A NEW TYPE OF HYBRID CHLOROSIS FOUND IN TETRAPLOID WHEATS¹⁾

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A type of chlorosis caused by two complementary genes, *Ch*₁ in A or B genome and *Ch*₂ on chromosome 3D of D genome, is known to occur in some F₁ hybrids between hexaploid wheats or between tetra- and hexaploid wheats (Tsunewaki and Kihara 1962, Nishikawa 1964, Hermesen 1966, Tsunewaki 1966). This type of chlorosis progresses with the age of plants and becomes finally lethal or semi-lethal. Distribution of the genes, *Ch*₁ and *Ch*₂, was extensively studied in polyploid wheats and *Aegilops squarrosa*, the D genome donor to common wheat (Sachs 1954, Tsunewaki and Kihara 1962, Nishikawa 1964, Tsunewaki and Hori 1967, and others). The study disclosed some phylogenetic relationships between common wheat and its ancestral species.

In the course of studying interspecific hybrids in wheat, the authors found a new type of chlorosis that occurred in reciprocal F₁ hybrids between *Triticum dicoccum* cultivar Hokudai and *T. timopheevi* var. *typicum* strain NIG. Since this chlorosis occurred in the F₁ hybrid with the genome constitution AABBG, the genic system responsible for it appeared to be different from that previously known.

In order to clarify the genetic basis of this new type of chlorosis, a conventional gene analysis has been carried out. At the same time, a preliminary investigation has been made of the distribution of the chlorosis genes in four species of tetraploid wheat. Results of those investigations are here reported.

MATERIALS AND METHODS

In 1964 three tetraploid wheats (2n = 28), *Triticum dicoccum* cultivar Hokudai (genome formula AABB), *T. durum* var. *reichenbachii* (AABB) and *T. timopheevi* var. *typicum* strain NIG (AAGG), were reciprocally crossed in diallele combinations. The result indicated that some complementary genes in *T. dicoccum* and *T. timopheevi* are responsible for chlorosis, and that the *T. durum* strain does not carry them. In order to determine the number of complementary genes in the *T. dicoccum* strain, the reciprocal F₁ hybrids between *T. dicoccum* cultivar Hokudai and *T. durum* var. *reichenbachii* were crossed as the female parents to *T. timopheevi* var. *typicum* strain NIG (hereafter, simply called as *T. timopheevi* strain NIG), and their offspring were examined for the occurrence of chlorosis.

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In order to study the gene in the *T. timopheevi* strain, transfer of the gene to emmer wheat was attempted, because no *T. timopheevi* strain free from the chlorosis gene was known to us, and the F_1 hybrids between *T. timopheevi* and *T. durum* were highly sterile due to the difference in their second genome. For this sake, the F_1 hybrids between *T. timopheevi* strain NIG and *T. durum* var. *reichenbachii* were backcrossed three times to the latter as the pollen parent. In each backcross generation, plants used for further backcrosses, were test-crossed to *T. dicoccum* cultivar Hokudai, and occurrence of chlorotic plants in their offspring was examined in order to determine whether they carried the chlorosis gene. In 1967, nine B_3 plants were cytologically investigated for chromosome pairing in meiosis in PMC's, and the results obtained were compared with the segregation data on chlorosis in their test-cross. Based on these results, it was decided whether the *T. timopheevi* gene for chlorosis is carried by a chromosome in A or G genome. Pedigrees of the materials used in this work are shown in Fig. 1.

Fig. 1. Pedigree showing successful transfer of the chlorosis gene from *T. timopheevi* NIG to *T. durum* *reichenbachii*. (Parentheses indicate carriers of the chlorosis gene.)

In the last part of this investigation, 19 strains of emmer wheat belonging to three different species, and 12 strains of *T. timopheevi* were crossed to both *T. dicoccum* cultivar Hokudai and *T. timopheevi* strain NIG as testers for the chlorosis genes. Most of the emmer strains used were collected in 1959 in Mediterranean countries by Yamashita and Tanaka (1960), and were maintained by self-pollination in our laboratory. Their species and variety were identified by Dr. M. Tanaka, Kyoto University. The *T. timopheevi* strains were obtained in 1964 from Max-Planck Institute, all of which were originally collected in Gruzia, Caucasus. The F_1 hybrids between these materials and the two testers were grown in the field, and occurrence of chlorosis was observed, and their genotype for chlorosis was determined.

RESULTS

(1) General observation

Result of the diallele crosses between *T. dicoccum* cultivar Hokudai, *T. durum* var. *reichenbachii* and *T. timopheevi* strain NIG is shown in Table 1; in no case the reciprocal F_1 's showed a difference in their phenotype, indicating that this type of chlorosis is not caused by cytoplasmic factors.

Table 1. Result of the diallele crosses between three species of tetraploid wheat

F_1 hybrids ($\text{♀} \times \text{♂}$)	Phenotype
<i>T. dicoccum</i> Hokudai $\times$ <i>T. durum</i> <i>reichenbachii</i>	normal
reciprocal	"
<i>T. dicoccum</i> Hokudai $\times$ <i>T. timopheevi</i> NIG	chlorotic
reciprocal	"
<i>T. durum</i> <i>reichenbachii</i> $\times$ <i>T. timopheevi</i> NIG	normal
reciprocal	"

Chlorosis appeared in early tillering stage, while in younger stage the plants did not differ from normal seedlings. When seeds were sown in October, chlorosis began to

Fig. 2. Chlorosis appeared in reciprocal F_1 hybrids between *T. dicoccum* Hokudai and *T. timopheevi* NIG. From left to right: *T. dicoccum*, *T. dicoccum* $\times$ *T. timopheevi* F_1 , *T. timopheevi* $\times$ *T. dicoccum* F_1 and *T. timopheevi*.

Fig. 3. Chromosome pairing in MI of PMC's in B_3 plants (*T. timopheevi* NIG $\times$ *T. durum* *reichenbachii**).
a. 14_{II} found in plant, 275-9.
b. $13_{II} + 2_I$ found in plant, 277-3.

appear in December; though tillering was almost normal, chlorosis progressed gradually from older leaves to younger ones, and from leaf tip to base. Chlorotic plants at this stage appeared pale-green rather than yellow-green, the common appearance of the previously known type. In April all leaves showed severe chlorosis and stunted growth, and as a consequence no plants headed. Appearance in the middle of May of the reciprocal F_1 hybrids between *T. dicoccum* cultivar Hokudai and *T. timopheevi* strain NIG is shown in Fig. 2 with their parents for comparison.

(2) *Analysis of the genes for chlorosis*

Results of the cross, (*T. dicoccum* cultivar Hokudai $\times$ *T. durum* var. *reichenbachii*) $F_1 \times$ *T. timopheevi* strain NIG, are summarized in Table 2. In all five test-crossed populations, segregation into normal and chlorotic plants fitted well to 1:1 ratio, indicating that *T. dicoccum* cultivar Hokudai carries a single chlorosis gene which interacts with the gene of *T. timopheevi*.

As described in "Materials and Methods", transfer of the *T. timopheevi* gene to emmer background has been attempted by backcross method. In the B_1 generation of

Table 2. Segregation of normal and chlorotic plants in the cross, (*T. dicoccum* Hokudai $\times$ *T. durum* *reichenbachii*) $F_1 \times$ *T. timopheevi* NIG

F_1 parent (cross combination)	No. of plants			χ^2 value (1:1)
	Total	Normal	Chlorotic	
266-4 (<i>T. dicoccum</i> $\times$ <i>T. durum</i>)	87	43	44	0.01
"-5 (")	106	53	48	0.94
267-10 (<i>T. durum</i> $\times$ <i>T. dicoccum</i>)	33	15	18	0.27
"-18 (")	80	46	34	1.80
"-19 (")	19	8	11	0.47
Total	325	170	155	0.69

Table 3. Results of the test-cross to *T. dicoccum* Hokudai and the backcross to *T. durum* *reichenbachii* of the B_1 plants, (*T. durum* *reichenbachii* $\times$ *T. timopheevi* NIG) $F_1 \times$ *T. durum* *reichenbachii*

Parent (B_1)	No. of plants from test-cross			No. of plants from backcross
	Total	Normal	Chlorotic	
204-1	2	1	1	1
"-2	1	1	0	0
"-3	2	2	0	2
"-4	17	17	0	12
"-7	4	4	0	7
"-8	17	17	0	13
"-10	3	1	2	3
206-2	7	2	5	13
"-3	3	3	0	0
"-4	4	4	0	2
"-5	5	5	0	5

the cross, (*T. durum* var. *reichenbachii* $\times$ *T. timopheevi* strain NIG) $F_1 \times T. durum$ var. *reichenbachii*, 11 plants were selected, and crossed to both *T. dicoccum* cultivar Hokudai and *T. durum* var. *reichenbachii*. Results of these crosses are summarized in Table 3. In three progenies of the test-cross, some chlorotic plants were obtained among normals. It is evident that the three B_1 plants, 204-1, 204-10 and 206-2, received the chlorosis gene from *T. timopheevi* strain NIG.

Backcrossed offspring of these B_1 's were grown, and 13 B_2 plants selected from them were again crossed to both *T. dicoccum* cultivar Hokudai and *T. durum* var. *reichenbachii*, the results being summarized in Table 4. Test-crossed offspring of nine B_3 plants segregated some chlorotic plants, suggesting that they were the carriers of the *timopheevi* gene.

Backcrossed offspring of these B_2 's were grown, and nine selected B_3 plants were

Table 4. Results of the test-cross to *T. dicoccum* Hokudai and the backcross to *T. durum reichenbachii* of the B_2 plants, (*T. durum reichenbachii* $\times$ *T. timopheevi* NIG) $F_1 \times T. durum reichenbachii^2$

Parent (B_2)	No. of plants from test-cross			No. of plants from backcross
	Total	Normal	Chlorotic	
1961- 2	8	3	5	8
" - 3	14	9	5	9
1963- 1	22	22	0	17
" - 3	21	21	0	21
" - 4	17	17	0	17
" - 5	8	4	4	7
" - 6	16	8	8	12
" - 7	22	10	12	18
" - 8	12	3	9	13
" - 9	1	1	0	14
" - 10	17	11	6	18
" - 11	15	6	9	12
" - 13	15	5	10	11

Table 5. Chromosome pairing in nine B_3 plants and occurrence of chlorosis in their test-crossed offspring to *T. dicoccum* Hokudai

Plant no. (B_3)	No. of PMC's observed	Chromosome association	No. of plants from test-cross		χ^2 -value (1 : 1)
			Normal	Chlorotic	
261-3	50	$1_{III} + 12_{II} + 1_I$	1	0	—
263-3	50	$13_{II} + 2_I$	1	2	—
277-3	50	$13_{II} + 2_I$	1	1	—
Total	150		3	3	0.0
263-8	50	14_{II}	5	0	—
271-4	50	14_{II}	2	0	—
273-1	50	14_{II}	1	0	—
273-4	50	14_{II}	2	0	—
275-7	17	14_{II}	2	0	—
275-9	50	14_{II}	1	0	—
Total	267		13	0	13.0**

** Significant at the 1 % level

further test- and backcrossed. At the same time, chromosome pairing in meiotic metaphase was observed with the results shown in Table 5. Three B_3 plants, 261-3, 263-3 and 277-3, showed a chromosome association, $1_{III}+12_{II}+1_I$ or $13_{II}+2_I$ (Fig. 3b), and two of them segregated chlorotic plants in test-crossed offspring. Plant 261-3 did not segregate any chlorotic plant, probably due to a very small size of the test-crossed offspring. One of the two univalents found in 263-3 and 277-3 must be a *timopheevi* chromosome, that can not form a bivalent with any chromosome of *T. durum* var. *reichenbachii*. Consequently, this chromosome should have belonged to the G genome. Formation of a trivalent in 261-3 suggests that this plant carried reciprocal translocations between an emmer chromosome of A or B genome and the G genome chromosome from *T. timopheevi*, because if the translocations were taking place between any two chromosomes of A and/or B genome, a quadrivalent instead of a trivalent plus a univalent should have been formed. Six other B_3 plants had all 14 bivalents (Fig. 3a), and none segregated chlorotic plants. Apparently, they did not receive any chromosome of G genome from *T. timopheevi*. All those informations point to the fact that the complementary gene present in *T. timopheevi* strain NIG is located on a single G-genome chromosome, which has been transferred, at least, to two B_3 plants with 28 chromosomes.

Segregation data in all test-crossed offspring, which segregated normal and chlorotic plants, are summarized in Table 6; their ratios in the B_1 , B_2 and B_3 generations fitted all to the 1:1 ratio. Since all nine B_3 plants, which were derived from B_2 plants carrying one G-genome chromosome, had 28 chromosomes somatically, a great majority of fertilized female gametes from $13_{II}+2_I$ plants seemed to have 14 chromosomes, *i.e.* either the full complement of emmer chromosomes or 13 emmer chromosomes plus one *timopheevi* chromosome. In other words, gametes having only 13 emmer chromosomes or

Table 6. Number of normal and chlorotic plants obtained in segregating lines of the test-cross in three backcross generations

Generation	Parent	No. of plants			χ^2 -value (1:1)
		Total	Normal	Chlorotic	
B_1	204 - 1	2	1	1	—
"	" - 10	3	1	2	—
"	206 - 2	7	2	5	—
"	total	12	4	8	1.33
B_2	1961 - 2	8	3	5	—
"	" - 3	14	9	5	—
"	1963 - 5	8	4	4	—
"	" - 6	16	8	8	—
"	" - 7	22	10	12	—
"	" - 8	12	3	9	—
"	" - 10	17	11	6	—
"	" - 11	15	6	9	—
"	" - 13	15	5	10	—
"	total	127	59	68	0.64
B_3	263 - 3	3	1	2	—
"	277 - 3	2	1	1	—
"	total	5	2	3	0.00
Grandtotal		144	65	79	1.36

Table 7. Occurrence of chlorosis in F₁ hybrids between 34 strains of tetraploid wheat and the two testers, *T. dicoccum* Hokudai and *T. timopheevi* NIG

Strain	Species	Variety	Testers		Designated genotype ¹⁾
			<i>dicoccum</i>	<i>timopheevi</i>	
3591	<i>T. dicoccoides</i>	<i>pseudo-rufovillosum</i>	+	+	<i>chl</i> ₁
4343	<i>T. dicoccum</i>	<i>brunneum</i>	+	+	"
4346	"	<i>farrum</i>	+	+	"
1684	"	(Hokudai)	+	c	<i>Chl</i> ₁
4060	<i>T. durum</i>	<i>aegyptianum</i>	+	+	<i>chl</i> ₁
4108	"	<i>affine</i>	+	+	"
4067	"	<i>africanum</i>	+	+	"
4351	"	<i>albo-provinciale</i>	+	+	"
4353	"	<i>apilicum</i>	+	+	"
4115	"	<i>hordeiforme</i>	+	+	"
4360	"	<i>italicum</i>	+	+	"
4361	"	<i>leucurum</i>	+	+	"
4053	"	<i>muriciense</i>	+	+	"
4057	"	"	+	+	"
4071	"	"	+	+	"
4098	"	"	+	+	"
4116	"	"	+	+	"
4123	"	"	+	+	"
4126	"	"	+	+	"
4055	"	<i>reichenbachii</i>	+	+	"
1685	"	"	+	+	"
3592	<i>T. timopheevi</i>	<i>viticulosum</i>	c	+	<i>Chl</i> ₂
3593	"	<i>typicum</i>	c	+	"
3594	"	"	c	+	"
3595	"	"	c	+	"
3596	"	"	c	+	"
3597	"	"	c	+	"
3598	"	"	c	+	"
3599	"	"	c	+	"
3600	"	"	c	+	"
3601	"	"	c	+	"
3602	"	"	c	+	"
3603	"	"	c	+	"
1703	"	"	c	+	"

+ : normal F₁, c : chlorotic F₁1) All *timopheevi* strains will possess *chl*₁, if *Chl*₁ locus is in A genome.

a full emmer complement plus a *timopheevi* chromosome (15 chromosomes in total), which are produced by 2:0 segregation of the two univalents, seem to be rarely functional in fertilization. Based on this consideration, the 1:1 segregation ratio obtained from the test-crosses in three successive backcross generations is reasonably understood.

(3) Distribution of the chlorosis genes

Results of the gene analysis described above demonstrated that the single gene of *T. dicoccum* cultivar Hokudai and the gene on a single chromosome of the G genome in *T. timopheevi* strain NIG are complementary to each other in causing chlorosis. A preliminary survey of these genes in emmer and *timopheevi* wheats was carried out; the results are summarized in Table 7. No emmer strains, except *T. dicoccum* cultivar Hokudai, showed chlorosis in their F₁ hybrids with either tester. On the contrary, all *timopheevi* strains gave rise to chlorotic F₁ hybrids, when crossed to *T. dicoccum* cultivar Hokudai. The result clearly indicates that *T. timopheevi* is distinctly different

from the emmer species by having one of the complementary genes for the new type of chlorosis.

DISCUSSION

The kind of chlorosis studied in the present investigation occurred in F_1 hybrids between *T. dicoccum* cultivar Hokudai (genome formula AABB) and *T. timopheevi* var. *typicum* strain NIG (AAGG). The former carried one of the complementary genes for this chlorosis, while a single chromosome of *T. timopheevi*, that belonged to G genome, carried the other complementary gene.

Chlorosis, that has been previously known to us, is controlled by two complementary genes, Ch_1 in A or B genome and Ch_2 on chromosome 3D of D genome. Chlorosis under the present investigation is controlled similarly by two complementary genes, but is clearly different from the previously known type, because one of the complementary genes for the present chlorosis is located in G but not in D genome, and the other complementary gene located in A or B genome of *T. dicoccum* cultivar Hokudai is different from Ch_1 , because F_1 hybrids between *T. dicoccum* cultivar Hokudai and *T. aestivum* cultivar Chinese Spring (Ch_2 -carrier) are completely normal. There is, also, some difference in expression of chlorosis between the two types.

The new type of chlorosis will be called as "chlorosis type 2", the name "chlorosis type 1" being given to the previously known type. The complementary genes for chlorosis type 2 will be symbolized by " Chl ", different from " Ch " for the genes of chlorosis type 1 by a third letter, both meaning chlorosis. The gene in *T. dicoccum* cultivar Hokudai will be designated as Chl_1 and that in *T. timopheevi* as Chl_2 .

The Chl_1 locus is located on a chromosome of A or B genome. If it were in A genome, *T. timopheevi* would possess this locus, if not it can be present only in emmer wheat. The Chl_2 locus is on a G-genome chromosome, therefore emmer wheat can not have it. From these considerations, the genotype for chlorosis type 2 can be designated for all emmer and *timopheevi* strains, which were investigated in the present study

Table 8. Frequency of three possible genotypes for chlorosis in four tetraploid wheat species

Species	No. of strains			
	chl_1	Chl_1	Chl_2	Total
<i>T. dicoccoides</i>	1	0	—	1
<i>T. dicoccum</i>	2	1	—	3
<i>T. durum</i>	17	0	—	17
Total	20	1	—	21
<i>T. timopheevi</i> ¹⁾	—	—	13	13

1) *T. timopheevi* will possess chl_1 in addition to Chl_2 , if Chl_1 locus is located in A genome.

(Table 7). The results are summarized in Table 8. Out of 20 emmer wheats, only *T. dicoccum* cultivar Hokudai possessed *Chl*₁ gene, while all the others carried its recessive allele. All strains of *T. timopheevi* had *Chl*₂ gene, that was, of course, never found in emmer wheat

T. timopheevi is distinctly different from emmer wheat as to some morphological characteristics as well as genome constitution and cytoplasm (Lilienfeld and Kihara 1934, Kihara and Tsunewaki 1967). The present result indicates, in addition, that *T. timopheevi* is genically different from emmer wheat. A future investigation on distribution of this gene in *T. araraticum* (AAGG) and *T. zhukovskyi* (AAAAGG) might throw a further light on the evolution of the *timopheevi* complex in polyploid wheat.

SUMMARY

1. A new type of progressive chlorosis was found to occur in reciprocal F₁ hybrids between *Triticum dicoccum* cultivar Hokudai (2n=28, genome formula AABB) and *T. timopheevi* var. *typicum* strain NIG (2n=28, AAGG); it was named "chlorosis type 2".

2. Two complementary genes cause chlorosis type 2; they were designated by *Chl*₁ and *Chl*₂. *T. dicoccum* cultivar Hokudai carries *Chl*₁ on a chromosome in A or B genome, while *T. timopheevi* strain NIG possesses *Chl*₂ on a chromosome in G genome.

3. Other 19 emmer strains belonging to three different species carried only *chl*₁ gene, while all 13 strains of *T. timopheevi* possessed *Chl*₂ gene; the species, *T. timopheevi*, is distinctly different from emmer wheat as to its population structure for the chlorosis genes.

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