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GENETIC DIVERSITY OF THE NOVI SAD WHEAT CORE COLLECTION REVEALED BY MICROSATELLITES

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Abstract: In recent years, considerable emphasis has been placed on the development of microsatellites to be used for a variety of objectives. Parental genetic diversity is a crucial requisition to derive desirable and superior progenies from crossing and selection. In order to determine desirable genotypes for hybridization, 710 wheat genotypes from the Novi Sad Core Collection, originating from 38 countries, have been evaluated during the 1993-2000 period. During those seven growth seasons, 54 agronomical, morphological, physiological and other traits have been evaluated in field and controlled conditions. In each year, the field experiment comprised 3-7 replications, while for each field replication the plot size was 1.2 m². Based on the results from this evaluation, 96 genotypes with the highest phenotypic variation for 26 of the very important traits for wheat breeding programmes in Yugoslavia and the UK, were identified for screening with microsatellites. A set of 36 microsatellite markers was used, covering all three wheat genomes and all 42 chromosomes. For the 36 microsatellites, a total of 46 loci and 366 alleles were detected, with the average number of 7.96 alleles per locus. For 35 loci, null alleles were detected. The association of microsatellite data with phenotypic data, for 6 important traits for wheat breeding (stem height, earliness, resistance to leaf rust and powdery mildew, sedimentation value and protein content), as well as the potential for their implementation in marker assisted selection (MAS) in wheat breeding programmes for both Yugoslavia and UK are discussed.

Key Words: Diversity, Genetic Resources, Microsatellites, SSRs, Wheat

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INTRODUCTION

It is widely accepted that evaluation of genetic resources (GR) is an essential preliminary to utilisation and the more information that is available the more valuable will be the collection.

In the two last decades molecular genetic techniques on their own and in combination with other biotechnological approaches are beginning to have a significant impact on GR conservation and use. As described by [12], the use of biotechnologies such as DNA molecular markers (RFLP, RAPD, AFLP, SSRs etc.) can assist the curator in acquisition, maintenance, characterisation and evaluation activities. [4] stated that apart from genetic mapping applications, a growing use of molecular markers in general, and SSRs (microsatellites) in particular, is in the measurement of genetic diversity of breeding material and cultivars. As microsatellites show a much higher level of polymorphism and informativeness in hexaploid bread wheat than any other marker system [2, 10, 13] they have been chosen as a tool for the evaluation of the Novi Sad Wheat Core Collection in a joint project involving the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia and the John Innes Centre, Norwich, UK. Field trials have been performed in Yugoslavia, and microsatellites were screened in the UK. This investigation is a rare example of so called "Forward Genetics", which implies detailed and comprehensive evaluation of data obtained in field conditions, followed by screening with microsatellites.

MATERIALS AND METHODS

Field evaluation of plant material

The wheat collection of the Institute of Field and Vegetable Crops in Novi Sad, Yugoslavia comprises about 2500 genotypes from more than 50 countries. The most divergent part of the collection is the Core collection which consists of 710 wheat genotypes originating from 38 countries. In order to obtain relevant data for crossing and establishment of different experiments, those genotypes have been evaluated during the 1993-2000 period for 54 agronomical, morphological, physiological and other traits in field and controlled conditions (Table 1). In each year, the field experiment comprised 3-7 replications, and for each field replication the plot size was 1.2 m². The total amount of data collected is about 1,250,000 measurements.

Based on the results from this evaluation, 96 genotypes with the highest phenotypic variation for 26 of the important traits (Table 1) for wheat breeding programmes both in Yugoslavia and the UK were identified for screening with microsatellites. The main idea was to accumulate as much variation as possible among 96 genotypes (Tables 2 and 3). In this paper the results obtained for plant height, earliness, resistance to leaf rust and powdery mildew, sedimentation value and protein content are discussed.

Tab. 1. Traits evaluated in the wheat Core Collection in Novi Sad

Traits evaluated periodically		Traits evaluated each year	
1	Coleoptile colouration	1	Winterhardiness in the field
2	Coleoptile length*	2	Number of leaves*
3	Growth habit	3	Heading time*
4	Tiller colouration*	4	Flowering time*
5	Winterhardiness. in cold chambers*	5	Plant height*
6	Colouration of auricles	6	Ear length*
7	Colouration of leaves	7	Ear density
8	Leaf position in flowering	8	No. ears/m ² *
9	Frequency of plants with recur.flag leaves	9	Lodging resistance*
10	Flag leaf glaucosity	10	Resistance to leaf rust*
11	Tillering capacity*	11	Resistance to stem rust*
12	Anther colouration	12	Resistance to powdery mildew*
13	Ear glaucosity	13	Resistance to fusarium
14	Culm glaucosity	14	No. of spikelets/ear*
15	Straw-pith in cross section	15	No. of sterile spikelets/ear*
16	Ear colouration	16	Ear weight*
17	Awn length	17	Biomass weight/m ² *
18	Ear shape in profile	18	Harvest index*
19	Apical rachis seg. hair. of convex surface	19	No. of grain/spike*
20	Lower glume shoulder width	20	Kernel weight/spike*
21	Lower glume shoulder shape	21	Spike index*
22	Lower glume beak length	22	1000 kernel weight*
23	Lower glume beak shape	23	Grain yield*
24	Lower glume ext. to internal hairs	24	Flour yield
25	Lowest lemma beak shape	25	Sedimentation value*
26	Grain colouration	26	Protein content*
27	Grain colouration with phenol		
28	Seasonal type		

* - key traits for choosing 96 genotypes with the highest phenotypic variation

Microsatellites

A set of 36 microsatellite primer pairs was used (Table 4) covering all three wheat genomes and all 42 chromosomes. The primers were made available from the John Innes Centre, Norwich, UK.

Genomic DNA was extracted using the Retsch MM300 Mixer Mill and the Dneasy 96 Plant Kit (Qiagen), using the protocol recommended in the Dneasy 96 Plant Kit Handbook 08/99. DNA was quantified according to the SYBR Green method. The standard protocol of sample preparation for an ABI 377 Sequencer was applied, and the gels were run using the standard ABI 377

Sequencer procedure. The results from this were analysed using Genescan® software which calculates the molecular size of each allele.

RESULTS AND DISCUSSION

In general, a high level of phenotypic variation was accumulated for the traits analysed (Table 3), indicating that the basic prerequisite for molecular screening – genetic diversity, had been successfully achieved with the selected material.

Tab. 2. The 96 genotypes and their origin.

Genotype	Origin	Genotype	Origin	Genotype	Origin
Acciaio	ITA	L - 1	HUN	Purdue 5392	USA
Ai-bian	JPN	Lambriego Inia	CHL	Red Coat	USA
Al KanTzao	CHN	Lr 10	USA	Renesansa	YUG
Ana	CRO	Lr 12	USA	Rusalka	BGR
Avalon	GBR	Magnif 41	ARG	Siete Cerros	MEX
Bankuty 1205	HUN	Mex.17 bb	MEX	Saitama 27	JPN
BCD 1302/83	MDA	Mex.3	MEX	Sava	YUG
Benni multifloret	USA	Mexico120	AUS	Semillia Eligulata	USA
Bezostaya 1	RUS	Minister Dwarf	AUS	Slavija	YUG
Brigand	GBR	Mina	YUG	Sofija	YUG
Cajeme 71	MEX	Mironovska 808	UKR	Sonalika	IND
Capelle Desprez	FRA	Nizija	YUG	Suwwon 92	IND
Centurk	USA	Norin 10/Brev.14	USA	Szegedi 768	HUN
Ching-Chang 6	CHN	Norin 10	JPN	Tibet Dwarf	TIB
Cook	AUS	Nov. Crvena	YUG	Timson	AUS
Don.polupatuljasta	RUS	Nova Banatka	YUG	TJB 990-15	GBR
Durin	FRA	NS 22/92	YUG	Tom Thumb	TIB
F 4 4687	ROM	NS 33/90	YUG	Tr. compactum	LV*
Florida	USA	NS 46/90	YUG	Tr sphaerococcum	USA
Gala	ARG	NS 55-25	YUG	Triple Dirk B	AUS
HAYS 2	USA	NS 559	YUG	Triple DirkB (cont.)	AUS
Helios	USA	NS 602	YUG	Triple Dirk S	AUS
Highbury	GBR	NS 63-24	YUG	UC 65680	USA
Hira	IND	NS 66/92	YUG	UPI 301	IND
Holly E	USA	NS 74/95	YUG	Vel	USA
Hope	USA	NS 79/90	YUG	Vireo "S"	MEX
Inia 66	MEX	Peking 11	CHN	WWMCB 2	USA
INTRO 615	USA	Phoenix	USA	ZG 1011	CRO
Ivanka	YUG	PKB Krupna	YUG	ZG 987/3	CRO
Kite	AUS	Pobeda	YUG	ZG K 238/82	CRO
L 1/91	YUG	Purd./Loras	USA	ZG K 3/82	CRO
L 1A/91	YUG	Purdue 39120	USA	ZG K T 159/82	CRO

* - Local variety

The results of PCR amplification of a number of microsatellite loci in 96 genotypes using 36 wheat microsatellite primer pairs are summarised in Table 4. A total number of 46 loci and 366 alleles were detected, with an average number

of 7.96 alleles per locus, which indicates that the appropriate, viz. very polymorphic, primers were used. For 35 loci, null alleles were detected. The maximum number of alleles was detected for primer pairs Gwm46 (25 alleles) followed by Gwm160, Gwm165, Gwm192 and Gwm484 (21 alleles), which indicates that wide genetic variation had been accumulated in the material.

Tab. 3. Range of phenotypic variation for the traits examined (7 year average).

Trait	Interval of variation
Stem height (cm)	18.7 – 123.1
Earliness (days from 1 st Jan. to flowering)	125 – 146
Leaf rust (% of total leaf area)	1-88
Powdery mildew (% of total leaf area)	1-53
Sedimentation value (Zeleny)	20.6 – 52.8
Protein content (%); (N x 5.7)	10.3 – 15.9

For four of the six of the traits examined here no significant relationship between phenotypic data and microsatellite scores was found. In contrast, for the two traits stem height and earliness interesting results were obtained.

Gwm161 identified three genotypes (Table 5) with the same allele. All three (Brigand, Minister Dwarf and Tom Thumb) are very late in Yugoslavian growing conditions, with 142-146 days from 1st January to flowering (Yugoslav standard variety Pobeda flowering on average after 133 days). At the same time, all three have very short stems (37-64 cm), belonging to so called “dwarf” wheats. The variety Tom Thumb is a well known source of the height reducing gene *Rht-B1c* (formerly *Rht3*), a class of gibberellin-insensitivity genes which have been used frequently in humid and cooler climates of Western Europe (UK, Germany etc.) for reducing stem height and at the same time increasing spike fertility in wheat. Studies with isogenic lines showed that the main pleiotropic effects of *Rht-B1c* are prolonged flowering and stem height reduction of about 50% [5, 7, 9]. In addition [8], reported that the variety Minister Dwarf possesses the same *Rht-B1c* as Tom Thumb, whilst due to its dwarf stature and late flowering, it is likely that Brigand also possesses an *Rht* gene. The *Rht* genes *Rht-B1a* (widely used in Northern European wheat breeding) and *Rht-B1c* are on chromosome 4BS, though our results indicated a interesting phenotypic value/marker score relationship with Gwm161 which is located on 3DS. In addition, these three genotypes were grouped in the same cluster using primer Gwm337 which is located on chromosome 1DS (Table 5). This raises the question – is it just coincidence or as well as *Rht-B1c* are there also DNA sequences on 3DS and 1DS strongly effecting short stature and late flowering in these genotypes ?

Using Gwm165, another interesting group was identified comprising three very early and dwarf wheat genotypes (Table 5). The genotype Ai-bian is known to

Tab. 4. Primer pairs, chromosome location, number of loci, number of alleles/locus, determined link between phenotypic value/marker score (PV-MS link), and number of genotypes in certain clusters.

Primer pair	Chromosome location	Number of loci	Number of alleles/locus	PV-MS link	No. of genotypes
Gwm3	3DL	1	6	-	-
Gwm11	1BS	1	9	-	-
Gwm46	7BS+?	3	5+17+3	-	-
Gwm99	1AL	1	9	-	-
Gwm130	7AS+?	2	5+6	-	-
Gwm155	3AL	1	9	-	-
Gwm160	4AL+?	2	8+13	-	-
Gwm161	3DS	1	7	+	3
Gwm165	4AS, 4BL, 4DL	3	5+5+11	+	3+3+3
Gwm186	5AL	1	11	-	-
Gwm190	5DS	1	8	-	-
Gwm192	4AL, 4BL, 4DL	3	5+5+11	-	-
Gwm257	2BS	1	5	+	9
Gwm292	5DL	1	6	-	-
Gwm295	7DS	1	10	-	-
Gwm325	6DS	1	10	-	-
Gwm337	1DS	1	15	+	3
Gwm357	1AL	1	5	-	-
Gwm369	3AS	1	18	-	-
Gwm389	3BS	1	13	-	-
Gwm458	1DL	1	4	-	-
Gwm484	2DS	1	21	-	-
Gwm540	5BS	1	9	-	-
Gwm626	6BL	1	4	-	-
Psp3009	6BS	1	4	-	-
Psp3030	7BL	1	5	-	-
Psp3050	7AS	1	8	-	-
Psp3071	6AL	1	9	-	-
Psp3078	3BL, 4BS	1	6	-	-
Psp3088	2AL	1	6	-	-
Psp3103	4DS+?	2	5+11	+	8+0
Psp3123	7DL	1	3	-	-
Psp3153	2AS+?	2	4+7	-	-
Psp3200	6DS	1	8	-	-
Wmc56	3BL	1	6	-	-
Wmc73	5BL	1	6	-	-
Total		46	366	5	32

be a donor of the *Rht-D1c* gene (formerly *Rht-10*), which reduces stem height by 60-75% [5, 6].

This group also contained the genotypes Ana and Timson which, because of their dwarf stature and earliness, may also possess the same *Rht-D1c* gene together with some additional stem height promoting genes. However, our results showed that this group had, with one exception for Timson, null allele scores for all homoeologous chromosomes on group 4 with Gwm165 (4AS, 4BL and 4DL). [1] reported that this primer identified polymorphic and dwarfing alleles on chromosomes 4B and 4D. Null SSR alleles are relatively common in wheat [3, 4, 10] and can arise from point mutation(s) in one or both of the priming sites, or even by deletions. It would be useful to examine these genotypes with other microsatellites known to map near the *Rht* loci on the group 4 chromosomes to confirm the associations we found with height.

Tab. 5. Primers, genotypes, characters and alleles

Primer	Genotype/origin		Character		Allele
			Earliness	Stem height	
Gwm161	Brigand	GBR	146	64	B
	Min. Dwarf	AUS	142	48	B
	Tom Thumb	TIB	146	37	B
Gwm165	-1 Ai-bian	JPN	131	27	null
	-2 Ana	CRO	132	66	null
	-3 Timson	AUS	131	64	null
Gwm257	Ching-Chang 6	CHI	128	85	D
	Hira	IND	127	54	D
	Kite	AUS	130	101	D
	NS 33/90	YUG	130	73	D
	NS 602	YUG	131	55	D
	Timson	AUS	131	64	D
	Triple dirk S	AUS	131	73	D
	UC 65680	USA	128	86	D
WWMCB 2	USA	127	64	D	
Gwm337	Brigand	GBR	146	64	A
	Min. Dwarf	AUS	142	48	A
	Tom Thumb	TIB	146	37	A
Psp3103	NS 22/92	YUG	134	86	A
	NS 33/90	YUG	130	73	A
	NS 55-25	YUG	130	73	A
	-1 NS 79/90	YUG	131	78	A
	-2 Pobeda	YUG	133	79	A
	Renesansa	YUG	132	74	A
	Sava	YUG	134	77	A
	Slavija	YUG	134	74	A

Finally, two groups detected by primers Gwm257 and Psp3103 (Table 5) are very early and with short to medium stature. The first group (Gwm257) comprised genotypes of different origin, all slightly earlier than the Yugoslav standard variety Pobeda, with stem height varying from 55 to 101 cm. This group could be useful for further wheat breeding in Yugoslavia, as one of the targets in wheat breeding is the creation of varieties at least 3-5 days earlier than present ones. A very interesting group of genotypes was identified using Psp3103, which comprised Yugoslav varieties with excellent yield potential. Pobeda and Renesansa are presently the best Yugoslav varieties concerning yield ability and stability. Variety Sava was the best Yugoslav variety about three decades ago, and even gave the world record yield at one stage with 10.6 t/ha. The remaining varieties in this group are also with excellent yielding potential and, in recent years, they have been frequently used as parents for crosses targeting increased yield performance. This result deserves further attention, in order to detect any connection between the allele scores of these genotypes and their excellent yielding performance.

In general, it may be concluded that such allelic and phenotypic data could benefit wheat breeding both in Yugoslavia and the UK, though the degree of association between alleles and phenotypes was lower than expected. A likely reason for this is the difficulty of carrying out statistically meaningful tests on allele associations when alleles for a given microsatellite are not suitably distributed amongst genotypes. In many cases the majority of varieties had only one or two alleles with rarely a second or third. Conversely, those markers that had very high numbers of alleles, such as Gwm46 resulted in many alleles having only one or two genotypes in that class. This limited the usefulness of the microsatellites that were tested.

Another reason for the lack of major associations reported here could be the relatively small number of microsatellite loci tested (only 46) for a genome over 3000 cM in length.

Thus, for microsatellites to be useful for association genetics in hexaploid wheat, many further primer combinations need to be examined with a wide diversity of wheats, such as those tested here, to identify not only those primer combinations that give simple banding patterns and that can be multiplexed for automation with, for example the ABI 377 sequencer used here, but also to identify those that generate a small number of alleles (ideally 4-8) distributed fairly evenly amongst genotypes.

Despite the fact that significant relationships have been found so far for only two traits, we are continuing our investigation on a further 20 traits, together with many additional traits - early vigour, polyethylene glycol (PEG) growth test, performance of the genotypes in stress conditions (drought and heat stress, salt tolerance), additional quality parameters (wet and dry gluten, crumb number, loaf volume, ...), grain filling duration and rate etc. We expect that such an approach will extend our knowledge on the association of allele scores for certain primer combinations with phenotypic performance of the genotypes.

Also, this kind of research should greatly improve our general knowledge of our GR and their genetic relatedness, as studies on variation and diversity in wheat using molecular markers have been relatively few so far [11].

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