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Gel Electrophoresis as a Tool to Study Polymorphism and Nutritive Value of the Seed Storage Proteins in the Grain Sorghum

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1. Introduction

Seed storage proteins of cereals constitute the basis of mankind nutrition. However, climate changes, especially, increased droughts that are distinctly observed in many regions all over the globe, hamper sustainable production of traditional cereals, such as wheat, maize and barley, and dictates necessity to cultivate drought resistant and heat tolerant crops. Among these crops, the grain sorghum, owing to its ability for sustainable grain production in conditions of minimal level of precipitation, takes one of the leading places. However, application of sorghum grain for food and feed purposes is limited by its relatively low nutritive value in comparison with other cereals.

One of the reasons of poor nutritive value of sorghum grain is the resistance of its seed storage proteins (kafirins) to protease digestion. Kafirins are alcohol-soluble prolamin proteins making up to 80% of endosperm sorghum proteins (Hamaker et al., 1995). As well as other prolamins, sorghum kafirins contain high levels of proline and glutamine and are deposited in protein bodies of endosperm cells during kernel development. According to differences in solubility in aqueous *tert*-butanol solutions, molecular weight, structure and immunochemical similarity to zeins (maize prolamins) the kafirins were classified into α-, β- and γ-kafirins (Shull et al., 1991; for review, see: Belton et al., 2006). The α-kafirins are highly hydrophobic prolamin proteins (soluble in 40-90% aqueous *tert*-butanol solutions), they comprise 66-84% of total kafirins, depending on the endosperm type (vitreous or opaque). By SDS-PAGE the α-kafirins usually are resolved into two proteins, 25 kDa and 23 kDa. The γ-kafirin accounts for 9-21% of total kafirins depending on the endosperm type (Waterson et al., 1993). According to immunochemical data, the γ-kafirin is a protein with molecular mass of 28 kDa (Shull et al., 1991) although the sequence of the γ-kafirin gene corresponds to the protein with molecular mass of about 20 kDa (De Barros et al., 1991). The β-kafirin, in different endosperm types, accounts for about 7-13% of the total kafirins, and is resolved by the SDS-PAGE into three bands of 20, 18 and 16 kDa (Shull et al., 1991; 1992) or produced one band of 20kDa (El Nour et al., 1998); such variability, perhaps, is due to genotype differences.

One of the main characteristic features of kafirin proteins is their ability to form olygo- or polymers of high molecular weight. These oligomers comprise α- and γ-kafirins linked

together by disulphide (S-S) bonds, which are formed by sulphur-containing amino acids (Nunes et al., 2005). In the native state, both mono- and oligomers are present, while in 'reduced' extracts (i.e. with addition of 5% 2-mercaptoethanol that destroys S-S bonds) only monomers were detected (El Nour et al., 1998).

The causes of the poor kafirin digestibility appear to be multi-factorial (Duodu et al., 2003). Among these factors are chemical structure of kafirin molecules, some of which (γ- and β-kafirins) are abundant with sulfur-containing amino acids that are capable to form S-S bonds, resistant to protease digestion; interactions of kafirins with non-protein components such as polyphenols and polysaccharides; and spatial organization of different kafirins in the protein bodies of endosperm cells.

Among the methods that were developed for investigation of sorghum protein digestibility (Pedersen & Eggum, 1983; Mertz et al., 1984; Aboubacar et al., 2003), pepsin digestion of the flour proteins with subsequent gel electrophoresis is the most informative. This method, originally applied by B. Hamaker and co-workers (Weaver et al., 1998; Aboubacar et al., 2001) has been used in a number of studies (Nunes et al., 2004; Wong et al., 2010). Application of this method allowed to isolate sorghum lines with high protein digestibility (Weaver et al., 1998) and to find out that γ-kafirin plays an important role in resistance of sorghum seed storage proteins to protease digestion, namely, γ-kafirin forms a disulfide-bound enzyme-resistant layer at the periphery of protein bodies that restricts access of proteases to the inferior-located and more easily digested α-kafirins (Oria et al., 2000).

In our investigations (Italianskaya et al., 2009), we studied the protein digestibility in different sorghum lines and hybrids using this method and revealed significant polymorphism for *in vitro* kafirin digestibility as well as the strong genetic bases of this trait. In this paper, we summarize the results of these studies, which allowed isolating sorghum lines and F_1 hybrids with increased nutritive value. In addition, we demonstrate that kafirin polymorphism may be used in genetic experiments, namely, in determination of genetic structure of endosperm in sorghum.

2. Material and methods

In vitro protein digestibility was studied in 10 lines and seven F_1 hybrids of the grain sorghum (*Sorghum bicolor* (L.) Moench) (Table 1).

To study *in vitro* protein digestibility the modified method of whole-grain flour pepsin treatment was used (Oria et al. 1995). For each variety 25 mg of flour was treated with 5 ml of 0.15% pepsin solution (P7000 Sigma-Aldrich) in the 0.1 M potassium-phosphate buffer (pH 2.0) for 120 min at 37 ^{0}C with repeated shaking. Analysis of seed storage protein (kafirin) spectra was performed before and after pepsin treatment by SDS-PAG electrophoresis (SDS-PAGE) in reducing conditions. SDS-PAGE was carried out in the 12.5% (w/v) acrylamide separating gel (0.375 M TRIS·HCl, pH 8.8) and 4% stacking gel (0.125 M TRIS, pH 6.8) according to modified Laemmli method (Laemmli, 1970). SDS-reducing buffer: 62.5 mM TRIS·HCl, pH 6.8, 20% glycerol, 2% SDS, 5% β-mercaptoethanol; running buffer: 25.0 mM TRIS·HCl, 192 mM glycine, 0.1% SDS, pH 8.3; spacer thikness 1.00 mm. Gels were electrophoresed at 20-23 ma for about 5 hr. Gels were stained with Coomassie Brilliant Blue G-250 or R-250 (Diezel et al, 1972).

Line, F_1 hybrid[1]	Grain color	Endosperm type
VIR-120	white	floury
Pishchevoe-614 (P-614)	light-brown	semi-vitreous
Volzhskoe-4	light-brown	floury
Volzhskoe-4 waxy (V-4w)	pink	semi-vitreous
Karlikovoe beloe (KB)	white	semi-vitreous
Milo-10	yellow	floury
KVV-45	white	semi-vitreous
KVV-97	white	vitreous
KVV-3	white	semi-vitreous
KP-70	creamy	semi-vitreous
Topaz	creamy	semi-vitreous
O-1237	white	semi-vitreous
Sudzern svetlyi (Sud)	creamy	semi-vitreous
F_5 [M35-1A] Pishchevoe-614/KVV-45	white	semi-vitreous
A2 Karlikovoe beloe/Pishchevoe-614 (A2 KB/P-614)	light-brown	semi-vitreous
A2 Karlikovoe beloe/KP-70 (A2 KB/P-614)	white-yellowish	semi-vitreous
M35-1A Karlikovoe beloe /KVV-45 (M35-1A KB/KVV-45)	white	semi-vitreous
A2 KVV-97/Pishchevoe-614	light-brown	semi-vitreous
A2 Sudzern svetlyi/Topaz (A2 Sud/Topaz)	creamy	semi-vitreous
A2 O-1237/ Pishchevoe-614 (A2 O-1237/P-614)	light-brown	semi-vitreous

In parenthesis: brief designation used in the paper. F_1 hybrids were obtained using male-sterile counterparts of fertile lines; they are designated as A2 or M35-1A depending on the type of male sterility-inducing cytoplasm.

Table 1. The grain sorghum entries used in this investigation

For quantitative estimation of kafirin digestibility the SDS-PAGE banding patterns were scanned by laser densitometer ULTROSCAN XL (LKB-Pharmacia) with wavelength 633 nm. The protein quantity in each fraction was expressed as the area (mm^2) of the appropriate peak on densitogram, which was calculated by Software LKB 2222 (Version 3.00). In some experiments, the SDS-PAGE banding patterns were analyzed by Scangel program (developed by Dr. A.F. Ravich). The protein quantity in each fraction and in each lane of electrophoregramm was expressed as the amount of dots in the appropriate protein band. Experiments were performed in two replications. The data on digestibility of kafirins (the ratio of protein peak area before and after pepsin digestion) were subjected to variance analysis using the program Agros (Version 2.09; Dr. S. Martynov, Wheat Genetic Resources Department, N.I. Vavilov Institute of Plant Production, St. Petersburg, Russia).

In some lines and hybrids, the dependence of *in vitro* protein digestibility from *in vitro* starch digestibility was studied. In this experiment, the flour, firstly, was subjected to amylolitic enzyme treatment according to the method of B.V. McCleary (McCleary et al., 2002) using Megazyme Resistant Starch Kit (Megazyme Co, Ireland). The pellet remained after removal of solubilised starch was used for pepsin treatment according to the method described above, and, after that, the protein spectrum of the sample was studied by SDS-PAGE.

In order to use kafirins as markers of genetic structure of endosperm the modified technique of SDS-PAGE was applied. In these experiments, AS-1a line of the grain sorghum, which is characterized by a low frequency of parthenogenic embryo formation (Elkonin et al., 2012)

was used. Emasculated panicles of this line were pollinated with the pollen of the line Volzhskoe-4w homozygous for dominant gene *Rs*, conditioning purple color of coleoptiles, seedling leaves and stem. To study the origin of the kernels (apomictic or sexual) with the aid of the kafirin polymorphism, the kernels were split into two parts. The part with an embryo was put in a tray on a moisture filter paper to study the phenotypic traits of a seedling (expression of the *Rs* gene). Another part was used in SDS-PAGE to study its kafirin spectrum. In these experiments, gels were electrophoresed at constant voltage (70 V) for about 15 hr. Gels were stained with $AgNO_3$ solution.

3. *In vitro* kafirin digestibility

SDS-PAGE spectra of the seed storage proteins of a number of lines used in our investigations, before and after pepsin digestion, are shown on Figures 1 and 2.

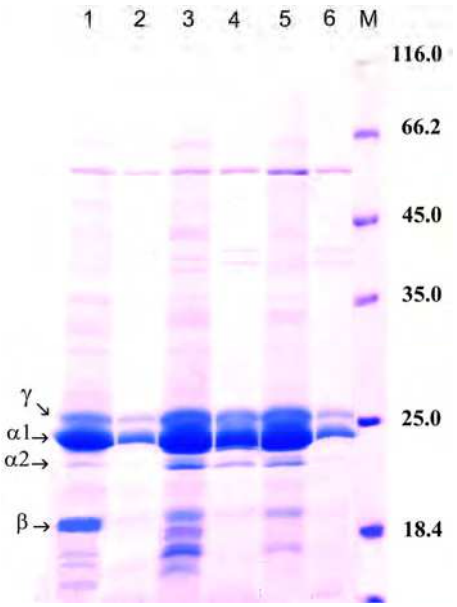

Fig. 1. Electrophoretic patterns of sorghum seed storage proteins before (1, 3, 5) and after (2, 4, 6) pepsin digestion. Lanes 1, 2 – Volzhskoe-4; 3,4 – Pishchevoe-614; 5,6 – F_5 [M35-1A] Pishchevoe-614/KVV-45; M – molecular weight markers (kDa).
α, β, γ – individual kafirin fractions. Gels were stained with Coomassie Brilliant Blue R-250.

In electrophoretic spectra of sorghum lines subjected to pepsin digestion, one could clearly distinguish the γ- (28 kDa), α1 (25 kDa) and α2 (23 kDa) kafirins and one or several bands of β-kafirin fractions (Fig. 1). These electrophoretic patterns correspond to kafirin spectra previously described in the literature (Shull et al., 1991; El Nour et al., 1998; Nunes et al., 2004). In our previous investigations (Table 2) we determined the relative content of different kafirin fractions and observed significant variation among different cultivars. The α1 and γ-kafirins were the most abundant in all lines and hybrids tested: 24-37% and 10-13% of all endosperm proteins, respectively; β-kafirins represent relatively small fractions

(4-10%) that is in concordance with the literature data (Shull et al., 1991; Waterson et al., 1993).

Line, F_1 hybrid	Protein fraction, % [1]			
	γ	α1	α2	β
KVV-45	13.2	37.3	2.0	3.9
Milo-10	12.8	30.7	5.3	7.2
A2 KVV-97	13.1	24.3	3.2	5.7
A2 KVV-97/P-614	13.3	31.4	4.4	5.3
P-614	10.7	26.9	5.5	5.5
A2 KVV-114	10.8	26.0	4.1	6.9
A2 KVV-114/V-4w	9.5	24.9	4.6	8.9
V-4w	10.3	33.1	3.4	10.2

[1] Relative content of each fraction is expressed as percentage of its peak area from the total endosperm proteins peak area sum. Mean data of two replications.

Table 2. Relative content of different kafirin fractions in some sorghum lines and F_1 hybrids (Italianskaya et al., 2009)

After pepsin digestion the amount of protein in kafirin fractions substantially reduced (Figs. 1; 2). Different sorghum lines and cultivars differed significantly by this trait. For example, among the entries presented in Figure 2 the highest digestibility level had VIR-120 – 90.8% (lanes 1 and 2), while the kafirins of line KVV-3 (lanes 9 and 10) were the most resistant to pepsin digestion (54.5% digestibility level) (Table 3).

In our previous study (Italianskaya et al., 2009), we observed significantly higher variation among the lines. For example, in the cultivar Volzhskoe-4 (V-4, registered standard), the amount of undigested γ- and α-kafirins after pepsin digestion was 80% and 73% from their initial contents, respectively. The total amount of undigested kafirins in cv. V-4 was 70% (digestibility level was 30%). At the same time, in the line KVV-45, the total amount of undigested proteins was 37% (digestibility level was 63%). Percentage of undigested α1 and γ-kafirins in the line KVV-45 was only 25% and 30%, respectively. The differences in kafirin spectra between this line and cv. V-4 before and after pepsin treatment are clearly seen in the Figure 3. Further investigation confirmed a high level of protein digestibility in this line (78.4%) (Table 3). Perhaps, the line KVV-45 contains mutation(s) in the genes encoding structure or deposition of kafirin molecules and, therefore, is of a great interest for future experiments.

Remarkably, in subsequent investigation it was found that in the line Topaz the digestibility level was even higher than in the KVV-45 and reached 89% (see chapter 4). This value is sufficiently high; it corresponds to digestibility level of whole grain flour protein of the best condenced-tannin-free sorghum entries (Axtell et al., 1981, and other reports, as cited in Duodu et al., 2003). One should expect that this line would have high nutritive value.

One should note high digestibility of the β-kafirin fractions in majority of lines. This fact contradicts to hypothesis that explains poor kafirin digestibility by formation of S-S bonds because β-kafirins as well as γ-kafirins contain a high amount of cystein, a sulfur-containing amino acid (Belton et al, 2006). In addition, in all lines, the polypeptides with molecular

weight approx. 42 and 46 kDa were prominent in electrophoretic spectra after pepsin digestion. These polypeptides, perhaps, represent kafirin dimers, which were formed as a result of association of kafirin monomers. Earlier, the formation of similar polypeptides (45 kDa) was observed after the cooking process (Duodu et al., 2003; Nunes et al., 2004).

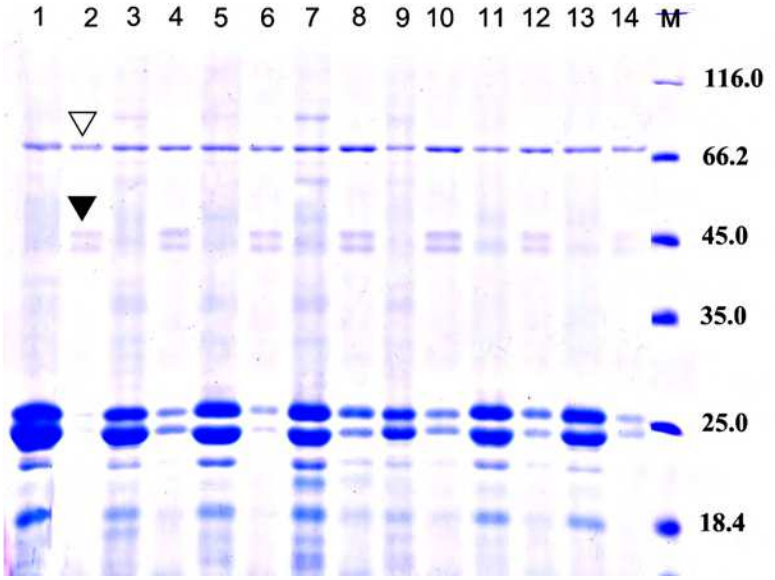

Fig. 2. Electrophoretic patterns of sorghum seed storage proteins before (1, 3, 5, 7, 9, 11, 13) and after (2, 4, 6, 8, 10, 12, 14) pepsin digestion. Lanes 1, 2 – VIR-120; 3, 4 – Volzhskoe-4w; 5, 6 – KVV-45; 7, 8 – KVV-97; 9, 10 – KVV-3; 11, 12 – Karlikovoe beloe; 13, 14 – KP-70; M – molecular weight markers (kDa). di- and trimers of kafirins are indicated by arrows, ◀ and ◁, respectively. Gels were stained with Coomassie Brilliant Blue R-250.

Lane number	Line	Total amount of dots in the lanes		Amount of undigested protein, %	Digestibility, %
		control	after pepsin digestion		
1,2	VIR-120	9769124	897710	9.2	90.8
3,4	Volzhskoe-4w	7285338	2692241	37.0	63.0
5,6	KVV-45	16465667	3554046	21.6	78.4
7,8	KVV-97	26995517	9483915	35.1	64.9
9,10	KVV-3	12242662	5571704	45.5	54.5
11,12	Karlikovoe beloe	13897393	4335642	31.2	68.8
13,14	KP-70	14462063	3651537	25.2	74.8

Table 3. Densitometry of electrophoretic patterns of seed storage proteins shown in Figure 2. The SDS-PAGE banding patterns were scanned and analyzed by Scangel program (developed by Dr. A.F. Ravich)

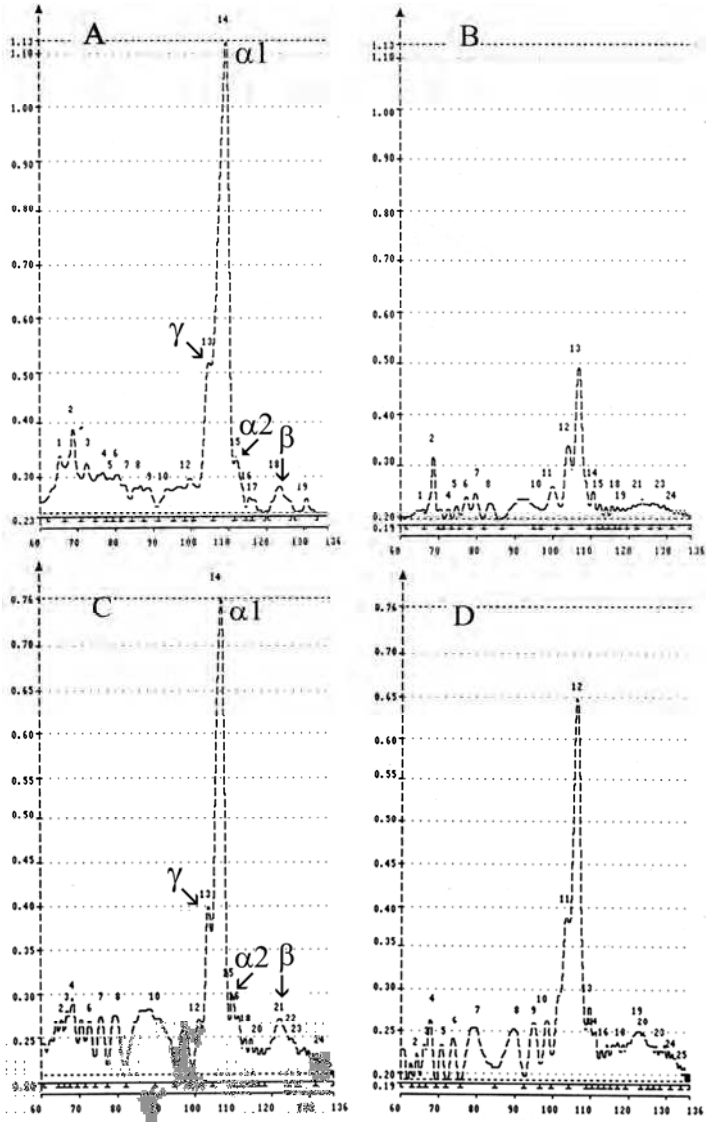

Fig. 3. Densitograms of electrophoretic spectra of endosperm proteins of sorghum line KVV-45 (a, b) and cultivar Volzhskoe-4 (c, d) before (a, c) and after (b, d) pepsin digestion. α1, α2, β and γ-kafirin fractions are indicated.

In order to explore the genetic basis of kafirin digestibility, we studied the expression of this trait in the F_1 hybrids between parental lines differing by resistance to pepsin digestion. Comparison of kafirin digestibility in the F_1 hybrids and their parental lines showed that different hybrid combinations had different mode of inheritance of resistance to pepsin affect (Table 4).

Line, F_1 hybrid[1]	Amount of undigested protein, percent from untreated sample[1]			
	γ	α1	β	Total proteins
KVV-45	24.4	24.6	32.2	24.5 a
M35-1A Karlikovoe beloe /KVV-45	36.2	33.9	34.2	26.8 ab
Karlikovoe beloe	21.3	37.2	26.0	32.1 bcd
A2 Karlikovoe beloe /KP-70	39.5	51.5	42.3	41.6 g
KP-70	22.4	29.5	22.3	26.1 a
A2 Karlikovoe beloe/Pishchevoe-614	41.1	51.3	42.3	40.4 efg
Pishchevoe-614	53.4	64.5	34.7	33.7 cd
A2 KVV-97/Pishchevoe-614	48.9	55.7	44.3	40.5 fg
KVV-97	40.4	30.3	20.4	34.2 d
$F_{0.05}$				14.76*
$LSD_{0.05}$				5.4

[1] Mean from two replications. Data followed by the same letter did not differ significantly (p<0.05) according to Duncan Multiple Range Test.
* Significant at p<0.05.

Table 4. *In vitro* protein digestibility of endosperm proteins in F_1 sorghum hybrids and their parental lines

The F_1 hybrids A2 KB/P-614, A2 KB/KP-70 and A2 KVV-97/P-614 had significantly lower kafirin digestibility than parental lines, which were characterized by its relatively high level. The reasons of such negative heterosis are unclear. Perhaps, genetic factors conditioning relatively high kafirin digestibility of KP-70, KB and P-614 are recessive and locate in different loci. At the same time, the F_1 hybrid M35-1A KB/KVV-45 did not differ from parental lines and retained high level of kafirin digestibility of the line KVV-45. Perhaps, high digestibility of KVV-45 contrary to other lines may be controlled by any dominant gene(s). This hybrid as well as the line KVV-45, is of great importance for fundamental investigation of factors influencing seed storage protein digestibility in sorghum (kafirin gene structure, structural organization of protein bodies and others) and for practical breeding.

Strong effect of genotype was also found on spectrum of high-molecular weight kafirins that were observed after pepsin digestion (Fig. 4). In some lines and F_1 hybrids two peaks di- and trimers) were found (Fig. 4, A-C), while in others only one peak (trimers) was seen (Fig. 4, D-F). Remarkably, densitograms of the F_1 hybrids in the peak area clearly resembled parental ones. One should note that while the peaks corresponding to trimers were observed in electrophoretic spectra already before pepsin treatment and their amount usually reduced after that, the dimers (45 kDa) were observed only after pepsin action. In some entries kafirin polymers were highly resistant to pepsin digestion, as in the KVV-45, while in others, as in the line P-614 and F_1 hybrid A2 KVV-97/P-614 (Fig. 5, A,B), these peaks were faint or almost absent. These data point on the genetic bases of formation of these molecules, which affect nutritive value of sorghum grain.

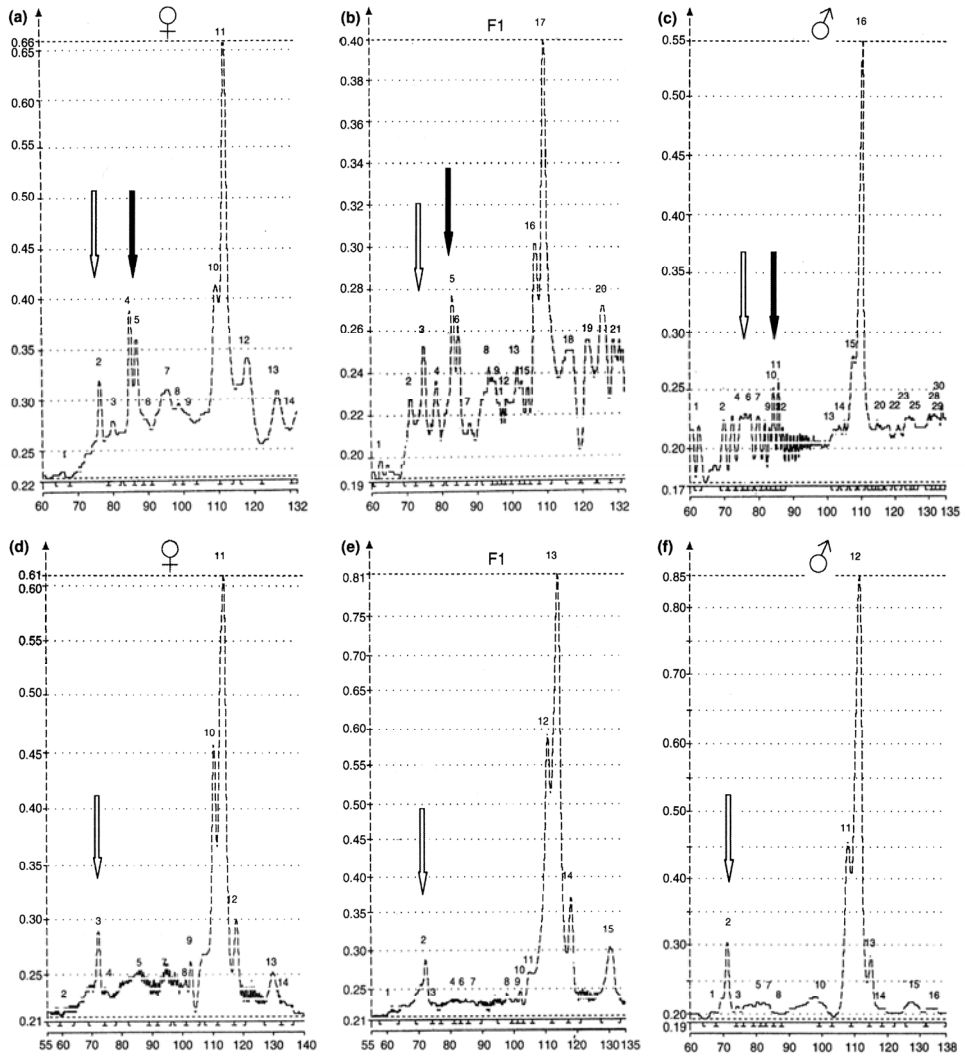

Fig. 4. Densitograms of endosperm proteins electrophoretic spectra of F_1 hybrids and their parental lines after pepsin digestion: a – A2 KVV-114, b – F_1 A2 KVV-114/V-4w, c – V-4w, d – A2 KB, e – F_1 A2 KB/KP-70, f – KP-70. Fractions of di- and trimers of kafirin proteins (45kDa and 66 kDa) are shown by arrows, ➡ and ⇨, respectively.

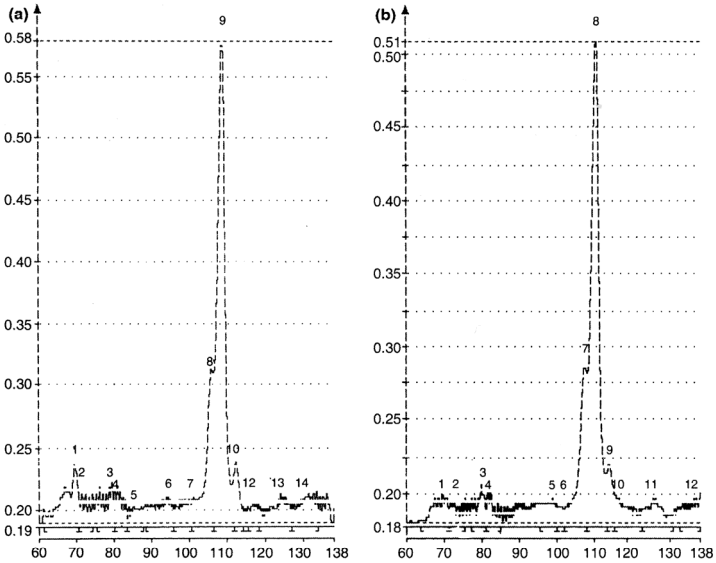

Fig. 5. Densitograms of endosperm proteins electrophoretic spectra of the line P-614 (a) and F_1 hybrid A2 KVV-97/P-614 (b) after pepsin digestion.

4. Interaction of starch and protein digestibility

In order to found out dependence of sorghum protein digestibility on starch digestibility the flour of several lines and F_1 hybrids was subjected to pepsin action after removal of digestible starch by the amylolytic enzymes treatment, and then was studied by SDS-electrophoresis for the presence of undigested proteins. It was found that after action of amylolitic enzymes the amount of protein in the kafirin fractions significantly increases (Fig. 6): in the lanes 3, 7 and 11 (samples after amylolitic enzyme action) almost all the protein is concentrated in the kafirin fractions, in comparison with the lanes 1, 5 and 9 (samples without amylolitic enzyme action). However, contrary to expectation that removal of starch will favor to kafirin digestion, the pepsin treatment of the samples treated before it with amylolitic enzymes (lanes 4, 8 and 12) were digested significantly fewer than samples digested by pepsin only (lanes 2, 6 and 10). Gel densitometry confirmed this visual conclusion (Table 5). Such phenomenon was observed in all F_1 hybrids studied (A2 Sud/Topaz, A2 O-1237/P-614, M35-1A KB/KVV-45) and their parental lines. Perhaps, partially digested starch molecules may interact with kafirin molecules by any physical or, probably, chemical way and prevent their protease digestion. One should not exclude that similar process might take place in *in vivo* conditions and thus decrease sorghum protein digestibility and reduce its nutritive value.

In addition, it was found that after amylolitic enzyme treatment the amount of di- and trimer fractions significantly reduced in comparison with the non-fermented control samples. In the F_1 hybrid A2 Sud/Topaz their amount was significantly fewer even in comparison with pepsin treatment only. Such a reduction of kafirin oligomers may be also responsible in increase of the level of kafirin monomers. These data testify that starch molecules might participate in formation of kafirin oligomer molecules. They are important for understanding the factors influencing kafirin and starch interactions in sorghum endosperm and their digestibility.

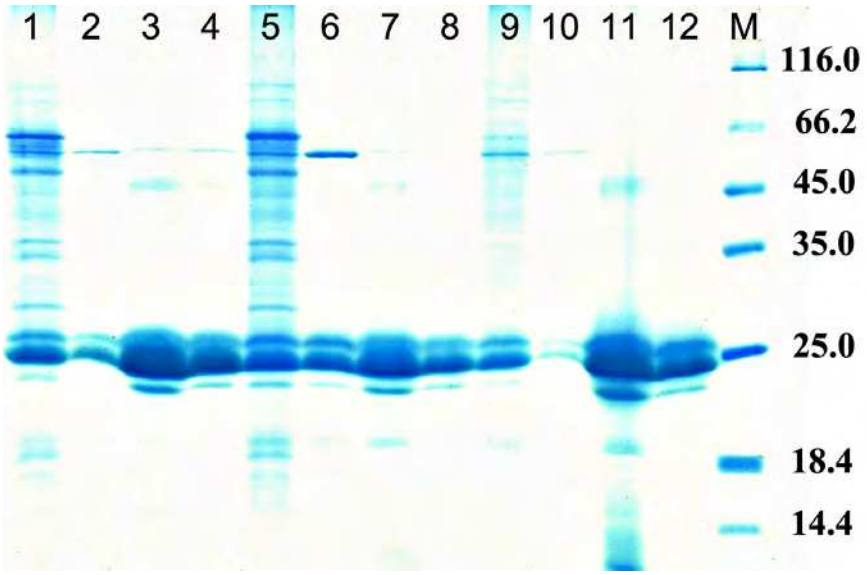

Fig. 6. Electrophoretic patterns of sorghum seed storage proteins from the flour before (1, 3, 5, 7, 9, 11) and after (2, 4, 6, 8, 10, 12) pepsin digestion; lanes 3, 4, 7, 8, 11, 12 – after removal of soluble starch by amylolitic enzymes before pepsin digestion; lanes 1, 2, 5, 6, 9, 10 – without this procedure. Lanes 1-4 – Sudzern svetlyi; 5-8 – F_1 A2 Sudzern svetlyi/Topaz; 9-12 – Topaz; M – molecular weight markers (kDa). Gels were stained with Coomassie Brilliant Blue G-250.

5. Kafirins as the markers of endosperm genetic structure

In addition to variation of a number of β-kafirin fractions in different sorghum entries described above, we have revealed polymorphism of the α-kafirins. The line Volzhskoe-4w (V-4w) that is used as a tester line to distinguish the hybrid seedlings from the maternal ones, possessed specific kafirin spectrum, which was rarely observed in other sorghum lines and cultivars. The α1 fraction was composed from three polypeptides: α1-1, α1-2, and α1-3; α2 fraction was composed from two polypeptides: α2-1 and α2-2 (Fig. 7, lanes 1-3). We hypothesized that this polymorphism could be used in studies of genetic structure of endosperm in apomixis research in sorghum.

To test this possibility we used the AS-1a line, which is characterized by ability for development of aposporous embryo sacs and parthenogenetic embryos (Elkonin et al., 2012). Gel electrophoresis showed that kafirin spectrum of this line differs from V-4w (Fig. 7). Two polypeptides were observed in the α1 fraction (α1 and α1-2), the α1-2 was in trace amount, and α1-3 was absent; the α2 fraction did not subdivide into two polypeptides (Fig. 7, lanes 4-6).

Genotype	Experimental treatment	Amount of protein in different kafirin fractions		Total proteins percent to the control
		Individual fractions (α+β+γ), percent to the control	Olygomers percent to the control	
Sudzern svetlyi	Control	100.0	100.0	100.0
	Pepsin	48.2	7.4	23.0
	Amylolitic enzymes	177.0	8.9	76.5
	Amylolitic enzymes, pepsin	102.9	5.5	44.2
A2 Sudzern / Topaz	Control	100.0	100.0	100.0
	Pepsin	66.5	19.1	35.6
	Amylolitic enzymes	111.2	4.5	52.4
	Amylolitic enzymes, pepsin	60.9	1.2	27.1
Topaz	Control	100.0	100.0	100.0
	Pepsin	18.1	10.2	11.1
	Amylolitic enzymes	225.6	24.9	139.4
	Amylolitic enzymes, pepsin	124.7	6.4	64.7
F_A (genotypes)		0.858	3.834	1.338
F_B (treatment)		6.340*	836.245***	7.945*
F_{AB}		0.995	5.964*	1.245
	In average for treatment			
	Control	100.0 a	100.0 c	100.0 b
	Pepsin	44.3 a	12.2 b	23.2 a
	Amylolitic enzymes	171.2 b	12.8 b	89.4 b
	Amylolitic enzymes + pepsin	96.1 a	4.4 a	45.3 a

Mean data of two replications; data followed by the same letter did not differ significantly (p<0.05) according to Duncan Multiple Range Test;
*, and *** significant at p<0.05, and p<0.001, respectively.

Table 5. Densitometry of seed storage proteins electrophoretic patterns of F_1 A2 Sudzern/Topaz and its parents after treatment with pepsin and/or α-amylase and amyloglucosidase

In order to use this polymorphism for identification of seeds formed via apomixis, the kernels obtained by pollination of emasculated panicles of AS-1a with the pollen of V-4w were split into two parts. The part with an embryo was used to study the phenotypic traits

of a seedling. Another part was used in SDS-PAGE to study its kafirin spectrum. In the case of autonomous endosperm development, no V-4w proteins should be found in the kafirin spectra of the kernels yielded maternal seedlings, while in the case of pseudogamous endosperm development, in the electrophretic spectra of these kernels, the SDS-PAGE must reveal V-4w proteins. It was found that kafirin spectra of kernels, which yielded maternal seedlings (Fig. 7, lanes 11,12) did not differ from the spectrum of AS-1a line (Fig. 7, lanes 4-6), while in the spectra of the kernels, which yielded hybrid seedlings the α1-3 protein was clearly distinguished (Fig. 7, lanes 7-10). These data support the results of our cyto-embryological observations of autonomous endosperm development in the AS-1a line (Elkonin et al., 2012) and are in accordance with the literature data on other sorghum lines with apomictic potentials (Rao et al., 1978; Wu et al., 1994; Ping et al., 2004).

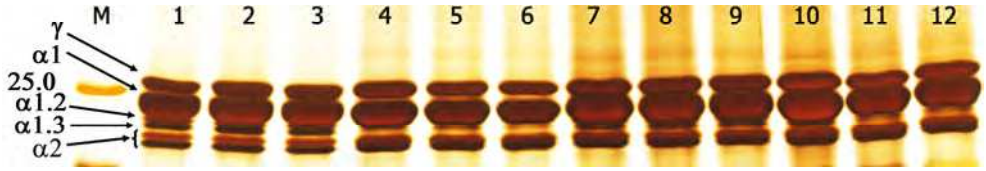

Fig. 7. Kafirin spectra of Volzhskoe-4w (lanes 1-3), AS-1a (4-6) and of the kernels, which were set on emasculated panicles of AS-1a pollinated with the Volzhskoe-4w pollen and yielded the F_1 hybrid seedlings (7-10) and maternal plants (11-12); M – molecular weight marker (kDa). Gels were stained with $AgNO_3$.

6. Conclusion

Summarizing, the results of our investigation demonstrate that gel electrophoresis of the seed storage proteins is a powerful instrument in researches on sorghum genetics and breeding that have both fundamental and applied orientation. It allowed to isolate of sorghum lines with individual kafirin fractions more sensitive to protease action, and, therefore, with increased protein digestibility – one of the main trait characterizing the nutritive value of sorghum grain. These lines may be used in breeding programs for developing new CMS-lines and F_1 hybrids. In addition, these lines (for example, KVV-45) may be used in future investigations on molecular organization of genes encoding structure and/or deposition of kafirins, their cloning and transfer into other sorghum lines by methods of classical genetics or genetic engineering.

Gel electrophoresis of the flour subjected to amylolitic enzyme action has demonstrated that starch digestion decreases content of kafirin polymers and reduces subsequent kafirin digestion by pepsin. This finding may explain the reduced nutrient value of sorghum grain, in comparison with other cereals. These data point on the complex mode of interactions of storage proteins and starch in sorghum endosperm.

Gel electrophoresis of the seed storage proteins allowed to determine genetic structure of endosperm in sorghum kernels with parthenogenic embryos developing in the line AS-1a with apomictic potentials and may be used in development of sorghum lines with high frequency and stable expression of this trait.

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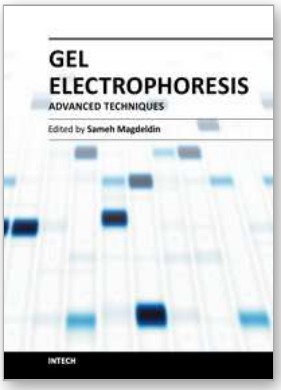

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As a basic concept, gel electrophoresis is a biotechnology technique in which macromolecules such as DNA, RNA or protein are fractionated according to their physical properties such as molecular weight or charge. These molecules are forced through a porous gel matrix under electric field enabling uncounted applications and uses. Delivered between your hands, a second book of this Gel electrophoresis series (Gel Electrophoresis- Advanced Techniques) covers a part, but not all, applications of this versatile technique in both medical and life science fields. We try to keep the contents of the book crisp and comprehensive, and hope that it will receive overwhelming interest and deliver benefits and valuable information to the readers.

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