Z. Pflanzenzüchtg. 95, ■—■ (1985)

ISSN 0044-3298 / InterCode: ZEPZAD

Department of Plant Pathology, Kansas State University, Manhattan (U.S.A.)

The Use of *phl* Gene in Direct Genetic Transfer and Search for *Ph*-Like Genes in Polyploid *Aegilops* Species

H. C. SHARMA and B. S. GILL

With 3 tables

Received November 19, 1984 / Accepted December 1, 1984

Abstract

Triticum aestivum cv. 'Chinese Spring' (CS) and its *phl* mutant were crossed to six *Aegilops* polyploid species-*columnaris*, *triaristata*, *triuncialis*, *variabilis*, *cylindrica* and *turcomenica* (= *juvenalis*), and the F_1 hybrids were backcrossed to CS and *phl* mutant to assess usefulness of *phl* mutant for the direct introduction of alien variation from these species into wheat and to detect genes in *Aegilops* species epistatic to the *Ph* locus. F_1 plants were self-sterile. CS⊗*Aegilops* F_1 hybrids readily produced backcross seed but *phl* mutant × *Aegilops* F_1 hybrids set no BC_1 seed on backcrossing, probably because of interference of *phl* gene with restitution division. Direct gene transfer from polyploid *Aegilops* species using the *phl* mutant may, therefore, not be feasible. It is proposed that a high-crossability wheat should be used in the initial cross, and that the *phl* mutant should be used in subsequent backcrosses to induce homoeologous pairing for alien gene transfer. Meiotic pairing was much higher in crosses involving the *phl* mutant than in CS. None of the CS-*Aegilops* F_1 hybrids showed pairing equal to or higher than *phl* × *Aegilops* F_1 hybrids and thus did not provide any evidence of gene(s) in the *Aegilops* species epistatic to the *Ph* locus.

Key words: *Aegilops* — *Triticum aestivum* — chromosome pairing — gene transfer — *Ph* gene

Diploid-like meiosis in the polyploid species of *Triticum* is due to the suppression of homoeologous chromosome pairing by the dominant gene *Ph* on chromosome 5BL (OKAMOTO 1957, RILEY and CHAPMAN 1958). In addition to *Ph*, genes suppressing homoeologous pairing are present on chromosome 3A, 3D and 4D, and genes promoting homoeologous pairing are found on 5BS and on both arms of chromosome 5A and 5D (SEARS 1976). The effect of *Ph* is also seen in hybrids of wheat with other species. In hybrids of *T. aestivum* and

Contribution no. 85-171-J, Department of Plant Pathology, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, Kansas.

other related species, homoeologous pairing among the wheat chromosomes and between wheat and alien chromosomes can be induced in the absence of 5B or through the suppression of the *Ph* activity by *Aegilops speltoides* (FELDMAN and MELLO-SAMPAYO 1967). Induced homoeologous pairing has been employed to transfer rust resistance from *Ae. speltoides* (DVOŘÁK 1977), *Ae. comosa* (RILEY et al. 1968) and *Agropyron elongatum* (SEARS 1972) into wheat. Recently, DARVEY (1984) has proposed construction of an alien gene bank by employing the *ph* mutant gene in a direct hybridization and breeding program with alien species.

Several authors have studied the origin and distribution of alleles similar to *Ph* in polyploid *Aegilops* species. MCGUIRE and DVOŘÁK (1982) crossed polyploid *Aegilops* species with *T. aestivum* cv. 'Chinese Spring' (CS) monotelosomics that were monosomic for 5B. Some *Aegilops* genotypes showed a suppressive effect on homoeologous pairing in hybrids lacking 5B whereas others had no effect. A number of genotypes promoted homoeologous pairing in the presence of 5B. ABUBAKER and KIMBER (1982) investigated chromosome pairing in F_1 hybrids involving eight polyploid and one diploid species of *Aegilops* with *T. aestivum* deficient for chromosome 5B. This allowed the recognition of genes in *Aegilops* species similar to those found on chromosome 5B but no such evidence was obtained.

The above studies involved 5B aneuploids. Our aim was to assess the usefulness of the *phl* mutant (SEARS 1984) for the introduction of alien variation from wild species into wheat by direct hybridization and to detect genes in *Aegilops* species epistatic to the *Ph* locus. Long-term objective of the wheat × *Aegilops* crosses is to create germplasm resistant to wheat pathogens (GILL et al. 1985).

Material and Methods

Five tetraploid and one hexaploid species of *Aegilops* (*Table 1*), and CS and its *phl* mutant were used in this study. One to four accessions of *Aegilops* species were used. The seed of these accessions came from Dr. J. G. WAINES, U. C. Riverside.

Hybrids were made between *Aegilops* species and CS, and between *Aegilops* species and *phl* mutant. The hybrids were raised from mature seed and grown in the greenhouse. Root tip counts of chromosomes were made to establish the genuineness of F_1 hybrids. Spikes from the F_1 hybrids were fixed overnight in Carnoy's solution at room temperature, anthers were squashed in 1 % acetocarmine and frequencies of various meiotic configurations were recorded.

The F_1 hybrids were backcrossed to CS and the *phl* mutant as the recurrent parents.

Results and Discussion

The wheat × *Aegilops* species hybrids were vigorous with spike morphology resembling the *Aegilops* parents. Spikes of the F_1 hybrids disarticulated like those of the *Aegilops* species and were non-free threshing. The F_1 hybrid seed was somewhat shrivelled when *phl* was used as the wheat parent.

Tab. 1 Aegilops species used, their genome symbols (after Kimber and Sears 1984), ploidy levels and accession numbers

Aegilops species	Genomes	Ploidy level	K-State Acc. No.	UC Acc. No. (PI No.)
Ae. columnaris	UM	4x	TA2105	G732
Ae. variabilis	US^v	4x	TA1893	G1026
Ae. triuncialis	UC	4x	TA1719	G392 (PI179192)
			TA1720	G393 (PI170193)
			TA1756	G624
			TA1758	G849
Ae. triaristata	UM	4x	TA1863	G623
Ae. cylindrica	CD	4x	TA1845	G413
Ae. turcomenica (= *juvenalis*)	DMU	6x	TA2115	G740

F_1 plants were self-sterile. No BC_1 seed was set on any of the *phl* mutant × *Aegilops* F_1 hybrids whereas CS × *Aegilops* F_1 hybrids set BC. seed on back-crossing (*Table 2*). Our experience has been the same with the diploid species *Ae. caudata* (genome C). The BC_1 is generally produced from rare restitution of female gametes of the F_1 hybrid and high homoeologous pairing in *phl* × *Aegilops* hybrids apparently interferes with restitution division. Also the *phl* mutant used in the present study has been maintained by selfing and may have accumulated some translocations causing sterility and poor seed set. Barring this effect, our results show that direct gene transfer using the *phl* mutant in wide crosses as proposed by DARVEY (1984) may not be feasible. We propose that high crossability lines such as CS should be used in the initial cross, and that *phl* mutant should be used in subsequent backcrosses to induce homoeologous pairing for alien gene transfer.

Tab. 2 BC_1 seed set on CS x Aegilops F_1 hybrids and on phl x Aegilops F_1 hybrids*

F_1 hybrids	No. of florets pollinated	No. of BC_1 seeds set
CS x *Ae. columnaris*	74	8
phl x *Ae. columnaris*	125	0
CS x *Ae. triaristata*	—	2
Ae. triaristata x *phl*	36	0
CS x *Ae. triuncialis*	—	5
phl x *Ae. triuncialis*	225	0
CS x *Ae. variabilis*	64	5
phl x *Ae. variabilis*	92	0
CS x *Ae. cylindrica*	~~224~~ 346	6
phl x *Ae. cylindrica*	136	0

* *Ae. turcomenica* x wheat hybrid not backcrossed
— Data not recorded

Tab. 3 Mean chromosome pairing (and range) in *Triticum aestivum* cv. 'Chinese Spring' (CS) (AABBDD) x Aegilops F_1 hybrids and in Chinese Spring mutant (phl) x Aegilops F_1 hybrids*

F_1 hybrid	Genome	Chromosome number	Cells scored	Chromosome association						Chiasma frequency
				I	Rod II	Ring II	Total II	III	IV	
CS x *Ae. columnaris*	ABDUM	35	57	29.78 (21–35)	2.41 (0–7)	0.02 (0–1)	2.43 (0–7)	0.12 (0–1)	0.00 (0)	2.69 (0–7)
phl x *Ae. columnaris*	ABDUM	35	12	18.83 (14–22)	5.42 (3–8)	0.75 (0–2)	6.17 (4–9)	1.17 (0–2)	0.08 (0–1)	9.50 (7–12)
CS x *Ae. triaristata*	ABDUM	35	35	33.04 (29–35)	0.95 (0–3)	0.00 (0)	0.95 (0–3)	0.02 (0–1)	0.00 (0)	0.99 (0–3)
Ae. triaristata x *phl*	ABDUM	35	10	22.47 (16–30)	4.74 (1–7)	0.38 (0–1)	5.12 (1–7)	0.67 (0–2)	0.07 (0–1)	7.05 (3–12)
CS x *Ae. triuncialis*	ABDUC	35	61	30.61 (24–35)	2.02 (0–5)	0.03 (0–1)	2.05 (0–5)	0.10 (0–1)	0.00 (0)	2.28 (0–6)
phl x *Ae. triuncialis*	ABDUC	35	6	18.00 (14–21)	7.00 (3–9)	0.50 (0–2)	7.50 (4–9)	0.67 (0–2)	0.00 (0)	9.34 (8–11)
CS x *Ae. variabilis*	$ABDUS^v$	35	18	30.00 (26–35)	2.33 (0–4)	0.00 (0)	2.33 (0–4)	0.11 (0–1)	0.00 (0)	2.55 (0–5)
phl x *Ae. variabilis*	$ABDUS^v$	35	5	8.60 (1–12)	9.00 (6–12)	1.40 (1–2)	10.40 (7–14)	1.60 (1–2)	0.20 (0–1)	15.60 (13–20)
Ae. turcomenica x CS	ABDDMU	42	35	30.95 (22–36)	4.76 (2–9)	0.66 (0–3)	5.42 (2–10)	0.07 (0–2)	0.00 (0)	6.22 (3–11)
Ae. turcomenica x *phl*	ABDDMU	42	23	22.15 (10–30)	6.19 (3–9)	1.17 (0–5)	7.36 (3–11)	1.39 (0–6)	0.24 (0–2)	12.03 (7–21)

* Data not recorded on wheat x Ae. cylindrica F_1 hybrid

Chromosome pairing was much higher in *phl* mutant × *Aegilops* F_1 hybrids than in CS × *Aegilops* F_1 hybrids (*Table 3*). There was a large increase in the mean number of chiasmata/cell in all the hybrids with the *phl* mutant. Our results on chromosome pairing are in agreement with those of the previous workers (ABUBAKER and KIMBER 1982, GIORGI and BARBERA 1981, LACADENA 1967, LACADENA and AZPIAZU 1969, MCGUIRE and DVOŘÁK 1982, RILEY and LAW 1965). Differences, if existing, may be due to tetraploid wheat (GIORGI and BARBERA 1981) or different *Aegilops* genotypes and 5B aneuploids (ABUBAKER and KIMBER 1982, LACADENA 1967, LACADENA and AZPIAZU 1969, MCGUIRE and DVOŘÁK 1982, RILEY and LAW 1965) used. The trend, however, is very much the same: meiotic pairing was much higher in crosses involving the *phl* mutant than in those with CS. Higher pairing in the *Ae. turcomenica* × CS hybrid than in other CS-*Aegilops* spp. hybrids (*Table 4*) could be due to the D genome being common to *Ae. turcomenica* and wheat. Pairing lower than expected may be due to differentiation of the D genome in *Ae. turcomenica* (KIMBER and ZHAO 1983).

As none of the CS × *Aegilops* F_1 hybrids showed pairing equal to or higher than *phl* × *Aegilops* F_1 hybrids, these results on chromosome pairing do not provide any evidence of gene(s) in the tested *Aegilops* species epistatic to the *Ph* locus and confirm the observations of RILEY and LAW (1965), ABUBAKER and KIMBER (1982), and MCGUIRE and DVOŘÁK (1982) that polyploid *Aegilops* species do not possess a gene that has an activity equivalent to that of *Ph*.

ABUBAKER and KIMBER (1982) tested *Ae. triuncialis*, *Ae. triaristata*, *Ae. columnaris*, *Ae. machrochaeta*, *Ae. ovata*, *Ae. kotschyi*, *Ae. cylindrica* and *Ae. juvenalis*. MCGUIRE and DVOŘÁK (1982) tested six, and we tested four, of these eight species. We additionally tested *Ae. variabilis*. This still leaves five more polyploid species of *Aegilops* yet to be tested for the presence of such genes.

Apparent absence of *Ph*-like genes in tested polyploid *Aeligops* species is enigmatic. This apparent paradox, and its resolution have been discussed in detail by MCGUIRE and DVOŘÁK (1982). It is also possible that genes regulating pairing in polyploid *Aegilops* may not be the same as in wheat, that they may not interact with *Ph* locus, or that they could be recessive to the *Ph* locus. It may also be noted that ours was only a small sample and should not be interpreted to mean that genes epistatic to *Ph* do not exist at all. Furthermore, genetic variation within each *Aegilops* species needs to be tested before the existence of such genes is ruled out.

Zusammenfassung

Die Verwendung des phl-Gens für direkten genetischen Transfer und für die Suche nach ähnlichen Genen in polyploiden *Aegilops*-Spezies

Triticum aestivum cv. 'Chinese Spring' (CS) und seine *phl*-Mutante wurden mit den sechs polyploiden *Aegilops*-Arten *columnaris*, *triaristata*, *triun-*

cialis, *variabilis*, *cylindrica* und *turcomenica* (= *juvenalis*) gekreuzt. Die sterilen F_1-Hybriden wurden mit Weizen zurückgekreuzt. Während mit der F_1 aus CS × *Aegilops* leicht Rückkreuzungssaatgut erhalten wurde, blieben die Versuche mit der F_1 aus der Mutante ergebnislos. Ein direkter Gentransfer aus polyploiden *Aegilops*-Arten mit Hilfe der *phl*-Mutante scheint somit nicht durchführbar. Es wird daher vorgeschlagen, die *phl*-Mutante nicht in der ersten Kreuzung, sondern erst in den Rückkreuzungen zu verwenden. Keine der CS × *Aegilops* F_1-Pflanzen zeigte gleichgroße oder stärkere Paarung als die *phl* × *Aegilops* F_1-Pflanzen, was nicht auf Gene in den *Aegilops*-Arten hindeutet, die epistatisch zu *ph* sind.

References

ABUBAKER, M., and G. KIMBER, 1982: Chromosome pairing regulators in the former genus *Aegilops*. Z. Pflanzenzüchtg. **89**, 130—138.

DARVEY, N. L., 1984: Alien wheat bank. Genetics **107** (Suppl.), 24.

DVOŘÁK, J., 1977: Transfer of leaf rust resistance from *Aegilops speltoides* to *Triticum aestivum*. Can. J. Genet. Cytol. **19**, 133—141.

FELDMAN, M., and T. MELLO-SAMPAYO, 1967: Suppression of homoeologous pairing in hybrids of polyploid wheats × *Triticum speltoides*. Can. J. Genet. Cytol. **9**, 307—313.

GILL, B. S., H. C. SHARMA, W. J. RAUPP, L. E. BROWDER, J. H. HATCHETT, T. L. HARVEY, J. G. MOSEMAN, and J. G. WAINES, 1985: Evaluation of *Aegilops* species for resistance to powdery mildew, leaf rust, hessian fly and greenbug. Plant Dis. ~~(in press)~~: 69, 314-316.

GIORGI, B., and F. BARBERA, 1981: Use of mutants that affect homoeologous pairing for introducing alien variation in both durum and common wheat. In: Induced Mutations — A Tool in Plant Breeding, pp. 37—47. Vienna: IAEA.

KIMBER, G., and E. R. SEARS, 1983: Assignment of genome symbols in the Triticeae. Sixth Int. Wheat Genet. Symp. ~~(in press)~~ Kyoto, Japan, pp. 1195-1196.

— —, and Y. H. ZHAO, 1983: The D genome of the Triticeae. Can. J. Genet. Cytol. **25**, 581—589.

LACADENA, J. R., 1967: Introduction of alien variation into wheat by gene recombination. I. Crosses between mono-V(5B) *Triticum aestivum* L. and *Secale cereale* L. and *Aegilops columnaris* Zhuk. Euphytica **16**, 221—230.

— —, and A. AZPIAZU, 1969: Introduction of alien variation into wheat. Action of the 5B genetic system on the meiotic behavior of *Triticum aestivum* × *Aegilops ovata* hybrids. Genet. Ibirica **21**, 1—10.

MCGUIRE, P. E., and J. DVOŘÁK, 1982: Genetic regulation of heterogenetic chromosome pairing in polyploid species of the genus *Triticum sensu lato*. Can. J. Genet. Cytol. **24**, 57—82.

OKAMOTO, M., 1957: Synaptic effect of chromosome V. Wheat Info. Serv. **3**, 6.

RILEY, R., and V. CHAPMAN, 1958: Genetical control of the cytological diploid behavior of hexaploid wheat. Nature **182**, 713—715.

— —, — —, and R. JOHNSON, 1968: The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic chromosome synapsis. Genet. Res. **12**, 199—219.

— —, and C. N. LAW, 1965: Genetic variation in chromosome pairing. Adv. Genet. **13**, 57—107.

SEARS, E. R., 1972: *Agropyron*-wheat transfers through induced homoeologous pairing. Can. J. Genet. Cytol. **14**, 736.

——, 1976: Genetic control of chromosome pairing in wheat. Annu. Rev. Genet. 10, 31—51.

——, 1984: Mutations in wheat that ~~enhance homoeologous~~ pairing. Stadler Genet. Symp. ~~(in press)~~.

Authors' addresses: Dr. H. C. SHARMA, Monsanto Agricultural Products Co., Research Division, 700 Chesterfield Village Parkway, Chesterfield, MO 63198 (U.S.A.); Dr. B. S. GILL, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506 (U.S.A.).

Please address reprint requests to the last author.