

Transfer of Wheat-Rye Translocation Chromosomes Conferring Resistance to Hessian Fly from Bread Wheat into Durum Wheat

B. Friebe,* R. G. Kynast, J. H. Hatchett, R. G. Sears, D. L. Wilson, and B. S. Gill

ABSTRACT

The Hessian fly, *Mayetiola destructor* (Say), is a damaging pest of bread wheat, *Triticum aestivum* L., and durum wheat, *T. turgidum* L. ssp. *durum* Desf. Husn., in many wheat production areas of the world. Breeding for host plant resistance is the most agronomically desirable way to control this pest. Twenty-seven major genes conferring resistance to Hessian fly have been identified and used in wheat improvement. These genes confer resistance to specific biotypes of the Hessian fly. Recently, new sources of Hessian fly resistance derived from cultivated rye, *Secale cereale* L., have been reported that confer resistance to all known biotypes of the Hessian fly. The resistance gene *H21* is present on the wheat-rye whole arm translocation T2BS-2R#2L. *H25* is present on an interstitial rye segment in the 4AL arm of the wheat-rye translocation chromosome Ti4AS-4AL-6R#1L-4AL. The objective of the present study was to transfer *H21* and *H25* to tetraploid durum wheat, thereby making these genes available for the improvement of durum wheat. Homozygous T2BS-2R#2L and Ti4AS-4AL-6R#1L-4AL translocation durum lines were recovered that expressed the *H21* and *H25* resistance. The *H25* durum translocation line was vigorous and set seeds similar to the durum wheat parental cultivar. Thus, the *H25* transfer can be used directly in durum wheat improvement. Plant vigor and seed set of the *H21* durum translocation line was drastically reduced, indicating that the missing 2BL arm in this translocation has genes that are essential for normal plant vigor and fertility. Further chromosome engineering is required to shorten the rye segment in this translocation before *H21* can be used in durum breeding.

THE HESSIAN FLY is a destructive pest of bread wheat (*T. aestivum* L., $2n = 6x = 42$, AABBDD) and durum wheat *T. turgidum* L. ssp. *durum* Desf. Husn., $2n = 4x = 28$, AABB) in most production areas of the world. To date, 29 major genes conferring resistance to Hessian fly have been identified and are being used in cultivar improvement (McIntosh et al., 1998). Most of these genes confer resistance only against specific biotypes of the Hessian fly.

Cultivated rye (*S. cereale* L., $2n = 2x = 14$, RR) is an important source of genes for insect and disease resistance in wheat. To date, 12 genes conferring resistance to various diseases and insects have been transferred from rye into wheat (Friebe et al., 1996). The designated genes include those conferring resistance to leaf rust, caused by *Puccinia recondita* f. sp. *tritici* Rob. ex Desm. (Lr25, Lr26, Lr45); stripe rust, caused by *P. striiformis* f. sp. *tritici* Westend. (*Yr9*); stem rust, caused

by *P. graminis* f. sp. *tritici* Eriks. & Henn. (*Sr27*, *Sr31*); powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* DC. E. O. Speer (*Pm7*, *Pm8*, *Pm17*, *Pm20*), greenbug (*Schizaphis graminum* Rond.) (*Gb2*, *Gb6*), and Hessian fly (*H21*, *H25*). Some of these genes are being used in cultivar improvement (Friebe et al., 1996).

Genes *H21* and *H25* confer resistance to biotypes Great Plains 'GP' and biotypes A through L of the Hessian fly (Friebe et al., 1990, 1991; Mukai et al., 1993; Ratcliffe and Hatchett, 1997). *H21* was derived from the rye cultivar Chaupon and was transferred to bread wheat via a wheat-rye translocation that apparently was induced by tissue culture of a wheat-rye hybrid. The resistance gene *H25* was derived from the rye cultivar Balbo and was transferred to wheat by radiation treatment.

The Hessian fly causes severe damage, especially in the main durum wheat producing areas in the Mediterranean and in North Africa. By screening thousands of accessions of durum wheat, only one source of resistance to Hessian fly was identified in a recent survey carried out in Morocco (Nsarellah et al., 1998). Average annual losses in grain yield caused by Hessian fly were estimated to 32% but severe Hessian fly infestation may result in total crop failure (Lhaloui et al., 1992; Nsarellah et al., 1998). At least in North Africa, *H21* and *H25* confer resistance to the prevalent biotypes of the Hessian fly (El Bouhssini et al., 1996). Thus, there is an interest in deploying genes such as *H21* and *H25* for improving durum wheat. The present study reports the transfer of *H21* and *H25* durum wheat improvement.

MATERIAL AND METHODS

The durum parent was the North American cultivar Cando, which was kindly provided by Dr. L.R. Joppa USDA-ARS, North Dakota State University, Fargo. The hard red winter wheat germplasm PI 549276, Hamlet (ND7532/Chaupon//4*ND7532) is the source of the Hessian fly resistance gene *H21*. *H21* is located on the long arm rye chromosome 2R#2, which is present in PI 549276 in the form of a compensating whole arm translocation T2BS-2R#2L (Sears et al., 1992). The hard red winter wheat germplasm KS92WGRC20, PI 592732 (TAM-101/4/Suwon92/Balbo rye//TAM-106/3/Amigo) (Sebesta et al., 1997) is the source of *H25*. *H25* is located on a 0.7- μ m long rye segment derived from the distal region of rye chromosome arm 6R#1L inserted into the long arm of wheat chromosome 4A in form of an interstitial Ti4AS-4AL-6R#1L-4AL wheat-rye translocation.

Chromosome identification was based on the standard karyotypes of wheat (Gill and Kimber, 1974a) and rye (Gill and Kimber, 1974b; Mukai et al., 1992) and was determined by the standard C-banding protocol described by Gill et al. (1991). Genomic in situ hybridization (GISH) analysis was according to Jiang et al. (1994). The resistance evaluations were according to Hatchett et al. (1981) and Friebe et al.

B. Friebe, R.G. Kynast, D.L. Wilson, and B.S. Gill, Dep. of Plant Pathology, Wheat Genetics Resource Center, Plant Sciences Building, Throckmorton Hall, Kansas State Univ., Manhattan, Kansas 66506-5502; J.H. Hatchett, USDA-ARS, Dep. of Entomology, Waters Hall, Kansas State Univ., Manhattan, Kansas 66506-5502; R.G. Sears, Dep. of Agronomy, Plant Sciences Building, Throckmorton Hall, Kansas State Univ., Manhattan, Kansas 66506-5502. Received 15 Dec. 1998. *Corresponding author (friebe@ksu.edu).

(1990). Plants in the seedling stage were evaluated for their reaction to biotype L of the Hessian fly. Biotype L is the most virulent biotype presently found in North America. The larvae can infest wheat with *H1* through *H8*, *H11*, and *H15*, but not wheat with *H9*, *H10*, *H12*, *H13*, *H14*, or *H16* through *H19*. To determine the seed set after self-pollination, the number of seeds per spikelet was determined in five spikes per line.

Cando was crossed as a female with PI 549276 and KS92WGRC20 and the F1 was backcrossed as male and female to Cando. Plants heterozygous for the wheat-rye translocations in the BC1F1 through BC3F1 were identified by C-banding. Plants homozygous for the wheat-rye translocations were recovered in the BC2F2 and BC3F2 generations, and their progenies were evaluated for their reaction to biotype L of the Hessian fly.

RESULTS

Transfer of *H21* into Durum Wheat

The crosses Cando/PI 549276 and Cando/KS92WGRC20 produced 4 and 10 seeds, respectively. Half of the F1 seeds were grown in the spring in a greenhouse. All plants had $2n = 35$ chromosomes but died as seedlings (Table 1). The remaining F1 seeds were grown in the greenhouse in the fall. Under cooler growing conditions, the plants with $2n = 35$ chromosomes flowered and were backcrossed as males and females with Cando.

Five BC1F1 seeds from the cross Cando//Cando/PI 549276 were grown, which had $2n = 30$ (1), 31 (1), 33 (2), and 34 (1) chromosomes and three of them were heterozygous for T2BS·2R#2L. Twelve BC1F1 seeds from the reciprocal cross Cando/PI 549276//Cando were grown, which had $2n = 30$ (1), 31 (2), 32 (3), 33 (4), and an unknown number of chromosomes (2). Only one of these plants was heterozygous for T2BS·2R#2L. All BC1F1 plants heterozygous for T2BS·2R#2L had severe, multi-branched spike deformations and were crossed with Cando as males and females. The BC2F1 seeds were plump and white when Cando was used as female and usually red and shriveled when Cando was used as male parent.

All 12 BC2F1 progenies obtained from the backcross with Cando as female were disomic for chromosome 2B, while among 17 progenies obtained with Cando as male, with $2n = 28$ (11) and 29 (6) chromosomes, six were heterozygous for T2BS·2R#2L. Plants heterozygous for T2BS·2R#2L had no spike deformations and were backcrossed as males with Cando and also selfed. All BC2F2-derived seeds were plump and white and sixteen were grown and analyzed cytologically. All plants had $2n = 28$ chromosomes; three were homozygous for T2BS·2R#2L, five were disomic for 2B, and eight were heterozygous for T2BS·2R#2L. Although the heterozygous plants were vigorous and fertile, the plant homozygous for T2BS·2R#2L were very weak and completely male sterile. Thus, plants heterozygous for T2BS·2R#2L were backcrossed as males to Cando. Eight of the BC3F1 seeds were grown and three were heterozygous for T2BS·2R#2L and these plants were selfed.

The BC3F2 seeds were plump and white. Twenty-

Table 1. Pedigrees, chromosome numbers, and status of the wheat-rye translocation chromosome T1BL·2R#2L in in backcross derivatives of the durum wheat cultivar Cando with the *T. aestivum* PI 549276, the source of the Hessian fly resistance gene *H21*.

Pedigree	$2n$		
	Number of plants	Chromosome number	Translocation chromosome
Cando/PI 549276 F1	4	35	+/-
Cando//Cando/PI 549276 BC1F1	1	30	-/-
	1	31	+/-
	2	33	+/-
	1	34	-/-
Cando/PI 549276//Cando BC1F1	1	30	-/-
	2	31	-/-
	3	32	-/-
	4	33	-/-
	1	unknown	+/-
Cando*2//Cando/PI 549276 BC2F1	1	unknown	-/-
	12	unknown	-/-
Cando/PI 549276//2*Cando BC2F1	3	28	+/-
	8	28	-/-
	3	29	+/-
	3	29	-/-
Cando/PI 549276//2*Cando BC2F2	3	28	+/+
	8	28	+/-
	5	28	-/-
Cando/PI 549276//3*Cando BC3F1	3	28	+/-
	5	28	-/-
Cando/PI 549276//3*Cando BC3F2	4	28	+/+
	11	28	+/-
	7	28	-/-

one plants with $2n = 28$ chromosomes were grown; four were homozygous for T2BS·2R#2L (Fig. 1a), seven were disomic for 2B, and 11 were heterozygous. The heterozygous plants again were more vigorous and set on average 2.5 seeds per spikelet, which is similar to the recipient durum wheat parent Cando that produced on average 2.2 seeds per spikelet. One of the three homozygous T2BS·2R#2L plants was male sterile, whereas the remaining two plants set on average 1.2 and 0.4 seeds per spikelet. The spikes of the plants homozygous for T2BS·2R#2L were smaller than the durum parent Cando (Fig. 2).

Homozygous T2BS·2R#2L BC3F3 plants were screened for their reaction to the Hessian fly biotype L. All 18 T2BS·2R#2L BC2F3 plants and nine plants of the *H21* donor germplasm PI 549276 were resistant and larvae died, whereas all the 36 plants of the recipient durum cultivar Cando were susceptible and had live larvae.

Transfer of *H25* into Durum Wheat

Nine BC1F1 seeds from the cross Cando//Cando/KS92WGRC20 were grown, which had $2n = 29$ (1), 30 (4), 31 (2), and 33 (2) chromosomes (Table 2) and four of them were heterozygous for Ti4AS·4AL·6R#1L·4AL. Ten BC1F1 seeds from the reciprocal cross Cando/KS92WGRC20//Cando were grown and four of them were heterozygous for Ti4AS·4AL·6R#1L·4AL, with $2n = 28$ (2), 30 (1), and 31 (1) chromosomes. The majority of the plants heterozygous for Ti4AS·4AL·6R#1L·4AL had no spike deformation. No BC2F1 seeds were obtained when Cando was used as female parent, and all BC2F1 seeds from the reciprocal cross were red and shriveled but germinated.

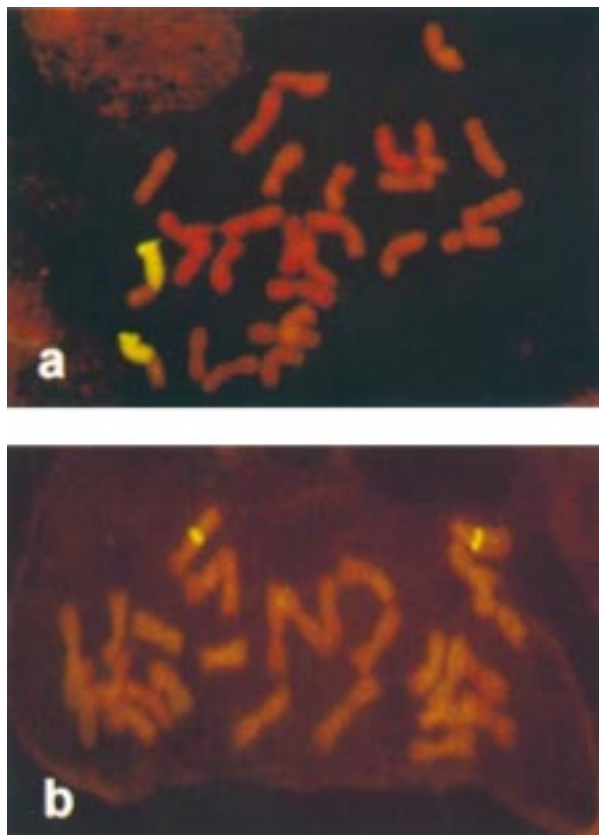


Fig. 1. Genomic in situ hybridization patterns of mitotic metaphase chromosomes of T1BL·2R#2L (a) and Ti4AS·4AL·6R#1L·4AL (b) durum wheat germplasms. Rye chromatin is visualized by yellow FITC fluorescence whereas wheat chromatin is counterstained with propidium iodide and fluoresce red.

Five BC2F1 plants were obtained, which had $2n = 28$ (3) and 29 (2) chromosomes and two of them were heterozygous for Ti4AS·4AL·6R#1L·4AL. These plants had normal spike morphology and were selfed. All BC2F2 seeds were plump and white and the chromosomal constitution was determined in 11 of them. All



Fig. 2. Spike morphologies of the recipient durum wheat cultivar Cando (left), the Ti4AS·4AL·6R#1L·4AL durum wheat germplasm (middle), and the T1BL·2R#2L durum wheat germplasm (right).

Table 2. Pedigrees, chromosome numbers, and status of the wheat-rye translocation chromosome Ti4AS·4AL·6R#1L·4AL in backcross derivatives of the durum wheat cultivar Cando with the *T. aestivum* germplasm KS29WGRC20, the source of the Hessian fly resistance gene *H25*.

Cando/KS29WGRC20 F1	10	35	+/-
Cando//Cando/KS29WGRC20 BC1F1	1	29	-/-
	2	30	+/-
	2	30	-/-
	2	31	-/-
	2	33	+/-
Cando/KS29WGRC20//Cando BC1F1	2	28	+/-
	1	30	+/-
	1	31	+/-
	6	unknown	-/-
Cando/KS29WGRC20//2*Cando BC2F1	1	28	+/-
	2	28	-/-
	1	29	+/-
	1	29	-/-
Cando/KS29WGRC20//2*Cando BC2F2	3	28	+/+
	5	28	+/-
	3	28	-/-

had $2n = 28$ chromosomes; three plants were homozygous for Ti4AS·4AL·6R#1L·4AL (Fig. 1b), three were disomic for wheat chromosome 4A, and five plants were heterozygous for Ti4AS·4AL·6R#1L·4AL.

Homozygous Ti4AS·4AL·6R#1L·4AL BC2F3 plants were vigorous and set on average 2.4 seeds per spikelet after self-pollination, which is similar to the seed set of the durum cultivar Cando. Spike morphology of the homozygous Ti4AS·4AL·6R#1L·4AL translocation line is similar to that of the durum wheat parent Cando, but the awns are more widely spread (Fig. 2).

Thirty-six of the homozygous Ti4AS·4AL·6R#1L·4AL BC2F3 plants together with 17 plants of the *H25* donor germplasm PI 592732 were evaluated for their resistance to the Hessian fly biotype L with the recipient durum cultivar Cando as a control. All the 58 Cando plants tested were susceptible with live larvae whereas all 36 durum wheat plants homozygous Ti4AS·4AL·6R#1L·4AL and the 17 plants of PI 592732 were resistant. All the resistant plants had dead larvae indicating that there were no escapes.

DISCUSSION

Wild relatives and related species are important sources for disease and pest resistance of cultivated bread wheat. A large number of agronomically useful alien genes have been transferred to hexaploid wheat, mainly by irradiation and induced homoeologous recombination. However, only a few of them have contributed to cultivar improvement because of the non-compensating nature of the transfers (Friebe et al., 1996).

The most successful wheat-alien transfers are the wheat-rye whole arm translocations T1BL·1R#1S and T1AL·1R#2S, which are still used worldwide in wheat improvement (Lukaszewski, 1990). The 1RS arm has genes conferring resistance to leaf rust, *Lr26*; stripe rust, *Yr9*; stem rust, *Sr31*; powdery mildew, *Pm8* and *Pm17*; and greenbug, *Gb2* (*Gb6* is present on T1AL·1R#3S) (Friebe et al., 1996). The 1RS arm in these translocations not only compensates for the loss of wheat chromosome arms 1BS and 1AS, but in some genetic backgrounds also has a heterotic effect on grain yield (Villareal et

al., 1991, 1995; McKendry et al., 1996; Singh et al., 1998). RFLP analysis showed that chromosome 1R is the only rye chromosome that is not structurally rearranged compared with the homoeologous groups of wheat (Devos et al., 1993).

So far, only a few wheat-alien translocations have been transferred to durum wheat. Rao (1978) introduced stem rust resistance derived from rye and *Agropyron elongatum* (Host.) P. Beauv. into durum wheat. The wheat-alien translocation chromosomes had no transmission through the pollen, and homozygous translocation lines were not recovered. Friebe et al. (1987, 1993), William and Mujeeb-Kazi (1993), and Mujeeb-Kazi et al. (1996) transferred the T1BL·1R#1S translocation to durum wheat. Homozygous translocation germplasm lines with normal plant vigor and seed set were recovered. Ceoloni et al. (1996) transferred *Pm13*, derived from *Aegilops longissima* chromosome 3S#1, to the short arm of wheat chromosome 3B of bread and durum wheat. The T3BL·3BS·3S#1S translocation has normal male and female transmission in durum wheat. Ceoloni et al. (1996) also transferred the T7AS·7Ae#1·S7Ae#1L wheat-*Agropyron elongatum* translocation (transfer No. 12, Sears, 1973) with the leaf rust resistance gene *Lr19* to durum wheat. However, the lack of male transmission prevented the recovery of homozygous translocation stocks.

In the present study, durum wheat germplasm lines homozygous for the T2BS·2R#2L and Ti4AS·4AL·6R#1L·4AL translocations were recovered that express *H21* and *H25* resistance to Hessian fly, respectively. Spike abnormalities present in earlier backcross generations were not observed in the homozygous translocation lines. The T2BS·2R#2L translocation chromosome was not transmitted through the pollen in BC1F1 plants, but similar to the Ti4AS·4AL·6R#1L·4AL translocation, had normal male and female transmission in the BC2 and BC3. The Ti4AS·4AL·6R#1L·4AL translocation stock is as vigorous as the recipient durum wheat cultivar and has a similar seed set. The *H25* resistant Ti4AS·4AL·6R#1L·4AL durum wheat germplasm can be used directly in breeding programs.

Plants heterozygous for T2BS·2R#2L have plant vigor and seed set similar to the durum wheat parent. Homozygous T2BS·2R#2L plants are less vigorous and set fewer seed. RFLP analysis indicated that the 2RL arm is not structurally rearranged compared with group 2 long arms of wheat (Devos et al., 1993). The 2RL arm in T2BS·2R#2L is expected to compensate well for the loss of wheat chromosome arm 2B. The T2BS·2R#2L translocation at the hexaploid level has no negative effect on plant vigor; however, it drastically reduces vigor and seed set at the tetraploid durum wheat level. Further chromosome engineering is required to shorten the rye segment in T2BS·2R#2L before *H21* can be exploited for durum wheat improvement.

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Isolation and Characterization of Two Promoters from Linseed for Genetic Engineering

Ravinder K. Jain,* R. Gail Thompson, David C. Taylor, Samuel L. MacKenzie, Alan McHughen, Gordon G. Rowland, Don Tenaschuk, and Maxine Coffey

ABSTRACT

Linseed (*Linum usitatissimum* L.) is an important oilseed crop worldwide and is cultivated for the high level of linolenic acid (18:3) in its seed oil. Currently, there is a concerted effort to improve linseed by genetic engineering. This will require appropriate transgenes and tissue-specific or constitutive promoters. We report the isolation and characterization of two linseed promoters from a two-member gene family encoding the enzyme stearoyl-acyl carrier protein desaturase (SAD). The *SAD1* and *SAD2* gene promoter were each fused transcriptionally with the reporter gene for β -glucuronidase (*uidA*; GUS) and were transferred to linseed to study their expression pattern. In transgenic linseed, GUS activity mediated by the *SAD2* promoter appeared to be constitutive and was detected in leaves, apices, stem, roots, flower buds, flowers, and seeds. In contrast, GUS activity mediated by the *SAD1* promoter appeared to be root- and seed-specific. In developing seeds, both the promoters exhibited a temporal expression pattern concomitant with protein and lipid biosyntheses. The GUS activity could be detected as early as 4 days after pollination (dap) and in mature seeds (~50 dap) with the highest activities around mid-development. The first pair of linseed promoters will be useful for manipulating the expression of indigenous as well as transgenes in linseed to create value-added cultivars.

Linseed is the third most important oilseed crop in Canada and an important crop worldwide. It is grown for the high linolenic acid (18:3) content in its seed oil. There is a concerted effort by several laboratories to diversify linseed as a crop by molecular genetic

manipulation. For example, there is a need for creating new linseed cultivars with a wider range of fatty acid composition to supplement the existing food and confections markets [17]. Also, there is commercial interest in the use of linseed as a vehicle for biofarming of pharmaceutically related products because of its self-pollinating nature [13]. A need for linseed varieties tolerant to various abiotic and biotic stresses has also been recognized [17]. A number of herbicide-tolerant linseed varieties that are useful in crop rotation programs are becoming available [17].

Molecular genetic manipulation of linseed can be achieved by expressing appropriate transgenes by means of tissue-specific or constitutive gene promoters. A limited number of promoters have been used in linseed to introduce novel characteristics. Whereas the constitutive promoters such as the CaMV 35S and *nos* gene promoters have been shown to function in linseed [10], the seed specific napin promoter is not effective [14]. Moreover, these promoters are protected by intellectual property laws, which cause unnecessary delays in reaching licensing agreements before the promoters can be used in a breeding program. We, therefore, set out to identify promoters from linseed that can be utilized in our breeding programs.

In linseed, SAD ($\Delta 9$ -18:0-ACP desaturase; EC 1.14.99.6) activity can be detected from about 10 dap to seed maturity (R. Jain et al., 1996, unpublished), suggesting that the promoter of this gene would be useful in manipulating gene expression during seed development. SAD is a soluble enzyme that catalyzes conversion of stearoyl-ACP (18:0-ACP) to oleoyl-ACP (18:1 $\Delta 9$ -ACP) by introducing a double bond at the car-

R.K. Jain, D.C. Taylor, S.L. MacKenzie, D. Tenaschuk, and M. Coffey, National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, SK, Canada S7N 0W9; R.G. Thompson, A. McHughen, and G.G. Rowland, Crop Development Centre, Univ. of Saskatchewan, Saskatoon, SK, Canada S7N 5A8. NRCC No. 42620 Received 2 Feb. 1999. *Corresponding author (Jravidr@pbi.nrc.ca).