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Full Length Research Paper

Assessing genetic diversity in a set of wheat (*Triticum aestivum*) genotypes using microsatellite markers to improve the yellow rust resistant breeding programs

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Evaluation of genetic diversity in plant germplasms is the first and fundamental step in plant breeding programs. For the fact that correct usage of it has lots of benefits, it decreases plants' damage associated with pests and diseases. In this study, genetic diversity and relationship of 70 genotypes of bread wheat were evaluated by using 60 microsatellite markers. 40 polymorphic markers were selected for clustering and evaluating of genotypes. In total, 309 polymorphic alleles were amplified with an average of 9.26 allele per microsatellite locus. Gene diversity according to Nei for the 42 microsatellite loci varied from 0.4 to 0.91 with an average of 0.74. Polymorphism information content (PIC) value ranged from 0.365 for the *barc* 87 and the second locus of barc 165 to 0.902 for the *Xgwm213*, with an average of 0.688. Clustering analyses based on Neibour-joinig algorithms and distance coefficient was used and all of the dendrograms indicated that most relative genotypes based on pedigree information, were grouped in the same cluster. In general, they could separate Bolani and MV17 genotypes that are susceptible and resistant parents in most rust breeding projects in the country. The dendrogram result of Rogers distance showed high concordance with available pedigree information of genotypes. Principle components analysis (PCA) also indicates similar results. With stepwise multivariate regression analysis according to the reactions of genotypes to yellow rust and their molecular result, 19 and 17 informative markers were detected for final infection coefficient and area under disease progress curve, respectively.

Key words: Bread wheat (*Triticum aestivum*), microsatellite markers, genetic diversity, yellow rust.

INTRODUCTION

Common wheat (*Triticum aestivum*) (2n = 6x = 42) is the most diverse and important specie of the plant, which produces large edible grain and provides about one-fourth of humans' food calories and a large part of their nutrient requirements. Wheat (*Triticum* spp.) is a worldwide cultivated and domesticated grass that is attacked by many pathogens such as bacteria, fungi, etc (Ijaz and khan, 2009; Singh et al., 2000). One of the substantial diseases is yellow rust or stripe rust that is caused by *Puccinia striiformis Tritici* which adapts to cool and humid weather (Eversmeyer and Kramer, 2000; Ma and Singh, 1996). Stripe rust is much more important than leaf rust or stem rust, because, in severe infection with the pathogen, the plant height, the number of seeds accompanied by their quality and weight, are decreased (Ma and Singh, 1996); so, current disease situations, plant pathologists, breeders, farmers, and governmental organizations have given much attention to the research on, and control of it (Kang et al., 2010). With attention to the role of yellow rust disease in reducing wheat yield, finding appropriate parents and detecting the loci of resistance genes and transferring them to produce resistant varieties are the best strategies to control the disease. Following this, in finding appropriate parents and

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achieving the number and type of resistance genes in plant population, we must be informed on the germplasm and gene diversity (Rodrigues, 2003; Zhang et al., 2005). As the knowledge of genetic diversity of germplasms is critical for their utilization in the improvement of crops, it is necessary to investigate the genetic diversity in wheat germplasm, to broaden genetic variation in wheat breeding. Today, molecular markers are the best tools used to determine the level of genetic diversity among plant and animal genotypes. They can also provide detailed characterization of genetic resources (Manifesto et al., 2001; Zhang et al., 2002). One kind of molecular markers is microsatellites which are simple sequence repeats (SSR) of 1 to 6 nucleotides. They are abundant, polymorphic (even among closely related genotypes) and distributed over the genome. These features, coupled with their ease of detection, their potential for automation and their inheritance in a co-dominant manner, have made them useful markers when compared with other types of molecular markers. In bread wheat, micro-satellites have been successfully applied for detection of genetic diversity (Huang et al., 2002; Wei et al., 2005; Zarkti et al., 2010), for genome mapping (Röder et al., 1998; Varshney et al., 2006) and for marker assisted selection of agronomical important traits (Ate-Sönmezo et al., 2010), because, they show a much higher level of polymorphism in wheat genomic research than other marker systems which have commonly used (Plaschke et al., 1995; Zhang et al., 2006; Mangini et al., 2010). They can also be used to characterize genetic diversity in wild relatives (Hammer et al., 2000) and in a seed bank collection of improved wheat germplasm (Börner et al., 2000; Huang et al., 2002). The objectives of this study were to (i) assess the level of genetic diversity and relationships among a representative sample of bread wheat genotypes, (ii) select appropriate parents that differ for the level of genetic material and the response of the yellow rust and (iii) determine the informative markers involved in resistance of yellow rust.

MATERIALS AND METHODS

Plant materials evaluated in this study include 70 wheat genotypes that were used in breeding programs, for resistance to yellow rust (Table 1). Assessment of response to yellow rust on genotypes was performed in Institution of seed and seedlings in Karaj. All of the genotypes were appraised with the spores of fungus 134E134 A+ in a completely randomized blocks design with three replications. In the end, two traits, area under curve disease progression (AUDPC), and coefficient infection were measured. AUDPC was calculated from the formula:

$$\mathrm{AUDPC}=\sum_{i=1}^{k}\frac{(X_{i+1}+X_i)}{2}(t_{i+1}-t_i)$$

where k is the number recorded, X_i is the percent (intensity) of disease, and t is the time scale (day) (Shaner and Fine, 1977) and infection coefficient measured from multiple infection percentage (0 to 100) with the coefficient of the host reaction, which was calculated based on improved Koub method (Peterson et al., 1948).

Genomic DNA of 70 wheat genotypes was extracted from fresh leaves, according to the method described by Saghaii-Maroof et al. (1984). Polymerase chain reaction was carried out in a 10 µ reaction volume. The amplification consisted of initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55 to 68°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 7 min. The amplification products were separated on 6% non-denaturing polyacrylamide gels and visualized by silver staining (CIMMYT, 2005). Electrophoresis was performed at 150 V constant power in 1 × TBE buffer as a running buffer, and stopped, depending on the expected product size of each primer set. Simple sequence repeat alleles per locus, polymorphism information content (PIC) and gene diversity (D) were calculated as genetic parameters of polymorphism. For all the parameters, the overall estimates are calculated as the averages across all the loci, whereas standard deviations were estimated by 1000 non-parametric bootstrapping samples across different loci using PowerMarker version 3.20 (Liu and Muse, 2005). Polymorphism information content (PIC) values were calculated for each microsatellite locus using the formula:

$$PIC=1-\sum p_i^2-\sum_{i=1}^{n-1}\sum_{j=i+1}^{n}2\,p_i^2\,p_j^2$$

where pi and pj are the frequencies of the ith and jth alleles of a given marker, respectively.

$$D_i=2n(1-\sum_{i=1}^{k}p_i^2)/(2n-1)$$

where pi is the frequency of the ith allele for each microsatellite locus in the sample of n genotypes studied (Nei, 1978). To investigate a possible population structure in the analyzed entries, genetic relationships were assessed using distance coefficients based on presence/absence of alleles as well as allele frequencies. Entries were grouped by cluster analysis according to their relationship using un weighted pair group method with arithmetic average (UPGMA) and neighbour joining (NJ) algorithms in MEGA3 (Kumar et al., 2004) and PowerMarker version 3.20 (Liu and Muse 2005) software. Support of clusters was evaluated by bootstrap analysis. Principle components analysis (PCo), with Jaccard coefficient, was accomplished as an alternative method for confirming the clustering method by using NTSYS V2.0 (statistical software), and eventually, a two-dimensional diagram of genotypes' distribution was drawn based on the first two components. To determine the genomic regions associated with resistance of yellow rust, the stepwise multivariate regression analysis was used, in which traits' value was considered as the dependent variable and genotypes' score for the markers as independent variables (regression analysis was done with SPSS V.14 software).

RESULTS

To assess the reaction of genotypes to yellow rust, they were inoculated with the spores of fungus 134E134 A + (the factor of disease). Before ANOVA, Log-normal distribution was used for standardizing of data, then data were analyzed based on completely randomized blocks design and the results displayed that significant difference there was among the genotypes in terms of response to disease (yellow rust). The comparison of

Table 1. List, pedigree and sources of bread wheat genotypes used in the present study.

Genotype	Pedigree	Genotype	Pedigree
Chinese 166	Chinese 166	N-75-16	shanghai7//Hahn's' *2/pvl 's'
Lee	Lee	Shiraz	Gv/D630//Ald"s"/3/Azd
Vilmorin 23	Vilmorin 23	Omid	Local variety
Hybrid 46	Hybrid 46	Roshan	Local variety
Jupateco ' 73R'	Jupateco ' 73R'	Ghods	Rsn/5/Wt/4Nor10/K54*2//Fn/3/Ptr/6/Omid//KalBb
Alamout	KVZ/Ti71/3/Maya"s"//Bb/Inia/4/Kj2/5/Anza/3/Pi/Ndr//Hys	Chanab	Chanab
Zarrin	PK15841	Bolani1	Bolani
Mv-17	Mv-17	M-78-16	MP151//Arvand/3/Brochis/Arvand
Gascogne	Gascogne	Mkh3	KAUZ//KAUZ/PVN
Pishtaz	Alvand//Aldan/Ias58	Mkh4	SERI/KAUZ
M-81-13	Hahn"S"//Mjl/Lira//2*Rsh	Mkh5	LIRA/SHA5
M-79-7	Bloyka ICW84-0008-013AP-300L-3AP-300L-0AP	Mkh6	BOW/FKG15
M-79-6	Bow"s"/Vee"s"//1-60-3	Mkh7	BOW/SERI
M-81-4	T.Aest/5/Ti/4/La/3/Fr/Kad//Gb/6/;F13471/Crow"	Bolani2	Bolani
M-82-6	Karawan 1//Sun640/M2512	CIMMYT1	WBLL1/FRET//PASTOR
M-82-12	Ald"s"/Snb"s"//Tjn	CIMMYT3	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
M-82-14	Ww33G/Vee"S"//Mrn/3/Attila/Tjn	CIMMYT5	FRET2*2/KUKUN
M-82-18	KASYON/GENARO.81//TEVEE-1 ICW92-0281-1AP-OL-2AP-…	CIMMYT7	FRET2/KURUKU//FRET2
WS-82-9	Ww33G/Vee"S"//Mrn/4/HD2172/Bloudan//Azd/3/San/Ald"s"//Avd	CIMMYT9	FRET2/KUKUN//FRET2
WS-82-13	Ww33G/Vee"S"//Mrn/3/Attila/Tjn	CIMMYT11	FRET2/KUKUN//FRET2
Crossing Block84	opata*2/wulp	CIMMYT12	FRET2/TUKURU//FRET2
Crossing Block87	catbird	CIMMYT13	FRET2/TUKURU//FRET2
Crossing Block86	yaco/parus//parus	CIMMYT16	WBLL1*2/KUKUN
Crossing Block97	Milan/sha7	CIMMYT17	WBLL1*2/KUKUN
Flat	Kvz/Buho"s"//Kal/Bb=Seri82	CIMMYT18	WBLL1*2/TUKURU
Hirmand	Byt/4/Jar//Cfn/Sr70/3/Jup"s"	CIMMYT19	WBLL1/4/HD2281/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KAMB1
S-78-11	Bow"s"/Cm34798/3/snb	CIMMYT21	WBLL1*2/KURUKU
Bezostaya	Bezostaya	CIMMYT22	WBLL1*2/KURUKU
Alvand	1-27-6275/CF1770	PRWYT-DT-2	SERI.1B//KAUZ/HEVO/3/AMAD
Niknejad	F13471/Crow"s"	PRWYT-DT-3	SERI.1B//KAUZ/HEVO/3/AMAD
Darab	Maya"s"	PRWYT-DT-7	SERI.1B*2/3/KAUZ*2/BOW//KAUZ
Tajan	Bow"s"/Nkt"s"(CM67428-GM-LR-5M-3R-LB-Y)	PRWYT-DT-14	HUW234+LR34/PRINIA
Shiroudi	Attila,(CM85836-4Y-OM-OY-8M-OY-OPZ)	PRWYT-DT-15	ATTILA*2/PASTOR
Chamran	Attila,(CM85836-50Y-OM-OY-3M-OY)	PRWYT-DT-17	ATTILLA*2/3/KAUZ*2/TRAP//KAUZ
Kavir	Stm/3Kal//V534/Jit716	PRWYT-DT-20	ATTILA*2/STAR

Table 2. Variance Analysis of genotypes' AUPDC.

Variant	Degree of freedom	Mean of square	F
Block	2	0.018	2.56^{ns}
Genotypes	69	2.61	378.58**
Error	138	0.0069	

^{ns} Non-significant; **, Significant in probability level of 0.01.

Table 3. Description of the SSR loci used in the present study including their number of alleles, gene diversity, PIC and chromosome location of each SSR.

Marker	Allele number	Gene diversity	PIC	Chromosome	Marker	Allele number	Gene diversity	PIC	Chromosome
Xgwm213	15	0.909	0.902	5B	Xgwm6	14	0.771	0.742	4B
Xgwm124	10	0.799	0.775	1B	Xgwm45	11	0.86	0.845	3A
Xgwm160	12	0.884	0.873	5A	Xgwm332	10	0.834	0.814	7A
Xpsp2999	11	0.694	0.679	1A	Xgwm118	4	0.527	0.47	4A- 5B
Xgwm161	9	0.686	0.656	3D	Xgwm118	5	0.668	0.611	4A- 5B
Xgwm299	7	0.669	0.65	3B	Barc87	3	0.401	0.365	3B-7D
Xgxm570	14	0.818	0.799	6A	Barc206	5	0.749	0.704	4A- 2B- 7D
Xgwm325	15	0.898	0.889	6D	Barc303	9	0.678	0.653	5A
Xgwm327	13	0.872	0.86	5A	Barc3	12	0.778	0.752	6A
Xgwm212	8	0.794	0.766	5D	Barc255	8	0.699	0.65	7B
Xgwm357	7	0.559	0.536	1A	Barc165	8	0.669	0.614	5A- 6A
Xgwm190	9	0.833	0.813	5D	Barc165	3	0.409	0.365	5A- 6A
Xgwm129	8	0.784	0.752	2B- 5A	Barc141	13	0.882	0.871	5A
Xgwm499	7	0.754	0.714	5B	Xgwm169	11	0.839	0.82	6A
Xgwm58	9	0.802	0.78	6B	Xgwm291	9	0.803	0.78	5A
Xgwm312	8	0.595	0.574	2A	CFA2155	6	0.745	0.71	5A
Xgwm408	11	0.84	0.822	5B	CFA2141	10	0.859	0.844	5A- 5D
Xgwm46	18	0.891	0.883	7B	CFA2028	5	0.691	0.644	7A
Xgwm30	8	0.818	0.797	2D- 3A	CFA2234	5	0.72	0.67	3A
Xgwm448	5	0.683	0.627	2A	Barc37	8	0.841	0.821	6A
Xgwm639	17	0.853	0.844	5A- 5B- 5D	Barc54	10	0.868	0.854	3A

mean genotypes' AUPDC is shown in Table 2. From 60 SSR primer pairs, forty screened polymorphic SSR primer pairs were used to assess the extent of genetic diversity among 70 wheat genotypes. A total of 390 alleles with an average of 9.26 allele per microsatellite locus were identified in each genotype. The second locus of Barc165 and Barc87 with 3 alleles and Xgwm46 with 18 alleles revealed the lowest and highest number, respectively. The average of polymorphic information content (PIC) was 0.688 with a range of 0.563 to 0.902. The second locus of Barc165 and gwm213 had the lowest and highest PIC value respectively. Gene diversity according to 42 microsatellite loci varied from 0.4 to 0.91 with an average of 0.74; the lowest value belonged to barc87 and the highest related to the gwm213. If all of the genotypes are homozygote, the PIC value and gene diversity will be the same, accordingly, we can conclude that, all of the samples in this study were not homozygote (Table 3).

Clustering analyses based on Neibour-joinig algorithms and distance coefficient was used and all of the dendrograms indicated that most relative genotypes, based on pedigree information, were grouped in the same cluster. In all the resulting dendrograms, sister genotypes like Bolani1 and bolani2, M-82-14 and WS-82-13, CIMMYT9 and CIMMYT11, and Shiroodi and Chamran, were grouped together. Also, the genotypes from CIMMYT (including CIMMYT and PRWYT-DT genotypes) were grouped closely, in the same cluster. Mkh and all of the Crossing Block genotypes except Crossing Block84 were grouped in the same branch too. However, the dendrogram resulting from Rogers distance showed the maximum concordance with available pedigree information for genotypes. In this dendrogram,

we had six clusters or six groups, where the first one consists of seven genotypes (Mv-17, Gascogne, Flat, Hirmand, Crossing Blocks 97, 89 and 87, and all of them had external parents in their pedigrees; except Flat and Hirmand genotypes, the rest were resistant to the rust. In the second group, there were four commercial genotypes (M-79-6, Alvand, Niknejad and darab) in which all, except M-79-6 genotype, were internal commercial genotypes and considered susceptible to rust. Many genotypes including, Shiroodi, Tajan, Chamran, Kavir, Omid, Ghods, Shiraz, Chnab, Bolani1, and Bolani2 with genotypes, Mkh and N-75-16, M-78-16, S-78-11 were in the third group. Most of them were susceptible to rust. All of them, except the final three genotypes (N-75-16, M-78-16, and S-78-11) which originated from Iran, were grouped with them for the Iranian genotypes in their pedigree. Eight external genotypes (Vilmorin23, Hybrid46, Chinese166, Lee and Jupateco73R, M-81-13, M-79-7 and M-81-4), and 3 Iranian genotypes (Zarrin, Alamut and Pishtaz) were separated in the fourth group. All 8 genotypes, except the three final genotypes derived from Iran, were external because of the external parents they had in Iranian genotypes pedigree, as such, the three final genotypes were assigned to the fourth cluster. All of the genotypes in the group in question, except genotypes Lee, Jupateco73R, Zarrin, and Alamout were resistant to rust. CIMMYT genotypes and total PRWYT-DT, which were derived from CYMMIT, were attributed to group 5. The majority of genotypes designated with CIMMYT have common parents in their pedigree, such as WBLL1, FRET2 and KURUKU. The PRWYT-DT lines, except PRWYT-DT-14, are sister lines having Seri or Attila as common parents in their pedigrees. Although PRWYT-DT-14 does not have these two genotypes in its pedigree, its female parent, HUW234, originated from Bluebird of which Seri and Attila are present in its pedigree. In terms of response to yellow rust, all of them were resistant. Group 6, consists of 8 genotypes (WS-82-9, Crossing Block 84, WS-82-13, M-82-14, M-82-6, M-82-12 and M-82-18) were external genotypes, except Bezustaya and Crossing Block84 that had a common parent in their family, and originated from temperate area. According to the conformity of response to yellow rust, all of the genotypes in this group except Bezustaya and Crossing Block 84 had a negative response and were resistant to yellow rust. Although none of the methods of clustering could not separate resistant and susceptible genotypes from each other definitively, in all clustering method, Bolani and MV17 genotypes are susceptible and resistant parents (respectively) in most rust breeding projects in the country and could be separated in different groups with efficient distance (Figure 1).

Figure 2 shows the average deviation of AUDPC for each group (cluster) from total mean of AUDPC. It can therefore be seen that the third group which includes the majority of Iranian genotypes is the most sensitive and the first group with resistant genotypes MV17 and Gascogne were the most resistant groups. According to this, we can conclude that genotypes are different for their resistance and their susceptibility, because cluster (group) 5, having all resistant genotypes, must be the most resistant group of all. It is therefore one of the reasons why the genotypes could not be separated for their different reactions to the disease.

Principle component analysis (PCA) revealed that the first two components of the PCA could explain almost 11% of the total variation. Principle component analysis as an alternative method of genotype grouping could confirm the clustering genotypes based on Rogers coefficient, because two-dimensional dendrogram based on two main components displayed similar result to the result of clustering separation, approximately. As PCA two-dimensional dendrogram revealed, the PRW and CYMMIT genotypes were distributed close each other; following this, Mkhs, Crossing Blocks, external genotypes (except the ones with Iranian parents), and Iranian genotypes (except Pishtaz, Alamout, and zarrin with external parents in their pedigree) could be dispersed separately. Also, in the present way, Bolani and Mv-17 as sensitive and resistant parents could be segregated efficiently.

Separation of genotypes according to their pedigree indicated that SSR markers are suitable for genetic diversity assessment in bread wheat (Figure 3).

To investigate the chromosomal locations involved in resistance of yellow rust, the stepwise multivariate regression was used with regard to the present or absent alleles of microsatellites used as the independent variable, and AUDPC as the dependent variable. To avoid statistical errors, the analysis of chromosomal markers for each group was performed separately. Analysis of genotype reaction to yellow rust and their molecular profiles association (19 and 17 informative markers), distributed on most chromosomes of wheat, were detected for final infection coefficient and area under disease progress curve, respectively. Based on these results, chromosomal locations which were located on chromosome 3A explain the greatest characteristic changes for resistance of yellow rust in this group of genotypes; these regions were located adjacent to 4 markers Barc54, Xgwm30, Barc45, and CFA2234. This result indicated that chromosome 3A has a main role in resistance of yellow rust. Table 4 shows that the most of informative markers are on chromosome A.

DISCUSSION

SSR or microsatellite markers are often chosen as the preferred markers for assessing genetic diversity among various molecular markers currently available because of their multi-allelic nature, level of polymorphism, relative abundance, and extensive genome coverage (Gupta and Varshney, 2000; Fu et al., 2005). According to the results,

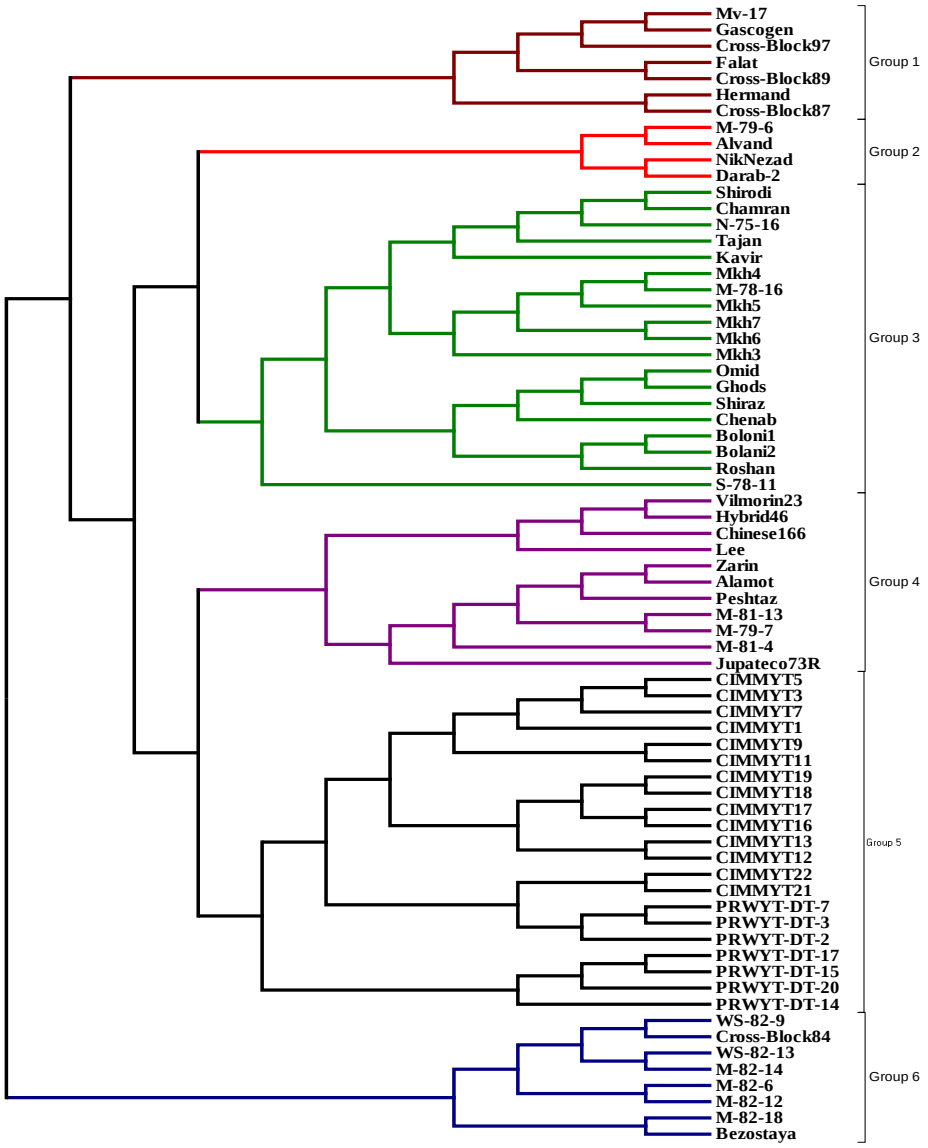

Figure 1. A dendrogarm of 70 wheat lines and genotypes based on microsatellite data using number of differences coefficient and neighbour joining algorithm.

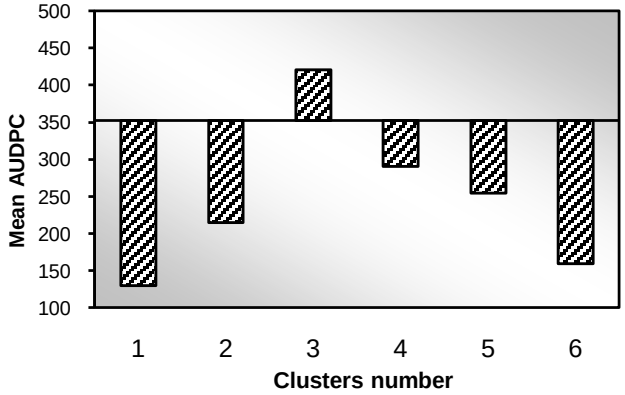

Figure 2. The average deviation of AUDPC for each group from total mean of AUDPC.

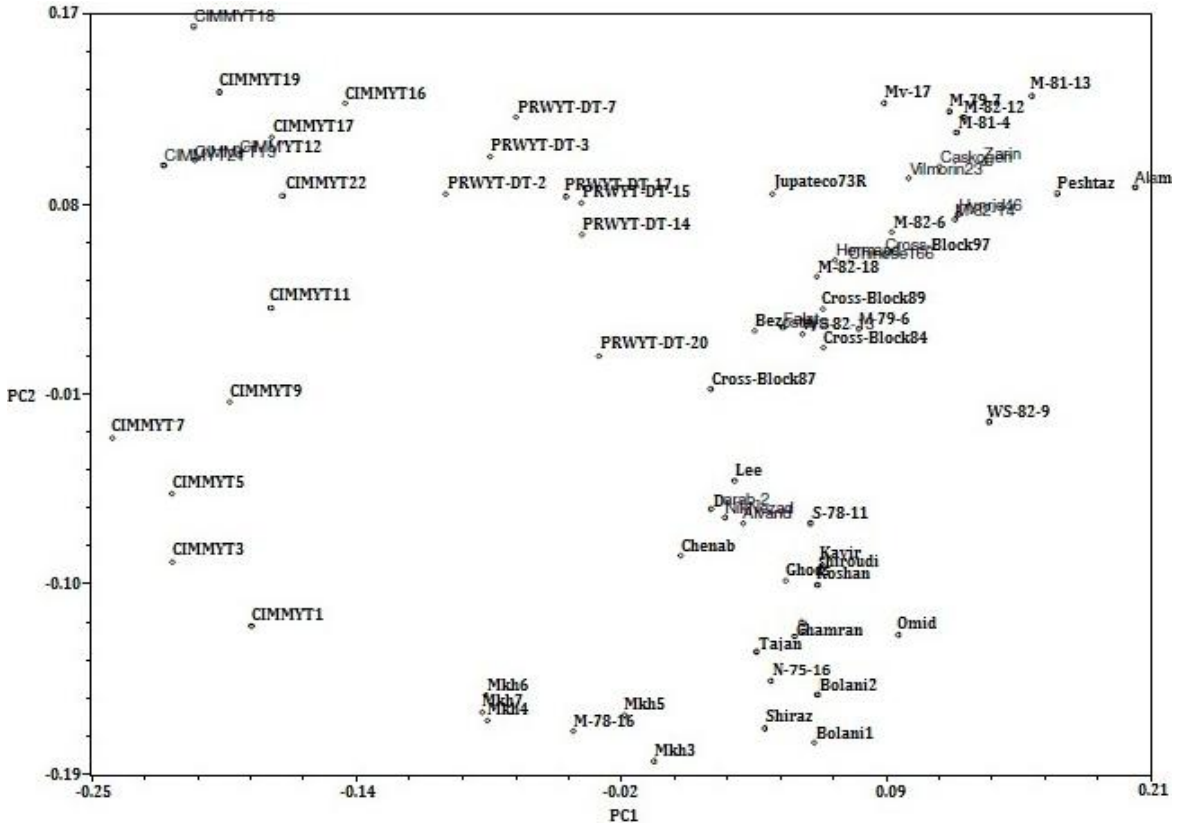

Figure 3.Two-dimensional diagram of principle component analysis (PCA) based on 42 SSR loci (40 markers)

Table 4. Locus, markers, and coefficient of determination and degree of freedom of informative markers.

	Locus	Marker	R^2	df
Final coefficient of infection	1A	Psp2999(d)*	0.63	0.02
	1B	Gwm124(a)	0.045	0.045
	1D	Barc255(c)	0.063	0.02
	3A	Barc54(c,e) Gwm30(a,f) Barc45(e)	0.335	0.000
	3B	CFA2234(b) Gwm299(a,d)	0.127	0.005
	3D	Gwm161(h)	0.1	0.004
	4A	Barc206(d,b)	0.252	0.000
	4B	Gwm6(h)	0.064	0.02
	5B	Gwm213(g)	0.129	0.005
	5D	Gwm499(c)	0.07	0.016
	6A	Barc3(a,b,d,h) Gwm169(a,j)	0.323	0.000
	6B	Gwm58(b)	0.06	0.025
	6D	Gwm325(m)	0.068	0.017

R^2= Coefficient of determination; df= degree of freedom.

the microsatellite primers generated 309 polymorphic alleles with an average of 9.26 allele per locus. The mean PIC (0.688), mean gene diversity (0.74) and the average allele per locus, indicate a high level of diversity compared

with other studies reported by SSR on bread wheat. Ribeiro-Carvaiho et al. (2004) and Christiansen et al. (2002) in the evaluation of 59 and 75 genotypes of European breed wheat, reported an average of 4.77 and 3.6 alleles per locus, respectively. Wei et al. (2005), Huang et al. (2002) and Manifesto et al. (2001) assessed genetic diversity of wheat genotypes originating from different countries with SSR markers and reported a variation in allele number per locus and gene diversity almost similar to that revealed in this study. These results suggest that a high level of polymorphism can be created in wheat by using microsatellite markers. So, high polymorphism of microsatellites made them useful and popular for evaluation of genetic diversity of bread wheat (*T. aestivum*) (Fahima et al., 2002; Singh et al., 2010) and other different applications in wheat breeding (Devos et al., 1995; Roder et al., 1995; Bryan et al., 1997; Roy et al., 1999; Lelley et al., 2000). In cluster analysis, most relative genotypes (based on pedigree information) were grouped in the same cluster. For instance, in all of the dendrograms, CIMMYT and PRWYT-DT genotypes which originated from CIMMYT, Mkh genotypes derived from the temperate Mediterranean, and all genotypes of Crossing Block except Crossing Block 84 were separated into different clusters (groups). The results showed that genetic relations of genotype, based on microsatellite markers, corresponded to their pedigree and their geographical distribution. Although none of the methods of clustering could separate the resistant and susceptible genotypes from each other definitively, in all the clustering methods, Bolani and MV17 genotypes which are susceptible and resistant parents (respectively) in most rust breeding projects in the country could be separated in the different groups with a reasonable distance. These results were similar to the result observed in tetraploid wheat samples which had different reactions to stripe rust. Although they could separated the genotypes according to their pedigree, but resistant and sensitive genotypes could not be separated completely. Principle component analysis as an alternative method of genotype clustering could confirm the clustering group based on Rogers coefficient, because the distribution of genotypes in two-dimensional dendrogram based on two main components displayed similar result as cluster grouping, approximately. These results are consistent with the one obtained by previous studies (Bai et al., 2000); their dendrodram derived from principle components analysis could verify their clustering analysis too. Distribution of markers throughout the genome, and different reactions of genotypes (susceptible and resistant reactions) to yellow rust were the main reasons why the sensitive and resistant genotypes could not be separated in the clustering analysis. The use of SSR markers made it possible for the informative markers which were used to investigate the chromosomal locations involved in resistance of yellow rust to be distinguished.

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