Distribution of the necrosis and chlorosis genes in two wild tetraploid wheats, *Triticum dicoccoides* and *T. araraticum*

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ABSTRACT

Distributions of the *Ne1* and *Ne2* alleles for type 1 hybrid necrosis, and *Ch1* allele for type 1 hybrid chlorosis in two wild tetraploid wheats, *Triticum dicoccoides* (wild emmer wheat) and *T. araraticum* (wild timopheevi wheat), have been investigated by crossing the collections of these species from their entire distribution areas with their appropriate testers. The frequencies of the *Ne1* and *Ch1* alleles were 53% and 13%, respectively, in *T. dicoccoides*; whereas they were 69% and 0% in *T. araraticum*. The *Ne2* allele was not found in both species. The *Ne1* allele of *T. dicoccoides* was mainly *Ne1^m*, while that of *T. araraticum* was mainly *Ne1^w*. The frequency of the *Ch1* allele differed significantly between the two species. These facts indicate that the emmer and timopheevi groups of tetraploid wheat had been differentiated genetically before their domestication. On the frequencies of the *Ne1* and *Ch1* alleles, Israeli *T. dicoccoides* revealed high genetic homogeneity within local populations. While there was extreme variability among these populations, they showed no geographical inclination of their frequencies. These alleles are assumed to be neutral for selection and have been randomly fixed in most populations. A limited numbers of Sitopsis accessions of the genus *Aegilops* were tested for the two alleles, *Ne1* and *Ne2*. None of them carried these alleles. Thus, the origin of the *Ne1* alleles found in tetraploid wheats could not be traced back to any Sitopsis species.

1. INTRODUCTION

Several types of hybrid weakness are known in polyploid wheats. They are hybrid necrosis, hybrid chlorosis, and hybrid dwarfism. Two types (types 1 and 2) distinguishable by phenotype and genetic basis are known in both the hybrid necrosis and hybrid chlorosis (hereafter simply necrosis and chlorosis). All of them are controlled by two complementary genes. This paper deals with type 1 necrosis controlled by *Ne1* and *Ne2* genes on chromosome 5B and 2B, respectively

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(Caldwell and Compton, 1943; Tsunewaki, 1960), and type 1 chlorosis determined by *Ch1* and *Ch2* genes on chromosome 2A and 3D (Tsunewaki and Kihara, 1961; Hermesen and Waninge, 1972).

The genotype for necrosis and chlorosis of a given accession can be determined by observing the phenotype of small numbers of its F_1 hybrids from the crosses with the appropriate testers. Due to the ease of determining the genotypes, the distribution of these genes has been subjected to extensive investigations, from which useful information on the origin and speciation of polyploid wheats has been obtained (Hermesen, 1963; Nishikawa, 1967; Tsunewaki and Nakai, 1973; Zeven, 1981; and many others).

Common wheat ($2n=6x=42$, genome constitution AABBDD) is known to have originated as an amphidiploid between emmer wheat ($2n=4x=28$, AABB) and *Aegilops squarrosa* ($2n=2x=14$, DD) (Kihara, 1944; McFadden and Sears, 1946). The work on type 1 necrosis and type 1 chlorosis has led to a conclusion that *Ne1* and *Ch1* alleles of common wheat have been derived from emmer wheat, and *Ch2* allele from *Ae. squarrosa*, whereas *Ne2* allele has originated by a mutation in common wheat (Tsunewaki, 1968, 1970).

Until now, no systematic investigation has been made on the distribution of the genes for both necrosis and chlorosis in the wild emmer and timopheevi species. Such investigation is expected to provide useful information on the origin of the two tetraploid wheat groups.

This paper reports the results on the distribution of the necrosis and chlorosis genes, both of type 1, in about 240 accessions of *T. dicoccoides* and *T. araraticum* which were collected across their entire distribution areas, and discusses the origin of the emmer and timopheevi groups of tetraploid wheat.

Because the *Ne1* allele is located on the 5B chromosome, the origin of this allele in tetraploid wheats is suspected to be in some Sitopsis species of the genus *Aegilops*, since it provided the second genome (B and G) to the tetraploid wheats. To prove this, different accessions of four Sitopsis species were tested for their genotype on necrosis. The result of this investigation is also reported.

2. MATERIALS AND METHODS

One hundred and fifty-two accessions of the wild emmer wheat, *Triticum dicoccoides* ($2n=4x=28$, genome constitution AABB) and 87 accessions of the wild timopheevi wheat, *T. araraticum* ($2n=4x=28$, AAGG) were used. They were collected from 52 sites in Israel, southeastern Turkey, northern Iraq, western Iran and Transcaucasus (Fig. 1, after Tanaka, 1983; Nevo et al., 1982).

To determine the genotypes for necrosis and chlorosis, six hexaploid wheats were used as testers (Table 1). These testers were crossed with all accessions of the two wild tetraploids, though some crosses were unsuccessful. The F_1 seeds obtained were sown in seed bed, then about 30-days later, the seedlings were

Fig. 1. Collection sites of the *T. dicoccoides* (●) and *T. araraticum* (○) accessions used, except those in Israel (after Tanaka, 1983).

Table 1. Necrosis and chlorosis testers of polyploid wheats employed

Tester		Allele detected
Taxon	Genotype ^a	
<i>T. aestivum</i> cv. Jones Fife	<i>ne1 Ne2^s ch1 Ch2</i>	<i>Ne1, Ch1</i>
<i>T. aesticum</i> cv. Chinese Spring	<i>ne1 ne2 ch1 Ch2</i>	<i>Ch1</i>
<i>T. aestivum</i> cv. S-615 ^b	<i>Ne1^m ne2 ch1 Ch2</i>	<i>Ne2, Ch1</i>
<i>T. aestivum</i> cv. S-615 ^b	<i>ne1 Ne2^s ch1 Ch2</i>	<i>Ne1, Ch1</i>
<i>T. aestivum</i> cv. Hosogara	<i>Ne1 ne2 ch1 Ch2</i>	<i>Ne2, Ch1</i>
<i>T. macha</i> var. <i>subletschchumicum</i>	<i>Ne1^w ne2 Ch1 ch2</i>	<i>Ne2, Ch2</i>
<i>T. durum</i> var. <i>reichenbachii</i>	<i>Ne1 ne2 ch1 —</i>	<i>Ne2</i>
<i>T. dicoccum</i> cv. Vernal ^b	<i>ne1 Ne2^s ch1 —</i>	<i>Ne1</i>

^a Superscripts, s, m and w, stand for strong, moderate and weak allele, respectively

^b Near-isogenic lines of the respective cultivars having the specified genotype

transplanted in the field plot. Their phenotypes on necrosis and chlorosis were observed both in the seed bed and the field plot. The genotypes, based on the phenotypes of individual accessions, have been determined, *Ne1ne2*, *ne1Ne2* or *ne1ne2* for necrosis, and *Ch1* or *ch1* for chlorosis. (The *Ch2* locus is located in the D genome, and therefore the alleles of this locus are not found in the tetraploid wheats.) In this article, the genotype is indicated in the haploid phase because both wild tetraploids are autogamous, and the accessions used have been maintained by self pollination.

3. RESULTS

Frequency and distribution of the Ne1 and Ne2 genes for necrosis

Numbers of *T. dicoccoides* and *T. araraticum* accessions having three genotypes for necrosis are given in Table 2. The *Ne2* allele was not found in either species. Frequency of the *Ne1* allele was 54% in *T. dicoccoides* and 69% in *T. araraticum*, and no difference was detected between them ($\chi^2=2.76$, $df=1$). In both species the *Ne1* allele showed wide geographical distribution.

Israeli accessions of *T. dicoccoides* were collected from ten local populations (Nevo et al., 1982). As shown in Fig. 2, frequencies of the two necrosis genotypes, *ne1ne2* and *Ne1ne2*, greatly differed among the populations. Most populations were fixed or almost fixed with either genotype. No geographical inclination of their frequency was detected.

Severity of the necrosis symptom varied among the hybrids of different accessions; almost all necrotic F_1 's of *T. araraticum* accessions showed a weak symptom, whereas a majority of the hybrids of *T. dicoccoides* showed a moderate symptom. These results suggest that the *Ne1* allele in *T. araraticum* is mostly *Ne1^w*, and that in *T. dicoccoides* is *Ne1^m*, because all testers used in detection of the *Ne1* alleles carried the *Ne2^s* allele.

Frequency and distribution of the Ch1 gene for chlorosis

Frequencies of the two alleles, *Ch1* and *ch1*, among the accessions of two wild tetraploids are given in Table 3. The *Ch1* allele was found in 13% of the *T.*

Table 2. Numbers of *T. dicoccoides* and *T. araraticum* accessions having three genotypes for necrosis

Species	Country	No. of accessions			
		<i>ne1 ne2</i>	<i>Ne1 ne2</i>	<i>ne1 Ne2</i>	Total
<i>T. dicoccoides</i>	Armenia	1	0	0	1
"	Iran	1	0	0	1
"	Iraq	8	3	0	11
"	Turkey	3	4	0	7
"	Syria	2	0	0	2
"	Israel	42	61	0	103
"	Total	57	68	0	125
"	(%)	(46)	(54)	(0)	(100)
<i>T. araraticum</i>	Armenia	2	0	0	2
"	Iraq	10	25	0	35
"	Turkey	1	4	0	5
"	Total	13	29	0	42
"	(%)	(31)	(69)	(0)	(100)

Fig. 2. Frequencies of the *Ne1*-carriers (■; genotype *Ne1ne2*) and *ne1*-carriers (□; genotype *ne1ne2*) in ten Israeli populations of *T. dicoccoides*. The locations of the populations were cited from Nevo et al. (1982).

Table 3. Numbers of *T. dicoccoides* and *T. araraticum* accessions having two genotypes for chlorosis

Species	Country	No. of accessions		
		<i>ch1</i>	<i>Ch1</i>	Total
<i>T. dicoccoides</i>	Armenia	1	0	1
"	Iran	1	0	1
"	Iraq	14	0	14
"	Turkey	11	0	11
"	Syria	2	0	2
"	Israel	104	19	123
"	Total	133	19	152
"	(%)	(88)	(13)	(101)
<i>T. araraticum</i>	Armenia	6	0	6
"	Iran	2	0	2
"	Iraq	67	0	67
"	Turkey	12	0	12
"	Total	87	0	87
"	(%)	(100)	(0)	(100)

Fig. 3. Frequencies of the *Ch1*-carriers (■) and *ch1*-carriers (□) in ten Israeli populations of *T. dicoccoides*. The locations were cited from Nevo et al. (1982).

dicoccoides accessions, whereas no *T. araraticum* accession carried it. The difference between the two species was statistically significant at the 1% level ($\chi^2=11.8$, $df=1$). We may conclude that *T. dicoccoides* contains *Ch1* allele, whereas *T. araraticum* does not.

In *T. dicoccoides*, a certain number of the *Ch1* allele was found in Israel, and its distribution among ten local populations was examined (Fig. 3). The frequency of *Ch1* was very high in population 2, but very low or zero in all other populations. Again, no clear geographical inclination was noticed.

Distribution of the necrosis genes in the section Sitopsis of Aegilops

Two emmer testers of the genotypes, *Ne1ne2ch1* and *ne1Ne2ch1* (Table 1) showed high cross incompatibility to all *Sitopsis* species. Only two accessions of *Ae. sharonensis*, four of *Ae. longissima*, four of *Ae. aucheri*, and two of *Ae. speltoides* were successfully crossed to one or both of the two testers. Phenotypes of the F_1 hybrids are shown in Table 4. The results indicated that none of the *Sitopsis* accessions tested carried *Ne1* or *Ne2* allele.

Table 4. Phenotypes of the F₁ hybrids between the *Ne*-testers^a and *Sitopsis* species

Species	Origin	Accession tested ^b	F ₁ 's phenotype		Assumed genotype
			× <i>Ne1</i> -carrier	× <i>Ne2</i> -carrier	
<i>Ae. sharonensis</i>	Israel	KU 5-3	normal	—	<i>ne2</i>
<i>Ae. sharonensis</i>	Israel	G 1321	—	normal	<i>ne1</i>
<i>Ae. longissima</i>	Jordan	KU 5752	normal	normal	<i>ne1ne2</i>
<i>Ae. longissima</i>	Israel	G 1303	chlorotic ^c	—	<i>ne2</i>
<i>Ae. longissima</i>	Israel	G 1307	normal	—	<i>ne2</i>
<i>Ae. longissima</i>	Israel	KU 4-1	normal	—	<i>ne2</i>
<i>Ae. aucheri</i>	Turkey	KU 2204A	normal	segregated ^d	<i>ne1ne2</i>
<i>Ae. aucheri</i>	Iraq	KU 7761	normal	normal	<i>ne1ne2</i>
<i>Ae. aucheri</i>	Iraq	KU 7804	—	normal	<i>ne1</i>
<i>Ae. aucheri</i>	Iraq	KU 7850	chlorotic ^c	normal	<i>ne1ne2</i>
<i>Ae. speltooides</i>	Turkey	KU 5725B	chlorotic ^c	chlorotic ^c	<i>ne1ne2</i>
<i>Ae. speltooides</i>	Iraq	KU 7786	chlorotic ^c	normal	<i>ne1ne2</i>

^a Genotype of the *Ne1*- and *Ne2*-carrier is *Ne1ne2*, and *ne1Ne2*, respectively

^b G and KU: Genetics and Plant Breeding Laboratory, Gifu University, and Plant Germplasm Institute, Kyoto University, respectively

^c Chlorosis observed in some hybrids differs from type 1 chlorosis of the present concern

^d Three chlorotic and 17 normal plants segregated

4. DISCUSSION

Distribution and origin of Ne1 and Ne2 genes in polyploid wheats

Tsunewaki (1968) hypothesized that *Ne1* allele of emmer and common wheats was originated from the B genome donor (a diploid ancestor), while *Ne2* allele occurred by mutation in common wheat. This hypothesis is based on a fact that large numbers of accessions of both common and emmer wheats carry the *Ne1* allele (22.2% of 2,157 common wheat accessions and 69.5% of 203 accessions of cultivated emmer wheat), while *Ne2* allele is found only in common wheat (17.1% of 2,157 common wheat accessions, but none among 203 emmer wheat accessions) (Tsunewaki and Nakai, 1973, for review). The present results that the frequency of *Ne1* allele was 54%, whereas the *Ne2* allele was not found among 125 *T. dicoccoides* accessions support the above hypothesis on the origin of *Ne1* and *Ne2* alleles in polyploid wheats.

The frequencies of *Ne1* and *Ne2* alleles observed here in two wild tetraploid wheats are in strong agreement with the results of Nishikawa (1967) and Tsunewaki (1970), who reported that 30% of 20 *T. dicoccoides* accessions and 30% of 39 *T. araraticum* accessions possess the *Ne1* allele, while none of them had the *Ne2* allele. All of these results suggest that the B genome donor to emmer wheat and G genome donor to timopheevi wheat carried *Ne1* allele. Because both the B and G genomes are assumed to have originated from some species of the section

Sitopsis of *Aegilops*, most likely from *Ae. speltoides* (Sarkar and Stebbins, 1956; Shands and Kimber, 1983; Dvorak, 1988; Tsunewaki, 1989), the carrier of *Ne1* allele was tested among all species of the Sitopsis section. Due to severe cross incompatibility between them and the testers for the *Ne* alleles, only a small fraction of the crosses attempted produced the hybrids. None of the accessions tested carried the *Ne1* or *Ne2* allele. The same attempt has already been made by Tsunewaki and Nakai (1973). Although on a smaller scale, they obtained essentially the same results as is reported here. We have still no evidence for the presence of *Ne1* allele in any Sitopsis species, though this is a critical key for identifying the B and G genome donor to tetraploid wheats.

Evidence on the diphyletic origin of the tetraploid wheats

Two groups of tetraploid wheats, emmer and timopheevi, differ from each other in their genome constitution, namely, AABB for the former and AAGG for the latter (Lilienfeld and Kihara, 1934). Their plasmons also differ distinctly from each other (Tsunewaki, 1989). As to their origin, there are two different views, i.e., monophyletic and diphyletic origin (Kimber and Sears, 1987). The former assumes that the two groups were originated from a common putative tetraploid, and the B and G genome were differentiated at the tetraploid level (Wagenaar, 1961; Tanaka et al., 1978). On the contrary, the latter assumes that they were derived from two different parental combinations (Tsunewaki, 1989).

The present investigation revealed that *T. dicoccoides* carries the *Ne1^m* allele for moderate necrosis at a high frequency (about 50%) and the *Ch1* allele at a low frequency (13%), whereas *T. araraticum* possesses *Ne1^w* allele for weak necrosis also at high frequency (about 70%) with no *Ch1* allele. Cultivated emmer wheat and *T. timopheevi* are known to have been domesticated from *T. dicoccoides* and *T. araraticum*, respectively. Therefore, the results obtained here imply that the putative form of emmer wheat carried the *Ne1^m* allele and the *Ch1* or *ch1* allele, while the timopheevi wheat carried the *Ne1^w* and *ch1* allele. These facts support the diphyletic origin of emmer and timopheevi wheats.

Interpopulational variation in the frequencies of Ne1 and Ch1 genes

Nevo and his coworkers studied allozyme polymorphisms of the Israeli populations of *T. dicoccoides* and obtained results suggesting multilocus differentiation adaptive to climatic conditions. They stressed distinct local adaptations through diversifying selection rather than genetic drift (Nevo et al., 1982, 1988; Golenberg and Nevo, 1987). The same Israeli populations of *T. dicoccoides* were studied here on the frequencies of the *Ne1*, *Ne2* and *Ch1* alleles. The *Ne2* allele was not found in any populations. As for the two other alleles, these populations showed extremely high or low frequencies, and very rarely showed moderate frequencies similar to the overall frequency. As for the *Ne1* allele, five populations showed 80% or higher frequencies, three populations less than 15%, and only two

populations showed moderate frequencies (64% and 36%), whereas overall frequency of this allele was 59%. As for the *Ch1* allele, one population showed 87% frequency but all other populations less than 20%, and overall frequency was 15%. In addition, the frequency of both alleles did not show any geographical inclination (Figs. 2 and 3). These facts are well explained by selective neutrality of those alleles resulting in random fixation in most populations. This interpretation is supported by the results of Tsunewaki and Koba (1979) who found no remarkable effects of the *Ne1* and *Ne2* alleles on 24 characters of a common wheat cultivar. Our interpretation, however, contradicts with the results of Gregory (1980) that suggested selective advantage of the *Ne1* over *ne1* allele. At any rate, it is clear that due to the autogamous nature, *T. dicoccoides* shows high genetic homogeneity within local populations and high variability among them.

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