Sources for resistance to Soil-Borne Cereal Mosaic Virus (SBCMV) among cultivated accessions of common wheat and its wild relatives

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INTRODUCTION

Soil-borne cereal mosaic virus (SBCMV) and related viruses are considered to be one of the most important pathogens of winter wheat in Europe, America and Asia [1], which can reduce grain yield of susceptible wheat variety on infected fields by up to 50-80% [2; 3]. SBCMV is exclusively vectored by the *Polymyxa graminis* [4] - eukaryotic zoosporic microorganism parasitising the plant roots. Viruses survive in soil in *P.graminis* resting spores that may remain viable for decades until suitable conditions for their germination will appear [5]. Using of resistant crop varieties currently the only practical, environmentally friendly and sustainable means of control [6]. Therefore, identification a new genetic source of resistance to SBCMV and its vector among world collection of common wheat varieties as well as wheat wild and cultivated relatives is a very important task. Preliminary investigations suggest that the soil-borne viruses of cereals are quite rare in Eastern European countries and particular in Ukraine. However, this could be partially explained by the fact that the disease is difficult to detect under the field condition otherwise an alternative explanation may be that the most commonly used Ukrainian wheat varieties are resistant to either viruses, or their vector. Last hypothesis is supported by the data from USA published on the Internet (see http://www.ars-grin.gov/npgs/**)** that show several older wheat varieties of the Ukrainian origin to be field resistant to *Soil-borne wheat mosaic virus* (SBWMV) (a virus related to the European SBCMV).

Several years ago in Rothamsted Research a gene, *Sbm1,* for resistance to SBCMV was identified using the population of doubled haploids developed by crossing of resistant common wheat variety Cadenza and susceptible Avalon. This gene was mapped to the gene-rich region on 5DL chromosome by application of microsatellite markers (barc110, barc144 and wmc765) and allele specific primers have been developed [7, 8]. In a separate study performed in Kansas State University on KS96WGRC40 a resistance to *Soil-borne wheat mosaic virus* was also detected on the long arm of chromosome *5D*. This gene was transferred into common wheat gene pool from *Aegilops taushii* (line TA2397) [9]. Therefore we infer that this particular region of chromosome 5D is of great importance for investigation of resistance of wheat and wheat wild relatives to the group of Soil-borne mosaic viruses transferred by *P.graminis*.

The aims of present study were: (i) to screen collection of common wheat varieties (old and current), which were cultivated in Ukraine, in respect to resistance to SBCMV; (ii) to study polymorphism between UK and Ukrainian varieties and analysis of diversity among accessions by application of target microsatellites (barc110, barc144 and wmc765) and allele specific primers (S12M61F2 and S12M61R2) linked to *Sbm1* (resistance to SBCMV) as well as cfd 10 which is linked to SBWMV resistance;(iii) to test collection of wild and cultivated wheat relatives kindly provided by National Centre of Plant Genetic Resources of Ukraine as potential new source of resistance to SBCMV and its vector and (iv) to study ability of specific *P. graminis* ribotypes to invalid roots of different monocotyledonous genotypes.

MATERIAL AND METHODS

Plant material. 53 accessions of common wheat varieties. 80 accessions of wheat cultivated and wild relatives, 39 accessions of genus *Triticum* and 22 of genus *Aegilops*, 1 accession of *Dasypyrum villosum* and 18 amphidiploids. These were kindly provided from collections of National Centre of Plant Genetic Resource of Ukraine (Kharkiv), Institute of Plant Physiology and Genetics NASU (Kyiv) and Institute of Genetics and Breeding UAAS (Odesa) *Detection of SBCMV.* Detection of SBCMV have been done by ELISA method according to the protocol published previously [7] (Fig. 1).

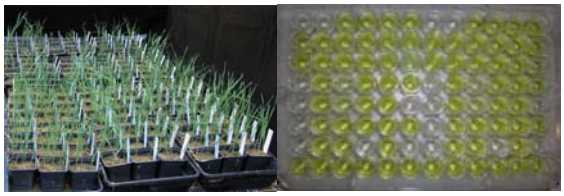

Fig.1 Left picture – plants tested under controlling conditions; Right picture - results of ELISA testing

PCR. Plant DNA extraction was done according the standard method. Allele specific primers S12M61F2 and S12M61R2 of gene *SbmCz1* of resistance to SBCMV recently were developed by Kanyuka (unpublished) by CAPS method. Microsatellite analysis and CAPS have been done as it was described in Bass et all., [8] and Hall et al [9]. Following microsatellite markers have been used barc110, barc144, wmc765 and cfd 10. *Detection of P. graminis*. To identify *P. graminis* resting spores roots were stained with 0.1 % acid fuchsin [10] and then were examined

under the light microscope. Detection of specific *P. graminis* ribotypes in plant roots by conventional PCR have been carried out according to protocol of Ward et al [11].

RESULTS AND DISCUSSIONS

Among accessions which have been tested three main groups according to their reaction of resistance to SBCMV were identified: 1. Highly susceptible accessions (group 1, table 1); 2. Resistant with high ELISA A_{405nm} values in root tissue (high concentration of virus in the roots) (group 4, table 1); 3. Resistant with low ELISA A_{405nm} values in root tissue (group 5 table **1**). A number of accessions were heterogeneous by those characteristics (groups 2, 3 and 6 table 1). The resistant variety Cadenza and susceptible Avalon were used as controls.

Table 1. Resulta of ELISA testing of plant accessions

Group	Absorbance values in ELISA, A405nm					
	after 7 weeks		*after 9 weeks*		*roots*	
	1	*2*	*1*	*2*	*1*	*2*
1	1,400-1,682	1,200-1,400	1,100-1,800	0,900-1,500	-	-
2	1,200-1,500	0,008-0,037	1,070-1,400	0,004-0,016	-	0,450-0,700
3	0,005-0,019	1,200-1,550	0,004-0,015	0,956-1,400	0,184-0,204	-
4	0,009-0,013	0,005-0,014	0,005-0,036	0,012-0,041	0,548-1,792	0,448-1,200
5	0,009-0,012	0,009-0,016	0,005-0,023	0,009-0,025	0,002-0,087	0,035-0,167
6	0,006-0,024	0,008-0,028	0,002-0,015	0,010-0,032	0,011-0,045	1,225-1,900

Group 1 was formed by Beltskaya 32, Priboi, Odeska 267, Tira, Strumok, Odeska chervonokolosa, Ukrainka 0246 (two accessions), Mironovskaya 808, Myronivska 61, Myronivska ostysta, Myronivska 33, Myronivska ranniostygla, Kyivska ostysta, Smuglyanka, Lutestsns 7, Poliska 90, Kopylivchanka, Khersonska bezosta, Ivanivska ostysta, Bezostaya 1, Kavkaz, Skyfianka, Lutescens 329, Kishinevskaya, Avalon Group 2: Krymka (accession1), Odesskaya 51, Albatros odesky, Odeska 162, Selyanka, Povaga, Myronivska 27, Myronivska 65, Podolyanka, Tsyganka, Yatran 60, Kharus; Species of genus *Triticum (AABB)*: *T.dicoccoides* IU016033, *T.dicoccum* UA 0300005, *T.dicoccum v. serbicum* UA0300009, *T.dicoccum v. volgense* UA0300014, *T.persicum* v. *osseticum* IR 00204, *T. persicum* v.*rubiginosum* UA 0300065; (*AA*): *T.monococcum* UA 0300104; Species of genus *Aegilops*: *Ae. umbellulata* IU 015892 (*UU*), *Ae.biuncialis* (Crimea 2) (*UUMM*), *Ae.neglecta* IU 015928 (*UUMM*) and amphidiploids: AD *Ae.ventricosa-T.dicoccum* UA 0500021. Group 3: Krymka (accession 1), Obrii, Panna; Hexaploid Species of genus *Triticum AABBDD*: *T.spelta* v *.caeruleum* , UA 0300074; Tetraploid species of genus *Triticum AABB: T.dicoccum* v. a*eruginosum haussknechtianum* UA 03000008, *T. persicum* v.*rubiginosum* UA 0300065; species of genus *Aegilops*: *Ae. umbellulata* IU 015905 *UU*, Ae. recta NCPGR *UUMMDD* and amphidiploids: MIT 346 (*T.durum* v.*muticoitalicum-Ae.taushii* k-346). Group 4: Ukrainka odes`ka, Kuyalnik, Cadenza; Species of genus *Triticum AABBDD*: *T.spelta* v.*arduini* IR00157, *T.spelta* v.*baulander v.duhamelianum* UA 0300101, *AABB: T.dicoccum v. volgense* UA0300031, *T.turanicum IR 000162, T. persicum v. persicum UA 0300062;* species of genus *Aegilops: Ae.triuncialis (NCPGR) UUCC, Ae.columnaris IU 015916 UUMM, and amphidiploids: AD T.durum-Hordeum chilense* IU 024318*, Haynatricum (Dasypyrum villosum-T.dicoccum)* UA 0500018. Group 5:.Odesskaya 4; Diploid an d Tetraploid species of genus *Triticum: T.boeoticum* d1814/96 (*AA*), *T.petropavlovlovskii* UA 0300106 (*AABB*), Other wheat related species: *Ae. tauschii* 23/2001 (*DD*), *Dasypyrum villosum* NCPGR (*VV*). Group 6:*:* Odesskaya 16, Nikoniya, Remeslivna; species of genus *Aegilops*: *Ae. vavilovii* IU 015922 *DDMMSS*, *Ae. geniculata* 26/93 *UUMM*.

Those accessions of groups 3, 5 and 6 which had low ELISA A_{405nm} values in root tissue have been tested in respect to presence in their roots plasmodium or resting spores of *P. graminis* using cytological methods and were confirmed to be present in all of those accessions. It is known that ribotypes I of *P. graminis* isolates were obtained from barley and most ribotypes II isolates reported have been from wheat and other cereals [11], therefore we tested roots of some wheat wild relatives accessions and detected that *P. graminis* of ribotype I can affect not only barley roots but roots of *Dasypyrum villosum* (Fig 2).

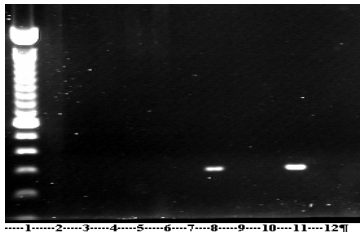

Fig 2. Detection *P.graminis* ribotype 1 in roots of some accessions: 1 – 100 bp marker, 2 – cv. Nikoniya, 3- cv Cadenza , 4 – *T.spelta v caeruleum*, 5 – *Ae. geniculata*, 6 – *Ae. umbellulata*, 7 – *Ae. taushii*, 8 - *Dasypyrum villosum*, 9 – *T. boeoticum*, 10 – *Ae. columnaris*, 11 – Positive control on ribotype 1, 12 – Positive control on ribotype II

The recently developed allele specific molecular marker S12M61F2 and S12M61R2 of gene *Sbm1* (resistance source to SBCMV in UK variety Cadenza Kanyuka unpublished) was applied for investigation of resistant varieties (table 1) in order to reveal presence of this gene (allele) in genome of those cultivars (Fig 3). It was observed that resistance of tested varieties was controlled by the same gene; however a few new alleles of this gene were identified which are different from that detected in Cadenza and their sequences should be studied in future.

The *Sbm1* locus was mapped to the gene-rich region on *5DL* (Fig. 4, A). There are three SSR markers (barc110, barc144 and wmc765) which are closely linked to this locus and exhibit polymorphism between resistant UK variety Cadenza (alleles 184bp, 239bp, 213bp respectively) and susceptible Avalon (alleles 175bp, 223bp, 199bp respectively). *Sbwmv*, the other gene inferred to be related to *Sbm1* (Fig. 4), which

controlled resistance to SBWMV in US line KS96WGRC40, is closely linked to the cfd 10 locus.

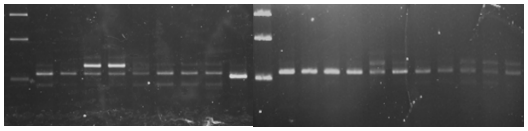

Fig.3 1, 11 – marker 200,300,400bp. (from the down to the top) 2-3 Remeslivna, 4-5 Ukrainka odesska, 6-7 Odesskaya 4,8-9 odesskaya 16, 10 Avalon; 12-14 Donskaya polukarlikovaya, 15-16 Kolumbiya, 17 – Povaga, 18-19 - Nikoniya, 20-22 Kuyalnyk

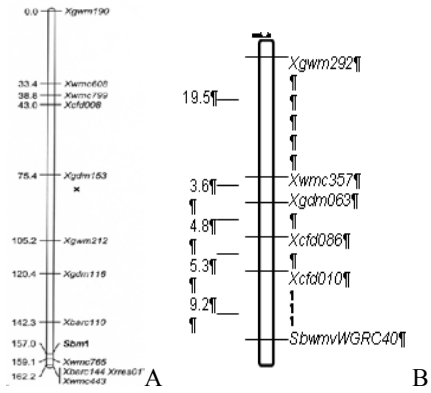

Fig. 4. Chromosome location of *Sbm1* (A), and *Sbwmv* (B)

We performed the screening of 30 resistant and susceptible varieties representing different regions by utilization of four SSR markers linked to *Sbm1* and *Sbwmv* genes in order (i) to study polymorphism between UK, and US and to compare with varieties tested in our work and (ii) the analysis of diversity among accessions. It was revealed that both UK and Ukrainian resistant and susceptible varieties have the same alleles of SSR markers for barc144 and wmc765, therefore polymorphism is low. Application of cfd 10 SSR marker did not show any differences between susceptible and resistant to SBCMV accessions, therefore not useful for MAS. For barc110 polymorphism was higher, at least 6 alleles were identified, however they did not separate precisely resistant and susceptible varieties. The highest frequency of varieties with resistant alleles is among cultivars of Southern region and the lowest appeared to be in the Northern. Mainly cultivars inherited their resistance from old landrace Krymka planted at the beginning of XX(20^{th} Century) and can be found in pedigree of a lot of cultivars all over the world [12].

Our results show that resistance to SBCMV accessions can be found among cultivated and wild wheat relatives with genomes (subgenomes) *AA*, *DD*, *UU*, *VV* and probably *MM*. Some tested Ukrainian varieties appeared to be resistant to SBCMV, especially the old cultivar Odesskaya 4 which has genetic system which prevents multiplication of *P. graminis* and virus in root tissues of wheat. Resistance to SBCMV in tested varieties is controlled by the same gene detected for UK variety Cadenza; however different alleles. *P. graminis* with ribotype I can affect not only barley roots, but as well the roots of *Dasypyrum villosum.* We have initiated work on association mapping of the loci carried by the genes *Sbm1* and *Sbwmv* in order to better understanding the genetic control of resistance to soil-borne viruses.

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