean lupine (*Lupinus mutabilis* Sweet), mutagenesis is a particularly important tool in the case of species whose natural gene pool is very narrow. The grass-pea is such a species, represented in Poland by only two cultivars and a small number of landraces cultivated in the South-Eastern part of the country.

The means of mutant traits (Table 2) indicate that for yield structure parameters a few mutants yielded higher as compared to their initial cultivars. The mutants K 3, K 7, K 56, K 63 and K 64 exceeded cultivar Krab in respect of the number and weight of seeds per plant. It was particularly visible for K 63 which is further characterized by plant height reduction, a higher number of branches, higher pod number per plant, longer and broader pod, as well as a higher number and weight of seeds per pod and earlier maturity. Still, the majority of mutants yielded below cultivar Krab. Nevertheless, a few of them can be interesting as initial material for crosses in a breeding programme. The K29 mutant, characterized by early ripening, lower susceptibility to lodging, and high number of pods per plant, belongs to such genotypes. A wide spectrum of morphological mutations have been found also, affecting plant habit, branching and maturity as well as stem shape, leaf size, stipule shape, flower colour structure, pod size and seed size and colour (Nerkar, 1976). Mutants such as dwarf, erect and giant forms have been also obtained. However, the mutants of cultivar Derek were characterized by lower parameters of yield structure, their breeding importance being mainly attributed to plant height reduction, improved lodging resistance, a lower number of lateral branches, biomass and earliness (mutants D 4 and D 13). As high levels of rainfall often occur in Poland in autumn, indeterminate growth grass-pea plants prolonged flowering and developing, making harvest difficult. Forms with early maturity and short vegetation period are thus desirable. Unfortunately, determinate mutants similar to lupine forms (Czerwiński and Święcicki, 1989) have not been found.

From the consumer point of view, the most interesting part of the plants are seeds. It was interesting whether, or to what extent, the mutation led to changes in seed properties. It was found that apart from the differences in plant properties discussed above (Tables 1 and 2), mutation evoked a great variability in seeds properties. The data presented in Table 3 show selected mass and geometrical properties of initial

T a b l e 2. Mean values of the analysed traits in mutants and original cultivars Krab and Derek

Cultivars and mutants	PH* (cm)	FLH (cm)	NBP	NPP	NFPP	NSPP	PL (cm)	PW (cm)	S/P	SW/P (g)	SNP	SWP (g)	DM
KRAB	108.1	24.8	10.4	64.1	52.3	11.8	3.63	1.27	3.44	0.39	176.8	19.2	126
K 3	107.0	22.1	15.3	91.2	86.2	5.0	3.46	1.24	3.10	0.33	211.1	21.4	130
K 6	92.5	25.9	14.0	77.1	68.3	8.8	3.45	1.22	3.31	0.29	177.5	14.0	120
K 7	100.9	26.9	16.2	81.3	76.8	4.5	3.35	1.23	3.31	0.32	208.5	20.6	124
K 10	106.7	23.8	13.5	90.7	72.9	17.8	3.40	1.24	2.59	0.25	146.2	13.6	120
K 11	99.7	26.2	13.9	60.1	53.9	6.2	3.58	1.31	3.13	0.39	146.3	14.2	124
K 12	91.3	26.3	13.5	72.9	56.9	16.0	3.46	1.21	3.10	0.26	144.2	11.6	128
K 13	102.4	26.5	12.4	70.2	61.5	8.7	3.34	1.28	2.52	0.33	130.5	15.7	122
K 14	89.9	19.3	12.0	78.3	67.0	11.3	2.89	1.14	2.86	0.20	181.1	13.9	120
K 25	127.9	41.9	11.2	62.0	50.7	11.3	3.31	1.29	2.32	0.25	96.3	10.3	119
K 29	105.5	25.7	14.1	76.8	68.0	8.8	3.10	1.17	2.84	0.25	161.8	13.0	120
K 37	103.6	26.1	8.9	45.1	45.1	0.0	3.45	1.23	4.14	0.33	169.7	21.6	118
K 46	97.2	28.3	9.7	37.1	36.0	1.1	3.70	1.31	3.18	0.46	112.3	13.9	116
K 50	129.4	35.9	12.5	94.7	79.0	15.7	3.61	1.25	2.55	0.33	174.4	22.2	124
K 56	100.4	22.8	8.6	63.4	57.0	6.4	3.72	1.29	3.40	0.37	212.8	23.2	124
K 59	103.8	27.0	10.8	73.7	58.3	15.4	3.43	1.23	3.04	0.30	154.4	16.6	122
K 63	100.4	22.7	13.1	95.4	89.0	6.4	3.80	1.30	3.28	0.43	250.6	32.0	119
K 64	96.3	24.1	11.4	73.4	65.0	8.4	3.61	1.32	3.24	0.40	179.3	22.4	126
DEREK	115.0	20.9	19.4	127.7	124.5	3.2	3.66	1.14	3.96	0.26	407.8	25.8	124
D 4	93.8	27.0	9.6	64.5	54.3	10.2	3.27	1.17	2.88	0.19	142.2	9.2	119
D 11	92.4	24.7	8.7	72.2	66.3	5.9	3.55	1.22	3.85	0.31	227.2	17.3	122
D 13	92.5	21.5	9.5	89.0	70.6	18.4	3.31	1.17	3.31	0.24	210.3	15.8	120
LSD $\alpha = 0.05$	5.98	2.98	1.92	3.88	5.45	8.86	0.25	0.06	0.21	0.06	17.83	2.60	1.96

*Explanations as in Table 1.

T a b l e 3. Mass and geometrical properties of mutant and initial cultivars Krab and Derek

Cultivars and mutants	1000 seeds weight (g)		Thickness (mm)		Diameter (mm)	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
KRAB	192.8	5.1	5.35	0.53	7.84	0.69
K 3	166.5	9.3	5.00	0.26	7.44	0.63
K 6	152.2	11.6	4.78	0.29	7.32	0.79
K 7	178.7	7.9	4.87	0.39	8.20	0.42
K 10	151.7	7.6	4.89	0.28	7.02	0.56
K 11	191.2	6.2	4.95	0.47	7.79	0.75
K 12	136.1	5.9	4.70	0.57	7.14	0.59
K 13	171.6	9.3	4.77	0.28	7.61	0.38
K 14	141.6	9.2	4.83	0.39	7.03	0.58
K 25	123.1	6.4	4.46	0.34	6.69	0.25
K 29	103.6	8.4	4.35	0.38	6.26	0.53
K 37	110.7	3.4	4.36	0.41	6.15	0.45
K 46	150.5	5.7	4.69	0.39	7.10	0.49
K 50	160.3	9.2	4.87	0.38	6.92	0.46
K 56	157.2	5.1	4.73	0.35	7.13	0.38
K 59	154.2	6.8	4.57	0.38	6.97	0.55
K 63	193.9	13.0	4.84	0.54	8.21	0.57
K 64	199.3	9.8	5.57	0.50	8.00	0.82
DEREK	117.0	7.3	4.56	0.37	6.29	0.45
D 4	69.2	4.7	3.63	0.48	5.32	0.29
D 11	98.6	5.5	4.49	0.20	5.85	0.39
D 13	103.0	5.8	4.34	0.25	5.79	0.48

cultivars and their mutants. Statistically significant differences were found for 1000 seed weight, seed diameter and thickness. The range of differences in weight: 192.8 and 103.6 g for Krab and K 29, respectively, were correlated with differences in seed diameter (7.84 and 6.26 mm) and thickness (5.35 and 4.35mm). Similar diversity was found for Derek and its mutants, however the mean values for all characteristics were distinctly lower.

Those differences could indicate that also the internal structure of seed could be strongly differentiated by chemical mutagenesis. Three main structural features of seeds were investigated. The seed coat is protective against mechanical damage, and influences water uptake during processing in the presence of water (soaking, cooking). According to Fornal (1998), surface of the seed coat can also be regarded as a factor discriminating the seed variation when seeds are of the same shape or weight, and influence, due to differences in roughness, seed behaviour during separation processes based on friction coefficient. Agbo *et al*. (1987), Swanson *et al*. (1985) and Fornal (1998) proved that surface morphology can be a good discriminating factor within such legumes as *Phaseolus vulgaris* L., *Vigna angularis* Willd. or *Lens culinaris* Medik. SEM pictures of seed coat surfaces of the experimental grass-pea cultivars revealed distinct differences not only between parental species but also within both groups (Fig. 1). A great similarity was observed between seed coat surface of Derek (Fig. 1a) and its mutant D 13 (Fig. 1c), as well as a striking resemblance of D 4 mutant (Fig. 1b) to control seed of Krab (Fig. 1d). The former are characterized by very clear, numerous and regular star-shaped elements sticking out from the surface. Their characteristic endings are spherical with average diameter of 1.5 μm. The latter are more 'condensed' with spherical endings of larger diameter (especially D 4). A close look at the control Krab seeds (Fig.1d) revealed distinctly marked fringes at their base. Striking differences were found between the two remaining mutants of Krab. Seed coat surface of K 63 (Fig.1f) was covered with a delicate network of connected filaments. Contrary to this, K 29 was totally covered with less ordered dense structures, probably waxy cuticle masking the original structural elements (Swanson *et al.* 1985). Cross-section through the seed coat (Fig. 2) revealed details of its structural elements similar to those reported earlier for pulse seeds (Agbo *et al*. 1987, Enquist and Swanson 1992, Enamuthu *et al*. 1993, and Swanson *et al*. 1985). This part of seed is composed of an epidermal layer of well organized, elongated palisade cells, columnar hour-glass cells being much shorter, and multi-layered parenchyma cells. As it can be seen from SEM microphotographs and Table 4, the investigated cultivars differed in the overall thickness and structural details. Among Derek seeds there were no differences in the overall thickness of the seed coat between parental form and D 4 mutant (Fig. 2a and b), however the former was characterized by a very

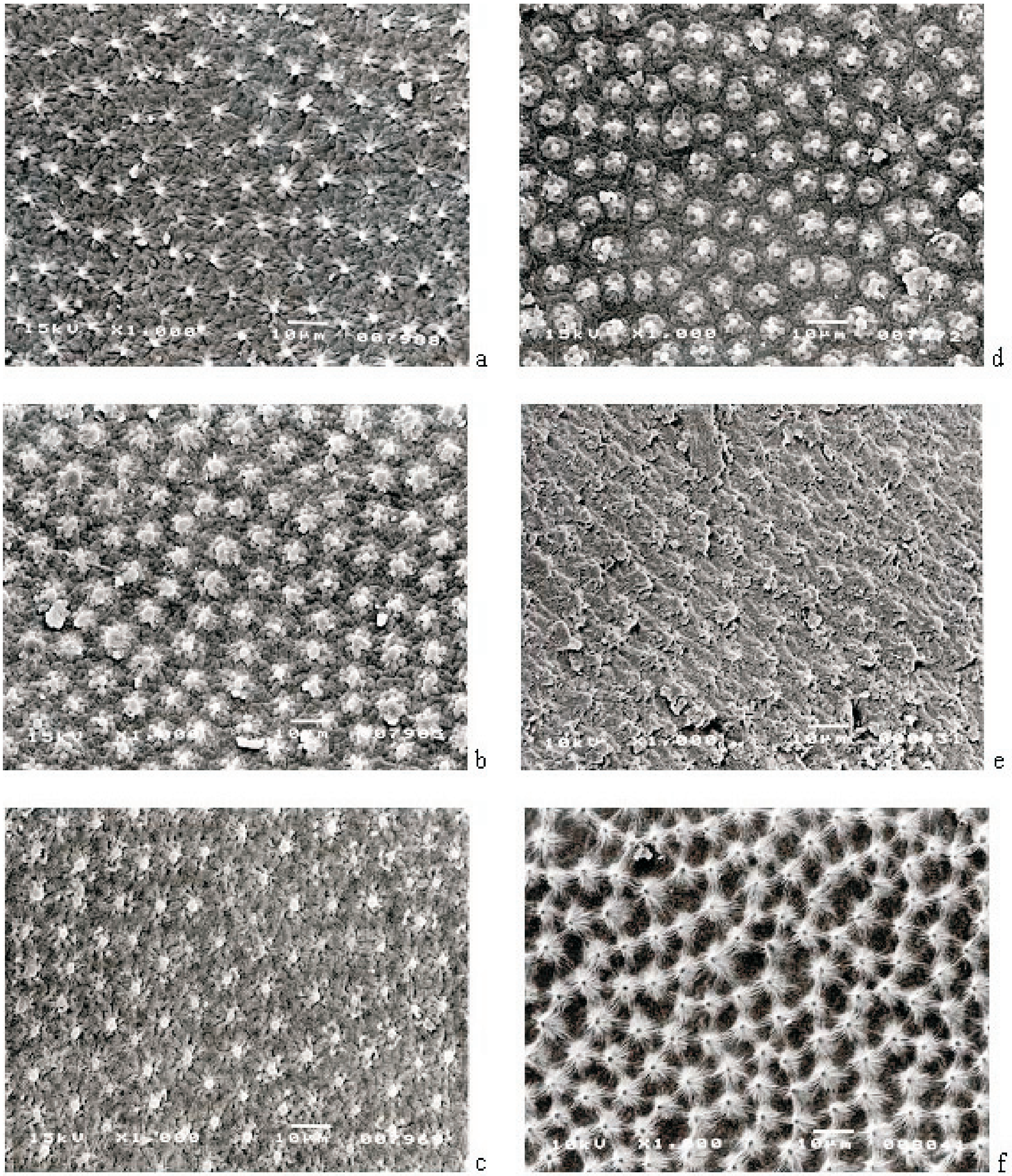

Fig. 1. Surface morphology of seeds of cv. Derek (a), its mutants: D 4 (b) and D 13 (c), and cv. Krab (d) and its mutants K 29 (e) and K 63 (f).

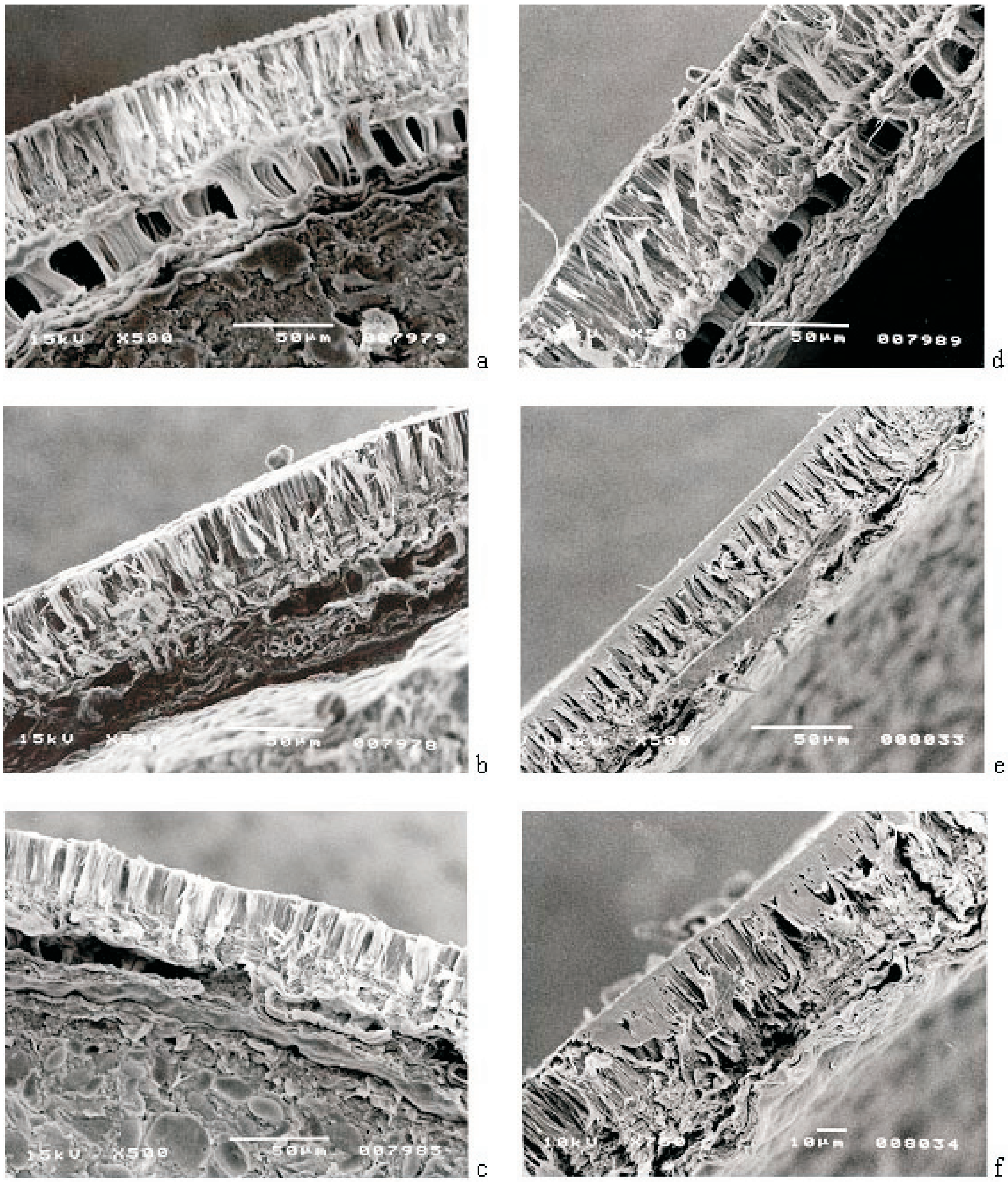

Fig. 2. Cross-section through the seed coat of seeds of cv. Derek (a), its mutants: D 4 (b) and D 13 (c), and cv. Krab (d) and its mutants: K 29 (e) and K 63 (f).

T a b l e 4. Average thickness of seed coat structural elements (μm) of parental cultivars Krab and Derek and their mutants

Parameter	DEREK	D 4	D 13	KRAB	K 29	K 63
Palisade	53 (2.7)	60 (3.1)	47 (1.8)	72 (3.1)	60 (2.6)	50 (3.7)
Hour-glass	30 (1.9)	12 (2.0)	10 (2.0)	19 (2.3)	0	0
Parenchyma	12 (1.8)	33 (2.0)	16 (2.3)	29 (1.8)	13 (3.0)	10 (1.7)
Total	95 (3.4)	95 (4.2)	73 (3.6)	120 (4.7)	73 (3.9)	60 (3.5)

distinct subepidermal layer of thick hour-glass cells and very thin, amorphous parenchyma layer. In D 4 mutant seed coat, single cells of this layer can be easily distinguished. The mutant form of D 13 has the thinnest seed coat with the smallest layer of hour-glass cells (Fig. 2c). The seeds of Krab also differed from each other as well as from Derek variety. The parental form, except for the highest of all the investigated forms of the seeds, was characterized by the highest palisade layer and a very dense layer of parenchyma cells (Fig. 3d). There was also a striking phenomenon observed in

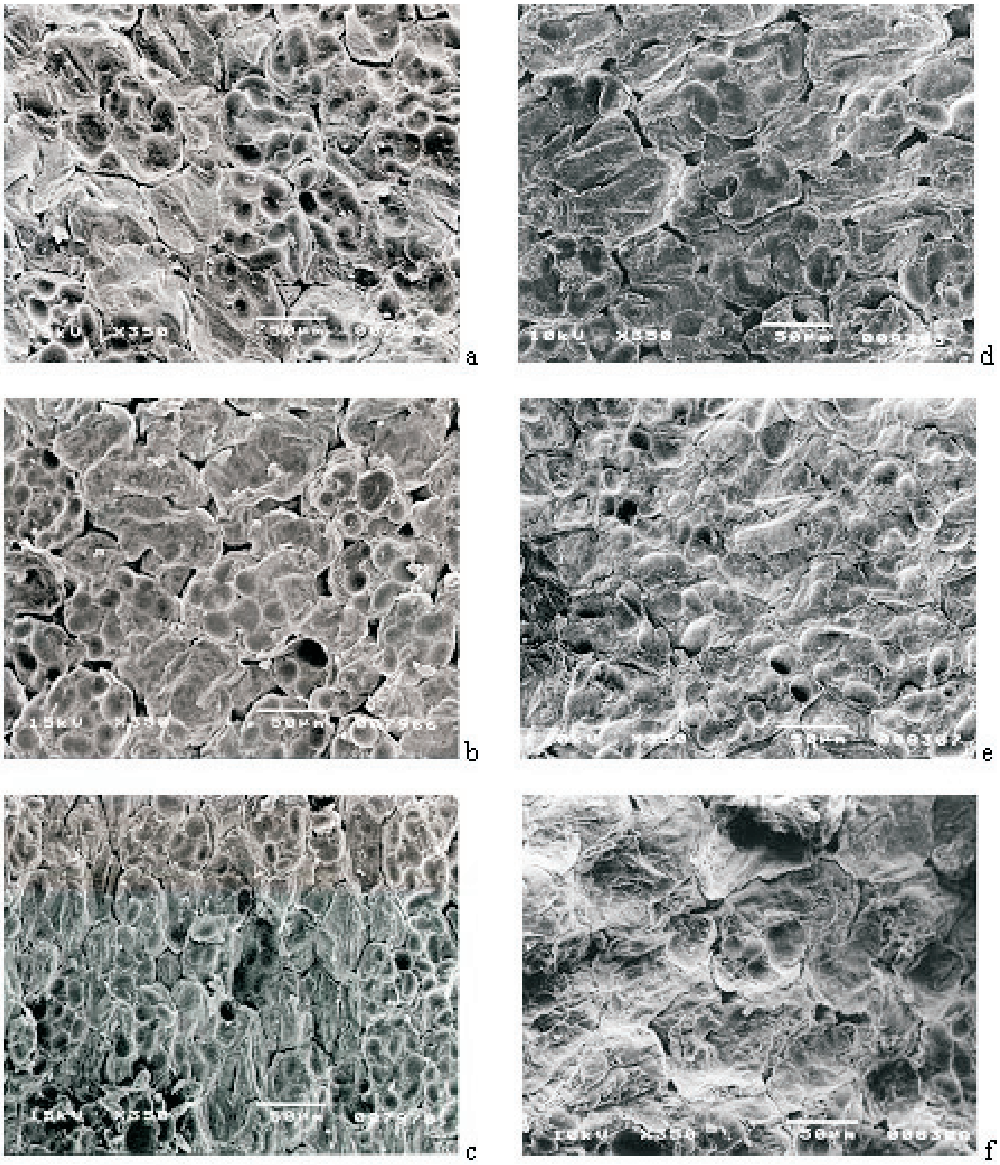

Fig. 3. Microstructure of cotyledon cells of seeds of cv. Derek (a), its mutants: D 4 (b) and D 13 (c), and cv. Krab (d), and its mutants: K 29 (e) and K 63 (f).

two mutant forms of this cultivar – lack of hour-glass cells (Fig. 2e and 2f). The cross-section of mutant K 29 is additionally characterized by a very thin peripheral layer which, due to its smearing properties, covers the outer epidermis. This corresponds with the cuticular waxes on the seed coat surface presented in Fig. 1e. The latter phenomenon was also observed in Fig. 2b and Fig. 2d. Similarity of surface elements reported earlier (Fig. 1b and 1d) can be linked to the resemblance in the structure of fibrilar, elongated palisade cells. The differences reported for seed coat thickness and structure may account for the rate of water imbibition which, as it is generally regarded, decreases with an increasing rate of seed coat thickness. The slow hydration of the seed coat leads to slow hydration of seed cotyledonary structures. On the other hand, inadequate water uptake may be responsible for insufficient heat transfer to inactivate anti-nutritional factors, lowering the quality of seeds.

Cross-section of grass-pea cotyledons revealed storage cells containing starch granules and protein matrices (Fig. 3). The accessions differed not only in the shape and size of cotyledon cells, but also in starch granule diameter as well as in binding forces of structural elements of cells (starch, protein and middle lamella). Regarding cells size, there was again a great similarity observed between D 4, Krab and K 63 seeds, characterized by the highest length and width (Fig. 3b and 3d, and 3f, respectively). The smallest size of cotyledon cells was found in D 13 mutant seeds. The D4 and Krab cotyledons were also alike in the number and size of intracellular spaces. Starch granules, the main storage compound of legume seeds, differed in diameter between the investigated varieties, being the largest in Krab, followed by D 4 mutant (Fig. 3b and 3d). The above-mentioned differences in the binding strength of cellular elements can be deduced with high probability on the basis of Fig. 3f. The cell content is covered by the middle lamella indicating that binding forces (pectic substances) are weaker between these structural elements of the cotyledon than between other cell components enabling the structural elements along lamellae. The differences observed in cotyledon structure can influence not only the mechanical properties but also the behaviour during soaking and cooking of seeds.

CONCLUSIONS

1. The studies performed showed that chemomutagens induced a broad variation of morphological and yield structure parameters in comparison to original cultivars. The range of traits variation indicated that, for the features analysed, mutants exceeded the variation observed for the original cultivars – Krab and Derek. This was particularly visible for such yield structure traits as pod number per plant, seed number and weight per pod. From agricultural point of view very promising are mutants with reduced plant height and biomass, decreased number of lateral branches, improved lodging resistance and earliness. Unfortunately, mutants with determinate growth type were not found.

2. Grass-pea may be improved in conventional breeding programs with the use of different crossing strategies. Furthermore, as shown in the presented paper, mutation induction (mutation breeding) can constitute a valuable supplement to above mentioned breeding strategy through mutant production characterized by desirable traits important from the agronomical point of view. Such mutants may constitute interesting initial material for breeding purposes with the aim to obtain new grass-pea cultivars as a good alternative to imported transgenic soya and other more popular legume crops cultivated in Poland. The most promising mutants described in the presented paper have been included in field trials at the Plant Breeding Station in Małyszyn.

3. Microscope pictures showed also considerable differentiation in the structural organization of seeds as compared to the control. Surface morphology can be a valuable factor for identification of lines not differing in geometrical and mass properties.

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