



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

International Journal of Recent Scientific Research
Vol. 6, Issue, 8, pp.5893-5900, August, 2015

**International Journal
of Recent Scientific
Research**

RESEARCH ARTICLE

MOBILE GENETIC ELEMENTS AS A TOOL FOR THE ANALYSIS OF GENETIC DIFFERENTIATION OF VARIETIES OF CULTIVATED PLANTS AND BREEDS OF FARM ANIMALS

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ARTICLE INFO

Article History:

Received 2nd, July, 2015
Received in revised form 10th,
July, 2015
Accepted 4th, August, 2015
Published online 28th,
August, 2015

Key words:

Mobile genetic elements, IRAP-PCR, polymorphic information content (PIC), gene pool "standard" of breed.

ABSTRACT

The paper discusses the possibilities of effective application of the terminal fragments of transposable elements of flowering plants and mammals as primers in IRAP-PCR to study the genetic structure of local breeds of sheep and horses in order to determine the gene pool "standard" of breed. Breed-specific loci differed in polymorphic information content (PIC) were found for each of the study groups of sheep and horses. Fragments specific in PIC of each of the studied groups from three different farms of Altaic horses were detected. Besides loci differentiated two inbreeding types of Edilbai sheep in different ecological and geographical conditions were found. Phylogenetic differentiation of breeds and inbreeding groups did not always coincide with the known history of the origin and depended on the primer. Variety-specific fragments have been identified as a result of genotyping wheat varieties using a site of LTR SIRE-1 as a primer. The dendrogram constructed on the basis of values of genetic distances reflects both the phenotypic characteristics of each of the varieties and their origin. The spectra of the fragments obtained by PCR with primer LTR SIRE-1 also distinguish two species of soybean (*G. max* and *G. soja*), as well as representatives of *G. max*.

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INTRODUCTION

Development of breeding programs requires an analysis of the processes taking place with the population's gene pool. Due to the sharp decline in plant and animal species, particularly relevant is the maintenance of local breeds and wild relatives of cultivated varieties of plants, is a valuable reservoir of genetic diversity. Learning the basics of population-genetic adaptation to ecological and geographical factors of habitat provides an opportunity to develop programs for the conservation breeds of farm animal and varieties of cultivated plants. Search and selection of genetic elements, which would allow genotyping in order to identify and control the gene pool of particular species (varieties), becomes relevant. The selection of molecular genetic markers, which polymorphism would be quite high, the estimation is reproducibility and low cost, the results were easily processed by mathematical programs of comparison and analysis the genetic structures of different groups of organisms, is of particular importance. For example, the estimation of polymorphisms of highly polymorphic genomic elements, such as regions flanked by inverted LTR retrotransposons (IRAP-PCR) (Kalendar et al, 1999), which allow to obtain the spectrum of DNA fragments of different lengths, unique in its characteristics for each group of organisms, are widely used for

genotyping cultivated plants (Alikhani et al, 2014; Khadivi-Khub and Soorni, 2014; Kuhn et al, 2014; Nasri et al, 2013; Boronnikova, 2009; Glazko et al, 2007; Khapilin and Raiser, 2013) and the parasitic fungi (Santana et al, 2012; de Queiroz et al, 2014). Using IRAP-markers to study genetic differentiation of groups of plants and fungi is possible due to the high prevalence of retrotransposons in their genomes. Plants retrotransposons occupy a considerable part of the genome: in *Arabidopsis thaliana* - a little over 7% (Pereira, 2004) in rice - 50% in wheat - 90% (Gao et al, 2004) in corn - 75% (Bousios et al, 2012). From 38 to 69% of the mammalian genome sequences are also generally associated with retrotransposons (Nellaker et al, 2012). The first and probably most important difference between them and other structural and functional elements of the genome is the ability to move. High speed of transpositions suggests their important role in the generation of genetic variability. In this regard, the mobile elements are promising molecular genetic markers for genotyping not only cultivated plants, but also farm animals. In this paper we analyzed the gene pools of indigenous breeds of farm animals (horses, sheep), cultivated plants (soft wheat) and wild (soybean) plants using IRAP-markers in order to search of breed- and variety-specific combinations of DNA fragments of different lengths, which can be used to determine

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the "gene pool standard" of studied breeds and varieties, as well as for differentiation of wild soybean populations.

MATERIALS AND METHODS

Genomic DNA was isolated using a commercial kit "DNA-Extran-1" (Syntol, Russia). Estimations of polymorphism of DNA fragments (IRAP-PCR markers) obtained using terminal sites of LTR (Long Terminal Repeats, LTR) retrotransposons of plants as PCR primers: terminal site of retrotransposon SIRE-1, a fifth of maize genome (GCAGTTATGCAAGTGGGATGAGCA), belonging to the genus *Sireviruses*, representatives of the family *Pseudoviridae*, whose members include env-like gene (Bousios and Darzentas, 2013), the site of retrotransposon PawS 5 (AACGAGGGGTTTCGAGGCC), belonging to the family R173, they are often associated with other retrotransposons, in the genome of diploid rye (Rogowsky et al, 1992), the fragment of barley retrotransposon BARE-1 (CCAAGTAGAGGCTTGCTAGGGAC), was used to study the genetic structure of populations used. Were also used terminal sites of LTR of endogenous retroviruses of mammals: -3 primer (GGACCTTCTCCTTCAAGGC), homologous to the sequence of terminal site of an endogenous retrovirus of cattle (Bovine endogenous retrovirus -3, BERV -3), k-1 primer (TATCAGGCCTCTCCGCATG), homologous to the terminal site of Bovine endogenous retrovirus K1, BERV k-1). BERV -3 and BERV k-1 belong to the genus *Betaretrovirus* and encode 4 basic proteins *GAG*, *PRO*, *POL*, and *ENV* (Xiao et al, 2008; Baba et al, 2011).

The polymerase chain reaction (PCR) was performed as IRAP-PCR (Inter-Retrotransposon Amplified Polimorphism). Amplicons represent the fragments of DNA which are situated between inverted repeats of a primer.

Amplification products were separated in a 1.5% agarose gel. Visualization of the amplification products was performed after coloration gel with ethidium bromide.

Mathematical processing was carried out using TFPGA. Calculation of the index of PIC (Polymorphic Information Content) performed by formula for diallelic loci for which $PIC = 2f(1-f)$, where f - frequency of one of the two alleles. Due to the dominant character of ISSR-PCR and IRAP-PCR, f was calculated by the formula: $f = R$, where R - occurrence frequency among the investigated animals (plants), in which amplification product of a given length was absence. R value was considered as a share of homozygotes for recessive allele.

RESULTS AND DISCUSSION

Genetic differentiation of indigenous fat-tailed sheep breeds

Three breeds of sheep (total 80 goals): Karachai, Kalmyk, Edilbai (two inbreeding types – Birlik and Suyunduk) were selected for study. Kalmyk fat-tailed sheep from Mongolia and western China appeared in Russia in the XVII century during the transition of Kalmyks. A part of livestock mixed over a huge area with local fat-tailed sheep as Kalmyks moved,

creating a number of breeds of fat-tailed sheep, of which Edilbai is the most valuable. Kalmyk sheep are adapted to the arid, semi-arid and hot climate, vegetation, year-round grazing and further transitions. The Edilbai sheep was created at the end of the XIX century in the Ural region (Kazakhstan) as the efforts of national selection. The Edilbai sheep are characterized by wide ecological valence and make good use of pastures in the desert, semi-desert and arid zones in different seasons. It is assumed that this breed is a product of crossbreeding of ordinary Kazakh sheep and Kalmyk rams. The Karachai sheep are bred in the mountainous area of the North Caucasus. This breed is bred more than two hundred years ago in the Karachay-Cherkess Republic by improving indigenous sheep in a year-round grazing (Erokhin and Erokhin, 2004).

The specific spectra of DNA fragments of three indigenous breeds of sheep were obtained using IRAP-primers (Fig 1). The most heterogeneous group was Kalmyk sheep ($PIC_{average}$ – an average value of PIC over the entire spectrum of amplicons) (P (a share of polymorphic loci) = 44%, 47%, PIC_{locus} = 0.187, 0.174, for primers LTR SIRE-1 and PawS 5, respectively). Karachai sheep was the most consolidated group according to the data obtained using primer BARE-1. Six conservative fragments from 710 to 1500 bp in length were visualized in the spectra of DNA of all studied animals obtained by using primer LTR SIRE-1. The rarest amplicons were 760 bp and 460 bp in length. The first was found only in Karachai (PIC_{locus} = 0.075), this fragment was absent in the Kalmyk and Edilbai sheep, and the second fragment was found only in the Kalmyk sheep (PIC_{locus} = 0.499). The fragment of 1130 bp in length was absent in Karachai sheep, a fragment of 1200 bp in length – in Kalmyk sheep. The fragment of 950 bp in length was present only in the spectra of Karachai sheep obtained using a primer PawS 5 (PIC_{locus} = 0.075). Spectrum of a primer homologous to a fragment of an endogenous retrovirus BERV -1 was polymorphic in all studied breeds of sheep only in the range of heavy fragments in length from 1000 to 1300 bp. The Suyunduk type of Edilbai sheep was the most homogeneous compared with The Birlik according to the main characteristic of the spectrum of the DNA fragments of a primer PawS 5 (P = 27% $PIC_{average}$ = 0.157 and P = 29% $PIC_{average}$ = 0.221, respectively). The features of the spectra of a primer BERV -3 also reflect the differentiation of inbreeding types of The Edilbai sheep. Thus The Birlik type was less heterogeneous than Suyunduk (P = 8% and 25%, $PIC_{average}$ = 0.041 and 0.112, respectively). Such differences have not been identified for the rest of the primers (Table 1).

Phylogenetic dendrograms were constructed based on the values of polymorphism of fragments obtained using each of the primers. The Birlik and Suyunduk types of the Edilbai sheep form a common cluster ($DN = 0.0104$) in the dendrogram obtained using genetic distance (DN) calculated on estimates of polymorphism of the amplification products of a DNA spectra of primer LTR SIRE-1 (Fig 2-a). Interestingly, the primer PawS 5 (Fig 2-b) and BARE-1 (Fig 2-c) – primers homologous to terminal sites of mobile element of monocots - show similar dendrograms, just in the case of primer PawS 5, the total cluster form The Birlik type and Kalmyk sheep, primer BARE-1 – The Suyunduk type and Kalmyk sheep. The Karachai sheep

form an autonomous cluster sheep in all three dendrograms. In general, sheep differentiation according to primers LTR SIRE-1, PawS 5 and BARE-1 corresponds to the known history of the origin of species.

Dendrogram constructed from the values of polymorphism of fragments' spectra of primers BERV (Fig 2-d) differs from the other three. Here The Kalmyk sheep stand out in a separate cluster and the Birlik type and Karachai sheep clustered together, and the genetic distance between them smaller than that between The Birlik Suyunduk sheep (DN = 0,0221 and DN = 0,0439). One of the possible reasons for this differentiation of two types of the Edilbi sheep may be that the original breeding plants from which they were taken on differed by environmental conditions. Thus, the breeding plant "Birlik", the source of the Birlik type is situated in Zhangalinsk region, and the breeding plant "Makash" from which the Suyunduk sheep were obtained is in the Kurmangazy district. Widely known Azgirsky nuclear test site is located in the same area. We can not exclude that the observed differentiation of the Edilbai sheep according to IRAP-PCR markers due to the fact that the sheep of the Suyunduk type were subjected to more intensive action of chronic environmental stress factors in connection with the initial greater closeness of their breeding places in the region with a high level of radionuclide contamination. Thus, the Suyunduk type of the Edilbai sheep has a higher capacity to adapt to adverse conditions of breeding compared with the Birlik type.

Results of search of breed-specific fragments of DNA spectra were as follows. The value of index PIC defines breed-specificity of locus for each studied groups. The largest number of loci (69%), specific to a particular group of sheep was obtained using primer LTR SIRE-1. About half of the fragments were breed-specific in spectra of primers k-1 (50%) and BARE-1 (44%). Spectra of primers PawS 5 and -3 were less informative (13 and 25%, respectively). For example, a fragment of 790 bp in length was not found in a spectrum of primer LTR SIRE-1 in Karachai sheep, it met with a frequency 1 in all representatives of other breeds of sheep. The fragment length of 1320 bp was absent in a spectra of primer BARE-1 of the Karachai sheep, whereas it met with varying frequency (0.88-0.94) in other breeds. The DNA spectra of primers -3, k-1 and PawS 5 differed in inbreeding types of the Edilbai sheep bred in different ecological and geographical conditions. The share of specific for each type was greatest in the spectrum primer PawS 5 (0.2). A fragment of 590 bp in length met in all representatives of the Birlik sheep ($PIC_{\text{average}} = 0$), while in the Suyunduk sheep the value of PIC of this locus was 0.457. Conversely, the value of PIC of locus length of 840 bp was equal to 0 in the Suyunduk sheep and 0.457 – in the Birlik. Thus, the most informative primer to differentiate groups of different breeds of sheep was primer LTR SIRE-1. Spectra primer PawS 5 most clearly reflect intrabreed differences of Edilbi sheep. Primers k-1 and BARE-1 are available for both inter- and intrabreed differentiation of sheep. A unique gene pool of the Suyunduk type of Edilbai sheep was formed under natural selection in chronic exposure to stressful environmental factors. Specifics of gene pool of the Suyunduk sheep can be identified using terminal fragments of transposable elements PawS 5, BERV and BARE-1 as primers.

Genetic differentiation of indigenous breeds of horses

A study was carried out on 88 blood samples of horses of different breeds and origin (the Karachai breed; the Altaic breed included 3 different farms, ("Dzhumbaev", "Enchi", "Genghis"; a group of trotters, the Orlov trotters, the Russian trotters, the American Standardbred).

The spectra of the amplicons obtained using different primers in a PCR, were not significantly different from each other in the limit of length of detected DNA fragments in all breeds of horses (Fig 3). The spectra of fragments of primer PawS 5 differs a bit (Fig 3 c), i.e. loci over 1500 bp in length were also visualized (Table 2). Each of the DNA spectra of different primers is unique and differs by share of polymorphic loci and their distribution. Spectra of DNA fragments of all length of Altaic horses from farm "Dzhumbaev" obtained using primers LTR SIRE-1 and PawS 5 were the most polymorphic. Polymorphic fragments of spectra of the other two groups of Altaic horses lie in the range of average lengths (from 5 to 10%). About a half of polymorphic loci of spectra of primer -3 in horses from farm "Enchi" and smaller amounts of polymorphic loci in the third group from farm "Genghis" include fragments from 500 to 1000 bp in length, in turn, polymorphism of fragments over 1000 bp in length was observed only in the first (18%) (Fig 3 a). A group of Karachai horses were homogenous in polymorphism and covers a range of both medium and heavy fragments lengths (from 5 to 10%). Spectra of fragments of primer k-1 were similar in all investigated breeds: 14% of polymorphic loci were fragments of the average length, and heavy fragments account 7%.

Representatives of Altaic horses from three different farms are significantly different from each other according to the values of the characteristics of the genetic diversity (PIC, P), calculated from the spectra of IRAP-markers. The most from the spectra of the Altaic horse farm "Dzhumbaev" was the most heterogeneous group according to spectra of primers LTR SIRE-1 and PawS 5 the least was horses from farm "Enchi" in which all the resulting DNA fragments were monomorphic in the range of primer PawS 5. Altaic horses from farm "Genghis" are close to those of Karachai horses by the values of PIC and P of spectra of amplicons of primer LTR SIRE-1, and in the case of spectra of primer PawS 5 they are close trotters. The latter are characterized by homogeneity of individuals, which is accompanied by a lower frequency of heterozygotes in the population and the lowest value of share of polymorphic loci in comparison with studied indigenous breeds of horses (except Altaic horses from farm "Enchi") (Table 3).

The Trotters have slightly higher values of PIC and P of DNA spectra of primer k-1 at the general background of low values of the spectrum of all breeds. This may be due to the heterogeneity of the study group trotters, which consists of representatives of The Orlov, Russian and American Standardbred trotters. At least a third of all loci were polymorphic in the spectra of -3 primer of the studied rocks. Interbreed differences of Altaic horses expressed less explicitly in the case of -3 primer, except for a few more consolidated horses from the farm "Genghis" whose characteristics are

similar to the spectra of Karachai horses. The trotters occupy an intermediate position.

On the dendrogram constructed based on the distribution of fragments obtained by applying the primer PawS 5 two clusters are formed, one subgroup includes Altaic horses from two different farms and a separate subgroup form trotting horses. The Karachai and Altaic horses (farm "Dzhumbaev") are combined into a separate group (Fig. 4-a).

As a result of differentiation of these same groups of animals on the values of polymorphism of DNA fragments obtained by PCR using a terminal fragment of retrotransposon LTR SIRE-1 as a primer Altaic horses from farm "Enchi" form an independent group, another group consists of clusters, in which representatives of the Altaic horse from farm "Dzhumbaev" are separated, and The Karachai, trotting horse and Altaic horses from farm "Genghis" are combined into a single cluster. The smallest genetic distance is observed between the Trotters and the Altaic horses (farm "Dzhumbaev") (0.001), and Karachai horses are equidistant from both groups (0.020 and 0.022) (Fig 4-b).

The differentiation of horses using terminal sites of endogenous retroviruses as primers is as follows: the Altaic horses from farms "Enchi" and "Genghis" are incorporated into one cluster, another one forms two separate groups. One forms the American and Russian trotters, another – the Karachai, the Altaic horses (farm "Dzhumbaev") and the Orlov trotters, with the last placed apart (Fig 4-c). Such distance of between representatives of the Altaic horses from three farms is observed with other IRAP-markers. This fact can be explained by the peculiarities of breeding work in each of the farms.

To establish a common phylogenetic relationships between grouped into three large groups of horse's breeds dendrograms were also constructed. Thus, according to the primer LTR SIRE-1 and primers homologous regions of ERV of mammals (-3 and k-1) the Karachai and trotting horses are grouped together, Altaic horses form a separate cluster. The Karachai and Altaic horse are clustered together (DN = 0.0767), and trotting horses forms a separate group according to the estimation of polymorphism obtained using primer PawS 5. This differentiation of Karachai horses observed when compared with the local horses of Tuva by analyzing biochemical polymorphism of proteins (transferrin, hemoglobin, albumin) and blood enzymes (carboxyl, serum arilesterase, alkaline phosphatase), where the distance between the groups was 0.0969. Another cluster is formed by Arabian horses (Olkhovskaya *et al*, 2011). It is known that to the Mongolian horse contributed in the formation of both the Altaic and Tuva horses, so such comparisons are legitimate (Lobanov and Trushnikov, 2005; Budyonny, 1952).

The greatest amount of breed-specific DNA fragments in the spectra of horses were prepared using fragment of retrotransposon LTR SIRE-1 (80%) as a primer, more than half - in the spectra of primer PawS 5 (53%) and -3 (64%). Polymorphic loci in the spectra of primer k-1 have not been identified. Intra-breed differences between groups of Altaic

horses from different farms reveals primer LTR SIRE-1. There were significantly fewer specific loci in the spectra of DNA fragments of primers PawS 5 (42%) and -3 (36%). Based on these data, we can talk about the universality of application of the primer LTR SIRE-1 in order to differentiate between and within different breeds of horses.

The findings lead to the following conclusion. Multi-locus genotyping using terminal sites of mobile elements allows revealing spectra of DNA fragments specific for each primer reflecting particular properties of the distribution of its inverted repeats in genomic DNA of studied species. That is, in the spectra of IRAP-PCR markers breed-specific combinations of DNA fragments of different lengths are detected, which can be used to determine the "standard gene pool" of the breeds.

Genetic differentiation of varieties of wheat and groups of wild soybean

The study was performed on monocotyledonous (*Triticum aestivum*) and dicotyledonous (*Glycine soja* and *Glycine max*) plants. Wheat was represented by two winter varieties (Moscovskaya 39 - soft winter, Mironovskaya 808 - soft winter, derived from the spring) and one of the springs (Omskaya 36 - soft spring), soybeans - five populations of wild Ussurian species (*G. soja*, Primorsky region, Russia) and one group of wild weedy form of soybean (*G. max*, China).

As a result of IRAP-PCR with a primer to a terminal site of retroelement soybean LTR SIRE-1 we obtained clearly replicating spectra of DNA fragments of soy and wheat, and such fragments were in the same size range: 22 loci length from 350 to 1240 bp in length, and 26 loci – from 220 to 1450 bp in length (Fig 5 and 6). The analyzed groups of *Glycine* had high polymorphism (PIC_{average} = 0.414, P = 91%) in comparison with *T. aestivum* (PIC_{average} = 0.120, P = 65%). The most polymorphic fragments of *Glycine* were fragments ranging in length from 350 to 490 bp and from 1010 to 1240 bp in length. The PIC_{average} values of fragments were respectively 0.449 and 0.364. In *T. aestivum*, conversely, high polymorphism was observed in the middle zone of lengths (from 520 to 720 bp and from 760 to 990 bp in length, PIC_{average} = 0.196 and 0.155, respectively).

We detected only one monomorphic locus length of 700 bp in *Glycine*, on average, the share of polymorphic loci in the spectrum was 93%, PIC_{average} = 0.414. This indicates a relatively high genetic diversity of the studied groups within the genus *Glycine*, and within one species *G. soja*. At the same time there was no locus length of 680 bp in the spectra of *G. max*, whereas the same locus was met in representatives of *G. soja*.

The unique for each variety of wheat spectra of polymorphic DNA fragments were obtained as a result of IRAP-PCR with a fragment of retrotransposon LTR SIRE-1 as a primer. So, DNA fragment of 790 bp length was present in all the studied samples of Moskovskaya 39, while the fragment of such length was not found in representatives of varieties Myronivskay 808 and Omskaya 36. Conversely, the locus corresponding to a

fragment length of 550 bp was not found only in Moskovskaya 39. Dendrogram was constructed on the basis of values of genetic distances (DN), calculated by the method of M. Nej (1972) based on the frequency of amplicons of different lengths in the obtained spectra of DNA fragments flanked by inverted sites of retrotransposon LTR SIRE-1 (Fig 7). There are two large separate clusters of monocots and dicots. The varieties Mironovskaya 808 and 36 Omskaya 36 are clustered together.

Such differentiation reflects their phenotypic characteristics and origin. For example, total phenotypic characteristic for varieties Mironovskaya 808 and Omskaya 36 is white beardless spike (The catalog of scientific and technical products, 2009; Remeslo and Kolomatsky, 1980), whereas Moscovskaya 39 differs from them in a white barbed spike (<http://www.nemchinovka.ru/sorta/yar>). In this winter variety Mironovskaya 808 was taken out of spring wheat by the group and mass selection of morphologically homogeneous plants (Remeslo and Kolomatsky, 1980).

It is shown that the physiological and phenotypic changes caused inbreeding can lead to activation of mobile elements, increasing the genetic diversity (Belyayev et al, 2012). This hypothesis may explain the high polymorphism of DNA spectra of *Glycine* and *Triticum*. In addition, there can be 10% of cross-pollinated plants in population of *Triticum* (Kozub et al, 2008). Probability of cross-pollination of representatives of *Glycine* can reach 7.7%, while the transfer of pollen can be carried out at a distance of 400 meters (Duncan et al, 2009). Consequently, high intravariety heterogeneity (high proportion of polymorphic loci and the increase in their index of PIC) of self-pollinating plants, such as *Glycine* and *Triticum* may be due to this fact.

Over a third of the fragments (38%) obtained in the spectra of wheat DNA resulting from use of a primer LTR SIRE-1, allow to distinguish varieties, about half (47%) loci of DNA fragments - the group of wild type of soybean (*G. soja*). A third of fragments were species specific that enables to distinguish the two groups of soybean species, *G. soja* and *G.max*.

Thus, these data indicate that fragments of mobile elements, in particular LTR SIRE-1 can be used in studies of the genetic structure of the groups and varieties as dicotyledonous (*Glycine*), and monocots (*Triticum*) plants.

It is assumed that a retrotransposon SIRE-1 was originally a retrovirus infection of invertebrates and later has been integrated into the genome of soybean by horizontal transfer (Laten et al, 1998). If we consider the fact that Sireviruses contain env-like gene horizontal transfer events between the families of monocots and dicots become less surprising. Furthermore, in sequenced genomes of GeneBank fragments homologous to fragments of mobile element LTR SIRE-1 was found in the genomes of soil bacteria *Artrobacteria*, and *Listeria monocytogenes*. The latter are animal pathogens, capable of infecting plants and penetrate the capillary system of roots and root hairs and further into leaf tissue from the soil (Vlasov and Pavlov, 2009).

From 200 to 500 sites of sequences homologous to terminal fragments of mobile elements of plant in the genome of *Ovis aries* was found, and 150 - in the genome of *Equus ferus caballus*. It is worth noting that the fragments of mobile elements of mammals are also found in the genomes of crop plants. Thus, it was found 200 such sites in the sequenced DNA of wheat and 120 - in the genome of soybean (*G. max*). In connection with this, it can be argued about generality of pool of mobile elements in the genomes of representatives not only of one department (angiosperms) or class (mammals) but as far as the kingdom of taxa. Accordingly, the mobile elements are a convenient tool to identify the genetic characteristics of the studied groups of organisms, population dynamics of gene pools and the definition of "standard" of the breed or variety.

Table 1 Values of polymorphic information content (PIC_{average}) and the share of polymorphic loci (P,%), obtained by genotyping breeds of sheep using IRAP-primers.

	LTR SIRE-1		PawS 5		BARE-1		k-1		-3	
	%	PIC	%	PIC	%	PIC	%	PIC	%	PIC
Birlik sheep	25	0,107	29	0,221	31	0,128	29	0,129	8	0,041
Suyunduk sheep	31	0,139	27	0,157	31	0,143	29	0,118	25	0,112
Edilbai sheep	25	0,092	50	0,195	13	0,050	31	0,140	8	0,041
Kalmyk sheep	44	0,187	47	0,199	25	0,154	23	0,107	17	0,037

Table 2 Detectable IRAP-spectra of DNA fragments of horse breeds

Primer	LTR SIRE-1	Paws 5	k-1	-3
range of lengths in a spectrum of DNA fragments (bp)	380-1500	370-2150	300-1300	490-1250

Table 3 Values of polymorphic information content (PIC_{average}) and the share of polymorphic loci (P,%), obtained by genotyping breeds of horses using IRAP-primers.

	LTR SIRE-1		PawS 5		k-1		-3	
	PIC	P	PIC	P	PIC	P	PIC	P
Altaic horse ("Dzhumbaev")	0,192	67	0,169	40	0,029	14	0,203	64
Altaic horse ("Genghis")	0,109	35	0,037	8	0,044	14	0,105	36
Altaic horse ("Enchi")	0,047	15	0	0	0,031	7	0,271	64
Karachai horse	0,145	33	0,048	11	0,057	21	0,138	36
Trotters	0,027	6	0,035	10	0,087	29	0,154	45

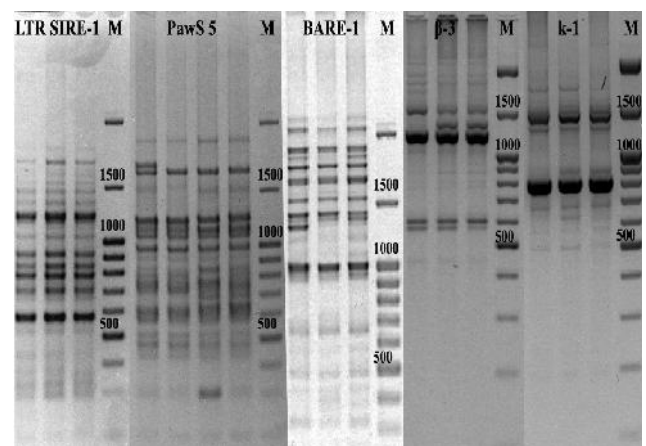


Figure 1 The spectra of DNA fragments derived from genotyping breeds of sheep (M, molecular weight marker).

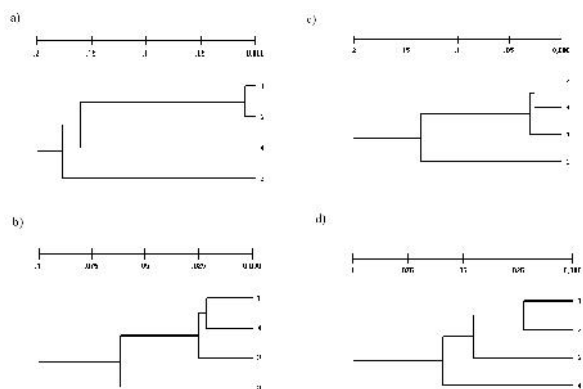


Figure 2 Dendrograms built between the two groups of sheep (1-Birlik type, 2-Suyunduk type, 3-Karachai sheep, 4-Kalmyk sheep) on the basis of values of genetic distances calculated from the primer: a) LTR SIRE-1, b) PawS 5, c) BARE-1, d) BERV.

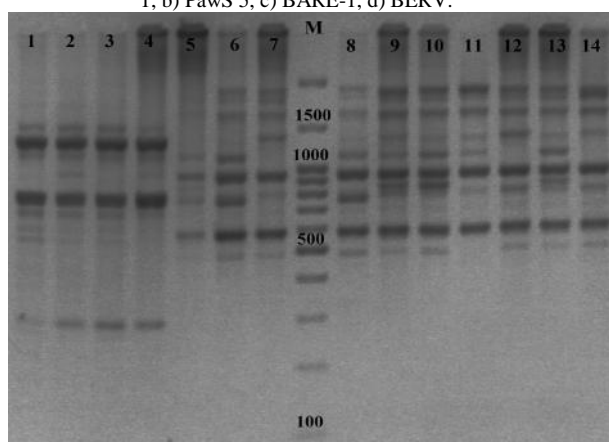
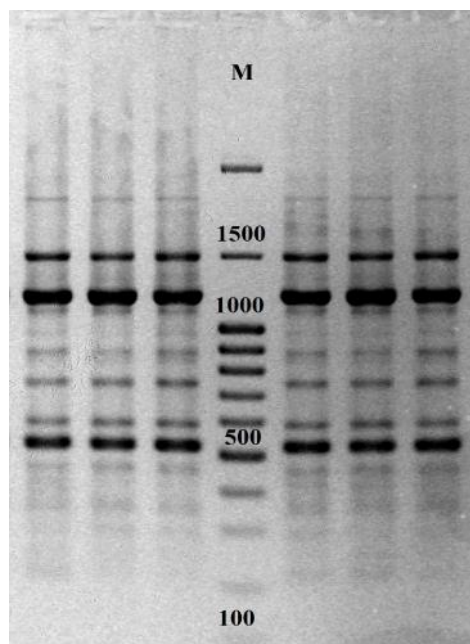
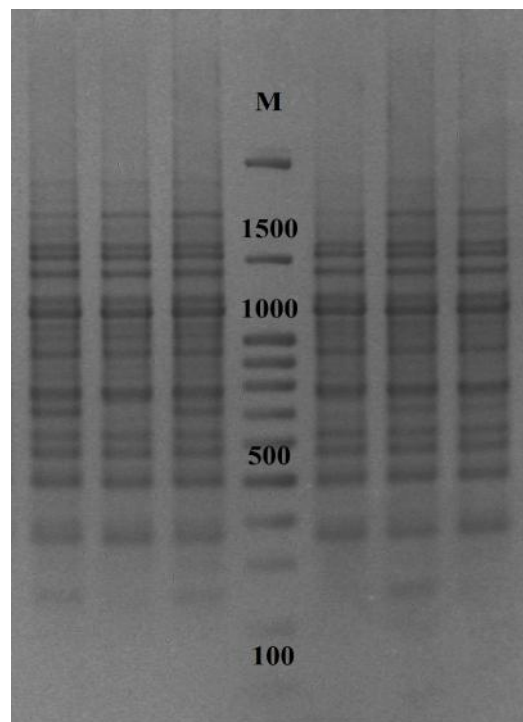


Figure 3 The spectra of DNA fragments derived from genotyping breeds of horses (M, molecular weight marker).

A
1 – 4 - the spectra of DNA fragments of the Altaic horse ("Genghis"), primer k-1
5 – 7 - the spectra of DNA fragments of the Altaic horse ("Genghis"), primer -3
8 – 14 - the spectra of DNA fragments of the Altaic horse ("Enchi"), primer -3



B the spectra of DNA fragments of the Trotters, primer LTR SIRE-1



C the spectra of DNA fragments of the Trotters, primer PawS 5

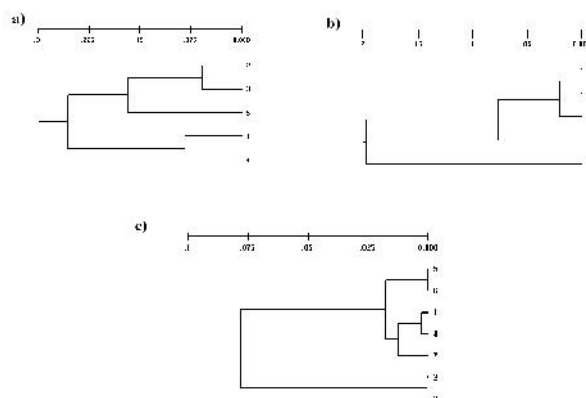


Figure 4 Dendrogram built between groups of horses (1-Altac horse ("Dzhumbaev"), 2-Altac ("Genghis"), 3-Altac horse ("Enchi"), 4- Karachai horse, 5-Trotting horse (for primers BERV: 5-American trotters, 6-Russian trotters, 7-Orlov trotters) on the basis of values of genetic distances calculated from the primer: a) PawS 5, b) LTR SIRE-1, c) BERV.

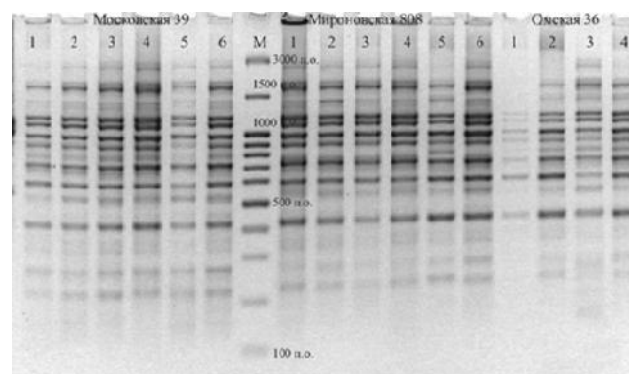


Figure 5. The spectra of DNA fragments derived from genotyping varieties of *T. aestivum* using a primer LTR SIRE-1 (M, molecular weight marker).

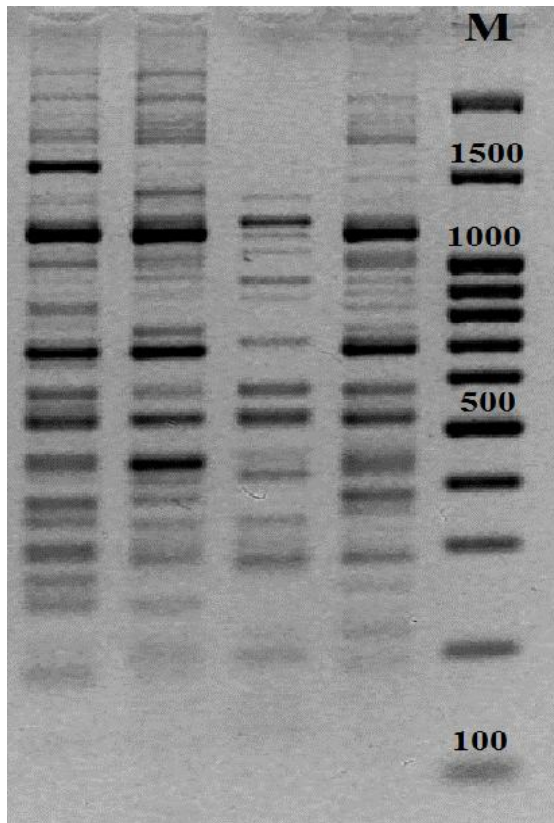


Figure 6 The spectra of DNA fragments derived from genotyping groups of *Glycine* using a primer LTR SIRE-1 (M, molecular weight marker).

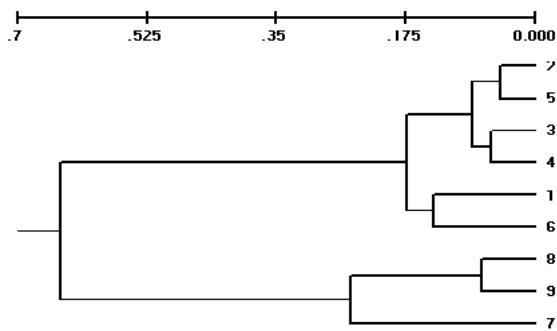


Figure 7 Dendrogram built between groups of plants (1-Glycine max (China); 2-6-Glycine soja (Primorsky region, Russia), 7-Moscovskaya 39; 8 Mironovskaya 808; 9-Omskaya 36) based on the values of genetic distances calculated from the primer LTR SIRE-1.

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How to cite this article:

Elkina, MA *et al.*, Mobile Genetic Elements As A Tool For The Analysis Of Genetic Differentiation Of Varieties Of Cultivated Plants And Breeds Of Farm Animals. *International Journal of Recent Scientific Research* Vol. 6, Issue, 8, pp.5893-5900, August, 2015
