Analysis of Genetic Diversity of Korean Wheat Cultivars Using Microsatellite DNA Polymorphisms

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ABSTRACT

Genetic background and phylogenetic relationships among 20 Korean wheat cultivars were assessed using microsatellites after amplifying with 13 SSR primer pairs. Average allele number per primer pair was 3.36. Genetic similarities for every pair of cultivars ranged from 0.42 to 0.97, with 0.69 of overall average. Korean cultivars were divided into two major groups based on microsatellite DNA polymorphisms. Group I consisted of relatively old cultivars developed until 1970s, and group II contained the recent cultivars developed during 1980s and 1990s. Amongst old elite cultivars/lines, 'Yukseung 3', 'Norin 12' and 'Norin 72' contributed most to the genetic background of cultivars belonging to group I, and 'Norin 4', 'Norin 12', 'Norin 43' and 'Norin 72' to group II, respectively. The phylogenetic relationship of Korean wheat cultivars was in accordance with the genealogical data of each cultivar. The genetic background of each cultivar was assessed from the point of breeding and germplasm management such as variety identification and duplicated accessions for assisting in developing a system for the registration of new variety based on the molecular characterization in future.

Keywords **:** *Triticum aestivum*, genetic background, microsatellite, phylogeny, genealogy

INTRODUCTION

Bread wheat (*Triticum aestivum* L., AABBDD) genome is very complex due to its allohexaploid nature, large genome size 16 × 10^9 bp/1C (Bennett & Smith 1976) and presence of highly highly repetitive sequences in the organization of genome (Lapitan 1992). It has extremely low level of polymorphisms in molecular marker system. Molecular technology is now well developed and has been applied in the genetic analysis and assessment of genetic diversity (Helentjaris et al. 1986, Bernatzky & Tanksley 1986, Landry et al. 1987, Devos et al. 1992, Devos et al. 1993, Marino et al. 1996). Anderson et al.(1992) used aneuploid sets of bread wheat to locate DNA clones on each chromosome.

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Table 1. Description of plant materials, in analysis of microsatellite DNA polymorphisms of Korean wheat cultivars along their pedigrees and year of development

Designation	Name of cultivars (Line ID)	Pedigree	Year of development	IT No.†
A	Yukseung 3	Quality/Suwon 13	1936	010793
B	Norin 4(Chugoku 2)	Eushyogi 347/Nongdosypre 3	1942	010739
C	Jinkwang(Sugye 108)	Yukseung 3/12 SE	1959	159623
D	Yongkwang(Sugye 118)	Yukseung 3/12 SE	1959	151086
E	Wongkwang(Sugye 146)	Suwon 85/ Norin 12	1969	151082
F	Namkwang(Sugye 147)	Suwon 85/ Norin 72	1969	151081
G	Sinkwang(Sugye 158)	Yongkwang/Norin 72	1973	165995
H	Olmil(Milyang 5)	Norin 72/Norin 12	1976	87053
I	Chokwang(Sugye 189)	Jaekwang/Norin 72	1976	176238
J	Dahongmil(Milyang 7)	Norin 72/Wonkwang	1979	175539
K	Cheonggemil(Milyang 9)	Norin 4/Sharbati-sonora	1979	87056
L	Naemil(Suwon 217)	Study/Scout//Strampelli/Bb-CNO	1980	151085
M	Saemil(Suwon 220)	Strampelli/69D-3607//Chokwang	1980	014335
N	Eunpamil(Suwon 224)	Chugoku 81///Tob-CNO/Yukseung 3//Suwon 185	1982	175521
O	Tapdongmil(Suwon 236)	Chugoku 81//Suwon 158 /Toropi	1986	175524
P	Namhaemil(Milyang 24)	Olmil/Calridad's	1988	87054
Q	Urimil(Suwon 250)	Geurumil/Olmil	1991	172221
R	Olgeurumil(Suwon 253)	SW76426-B77//Seohae 143	1993	175570
S	Alchanmil(Suwon 257)	Suwon 210/Tapdongmil	1994	175574
T	Saeolmil(Milyang 29)	Sirogane//Norin 43/Sonalika	1997	87057

† IT No. : Accession numbers of RDA genebank in the Rep. of Korea.

Microsatellite markers, consisting of 2-6 base pair repeats, have been developed in major crop species, and evaluated as much more reliable and polymorphic DNA marker system in many crop species including wheat (Baryan et al. 1994, Diwan & Cregan 1997, Smith et al. 1997, Taramino 1997). These microsatellite markers are revealed as genome specific markers and distributed in the linkage map of A, B and D genome of bread wheat. Also, microsatellite markers has been successfully undertaken in hexaploid wheat (*T. aestivum*) and also applied in assessing genetic diversity of germplasm including European commercial varieties (Röder et al. 1995, Plaschke et al. 1995, Röder et al. 1998) and a large number of accessions (Huang et al. 2002).

Until recently, the identification of cultivars has been done, mainly by investigating phenotypes using qualitative and quantitative traits. Many varieties have been developed using limited number of elite lines, so that modern cultivars have narrow genetic base. Therefore, it is likely that the discrimination of one variety from all the others by morphological characters is not easy to meet plant variety registration requirements. In addition, the need for broadening the base of newly developed cultivars either through conventional breeding programmes or through the use of molecular genetic tools, is well recognized. A

descriptive tool that uses molecular characterization along with conventional check lists for variety registration may be more effective way to protect breeder's right in the seed industry. In addition, use of molecular markers may prove to be very effective method to assess genetic diversity of each cultivar and provide information to breeders to focus on broadening genetic base of new cultivars.

This study was conducted to assess genetic diversity among Korean wheat cultivars using microsatellite markers. It will be helpful in identifying genetic background of each cultivar and their proper utilization in future breeding program and plant variety protection system.

MATERIALS AND METHODS

Plant materials

Twenty wheat cultivars were used for DNA isolation. The materials include the earliest varieties such as

Table 2. Primer sequences, chromosomal locations and number of alleles in the microsatellite analysis of Korean wheat cultivars

Primer ID	Primer sequences(5'→3')	Microsatellite composition†	Fragment size (bp)	Chr. Location‡	No. of alleles
WMS5	GCCAGCTACCTCGATACAACTC AGAAAGGGCCAGGCTAGTAGT	$(TC)_{23}(T)_4(GT)_{12}$ $(GA)_{10}$	172	2AS	3
WMS18	TGGCGCCATGATTGCATTATCTTC GGTTGCTGAAGAACCTTATTTAGG	$(GT)_{17}CT(AT)_4$	186	4BS	4
WMS24	CACACAAGGCACCATTGC CAATGGACATAGTTGTGTGCG	$(GT)_9GCA(TG)_8$	172	1BL	2
WMS46	GCACGTGAATGGATTGGAC TGACCCAATAGTGGTGGTCA	$(GA)_2GC(GA)_{33}$	187	7BS	5
WMS52	CTATGAGGCGGAGGTTGAAG TGCGGTGCTCTTCCATTT	$(GT)_4AT(GT)_{20}$	150	3DL	2
WMS82	ACGTTAGAAGGTGCAATGGG AGTGGATGCACCGACTTTG	$(GT)_{10...}(GT)_4T$ $(AG)_4$	152	6AL	3
WMS88	CACTACAACTATGCGCTCGC TCCATTGGCTTCTCTCTCAA	$(GT)_{18}TT(GA)_4$	121	6BL	3
WMS106	CTGTTCTTGCGTGGCATTAA AATAAGGACACAATTGGGATGG	$(GA)_{24}$	139	1DS	1
WMS111	TCTGTAGGCTCTCTCCGACTG ACCTGATCAGATCCCACTCG	$(CT)_{32}(GT)_{17}$	205	7DS	10
WMS120	GATCCACCTTCCTCTCTCTC GATTATACTGGTGCCGAAAC	$(GT)_{16}(GA)_{11}$	139	2BS	0
WMS154	TCACAGAGAGAGAGGGAGGG ATGTGTACATGTTGCCTGCA	$(GA)_4(GGGA)_4$ $(GA)_{25}$	102	5AS	0
WMS155	CAATCATTTCCCCCTCCC AATCATTGGAAATCCATATGCC	$(GA)_{19}$	144	3AL	4
WMS174	GGGTTCCTATCTGGTAAATCCC GACACACATGTTCCTGCCAC	$(GA)_{22}$	173	5DL	2

†‡ The data for microsatellite repeats and chromosome locations from Plaschke et al (1995).

'Yukseung 3' and 'Norin 4', developed in 1936 and 1942, respectively. Most of cultivars were used as parents in succeeding breeding programmes to develop new cultivars. All the accessions listed in Table 1 were provided from the genebank of Rural Development Administration in Rep. of Korea.

DNA isolation and analysis of microsatellites DNA polymorphisms

Total genomic DNA was extracted from six week old seedlings of each cultivar according to the phenol/chloroform method described by Devos et al. (1992). Analysis of microsatellite polymorphism was conducted using 13 primer pairs including WMS 5 (Table 2). PCR reaction was performed in a total volume of 50μl with a PTC-100 (MJ Research). The reaction mixture contained 80ng template DNA, 0.2mM deoxynucleotides, 2.5mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl, pH 9.0, 0.1% Triton X-100, 1U *Taq* polymerase and 0.2μM of each primer. The reaction mixture was subjected to initial denaturation for 3min at 94℃, and DNA amplification of 45 cycles. Each amplification cycle consisted of 94℃ 1 min, 55℃ 1 min and 72℃ 2 min. Final extension was for 10 min at 72℃. Amplified fragments were visualized using 6% polyacrylamide denaturing gels and Silver Staining Kit (Promega USA).

RESULTS

The microsatellite DNA polymorphisms among 20 Korean wheat cultivars including 'Yukseung 3' (earliest variety) and 'Saeolmil' (developed in 1997) were analyzed. Thirteen primer sets were selected based on their chromosomal groups and high polymorphisms. Two primer pairs from 1-7 homoeologous groups

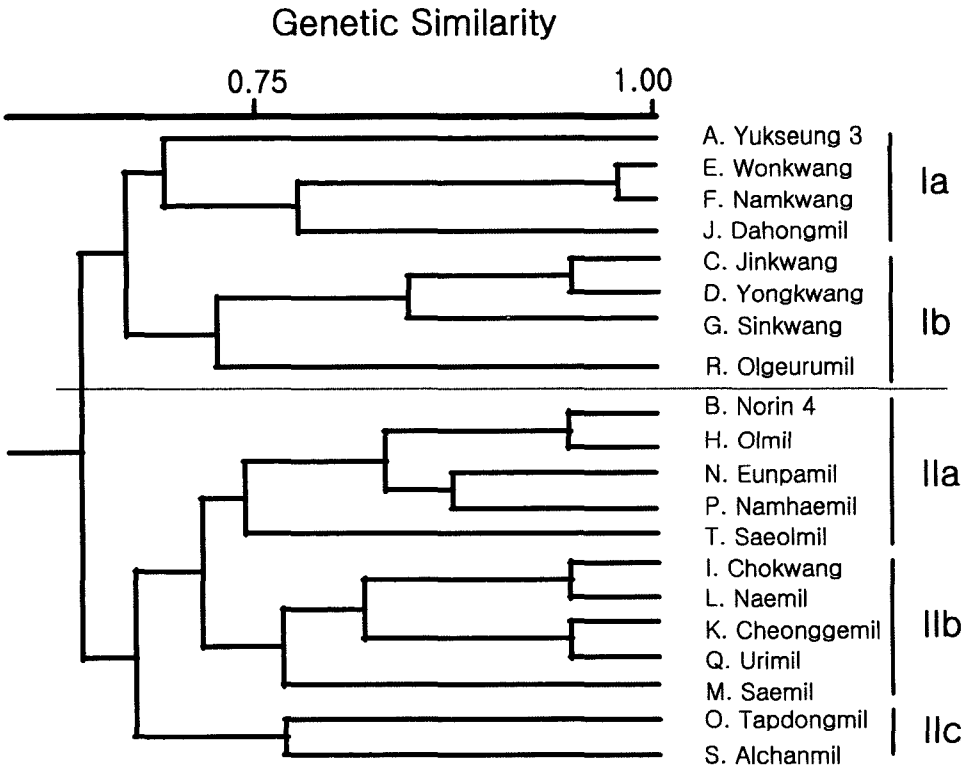

Fig 1. Dendrogram of 20 Korean wheat cultivars, based on the genetic similarity calculated from data of microsatellite DNA polymorphisms using 11 primer sets.

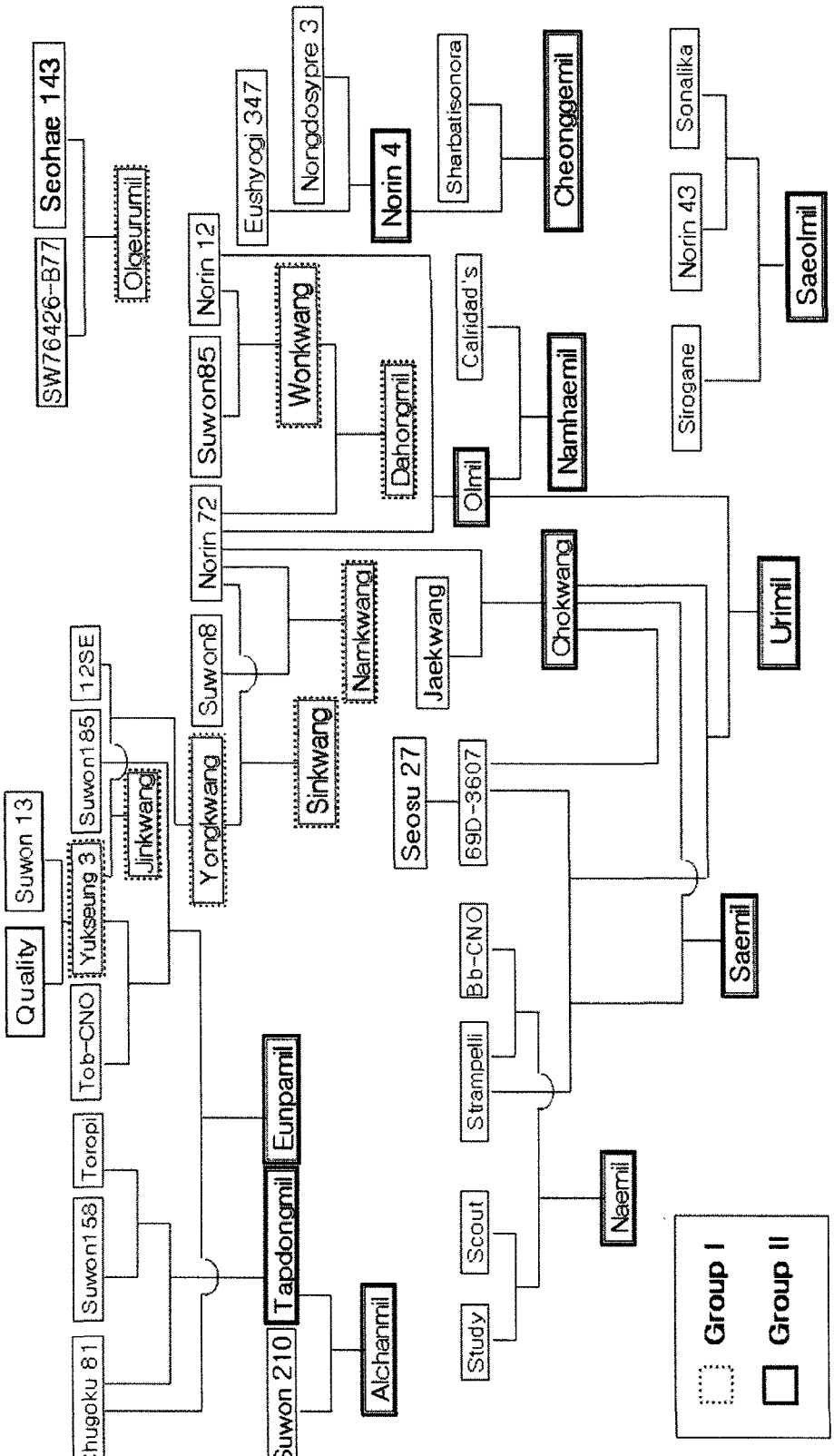

Fig 2. Genecological relatiohships of 20 Korean wheat cultivars. Group I & group II, developed by microsatellite DNA polymorphisms, are distinguished by different colors.

except for group 4 from which one primer pair of WMS18 was selected. A total of 37 alleles were detected and average number of alleles per primer pair was 3.36 (Table 2). The degree of polymorphism among Korean varieties was lower than that of European ones, 6.2 alleles per primer pair (Plaschke 1995). Also, the materials used for the previous study included 12 countries including China as their origins and both spring and winter sowing types. The degree of diversity of crop species depends on geographical diversity and cropping system (Huang et al. 2002). The low diversity in the case of Korean cultivars might be explained by much narrower geographical diversity and Korean cultivation system of bread wheat, which is restricted to winter type only, in contrast to European countries. Amongst 13 primer sets, WMS120 and WMS154 have no allele in Korean cultivars and WMS106 has no polymorphism in the cultivars analyzed. Null alleles caused by sequence variation of primer region in genome were observed in the previous report (Röder et al. 1995).

The genetic similarities between cultivars were analyzed using UPGMA (Unweighted Pair Group Method using Arithmetic means) of NTSYS program (Rohlf 1997), based on Nei's genetic distance (Nei 1972). The cluster analysis produced two main groups (Group I & Group II) (Fig. 2). Group I included most of cultivars developed before 1980 except for 'Olgeurumil'. The genetic background of this group might have been mainly derived from 'Yukseung 3', 'Norin 12' and 'Norin 72' (Fig. 2). One sub-group (Ia) included 'Yukseung 3', 'Wonkwang', 'Namkwang' and 'Dahongmil' whereas the other sub-group (Ib) included 'Jinkwang', 'Yongkwang', 'Sinkwang' and 'Olgeurumil', indicating 'Norin 72' was a central to this group. 'Suwon 8' and 'Suwon 85' also contributed to the genetic background of cultivars in group I. Also, 'Dahongmil', which was developed from a cross between 'Norin 72' and 'Wonkwang', was grouped in the same sub-group (Figs. 1 & 2). In the second sub-group (Ib), 'Jinkwang' was very close to 'Youngkwang', developed from same cross combination (Yukseung 3/12SE). 'Sinkwang' belonged to this sub-group (Ib) as this was derived from 'Yongkwang' as one parentage (Fig. 2). It was noticeable that 'Olgeurumil' belonged to Group I because it had no direct relationship with the cultivars in Group I. Many cultivars developed from 'Norin 72' including 'Sinkwang', 'Namkwang' and 'Dahongmil' were belonged to Group I.

Most of the cultivars developed after 1980 clustered together as group II. It consisted of three sub-groups (IIa, IIb & IIc). Sub-group IIa contained 'Norin 4', 'Olmil', 'Eunpamil', 'Namhaemil' and 'Saeolmil'. 'Olmil' was used as a parent for developing 'Namhaemil' and 'Urimil', but other cultivars were not directly related in their parentage. Nevertheless, these cultivars in sub-group IIa were genetically close. Sub-group IIb consisted of 'Chokwang', 'Naemil', 'Cheonggemil', 'Urimil' and 'Saemil'. 'Saemil' was close to 'Naemil' and 'Chokwang' in term of their genealogy (Fig. 2). But 'Cheonggemil' derived from 'Norin 4', was not directly related to 'Urimil' based on its pedigree. Sub-group IIc consisted of 'Tapdongmil' and 'Alchanmil'. Their close relationship might be easily explained by the fact that 'Alchanmil' was developed from 'Tapdongmil'. Based on the analysis of genetic diversity of Korean wheat cultivars, it is clear that 'Yukseung 3', 'Norin 12' and 'Norin 72' contributed most to developing cultivars belonging to Group II, and 'Norin 4', 'Chugolu 81', 'Norin 72' and 'Strampelli' to Group I. In particular, 'Norin 72' is an important cultivar that contributed to both the groups. In Korean wheat cultivars, the genetic relationships based on the microsatellite DNA polymorphisms confirm very well with genealogical background.

The mean similarity of each cultivar relative to all the other cultivars was calculated and their distribution

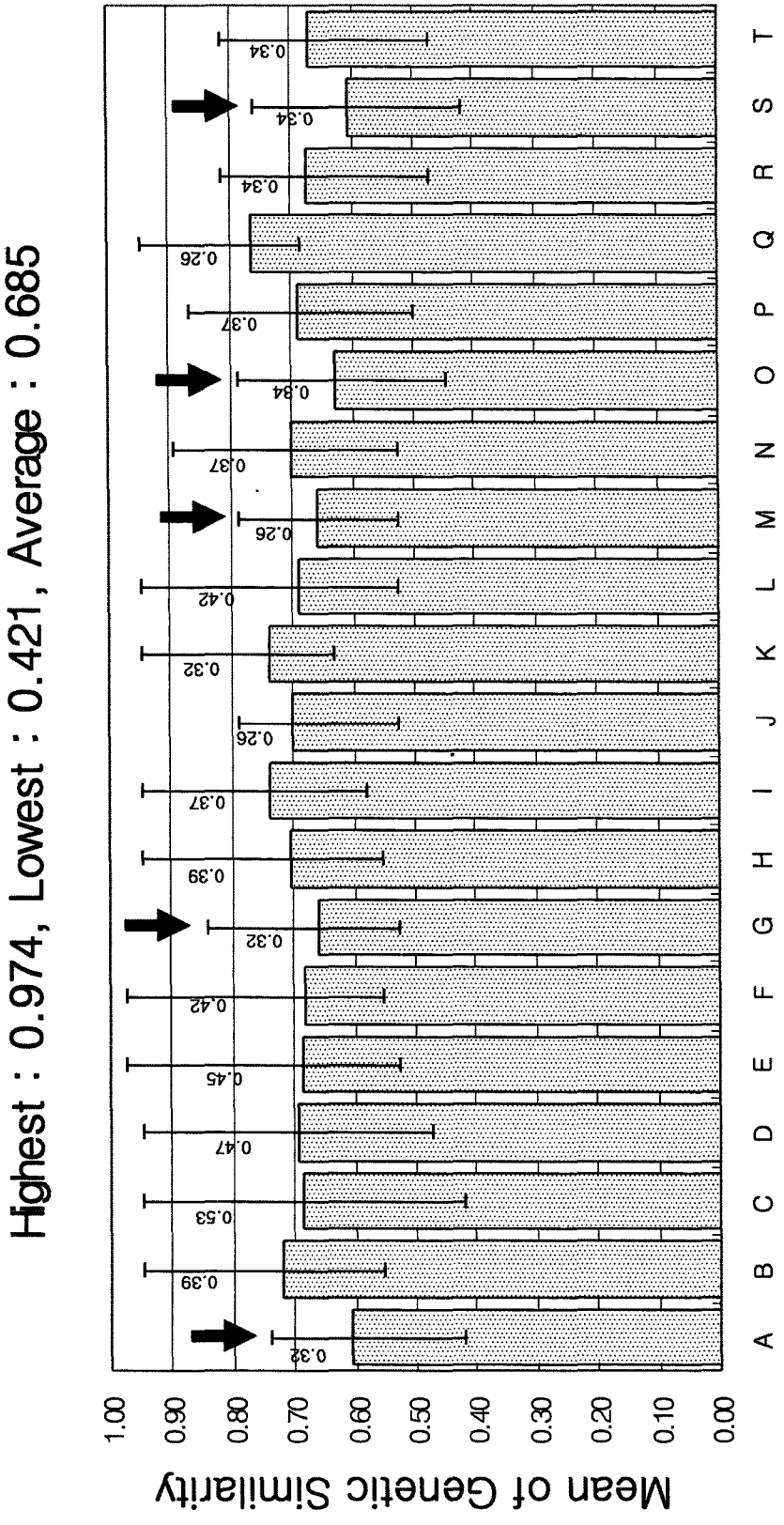

Fig 3. The distribution of average genetic similarities of Korean wheat cultivars. Arrows indicate cultivars with low means of genetic similarity. A°≠T are referred from Table 1.

is shown as Figure 3. According to pair-wise comparison of genetic similarities, the range was 0.421~0.974 with 0.685 of overall average. The variety pairs with the similarities below 0.5 were 'Jinkwang'/ 'Alchanmil' (0.421), 'Yukseung 3'/ 'Tapdongmil' (0.447), 'Norin 4'/ 'Alchanmil' (0.474) and 'Olgeurumil' / 'Alchanmil' (0.474), indicating genetically distant between each paired cultivars. The variety combinations with extremely high genetic similarities (>0.9) were 'Wongkwang'/ 'Namkwang' (0.974), 'Urimil'/ 'Cheonggemil' (0.947), 'Norin 4'/ 'Olmil' (0.947) and 'Jinkwang'/ 'Yongkwang' (0.947). The interval of each cultivar to all other ones ranged from 0.263 to 0.526. 'Saemil' and 'Urimil' had very narrow interval whereas 'Jinkwang' and 'Youngkwang' had wide intervals in their spectrums of genetic similarities to other cultivars. This result reflects the closeness between the cultivar pairs and common parentage in their pedigrees (Fig. 2). It is noticeable that 'Norin 4', which was developed in 1936, contributed most to the Korean wheat cultivars, with 0.72 overall average genetic similarity indexes. It can also be seen that this variety was genetically very close to 'Norin 72' or 'Norin 12' because of its close relationship to 'Olmil' (Fig. 1) as 'Olmil' was developed from the cross between 'Norin 72' and 'Norin 12'. The narrow genetic background of Korean cultivars is an indication of genetic vulnerability in the near future. Hence, more efforts are needed to breed new varieties genetically divergent from previous cultivars with good adaptation and high yield. To do so, it is very important to introduce more diversity from relatively distant germplasm nevertheless the implementation of earliness in wheat breeding restricted in the cropping system of wheat before rice cultivation in the southern paddy land for main barley production, that is broadening of genetic base and increasing genetic diversity in newly released commercial cultivars.

DISCUSSION

Microsatellite markers in wheat have been shown to be more variable than any other molecular marker system (Röder et al. 1995), and very effectively applied in mapping of bread wheat (Röder et al. 1998). The earlier developed wheat cultivars in Korea include 'Jinkwang', 'Yongkwang' and 'Jangkwang' were tall before the introduction of dwarf gene. The second generation of cultivars are characterized as earliness and carrying dwarf gene such as 'Wongkwang' and 'Namkwang'. The recent cultivars, 'Saemil' and 'Geurumil', have been focused on earliness than those in the second generation. The similarity of Korean wheat cultivars ranged from 0.42 to 0.97, with an average of 0.68. Cultivars 'Chokwang', 'Cheonggemil', and 'Urimil' had an average similarity of 74%, 74% and 77%, respectively. These cultivars share much of their genetic background with all the other cultivars. Whereas, 'Yukseung 3', 'Alchanmil', and 'Tapdongmil' had an average similarity of 61%, 61% and 63%, indicating that this variety was more diverse than all the others, based on microsatellite DNA analysis.

Huang et al. (2000) demonstrated that microsatellite marker is one of the fast and high throughout fingerprinting methods for analysing large number of accessions in genebank. The duplication is of accessions in germplasm collection of genebank is one of serious problem world-wide from each other. All the cultivars used in this experiment could be distinguished one from the others by the fingerprints developed with 11 pairs of microsatellite markers. We successfully screened out three different accessions of same variety (data not shown), where no variation was found in the duplicated accessions of same cultivar. But some special care is needed in out-breeding crops and selfing crops for segregating generation even through highly homogenized generation after releasing to farmers,

since some variations may present by segregation, mutation and physical/genetical contamination as shown in same inbred line of maize (Gethi et al. 2002) and variety identification for soybean by microsatellite marker (Diwan & Cregan 1997).

According to the microsatellite polymorphisms of Korean cultivars, 'Yukseong 3', 'Tapdongmil' and 'Alchanmil' were relatively low in their average values of genetic similarities to other cultivars. These cultivars might contribute to expand genetic spectrum of wheat cultivars in wheat breeding in Korea. 'Norin 4', 'Chokwang', 'Cheonggemil' and 'Urimil' were main cultivars with highly sharing their genetic backgrounds with other wheat cultivars, especially cultivars belonged to Group II. The cultivars developed in the early generation were belonged into Group I. The main cultivars, which contributed as these parentages in their genetic backgrounds, were 'Yukseong 3', 'Norin 72'. Later on, more diverse parents were used for breeding program. Most of the recent developed cultivars were belonged to Group II. 'Chugoku 82' and 'Norin 4', 'Norin 43' and 'Norin 72' were mainly contributed in the wheat breeding of this stage. 'Norin 72' is a variety contributing to both the groups. According to the pair-wise comparison of genetic similarities, some varieties have very wide spectrums of intervals in genetic similarities to other cultivars. This means that they have potentially contributed to development of other cultivars. It was revealed that most of the cultivars developed after 1980' were classified into Group II. This may be explained by sharing their parentages and same breeding target. The earliness in heading and ripening, and the tolerance to wet injury have been two major targets of wheat breeding to develop new cultivars for cropping system with rice in paddy field.

The assessment of diversity by molecular marker system could be very useful in understanding the genetic backgrounds and evolutionary relationships among cultivars (Hammer et al. 2000). Hence, it could be used for providing molecular information of a new variety in plant breeding as shown in other crop species (Hang et al. 2000, Virk et al. 2000; Heckenberger et al. 2002, Kim et al. 2002). Also, it could be applied for managing genetic resources, for example, duplicate accession identification, developing core collections, etc. (Kim & Ward 1997, 2000). It is important to note that special care needs to be taken in applying microsatellite marker system for variety registration because of its nature of slippage of repeat units, resulting in variation of their polymorphism. But still it is one of the more reliable methods to support characterization system for new variety registration in future.

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