Gene transfer between three groups of wheat. II. Differential transmission rates of deleterious genes in 6x and 4x wheats¹⁾

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ABSTRACT

Three genes representing an indigenous (*B1* gene for awn suppression), an alien (*Hp* gene for hairy peduncle from rye), and a lethal mutant gene (*v1* for virescence), were transferred from common sources to a 6x (*Triticum aestivum* cv. S-615) and 4x wheat (*T. durum* var. *reichenbachii*), by repeated backcrosses using the latter as recurrent pollen parents. In every backcross generation, heterozygotes of each gene were backcrossed as well as self-pollinated, and the transmission rates of these genes to offspring were determined. The *B1* gene showed normal transmission in both 6x and 4x wheats. The *Hp* gene was transmitted normally in 6x wheat, while its transmission rate was slightly depressed (ca. 10%) through both female and male gametes in 4x wheat. The *v1* gene was transmitted at slightly lower frequency (about 10%) than its normal allele in 6x wheat, while its transmission rate was greatly reduced through both female (ca. 40%) and male gametes (ca. 70%) in 4x wheat. Thus, it was concluded that polyploidy increases tolerance of plants to deleterious genes.

1. INTRODUCTION

Polyploid species are known to carry large numbers of duplicated genes (Stephens 1951, McIntosh 1973), for which reason they are believed to be more tolerant than their related diploid species for deleterious genes (Dobzhansky 1951). In fact, it has long been known that polyploids are more resistant to artificially induced, deleterious mutations than diploids (Stadler 1929, and others).

If polyploid species are really more tolerant to deleterious genes than their relatives of lower ploidy, then we should expect higher transmission rates of the deleterious gene in the higher than in the lower ploidy species. A comparison of transmission rates of deleterious genes among related but different ploidy species is another experimental approach to show differential tolerance of polyploids to deleterious genes. In the present investigation, transmission rates of two supposedly deleterious genes were compared between a hexaploid

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common wheat ($2n=6x=42$) and a tetraploid emmer wheat ($2n=4x=28$). As a control, the transmission rate of one of most prevalent genes in common wheat was investigated in the same genetic backgrounds. The results are reported below.

2. MATERIALS AND METHODS

(1) *Gene sources*

(a) Awn suppressor, *B1*: Awnlessness of the common wheat cultivar, Jones Fife, is under control of a single dominant gene, *B1* which is located on the long arm of chromosome 5A (Sears 1954, Tsunewaki and Jenkins 1961). This cultivar was used as the donor of the *B1* gene.

(b) Hairy peduncle gene, *Hp*: The hairy peduncle character had originally derived from rye through a spontaneous translocation of the chromosome segment carrying the gene, *Hp*; the gene was translocated to the left arm of chromosome 4A of the wheat line, Cornell Wheat Selection 82a1-2-47, and subsequently was transferred to the common wheat cultivar, Chinese Spring, by backcrosses (Driscoll and Sears 1965). The distance between the centromere and the breakpoint of the translocation of the rye chromosome segment is estimated to be about 30 map units (Driscoll and Sears 1965). A strain of Chinese Spring carrying this translocation, provided by Dr. E.R. Sears, was used in the present investigation as the donor of the *Hp* gene.

(c) Virescent gene, *v1*: A virescent seedling was found by Neatby (1933) in the F_2 generation of a cross, Garnet x Double Cross, both belonging to common wheat, *T. aestivum*. This virescent character was transferred to cv. Chinese Spring, and the gene *v1* responsible for it was shown to be located on the short arm of chromosome 3B (Sears 1954, Steinitz-Sears 1966). A heterozygote (*V1v1*) of Chinese Spring, also provided by Dr. E.R. Sears, was used in the present investigation as the donor of this gene.

(2) *Transfer of the three genes to a 6x and 4x wheat*

The donors of the three genes (*B1*, *Hp* and *v1*), all being hexaploid ($2n=6x=42$, genome constitution AABBDD), were repeatedly backcrossed to a hexaploid common wheat, *Triticum aestivum* cv. S-615 ($2n=6x=42$, AABBDD) and to a tetraploid emmer wheat, *T. durum* var. *reichenbachii* ($2n=4x=28$, AABB) as recurrent pollen parents. Since all three genes are located on an A or B genome chromosome, their transmission should not be affected by the presence or absence of D genome chromosomes.

In each backcross generation, a variable number of heterozygotes were self-pollinated as well as backcrossed to the recurrent pollen parent, and their offspring were grown and examined for segregation of the characters controlled by these genes. In the case of the *v1* gene, segregation of the virescent

character could be examined only among the self-pollinated offspring.

(3) *Transmission of the genes through female and male gametes*

In the main parts of this investigation, the transmission rates of the three genes were examined using backcrossed and selfed offspring of heterozygotes, as mentioned above. However, in order to estimate the transmission rates through the female and male gametes of 4x wheat, the heterozygotes were reciprocally test-crossed to *T. durum*, and the segregation patterns of the characters controlled by these genes were observed. In the case of the *v1* gene, however, individual plants raised from these test-crosses were selfed, and the transmission rates were determined by the frequency of segregating lines for virescence.

3. RESULTS

(1) *Transfer of the B1 gene, and its transmission rates in 6x and 4x wheats*

The *B1* gene for awn suppression in *T. aestivum* cv. Jones Fife (genotype *B1B1*) was transferred to fully awned *T. aestivum* cv. S-615 and *T. durum* var. *reichenbachii* (both *b1b1*) by repeated backcrosses, using the latter as recurrent pollen parents. During the course of transferring the *B1* gene to 4x wheat (*T. durum*), plants having abnormal chromosome number should have segregated. Therefore, in the B_3 generation plants having 14 bivalents at meiosis (Fig. 1a) were saved and used as the backcross parent. Ears of S-615, *T. durum* and the awnless types produced by repeated backcrosses are shown in Fig. 2.

The results of segregation of awnless (*B1*-carrier) and awned plants (non-carrier) in the backcrossed as well as selfed progeny of the heterozygotes are summarized in Table 1. The results of χ^2 -tests on the segregation ratio of awnless vs. awned ear type revealed that the transmission rates of the *B1*

Fig. 1. Chromosome pairing in MI of PMC in B_3 plants of *T. durum* var. *reichenbachii* (recurrent parent) backcrossed to the donor (common wheat, $2n=42$) of the *B1*, *Hp* and *v1* gene.

(a) *B1b1* heterozygote ($2n=28, 14''$), (b) *Hphp* heterozygote ($2n=28, 14''$), and (c) *V1v1* heterozygote ($2n=29, 14''+1'$).

Fig. 2. Ears of *T. aestivum* cv. S-615, *T. durum* var. *reichenbachii*, and their *B1*, *Hp* and *v1* carriers (the *B1* and *Hp* carriers are homozygote, and the *v1* carrier is heterozygote of the respective gene).

(a) Normal line and *B1*, *Hp* and *v1* carriers of *T. aestivum* cv. S-615, and (b) normal line and *B1*, *Hp* and *v1* carriers of *T. durum* var. *reichenbachii* (both left to right).

gene in both the female and male sides did not differ from those of its allele, *b1*, in 6x or 4x wheats.

Transmission of the *B1* gene in 4x wheat, through female and male gametes, was further tested by reciprocal crosses between the heterozygote and *T. durum*, as shown in upper part of Table 2. The results indicated that transmission of the *B1* gene through the female and male gametes was not handicapped at all, but slightly favored compared to its normal allele *b1*. In fact, the transmission rate of *B1* in the backcrossed offspring of 4x wheat was significantly higher than that in 6x wheat ($\chi^2=6.45$, $df=1$), although the rate in the selfed offspring did not differ between 4x and 6x wheat ($\chi^2=0.45$, $df=1$) (ref. Table 1).

(2) Transfer of the gene *Hp*, and its transmission rates in 6x and 4x wheats

A translocated rye chromosome segment carrying the *Hp* gene for hairy peduncle in *T. aestivum* cv. Chinese Spring (genotype *HpHp*) was transferred to non-hairy *T. aestivum* cv. S-615 and *T. durum* var. *reichenbachii* (both *hphp*) by repeated backcrosses, using the latter as recurrent pollen parents. During the course of transferring the *Hp* gene to 4x wheat (*T. durum*), plants forming 14 bivalents at meiosis in the B_3 generation (Fig. 1b) were saved and used as the female parent for further backcrosses. Ears of S-615, *T. durum*

Table 2. *Transmission rates of the B1, Hp and v1 genes through the female and male gametes in 4x wheat, T. durum*

Gene	Gamete	Cross combination	No. plants			% Transmission rate	χ^2 -value (1:1)
			Total	Carrier	Non-carrier		
B1	Female	<i>B1b1</i> × <i>durum</i> (<i>b1b1</i>)	239	133	106	55.6	3.05
"	Male	<i>durum</i> (<i>b1b1</i>) × <i>B1b1</i>	165	89	79	53.9	1.02
"	—	Total	404	222	182	55.0	3.96*
Test for heterogeneity: $\chi^2=0.11$, $df=1$							
Hp	Female	<i>Hphp</i> × <i>durum</i> (<i>hphp</i>)	219	96	123	43.8	3.33
"	Male	<i>durum</i> (<i>hphp</i>) × <i>Hphp</i>	114	50	64	43.9	1.72
"	—	Total	333	146	187	43.8	5.05*
Test for heterogeneity: $\chi^2=0.00$, $df=1$							
v1	Female	<i>V1v1</i> × <i>durum</i> (<i>V1V1</i>)	127	47	80	37.0	8.57**
"	Male	<i>durum</i> (<i>V1V1</i>) × <i>V1v1</i>	75	16	59	21.3	24.65**
"	—	Total	202	63	139	31.2	28.59**
Test for heterogeneity: $\chi^2=4.63^*$, $df=1$							

Note) For the *v1* gene, the progeny test was carried out in order to distinguish *V1v1* (carrier) from *V1V1* (non-carrier).

and the hairy types produced by repeated backcrosses are shown in Fig. 2.

The data on segregation of hairy (*Hp*-carrier) and non-hairy plants (non-carrier) in the backcrossed as well as selfed progeny of the heterozygotes are summarized in Table 3. The results of χ^2 -tests on the segregation ratio of hairy vs. non-hairy peduncle types indicated that transmission rates of the *Hp* gene in both female and male gametes did not differ from those of its normal allele *hp* in 6x and 4x wheats.

The transmission of the *Hp* gene in 4x wheat, through female and male gametes was tested by reciprocal crosses between the heterozygote and *T. durum*, as shown in the middle part of Table 2. The results confirmed that transmission rates of the *Hp* gene (or more correctly the translocated rye chromosome segment) through both the female and male gametes were not different from those of its normal allele *hp* (on normal wheat chromosome). However, when the data from reciprocal crosses were pooled, the *Hp* gene revealed a slightly lower transmission rate than its normal allele, their difference being significant at the 5% level.

(3) *Transfer of the v1 gene, and its transmission rates in 6x and 4x wheats*

The *v1* gene for virescence in *T. aestivum* cv. Chinese Spring (genotype *V1v1*) was transferred to normal *T. aestivum* cv. S-615 and *T. durum* var. *reichenbachii* (both *v1v1*) by repeated backcrosses, using the latter as recurrent

Table 3. Frequency of the hairy peduncle type (Hp-carrier) in the backcrossed and selfed progeny of the Hphp heterozygotes

Generation	No. pro- geny	6x wheat			No. pro- geny	4x wheat			No. plants	% Hairy	χ^2 -value	χ^2 -value
		No. plants				No. plants						
		Total	Hairy	Non-hairy		Total	Hairy	Non-hairy				
a) Backcrossed with the recessive homozygote												
B ₁	3	36	17	19	—	—	—	—	—	—	—	—
B ₂	3	42	19	23	—	—	—	—	—	—	—	—
B ₃	1	16	7	9	2	19	13	6	68	54	2.58	0.60
B ₄	3	43	22	21	4	81	44	37	54	20	0.21	5.05*
B ₅	11	377	190	187	3	43	23	20	187	44	1.38	1.09
B ₆	3	46	31	15	7	333	146	187	42	—	—	—
B ₇	3	54	25	29	3	26	16	10	62	—	—	—
B ₈	3	28	17	11	3	45	19	26	—	—	—	—
B ₉	3	44	22	22	—	—	—	—	—	—	—	—
Total	33	686	350	336	22	547	261	286	47.7	1.14		
Test for heterogeneity: $\chi^2=7.65$, df=8												
b) Selfed												
F ₂	2	28	19	9	—	—	—	—	—	—	—	—
B ₁ F ₂	3	60	47	13	1	5	3	2	60	0.07	0.82	1.05
B ₂ F ₂	3	48	38	10	2	49	34	15	69	3.23	2.26	0.05
B ₃ F ₂	3	60	49	11	4	62	50	12	81	—	—	—
B ₄ F ₂	3	56	41	15	3	50	32	18	64	—	—	—
B ₅ F ₂	56	1467	1105	362	7	661	479	182	72	—	—	—
B ₆ F ₂	3	50	39	11	3	59	45	14	76	—	—	—
B ₇ F ₂	3	58	47	11	—	—	—	—	—	—	—	—
B ₁₀ F ₂	3	59	42	17	—	—	—	—	—	—	—	—
Total	79	1886	1427	459	20	886	643	243	72.6	2.78		
Test for heterogeneity: $\chi^2=4.54$, df=8												

Note) χ^2 -test was made against the 1 : 1 or 3 : 1 ratio for the backcrossed and selfed progeny, respectively.

pollen parents. The heterozygote, *V1v1*, was used as the gene donor because the homozygote, *v1v1*, is inviable due to a chlorophyll deficiency. During the course of transferring the *v1* gene to 4x wheat (*T. durum*), plants having abnormal chromosome numbers should have segregated. A few heterozygous plants (*V1v1*) were selected for cytological examination, and were found to have a single D-genome chromosome in addition to the normal chromosome complements of *T. durum*, i.e., $2n=29$, showing $14''+1'$ or $13''+1'''$ configurations (Fig. 1c). However, later backcross generations were not examined cytologically.

Since this gene is recessive, segregation of the virescent character occurs only in selfed progeny of heterozygotes. The results of segregation are summarized in Table 4. In 6x wheat, segregation of virescents vs. normals did not show significant deviation from a 1:3 ratio in any generation; however, pooled data revealed a significantly (5% level) lower frequency (22.5%) of virescents than the expected 25%, while, no heterogeneity was observed among F_2 to $B_{10}F_7$ generation progeny. These facts indicate that the transmission of this gene is slightly depressed; the degree of depression is estimated to be about 13% by comparing the actual number of virescents (420 plants) to that expected from the frequency of normals, i.e., one-third of the normals (482 plants). In 4x wheat, the segregation ratio deviated significantly from the expected 1:3 ratio in all generations as well as in the pooled data, and, again, no heterogeneity of that ratio was exposed among seven generations of progeny. The frequency of virescents rarely reached 10%, the average for all generations being about 5%. The degree of depression of *v1* gene transmission was estimated to be about 86%. A χ^2 -test revealed a significant (1% level) difference between 4x and 6x wheats measured by the segregation ratio of virescents ($\chi^2=196.5$, $df=1$). These facts clearly indicate that the transmission of the *v1* gene is depressed six to seven times more in 4x wheat than in 6x wheat. In fact, the growth of virescent seedlings of 4x wheat was more retarded than that of 6x wheat, as shown in Fig. 3.

The transmission of the *v1* gene in 4x wheat through female and male gametes was tested by reciprocal crosses between heterozygotes and *T. durum*, as shown in lower part of Table 2. In this test, all plants raised from the crosses were self-pollinated, and the segregation of virescent plants among the selfed progenies was observed. The results demonstrated that the transmission of the *v1* gene is greatly depressed in both female and male gametes, and that the depression is more severe on the male (ca. 73% depression) than on the female side (ca. 41% depression). From the transmission rates of the *v1* gene through female and male gametes, we should expect about 7.8% virescent plants in the selfed offspring of heterozygotes, a somewhat higher rate than observed (4.6%) (Table 4). This discrepancy is probably due to a zygotic weakness that leads to partial failure of germination.

Table 4. *Frequency of the virescent type (v1v1 homozygote) in the selfed progeny of the V1v1 heterozygote*

Generation	No. progeny	6x wheat				4x wheat			
		No. plants		% Virescent	χ^2 -value	No. plants		% Virescent	χ^2 -value
		Total	Normal			Total	Normal		
F ₂	4	90	67	23	0.01	—	—	—	—
B ₁ F ₂	6	115	89	26	0.02	125	117	8	23.06**
B ₂ F ₂	7	96	78	18	2.00	34	34	0	11.83**
B ₃ F ₂	4	78	57	21	0.15	44	39	5	4.36*
B ₄ F ₂	4	171	138	33	2.96	64	59	5	10.08**
B ₅ F ₂	8	155	116	39	0.00	440	424	16	107.10**
B ₆ F ₂	5	97	77	20	0.99	599	573	26	136.35**
B ₇ F ₂	9	174	140	34	2.77	40	38	2	8.35**
B ₈ F ₂	5	95	73	22	0.17	—	—	—	—
B ₉ F ₂	1	16	11	5	0.08	—	—	—	—
B ₁₀ F ₂	3	59	46	13	0.28	—	—	—	—
B ₁₀ F ₃ -B ₁₀ F ₇	35	720	554	166	1.45	—	—	—	—
Total	91	1866	1446	420	22.5	1346	1284	62	298.56**

Test for heterogeneity: $\chi^2=4.70$, df=11 $\chi^2=2.25$, df=6(Note) χ^2 -test was made against the 3 : 1 ratio.

Fig. 3. Virescent seedlings segregated in the selfed offspring of the heterozygote, V1v1, of *T. durum* var. *reichenbachii* (left) and *T. aestivum* cv. S-615 (right).

4. DISCUSSION

A single dose of the *B1* gene in the A genome of wheat greatly suppresses awn development, resulting in awnless or awnletted phenotypes of ears. This gene is found frequently among common wheat cultivars (ca. 36%), and at a higher frequency within advanced than within old cultivars (Tsunewaki 1966). On the contrary, carriers of this gene are never found in wild and cultivated einkorn wheats or in wild emmer wheat, and only rarely in cultivated emmer wheat (ca. 5%). In order to identify the presence of the *B1* gene in a rare awnless type of emmer cultivars, the *B1* homozygote of *T. durum* produced here was crossed to an awnless emmer cultivar, and the segregation of fully awned types in the F_2 generation was observed; however, no awned segregants were found among 95 F_2 plants. So, it was concluded that a rare awnless emmer cultivars carry the *B1* gene. This gene is thus considered to be indigenous to both 6x and 4x wheats, and normally transmitted to offspring through both female and male gametes, in both 6x and 4x wheats.

The *Hp* gene carried on a rye chromosome that was spontaneously translocated to the 4A wheat chromosome, is literally alien to wheat. This gene has been normally transmitted to offspring through both female and male gametes in a 6x wheat. In 4x wheat, on the contrary, the transmission rate of this gene was significantly (5% level) lower than the expected 50% in one of the backcross generations (B_6). In the pooled data of all backcross generations, the frequency of hairy type (*Hp*-carrier) was 9% lower than that of non-hairy type (non-carrier), although their difference was not statistically significant. The same tendency was recognized among the selfed offspring, i.e., the frequency of the hairy type was 10% lower than that expected based on the frequency of the non-hairy type. These facts suggest that the transmission of the *Hp* gene is about 10% depressed in 4x wheat, compared with its normal transmission behavior in 6x wheat. The depression clearly occurs

equally on both the female and male sides; the data shown in Table 2 indicate about 12% depression of transmission on both sides.

The *v1* gene located on chromosome 3B is a mutant gene that occurred spontaneously in common wheat; it causes inviability of homozygotes due to a chlorophyll deficiency. Two loci homoeoallelic to the *v1* locus were found on chromosome 3A and 3D, and were designated *V2* and *V3*, respectively (Sears 1957). S-615 carries both *V2* and *V3* alleles, while *T. durum* lacks *V3*. In 6x wheat, the transmission rate of the *v1* gene was slightly lower (10% if the rate is the same in female and male gametes) than that of its normal allele (*V1*). In 4x wheat, on the other hand, the transmission rates of *v1* through female and male gametes were 59% and 27%, respectively, of that of *V1*. Furthermore, the germination rate of the *v1* homozygote was about 60% of that of normal seeds (*V1v1* and *V1V1*, inclusively). These considerations lead to the conclusion that the transmission of *v1*, through both gametes, and the viability of *v1v1* homozygote are greatly reduced in 4x wheat, compared to 6x wheat.

These results show that the indigenous gene *B1* exhibits normal transmission behavior in both 6x and 4x wheats, but that the alien gene *Hp* and the lethal mutant gene *v1* exhibited a more or less pronounced reduction of transmission rates in 4x wheat, compared to 6x wheat. Neither 6x nor 4x wheats carry isoalleles to *B1*, *Hp* and *v1*. Thus, it is concluded that polyploidy increases the tolerance of plants to deleterious genes, probably because of an increased number of homoeoalleles of the deleterious genes.

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