UDC 577.2:631[633.111:664.64.016.8]

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DNA TYPING OF AGRICULTURAL CROPS BY MICROSATELLITE ANALYSIS FOR THE PURPOSES OF GENOTYPE DIFFERENTIATION, IDENTIFICATION AND REGISTRATION

Principles of microsatellite marker systems for breeding and varieties investigation needs were analyzed basing on material of bread winter wheat (Triticum aestivum L.*), barley (Hordeum vulgare* L.*), maize (Zea mays* L.*), sunflower (Helianthus annuus* L.*), hop (Humulus lupulus* L.*), rice (Oriza sativa* L.*), sorghum (Sorghum bicolor* L.*). The key features of such microsatellite systems are their multi-allelic nature, reproducibility, high polymorphism, easy automation, and co-dominant inheritance. Attention is paid to determination of allelic state of agriculturally important genes that can be used as supplementary part of the existing molecular genetic formulas.*

Key words: *molecular markers, microsatellite analysis, variety investigation, identification, differentiation.*

Introduction. Nowadays the application of modern biotechnology to plant breeding is acknowledged to be more effective and quicker than conventional breeding techniques in the creation of new stable and more resistant crop varieties. Molecular markers are the modern strategy used to characterize the genetic diversity and redefine the plant genetic resources. Genetic improvement of wheat varieties depends on the existence of different diversified and preferable genes or alleles suitable for the environmental conditions of Southern steppe. However modern intensive plant breeding practices led to narrowing of genetic diversity in crop varieties. Knowledge of the genetic diversity, allelic composition of economically important loci, the genetic relationship between genotypes and their pedigrees is essential for breeding as well as for the best protection and saving of plant varieties in genetic collections and gene banks all over the world.

Enforcement of Trade Related Aspects of Intellectual Property Rights Agreement (TRIPs) under World Trade Organization (WTO) has resulted in

worldwide shift from free exchange and unhindered exploitation to controlled access to plant genetic resources. Intellectual property rights of plant breeders need to be protected. A new plant variety requires novelty, denomination, distinctness, uniformity, and stability (DUS) to get registered for protection of plant breeder rights based on the International Union for the Protection of New Varieties of Plants (UPOV) regulations. The estimation of morphological characters is used for the DUS test and it is obvious to conduct several growing cycles at the fields or greenhouse for growth trials and to cultivate plants to complete ripeness.

A significant problem becomes determining the features of the variety structure, as well as the genetic monotony of lines and the typicalness of hybrids by assessment of plant morphological traits and conducting phenotypical tests in fields. Moreover, estimation of morphological traits has restrictions, such as subjectivity in the analysis of a morphological trait; their expression is affected by environmental factors or the technique of treatment, inability to reveal the distinctions between closely related genotypes, the opportunity of testing only adult plants, etc. The advantages of DNA markers are as follows: independence of the DNA sequence from the environmental conditions, presence of the same DNA in each plant cell which allows one to conduct studies on any tissues, a potential opportunity to receive an unlimited quantity of informative DNA markers, and an opportunity to analyze practically entire genome [1].

UPOV has constituted a Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular (BMT) to study the utility of molecular markers in the variety registration system which recommended [2–4] to use molecular techniques in establishing distinctness between candidate varieties. Molecular marker system is one of the most effective methods for DNA profiling of crop genotypes and assessing genetic diversity and relatedness among them [5]. Thus, a rapid and robust DNA marker technique has been used to identify cultivars for the DUS test [6]. DNA markers have many advantages to identify varieties due to their independence from environmental influences. The UPOV suggests that simple sequence repeat (SSR) markers are suitable for a DNA profiling database due to their multi-allelic nature, reproducibility, high polymorphism, easy automation, and codominant inheritance [4]. SSRs are present in both coding and noncoding regions [7, 8]. At the moment, among other molecular techniques SSR markers are widely used in plant breeding and genomic research and are the bases for mapping of genes and qualitative trait loci (QTLs), marker assisted breeding, phylogenetic studies and comparative genomics [9–11]. Microsatellite markers have been integrated into the molecular genetic maps of a number of plant species, and they have been successfully used to perform gene-mapping, population and evolutionary studies for the purpose of variety development [12].

In recent years, microsatellite markers have been widely used to screen, characterize and evaluate genetic diversity in cereal species [13–19]. In par-

ticular, microsatellite-based methods offer an attractive high-throughput and non-labor-intensive way to identify, differentiate and registry agricultural crop varieties, lines and hybrids according to fulfilling breeding programs. Furthermore, the development of molecular methods to efficiently identify additional agriculturally important genes has the potential to greatly improve modern varieties, and such methods would help accelerate the application of marker assisted selection (MAS) breeding in crop improvement programs.

The main objectives of this study are the following: (i) to outline the way of applying of DNA typing for the purposes of identification and registration of varieties; (ii) to provide the quantity of varieties, lines and hybrids of different agricultural crops which were DNA-typed by microsatellite markers; (iii) to outline the examples of molecular-genetic formulas given as passports to protect plant breeder's rights; and (iv) to provide examples of how markers of agriculturally important genes have been used to supply the existing molecular genetic formulas.

Material and methods. The analyzed material consists of varieties, lines and hybrids of such agricultural crops as bread winter wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), hop (*Humulus lupulus* L.), rice (*Oriza sativa* L.), five sorghum (*Sorghum bicolor* M.) species: grain sorghum (*S. bicolor*), sugar sorghum (*S. saccharatum*), broom sorghum (*S. technicus*), sudanka (*S. sydanense*), soryz (*S. orysoidum*).

Genomic DNA was extracted from seedlings using modified CTAB method [20]. Polymerase chain reactions (PCR) was performed on a Tertsyk thermocycler (DNA Technology, Russia) according to [13, 15, 16, 19, 21–28], with 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 50°C (55 or 60°C depending on the primer) and extension for 1 min at 72°C followed by a final extension step for 5 min at 72°C. PCR was carried out in a final reaction volume of 20–25 μL containing: 60 ng of DNA; 0.25 μM of each primer; 1 x PCR-buffer (40 mM Tris-HCl pH 8,4; 25 mM KC1; 1.5 mM of $MgCl_2$; 0,01 % Tween-20); 0,2 mM of each dNTP; 1–2 unit of Taq-polymerase. The amplification products (10-μL aliquot of the PCR mixture) were separated in 7 % polyacrylamide gel in 1 x TBE using Hцfer scientific instruments (USA). Visualization of PCR products was performed by the staining of gels in $AgNO_3$ according to «Silver sequence TM DNA Sequencing System Technical Manual» [29]. Image Master VDS video system (Amersham Pharmacia Biotech, USA) was used to assess the fragment size of the alleles at each microsatellite locus. The pUC19 DNA/MspI and 100 bp DNA Ladder were used as standard ladders. Statistical processing of the results obtained was carried out by standard methods [30].

Results and discussion

The way of applying of DNA typing for the purposes of identification and registration of varieties. A method for the identification of plant varieties by DNA typing has been first developed in South Biotechnology Center (National Academy of Agricultural Sciences) [31], which later be-

comes the department of general and molecular biology of Plant breeding and genetics Institute — National center of seed and cultivar investigation (PBGI–NCSCI). There the polymerase chain reaction (PCR) analysis was introduced by Acad. Yu. M. Sivolap to investigate the molecular–genetic polymorphism and the technology of the application of DNA typing for the identification and registration of varieties was developed and reported on the Seventh Session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular of UPOV (Hanover, Germany. 21–23.11.2001) [32].

A molecular genetic formula of a variety is proposed [33]. In the mentioned formula, the tested variable locus is coded by a Latin letter, and the molecular mass of a detected allele is presented in the lower index. Thus, the formula consists of two parts. One of them is differentiating, which gives the opportunity to identify a variety, a line, or a hybrid. The second part contains data concerning the allelic condition of microsatellite loci. Such formula gives representation of the genetic structure of variety and the conformity of a variety to the UPOV requirements for monotony. The variety definition by DNA technology has incomparably larger productivity, opportunity for the objective evaluation of data, testing at all stages of ontogenesis, and other advantages in comparison to traditional methods based on phenotypic analysis (DUS tests). The use of 12–15 SSR loci gives the possibility to differentiate uniquely, identify and register varieties of wheat [34], barley [35–37], maize [38], sunflower [39, 40], sorghum [15, 41], rye [42], hop [19] and rice [13].

Number of varieties, lines and hybrids of different agricultural crops which were DNA typed by microsatellite markers. A variety that consists of several genotypes and all the genotypes differ in the allelic composition, has a combination of biotypes and each of them differs in the molecular mass of alleles; each biotype of such variety is characterized by microsatellite loci that have certain alleles detected at appropriate loci; and it is desirable to determine the frequency of the occurrence of each of them in the variety [33]. A list of varieties, lines and hybrids which were DNA typed in the department of general and molecular biology (PBGI–NCSCI) is given in Table 1.

Information about genetic diversity pattern among modern varieties is essentially important for understanding direction of contemporary breeding and for further genetic improvement of varieties or creation new ones. The effectiveness of the new improvement technologies of crops depends largely on studies of the genetic diversity in collections of cereals on the basis of the molecular genetic markers. The allele characterization of the microsatellite loci of varieties allows to evaluate genetic variability level in the pool of the modern Ukrainian agricultural crops as well as cataloging of different varieties. The evaluation of genetic heterogeneity makes it possible not only to characterize the structure and composition of varieties, but also allows to control the quality of seeds and genetic purity of varieties.

Table 1

Consolidated list of varieties, lines and hybrids DNA typed in department of general and molecular biology, PBGI–NCSCI

Crop	Number of varieties / lines / hybrids	Studied SSR loci	Overall number of detected alleles
Wheat	Varieties Hospodynia, Scarbnytsia, Kosovytsia, Antonivka, Zamozhnist', Blahodarka odes'ka, Misiia odes'ka, Dal'nyts'ka, Yednist', Kiriia, Liona, Kuial'nyk, Poshana, Zaporuka, Bunchuk, Podiaka, Oksana, Zahrava odes'ka, Epokha odes'ka, Lytanivka, Sluzhnytsia odes'ka, Hoduval'nutsia odes'ka, Istyna odes'ka, Zmina, Dovira, Krasen', Otaman, Albatros odes'kyi, Bezosta 1 (two samples), Borvii, Turunchuk, Diuk, Nebokrai, Khyst, Pylypivka, Zorepad, Zhaivir, Uzhynok, Hurt, Dobrochyn, Vatazhok, Pol'ovyk, Holubka odes'ka, Kniahynia O'lha, Lebidka odes'ka (two samples), Zhuravka odes'ka, Bezmezhna, Lastivka odes'ka (48 varieties, 172 biotypes)	*Xgwm357*–1A, *Xgwm18*–1B, *Xtaglgap*-1B, *Xgwm095*–2A, *Xgwm155*–3A, *Xgwm389*–3B, *Xgwm3*–3D, *Xgwm165/l*-4D, *Xgwm186*–5A, *Xgwm408*–5B, *Xgwm190*–5D, *Xgwm325*–6D, *Xgwm577*–7B, *Xgwm437*–7D, *Xbarc126*–7D, *Xgwm44*–7D, *Xwmc405*–7D,	114
Barley	Pallidum 32, Medykum 46, Odeskyy 9, Odeskyy 14, Odeskyy 18, Uzhnyy, Stepovyy, Nutans 106, Odeskyy 36, Chornomorets, Nutans 244, Slavutich, Odeskyy 69, Odeskyy 70, Odeskyy 82, Nutans 518, Druzhba, Pervenets, Odeskyy 100, Visnyk, Odeskyy 111, Nutans 778, Romantyk, Typhun, Eney 1, Odeskyy 115, Ityl, Gelios 1, Preriya, Poss, Pallidum 107, Odeskyy 131, Odeskyy 151, Spomyn, Prestij, Deribas, Peremozhnyy, Gambrynus, Stalker, Edem, Nezalezhnyy, Adapt, Galateya, Galactyk, Zorianyy, Pivdennyy, Getman, Obolon, Chudovyy, Vacula, Selenit, Charivnyy, Kazkovyy, Vodogray, Gelios 2, Komandor, Eney 2 (57 varieties)	EBmac501, UMB503, AWBMS56, Bmac93, Bmag225, EBmac701, Bmac310, Bmac96, EBmac602, Bmag321, Bmag341, Bmag120	126
Sorghum	grain sorghum (NK-180, K 35-E5, NK-5418, NK-2517, NK-1486); sugar sorghum (Odesskiy 1800, 1969 Budzhak, Odesskiy 2111, Odesskiy 2113, 2179 Budzhak); broom sorgum (2645 Budzhak, 2806 Budzhak, 2778 Budzhak);	p 13, p 32, p 37, p 39, p 43, p 44, p 48, p 49, p 57, p 83, Sb4–32, Sb4–12, Sb6–36, Sb6–57, Sb6–84,	50

Table 1 continued

Crop	Number of varieties / lines / hybrids	Studied SSR loci	Overall number of detected alleles
Sorghum	sudanka (2810 Budzhak, Sudanka 1); soryz (4005 Budzhak, 2265 Budzhak, 721/I, 1/II, Odesskiy 302) (20 lines)	Xtxp 18, Xtxp 250, Xtxp 400, Xtxp 406	
Maize	Lines GK11,GK26, DK2/427–5, DK66, DK185, DK266/417, DK267, DK267–4, DK277–9, DK277–10, DK279, DK293, DK312, DK315, DK322, DK347, DK366, DK374/707, DK403, DK411–12, DK417–32, DK420–17, DK427, DK429, DK437, DK487, DK507, DK517, DK633/619–5, DK710, DK714, IK107–1, IK205–2, Odes'ka 7, Odes'ka 17, Odes'ka 18, Odes'ka 24, Odes'ka 141, Odes'ka 221, Odes'ka 308, Odes'ka 329, Odes'ka 384, Odes'ka 386, A654, BAM97, CA33, F2, F564, F564–12, HMV404, Oh43, OK109, OM74, P101, P346, P502, PLS61, 156Rf. Hybrids DAR 347, Dneprovs'kiy 181, Dneprovs'kiy 196, Dneprovs'kiy 197, Dneprovs'kiy 223, Dneprovs'kiy 227, Dneprovs'kiy 293, Dneprovs'kiy 335, Dneprovs'kiy 407, Dneprovs'kiy 453, Kadr 195, Kadr 217, Kadr 307, Kadr 443, Kedr, Kross 403, Melodiya, Odes'kiy 346, Odes'kiy 360, Odes'kiy 375, Odes'kiy 385, Odes'kiy 480, OdMa 310, OdMa 338, Platan, Roza, SiD 247, SiD 357, Siren', Smena, Stozhar, Suvenir, Syurpriz, P 3978 (58 lines; 34 hybrids)	phi055, phi064, phi083, phi127, phi029, phi073, phi021, phi079, phi093, phi024, phi070, phi078, phi034, phi116, phi115, phi015, phi022, phi027, phi062, phi084	65
Sunflower	inbred lines: Orange, Odol1, Od391, Od973, Od1318, Od1295; parental lines of hybrids: Siver, Kovcheg, Noy, Etyud, Dariy; hybrids: Odor, Oliver, Sapfir, Karat, Siver, Jason, Vsesvit, Dariy, Etyud, Kovcheg, Noy, Oskil, Psyol, Oberig, Slavutych, Lakomka, Master, Rodnik, Flagman, Oniks, Chas, Zlatibor, Serzhan, Iberiko, Latino, PR64G, Tayfun, KVS Geliya (13 inbred lines; 28 hybrids)	*ORS 409, ORS 509, ORS 78, ORS 1024, ORS 3, Ha 1796, ORS 546, ORS 595, ORS 599, ORS 4, Ha 1608, ORS 815, ORS 307, ORS 533,* Ha 1209,	74

Table 1 continued

Crop	Number of varieties / lines / hybrids	Studied SSR loci	Overall number of detected alleles
Hop	Al'ta, Zmina, Ksanta, Kumyr, Nadiya, Nazaryi, Obolons'kyi, Poles'kyi, Promin', Chaklun (bitter varieties); Vydybor, Haidamats'kyi, Zhytomyrs`kyi 75, Zahrava, Klon 18, Oskar, Pyvovar, Polisianka, Slavianka, Khmeleslav (aroma varieties), Granit, National'ny (22 varieties)	HIGA3, HIGA4, HIGA9, HIGA29, HIGT1, HIGT2, HIGT4, HIGT5, HIGT9, HIGT10, HIAGA7	44
Rice	Ukraina_96, Ontario, Pam'yati Gichkina, Vicont, Agat, Prestizh, Debut, Serpnevy, Yantarny, Premium, Antey (11 varieties)	RM1, RM20, RM21, RM161, RM222, RM259, RM283, RM307, RM316, RM474, RM510, RM552	31

*PBGI–NCSCI is abbreviation from Plant breeding and genetics Institute — National center of seed and cultivar investigation.

Thus, DNA typing holds considerable promise as a reliable tool of intellectual property protection of crop varieties and germplasm. Moreover, a system of codominant molecular markers is able to fix the genotype of a hybrid. For the definition of novelty, the molecular genetic formula of a variety is compared with data, which are available in an information base. Depending on the used equipment and the uniformity of the variety, the DNA typing procedure can take several weeks, instead of two or three years, which are required for field experiments according to the DUS test.

Creation of molecular-genetic formulas given as passports to protect plant breeder's rights. Based on the results of an SSR analysis of a variety, a molecular genetic registration certificate of a variety is created. This document certifies the distinctive features of the type of DNA of a variety, a line, or a hybrid [34–42]. The typing of DNA is the specific distribution of DNA fragments as amplification products in an electrophoretic gel or peaks on a densitogram. A molecular genetic passport reflects the features of the DNA structure of a variety, a line, or a hybrid, which allows identifying it and differing from other variety, line or hybrid. Table 2 contains examples of molecular genetic formulas of studied crop varieties, lines and hybrids which can serve as molecular genetic passports.

From the given wheat formulas we can see that varieties Podiaka and Nezalezhnyy are heterogeneous, consisting of five and two biotypes, respectively. On the contrary varieties Oksana and Vacula are presented by only one biotype and meet the requirements of the UPOV in the parameter of uniformity.

The universality of the approach of DNA typing by microsatellite loci lies in the possibility of registration of any agriculture crop variety on condition of availability of microsatellite markers panel with high discriminatory potential. The examples of applying of DNA typing by microsatellite loci on other agriculture crops in different institutions of Ukraine and abroad are represented in scientific works [14, 43].

Table 2

Examples of molecular genetic formulas of studied crop varieties, lines and hybrids

Crops		Molecular genetic formulas*
Wheat	Oksana (homogenious variety)	$A_{123}B_{110}C_{149}D_{195}E_{115}F_{188}H_{138}L_{86}M_{204}O_{107}P_{144}Q_{164}R_{185}S_{218}T_{188}U_{173}V_{215}$
	Podiaka (biotype 1)	$A_{125}B_{120}C_{129}D_{193}E_{125}F_{186}H_{136}L_{79}M_{208}O_{107}P_{142}Q_{156}R_{187}S_{216}T_{188,192}U_{173}V_{218}$
	Podiaka (biotype 2)	$A_{123}B_{122}C_{141}D_{193}E_{139}F_{186}H_{138}L_{77}M_{210}O_{107}P_{142}Q_{164}R_{185}S_{218}T_{188,192}U_{173}V_{218}$
	Podiaka (biotype 3)	$A_{123}B_{110}C_{141}D_{193}E_{129}F_{192}H_{138}L_{77}M_{212}O_{107}P_{142}Q_{164}R_{185}S_{216}T_{188,192}U_{137}V_{218}$
	Podiaka (biotype 4)	$A_{123}B_{120}C_{129}D_{193}E_{125}F_{188}H_{117}L_{77}M_{208}O_{107}P_{142}Q_{164}R_{185}S_{218}T_{188,192}U_{173}V_{218}$
	Podiaka (biotype 5)	$A_{123}B_{120}C_{129}D_{193}E_{125}F_{188}H_{117}L_{79}M_{208}O_{111}P_{138}Q_{164}R_{187}S_{216}T_{188,192}U_{137}V_{218}$
Barley	Vacula (homogenious variety)	$A_{146}B_{143}C_{222}D_{156}E_{148}F_{142}G_{136}H_{173}L_{148}M_{208}O_{224}P_{258}$
	Nezalezhnyy (biotype 1)	$A_{146}B_{134}C_{230}D_{154}E_{148}F_{146}G_{138}H_{173}L_{172}M_{208}O_{218}P_{234}$
	Nezalezhnyy (biotype 2)	$A_{146}B_{134}C_{230}D_{154}E_{160}F_{146}G_{138}H_{173}L_{172}M_{208}O_{218}P_{234}$
Maize	A 654	$A_{109}B_{101}C_{121}D_{132}E_{150}F_{205}G_{134}H_{180}I_{290}J_{162}K_{78}L_{122}M_{128}N_{157}O_{97}P_{98}Q_{376}R_{142}S_{167}T_{156}$
	Roza	$A_{109}B_{101,117}C_{121}D_{128,132}E_{146}F_{202,205}G_{134}H_{180}I_{286,290}J_{159,162}K_{78,83}L_{122}M_{131}N_{162}O_{97,101}P_{94,98}Q_{376}R_{142,157}S_{167}T_{159}$
Sunflower	Od1036 (inbred line)	$A_{216}B_{204}C_{140}D_{175}E_{223}F_{172}G_{165}H_{228}I_{149}J_{154}K_{150}L_{114}M_{189}N_{178}O_{228}$
	Od122 (hybrid F_1)	$A_{204}A_{216}B_{204}C_{140}C_{185}D_{175}D_{190}E_{223}F_{172}G_{165}G_{252}H_{228}H_{263}I_{149}I_{152}J_{154}J_{181}K_{150}K_{153}L_{114}M_{189}N_{178}O_{228}$
Hop	Al'ta	$A_{194}B_{198}C_{194}D_{186}E_{186}F_{228,230}G_{201}H_{195}I_{204}J_{200,}K_{247,267}L_{248,264}M_{580,580}N_{230,240}O_{188,188}P_{917,1303}R_{183,207}$
	Khmeleslav	$A_{184,194}B_{198}C_{194}D_{182}E_{186,196}F_{232}G_{201}H_{195}I_{204,208}J_{208}K_{247,267}L_{248,264}M_{580,580}N_{237,237}O_{175,185}P_{1303,1303}R_{183,207}$
Rice	Premium	$A_{91}B_{204}C_{143,130}D_{172}E_{184}F_{159}G_{157}H_{130}I_{198}J_{253}K_{121}L_{215}$
	Ukraina_96	$A_{91}B_{201}C_{143}D_{176}E_{184}F_{159}G_{157}H_{130}I_{198}J_{253}K_{121}L_{215}$

Table 2 continued

Crops		Molecular genetic formulas*
Sorghum	Odesskiy 1800	$A_{201,}$ $B_{197,}$ $C_{299,}$ $D_{177,}$ $E_{140,}$ $F_{412,}$ $G_{450,}$ $H_{373,}$ I_{169}
	Sudanka 1	$A_{201,}$ $B_{185,}$ $C_{293,}$ $D_{192,}$ $E_{137,}$ $F_{415,}$ $G_{453,}$ $H_{373,}$ I_{163}

*Letters A — V encode microsatellite loci as follows: for wheat varieties A — *Xgwm357*–1A, B — *Xgwm095*–2A, C — *Xgwm155*–3A, D — *Xgwm165/1*–4A, E — *Xgwm186*–5A, F — *Xgwm18*–1B, H — *Xgwm389*–3B, L — *Xgwm3*–3D, M — *Xgwm190*–5D, O — *Xgwm437*–7D, P — *Xgwm325*–6D, Q — *Xbarc126*–7D, R — *Xgwm44*–7D, S — *Xwmc405*–7D, T — *Xgwm408*–5B, U — *Xgwm577*–7B, V — *Xtaglgap*-1B; for barley varieties A — EBmac501, B — UMB503, C — AWBMS56, D — Bmac93, E — Bmag225, F — EBmac701, G — Bmac310, H — Bmac96, L — EBmac602, M — Bmag321, O — Bmag341, P — Bmag120; for maize lines and hybrids A — phi055, B — phi064, C — phi083, D — phi127, E — phi029, F — phi073, G — phi021, H — phi079, I — phi093, J — phi024, K — phi070, L — phi078, M — phi034, N — phi116, O — phi115, P — phi015, Q — phi022, R — phi027, S — phi062, T — phi084; for sunflower lines and hybrids A — *ORS 409*, B — *ORS 509*, C — *ORS 78*, D — *ORS 1024*, E — *ORS 3*, F — *Ha 1796*, G — *ORS 546*, H — *ORS 595*, I — *ORS 599*, J — *ORS 4*, K — *Ha 1608*, L — *ORS 815*, M — *ORS 307*, N — *ORS 533*, O — Ha 1209; for hop varieties A — J — microsatellite loci; K–R — the list of genes encoding chalcone synthases; for rice varieties A — RM1, B — RM307, C — RM316, D — RM474, E — RM552, F — RM20, G — RM21, H — RM161, I — RM222, J –RM259, K — RM283, L — RM510; for sorghum varieties A — Sb4–32, B — Sb4–12, C — Sb6–57, D — Sb6–84, E — Xtxp 18, F –Xtxp 250, G — Xtxp 400, H — Xtxp 406, I — Sb6–36.

Markers of agriculturally important genes used to supply the existing molecular genetic formulas. Markers of a number of agriculturally important genes, such as the quality of reserve proteins [44] and the Wx-genotypes [45] for wheat, have been created and added as an informative part to the existing molecular genetic formulas of crop varieties. As for hop (*Humulus lupulus* L.), the most important genes in its genome are genes *chs*_H1, *chs*2, *chs*3, *chs*4 and *vps* (*chs*5), which encode chalcone synthases catalyzing biosynthesis of aroma and bitter substances. Depending on content of aroma and bitter substances hop varieties are derived in aroma and bitter samples. Table 2 contains information about allelic state of chalcone synthase genes of both aroma and bitter hop varieties. Application of molecular markers for detection of aroma and bitter substances levels in hop is cheaper and needs less time than applying of biochemical methods. Creating of hop genome database is important for studying of hop diversity, evolution and identification of varieties, members of population and individual samples of hop.

In result, DNA technology for the identification of varieties of agricultural cultures has a number of advantages, among them obtaining of a molecular genetic passport of a variety, which can serve as a protection document of breeders' copyrights as well as a significant decrease in time necessary for the determination of the novelty, uniformity and stability of varieties.

Conclusions

The analyzed material was characterized using SSR markers and molecular genetic formulas of the examined varieties, lines and hybrids were designed according to the allelic state of microsatellite loci. Notwithstanding that genetic differentiation of the varieties was sometimes low, the use of the SSR markers was found to be an effective tool to make assessment of genetic diversity and to genotyping within varieties of different breeding origin. The results provide guidance for future efficient use of the selected microsatellite markers in molecular genetic analysis to characterize genetic diversity, perform their differentiation, identification and registration which allows appearance of new and diverse varieties on the Ukrainian and world market suitable for cultivation under a variety of biotic and abiotic stresses.

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Received 26.05.2015.

УДК 577.2:631[633.111:664.64.016.8]

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ДНК-ТИПУВАННЯ СІЛЬСЬКОГОСПОДАРСЬКИХ КУЛЬТУР ЗА ДОПОМОГОЮ МІКРОСАТЕЛІТНОГО АНАЛІЗУ ДЛЯ ДИФЕРЕНЦІАЦІЇ, ІДЕНТИФІКАЦІЇ ТА РЕЄСТРАЦІЇ ГЕНОТИПІВ

На матеріалі пшениці м'якої озимої (*Triticum aestivum* L.), ячменя (*Hordeum vulgare* L.), кукурудзи (*Zea mays* L.), соняшнику (*Helianthus annuus* L.), хмелю. (*Humulus lupulus* L.), рису (*Oriza sativa* L.), сорго (*Sorghum bicolor* L.) проаналізовано шляхи функціонування систем, заснованих на використанні мікросателітних маркерів для ідентифікації сортів, а також для задач селекції та сортовивчення. Ключовими особливостями таких систем є їх мультиалельна природа, відтворюваність, високий поліморфізм, легкість автоматизації та кодомінантне успадкування. Приділяється увага визначенню алельного стану агрономічно важливих генів, що можуть бути використані як додаток до існуючих молекулярно-генетичних формул.

УДК 577.2:631[633.111:664.64.016.8]

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ДНК-ТИПИРОВАНИЕ СЕЛЬСКОХОЗЯЙСТВЕННЫХ КУЛЬТУР С ПОМОЩЬЮ МИКРОСАТЕЛЛИТНОГО АНАЛИЗА С ЦЕЛЬЮ ДИФФЕРЕНЦИАЦИИ, ИДЕНТИФИКАЦИИ И РЕГИСТРАЦИИ ГЕНОТИПОВ

На материале пшеницы мягкой озимой (*Triticum aestivum* L.), ячменя (*Hordeum vulgare* L.), кукурузы (*Zea mays* L.), подсолнечника (*Helianthus annuus* L.), хмеля (*Humulus lupulus* L.), риса (*Oriza sativa* L.), сорго (*Sorghum bicolor* L.) исследованы пути функционирования систем, основанных на использовании микросателлитных маркеров для идентификации сортов, а также для задач селекции и сортоизучения. Ключевыми элементами таких систем являются их мультиаллельная природа, воспроизводимость, высокий полиморфизм, легкость автоматизации и кодоминантное наследование. Уделяется внимание определению аллельного состояния агрономически ценных генов, которые могут быть использованы в качестве дополнения существующих молекулярно-генетических формул.