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PLANT GENETICS

Study of Genetic Diversity in Potato Cultivars Using PCR Analysis of Organelle DNA

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Abstract—The genetic diversity of 98 potato cultivars of Russian and foreign breeding was studied using of PCR with organelle-specific primers. The polymorphism of both plastid (*atpE, trnG*/*trnR*) and mitochondrial (*rps10, atp6*) loci was revealed. Eight different haplotypes were detected in the sample of cultivars studied. Comparatively low polymorphism of organelle DNA in the potato cultivars was demonstrated: most cultivars (91 or 92.9%) possessed only two haplotypes (I and II); 62 cultivars of them had the same "cultural" cytoplasmic type (haplotype I). The breeding cultivars of the Russian and foreign origin did not differ from each other in frequency of basic haplotypes.

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INTRODUCTION

The genetic diversity of potato species (genus *Solanum* L., section *Petota* Dumort.), growing on territory from southwestern United States regions to southern boarders of Chili, includes 228 wild and seven cultivated species together with common potato *Solanum tuberosum* L. [1]. The potato occupied the fourth place in the world among the most significant food crops after wheat, maize and rice [1]. At the same time potato can be included in the list of agriculturally valuable plants that need the most intensive usage of chemical treatment to protect against diseases and pests [2]. Such situation can be explained by low genetic diversity of common potato [3, 4], which may have the following explanations:

(1) limited number of introductions of South-American cultivated species in Europe [5] and limited group of initial forms used in breeding programs: e.g., more than 1000 cultivars were derived from cultivar Early Rose [6];

(2) low level of introgression of genetic material from wild species into newly bred cultivars: due to incompatibility barriers, less than 10% of wild potato species are involved in breeding process [7];

(3) limited possibilities of genetic and breeding investigations as a result of high heterozygosity and the tetraploid nature of the genome of potato *Solanum tuberosum* L., as well as absence of collections of inbred genetically marked lines.

Analysis of polymorphism of chloroplast (cpDNA) and mitochondrial (mtDNA) DNA loci is one of the approaches to estimating inter- and intraspecific genetic diversity. In wild and common potato species, high variability of haplotypes was revealed [8–11]. Five basic (T, W, C, S, A) [12] and few minor types of plastid genomes [11] were described in wild species. W, or "wild," plastome type was found in most wild species studied [12]. T (*tuberosum*) type, associated with 241-bp deletion in cpDNA, is unique, as it was found only in two (*S. tuberosum* L. and *S. tarijense* Hawkes) of 235 potato species [14]. The T type of cpDNA characterizes the most part of studied European cultivars and aboriginal forms of *S. tuberosum* subsp. *tuberosum* from Chili [15–18]. In the most of German cultivars studied, β and α chondriome types were revealed, with β prevailing [9, 13]. Consequently, in common potato T/β type of cytoplasm is called "cultural" and W/α, "wild" [9, 13]. Nowadays, the specific primers were designed, giving an opportunity to distinguish the most widespread types of organelle genomes via polymerase chain reaction (PCR) [9, 13].

Cytoplasmic genome variability of cultivars of foreign origin is studied in many works [9, 13, 16–18]. No research of such kind was performed for cultivars of Russian breeding. The present work was aimed at investigating genetic diversity of potato cultivars of different origin, using PCR with primers specific for a number of loci of cpDNA and mtDNA.

MATERIALS AND METHODS

We examined 98 accessions of common potato *Solanum tuberosum* L. from the collection of Vavilov Institute of Plant Industry representing two groups of cultivars of different origin : (1) 63 cultivars of Russian breeding and from countries of the former Soviet Union; (2) 35 cultivars bred abroad (European countries and United States) including old cultivars that are progenitors of many hundreds of modern ones.

The extraction of total DNA was performed from young leaves according to Wienand and Feix [19] with

some modifications. Analysis of plastid genome polymorphism was carried out using PCR with primers ALC_1/ALC_3 and NTCP9, specific for loci *atpE* and *trnG*/*trnR* of cpDNA, respectively [9, 20]; study of mtDNA polymorphism was performed using primers AL_Mt2/ALM_3 and ALM_4/ALM_5 specific to loci *atp6* and *rps10*, respectively [9, 13]. The specific primers used in the present work permit identification of the basic cytoplasm types in potato. For instance, amplification of potato DNA with primer pair ALC_1/ALC_3 and fractionation of the products in agarose gels produced two types of fragments with sizes of 380 and 620 bp, which are markers of the "cultural" (T) and "wild" (W) types of plastid genomes, respectively [9, 13]. The fractioning of the amplification products with primer pair ALM_4/ALM_5 reveals two types of fragments 2.4 and 1.6 kb in size, which mark α and β types of potato mitochondrial genomes, respectively [9, 13]. The designations of alleles of loci listed above correspond to classification of Lössl [9, 13] with addition according to Antonova and Gavrilenko [8].

The PCR conditions corresponded to the protocols of the authors who designed the primers [9, 13, 20]. Fractionation of the fragments was carried out by electrophoresis in agarose gel in TBE buffer, and also in 6 and 10% PAAG in both denaturing and nondenaturing conditions.

Significance of differences between groups of cultivars was estimated pairwise using t_φ test [21].

RESULTS

PCR with specific primers gives an opportunity to evaluate intraspecific polymorphism in cpDNA and mtDNA loci. Here, the variability of organelle DNA was analyzed in 98 potato cultivars at two plastid (*atpE* and *trnG*/*trnR*) and two mitochondrial (*rps10* and *atp6*) loci.

Only two alleles of plastid locus *atpE* were found in the subset of cultivars studied (Fig. 1a). The most part of cultivars (63 of 98) contained T allele, i.e., were of the *tuberosum* plastome type, while 35 cultivars carried the W allele ("wild" plastome type) (table).

Three alleles were revealed in plastid locus *trnG*/*trnR* among the cultivars examined (Fig. 1b). The most cultivars (65 of totally 98) carried allele 6; allele 8 was found in 32 cultivars, and only one cultivar, Flourball, had allele 7 (table).

Two alleles (1 and 0; Fig. 1c) were revealed in mitochondrial locus *atp6*. In case of null allele, the absence of amplification products was confirmed in three independent experiments. Allele 0 was found in 65 cultivars of 98 studied, while the remaining 33 possessed allele 1 of this locus.

In the cultivars studied, the mitochondrial locus *rps10* had three alleles, α, β, and 0 (Fig. 1d); allele β was the most widespread (66 cultivars) (table).

No differences in allele frequency at the loci studied were found between the groups of cultivars of the Russian and foreign breeding.

Data analysis revealed that organelle genomes of cultivars are characterized with stable combinations of alleles. In most cultivars (93 of 98 studied), two variants of allele coupling were identified in plastid loci *atpE* and *trnG*/*trnR*: either T and 6 ("cultural" cpDNA type) or W and 8 ("wild" cpDNA type). Similarly, in 93 cultivars of 98 studied, in studied mitochondrial loci *atp6* and *rps10* the following allele combinations were found: either 0 and β ("cultural" mtDNA type) or 1 and α ("wild" cpDNA type) (table).

The comparison of results of analysis of cpDNA and mtDNA revealed eight haplotypes among 98 studied cultivars, while the most part of cultivars (91 cultivars, or 92.9%) corresponded to two haplotypes only, I and II (table). The most widespread haplotype I, found in 62 cultivars, was characterized by combination of cpDNA and mtDNA of the "cultural" type. In case of haplotype II, the presence of cpDNA of the "wild" type was associated with ("wild") type of mtDNA (table). Thus, two prevailing haplotypes I and II corresponded to cultural (T/β) and wild (W/α) cytoplasmic types [9]. The haplotypes III (W/0) and IV–VIII with unusual allele combinations were found only in a few cultivars such as Aksamit, Hilta, Paul Wagner, Flourball, Alaska Frostless, Malinovka, and Bryanskaya Novinka (table).

As for frequency of the main haplotypes (I and II), the cultivars of the Russian and foreign breeding showed no differences between each other, while the percentage of rare haplotypes in Russian cultivars appeared to be twofold lower than in the foreign ones (4.8 and 11.6%, respectively) (table).

DISCUSSION

Our results indicate a low level of polymorphism of organelle DNA among potato cultivars. Most of the cultivars (91 of 98, or 92.9%) belonged to two haplotypes only (I and II), 62 of them (63.3%) having the same "cultural" cytoplasmic type (haplotype I).

The degree of polymorphism in potato cultivars revealed in given work was lower than diversity of the same loci studied in species of the genus *Solanum* L. earlier [8]. While only three alleles (6, 7, and 8) of plastid locus *trnG*/*trnR* were earlier revealed, eight alleles (1 to 8) of this locus were found in species of *Solanum* L. [8]. Similarly, only two alleles of plastid locus *atpE* (T and W) were found in potato cultivars, while four alleles of this locus were described in the species studied [8]. Only three alleles of mitochondrial locus *rps10* (α, β, and 0) were found in potato cultivars, as compared with species of the genus *Solanum* L. having eight alleles [8]. Finally, two alleles of locus *atp6* of mtDNA (0 and 1) were revealed in cultivars, while three alleles (0, 1, and 2) were present in the species examined [8]. Note that the subsets of the Russian and

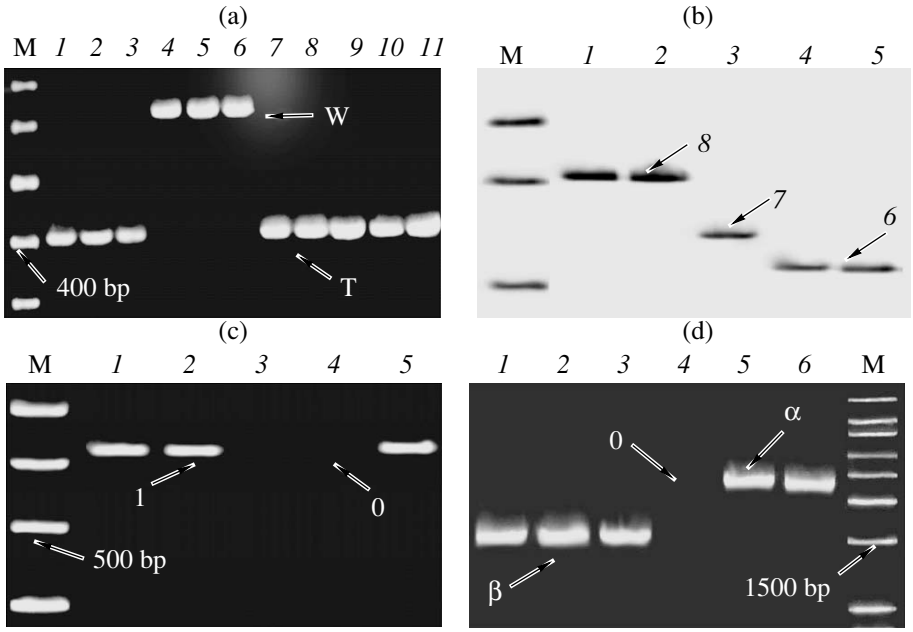

Fig. 1. Products of amplification with organelle–specific primers in cultivars of common potato. Arrows indicate different variants of revealed alleles. (a) Primers ALC_1/ALC_3 (plastid locus *atpE*). Cultivars: *1*, Early Rose; *2*, Jubel; *3*, Sante; *4,* Skarb; *5,* Lazurit; *6*, Nevskii; *7*, Kameraz; *8*, Zarevo; *9*, Temp; *10*, Elizaveta; *11*, Istok. M, marker of molecular weight EZ100 bp (BioRad). Electrophoresis in 1.4% agarose gel. (b) Primer NTCP 9 (plastid locus *trnG*/*trnR*). Cultivars: *1*, Apta; *2*, Vytok; *3*, Flourball; *4*, Ella; *5,* Gorizont. M, marker of molecular weight EZ100 bp. Electrophoresis in 10% PAAG in denaturing conditions. (c) Primers AL_Mt2/ALM_3 (mitochondrial locus *atp6*). Cultivars: *1*, Adretta; *2*, Utyonok; *3*, Epicure; *4*, Simfonia; *5,* Rozhdestvenskii. M, marker of molecular weight EZ100 bp, Bio-Rad. Electrophoresis in 1.4% agarose gel. (d) Primers ALM_4/ALM_5 (mitochondrial locus *rps10*). Ciltivars: *1,* Early Rose; *2*, Provita; *3*, Lugovskoi; *4*, Hilta; *5*, Dorisa; *6*, Lazurit. M, marker of molecular weight Jetway 5000 (Genomed GmbH). Elecrophoresis in 0.8%.

foreign cultivars did not differ significantly in allele frequency of cpDNA and mtDNA loci; the haplotype I prevailed in both groups (table).

The prevailing of the "cultural" cytoplasmic type (haplotype I) in the cultivars can be explained by analysis of their pedigrees [6]. As noted earlier, more than 1000 cultivars originate from old cultivar Early Rose, which comes from a seedling of cultivar Garnet Chili, originated from cultivar Rough Purple Chili, which is assumed to be a Chilean landrace sample (introduced by Goodrich in 1853) with the "cultural" cytoplasm type. The same type of cytoplasm characterizes the most part of Chilean landraces of *S. tuberosum* subsp. *tuberosum* [10, 15, 22].

The investigation of reciprocal hybrids, obtained from intra- (with *S. tuberosum* subsp. *andigena*) and interspecific crosses of Chilean landraces, revealed the positive influence of "cultural" cytoplasmic type on tuber yield [23, 24], on one hand, and on induction and pollen sterility in the formed hybrids, on another hand [25, 26]. These traits were transferred from Chilean landraces of *S. tuberosum* subsp. *tuberosum* as well as cultivar Early Rose to all their descendants derived from the maternal progenitor [25, 26]. For this reason Early Rose was preferably used in breeding of highly yield cultivars as the maternal form, and hybrids originating from crosses with Early Rose possessed sterile pollen and thus could be used only as the female parent in further crosses. Hence, influnce of "cultural" cytoplasmic type on tuber yield and pollen sterility in hybrids were the reason of the wide occurence of this cytoplasmic type among potato cultivars.

Rare haplotypes of old cultivars are connected with other independent introductions of nonrelated samples of South–American potato. For example cultivar Paul Wagner (haplotype VI) received its cytoplasm in maternal line from cultivar Industrie, descending in maternal line from Erste von Nassengrund, belonging to an old continental European group arising from first Spanish introductions of *S. tuberosum* subsp. *antigena* in 1580–1600 [6]. Landrace Flourball having haplotype VII is close to Daber group of earlier introduction of the 1830s. This cultivar is fertile and was used in crosses as the paternal plant mostly [6]; thus, its cytoplasm does not frequently occur among modern range of cultivars.

Analysis of pedigrees of cultivars with the "wild" cytoplasmic type (haplotypes II and III) and rare haplotypes (IV to VIII) demonstrated that in maternal line these cultivars usually originate from hybrids of *S. tuberosum* subsp. *tuberosum* with one or few cultivated and/or wild species: *S. tuberosum* subsp. *andigena* (Paul Wagner), *S. acaule* (Alaska Frostless), *S. demissum* (Veselovskii 2-4, Nevskii, Arina, Bryanskaya Novinka) and some other. The most of cultivars with the "wild"

Distribution of haplotypes in potato cultivars of different origin

Haplotype*	Total (%)	Number of foreign cultivars (%)	Number of native cultivars (%)
I. T/6/β/0	62 (63.3%)	22 (62.9%)	40 (63.5%)
		Agave, Baltica, Bintje, Cobbler, Deodara, Desiree, Early Rose, Ella, Epicure, Garnet Chili, Hindenburg, Jubel, Paterson Viktoria, Provita, Reichskanzler, Quarta, Russet Burbank, Sante, Sonate, Xenia, Simfonia, Sinyukha	Agronomicheskii, Atlant, Bronnitskii, Gatchinskii, Gorizont, Detskosel'skii, Elizaveta, Zhuravinka, Zaravshan, Zarina, Zarevo, Imandra, Istok, Kameraz, Katunskii, Komsomolets 20, Lorkh, Loshitskii, Lugovskoi, Lybid', Matryoshka, Milavitsa, Murmanskii, Naroch', Oredezhskii, Peterburgskii, Pobeda, Povirovets, Pramen', Priekul'skii rannii, Priobskii, Raduga, Severnaya Roza, Sineglazka, Suzorie, Talisman, Temp, Fitoftoroustoichivyi, Energiya, Yantarnyi
II. W/8/α/1	29 (29.6%)	9 (25.7%)	20 (31.8%)
		Adretta, Apta, Carla, Delikat, Dorisa, Galina, Karlena, Rasant, Turbella	Al'pinist, Arina, Veselovskii 2–4, Vesna Belaya, Vytok, Del'fin, Dina, Zhavoronok, Zhukovskii rannii, Zov, Lazurit, Lasunok, Mars, Nevskii, Rozhdestvenskii, Rumyanka, Svitanok Kievskii, Skarb, Utyonok, Charodei
III. W/8/0/1	1 (1.0%)	0	1 (1.6%)
			Aksamit
IV. W/6/0/1	1 (1.0%)	1 (2.9%)	0
		Hilta	
V. W/6/β/0	2 (2.0%)	0	2 (3.2%)
			Bryanskaya novinka, Malinovka
VI. W/8/α/0	1 (1.0%)	1 (2.9%)	0
		Paul Wagner	
VII. W/7/β/1	1 (1.0%)	1 (2.9%)	0
		Flourball	
VIII. T/8/β/1	1 (1.0%)	1 (2.9%)	0
		Alaska Frostless	

* Alleles revealed in loci *atpE/trnGtrnR/rps10/atp6*, respectively, are indicated.

cytoplasm of were bred during last 40 years when interspecific hybridization began to be used widely in breeding programs. However, as mentioned above, the number of wild species able to cross with common potato is limited due to incompatibility barriers. Enrichment of genetic diversity of potato cultivars (including cytoplasmic factors) can be achieved by using of modern methods of biotechnology, e.g., somatic hybridization of plants [27].

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