Analysis of the functional relationships of gametocidal genes

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ABSTRACT

We have recently produced EMS-induced knock-out mutations at the *Gc2* locus that were derived from *Ae. sharonensis*. Our data suggest that the *Gc2* mutant is a knock-out of the gene encoding for the breaking agent. For analyzing the interactions of the *Gc2* mutants with other Gc genes from different *Aegilops* species, we have crossed stocks with functional and mutant *Gc2* genes with stocks having Gc genes from *Ae. speltoides* (T2B-2S, *Gc1*), *Ae. cylindrica* (DA2C^{c}, *GcAe.*$^{cyl.}$), *Ae. triuncialis* (DtA3C^{t}L, *Gc3*), and *Ae. geniculata* (DA4M^{g}, *GcAe.*$^{genic.}$). If the mode of action of *Gc1*, *Gc3*, *GcAe.*$^{cyl.}$, and *GcAe.*$^{genic.}$ is similar to that of *Gc2,* the introduction of a mutant *Gc2* allele that lacks the factor for the breaking agent and only encodes for the protecting agent might restore the fertility in these plants. The presented data show that in none of the cross combinations analyzed does the introduction of a *Gc2* mutant allele rescue plant fertility suggesting that their modes-of-action are different.

INTRODUCTION

Gametocidal genes are selfish genetic elements that have been introduced from related *Aegilops* species into common wheat, *Triticum aestivum* L. during the production of chromosome addition and alloplasmic lines (1, 8). Gc genes are known to induce chromosome breakage in gametophytes lacking them, leading to preferential transmission of the Gc-carrier chromosome (2, 4, 6). We have produced two EMS-induced knockout mutations at the *Gc2* locus that were transferred from *Ae. sharonensis* Eig to wheat in the form of 4S^{sh} addition/substitution and in the form of the translocation stock T4BS·4BL-4S^{sh}#1L. Because of the selfish transmission behaviour of 4S^{sh}, it was designated as 'cuckoo' chromosome (5). Heterozygous plants with either the *Gc2*^{mut}*#1* or *Gc2*^{mut}*#2* allele and a functional *Gc2* allele do not suffer from gametophytic chromosome breakage and are fully fertile, indicating that the mutant alleles are dominant over the functional *Gc2* allele (3, and unpublished). These data also suggested that *Gc2* encodes for two agents, one causing chromosome breaks in gametophytes lacking *Gc2* and another that protects *Gc2* carriers from breakage and that both mutants are knockouts of the gene encoding for the breaking agent. The present study was initiated to analyze the functional relationships of the *Gc2* mutant alleles with other Gc genes from different *Aegilops* species. If the mode-of-action of *Gc2* is similar to those of other Gc genes, one might expect that the introduction of a mutant *Gc2* allele that lacks the factor for the breaking agent and only encodes for the protecting agent might restore the fertility in these plants.

MATERIAL AND METHODS

T4BS·4BL-4S^{sh}#1L with either the functional *Gc2* allele or the mutant alleles *Gc2*^{mut}*#1* and *Gc2*^{mut}*#2* were crossed with stocks having Gc genes derived from *Ae. sharonensis*, *Gc2*, located on chromosome 4S^{sh}#1; *Ae. speltoides* Tausch, *Gc1*, located on the translocation chromosome T2B-2S; *Ae. triuncialis* L., *Gc3*, located on the telosome 3C^{t}L; *Ae. cylindrica* Host, *GcAe.*$^{cyl.}$, located located on chromosome 2C^{c}; and *Ae. geniculata* Roth, *GcAe.*$^{genic.}$, located on chromosome 4M^{g}. Gametophytic chromosome breakage was analysed in ana-/telophases of the first postmeiotic pollen mitosis according to Nasuda et al. (6). Plant fertility was analysed in three spikes per plant and three plants per genotype and determined as the number of the seeds per spikelet. Fluorescence *in situ* hybridisation (FISH) using clone pGc1R-1, which hybridises to S-genome chromosomes of species belonging to the section Sitopsis but not to any A-, B-, and D-genome chromosomes of wheat was according to Friebe et al. (3).

RESULTS

In cross combinations of DA4S^{sh}#1 with translocation stocks T4BS·4BL-4S^{sh}#1L having either the functional *Gc2* or mutant *Gc2*^{mut}*#1* or *Gc2*^{mut}*#2* alleles, about 10 to 14 % of the ana-/telophases of the first pollen mitosis had chromosome breaks and the fertility ranged from 1.9 to 2.8 seeds per spikelet (Table 1). On the contrary, in cross combinations using the translocation stock T4BS·4BL-4S^{sh}#1L as the carrier of the functional *Gc2* allele and T4BS·4BL-4S^{sh}#1L stocks with *Gc2*^{mut}*#1* or *Gc2*^{mut}*#2* alleles no chromosome breakage was observed in 391 and 565 ana-/telophases analysed, respectively. The low level of breakage observed in combinations using the disomic addition line DA4S^{sh}#1 as the carrier of the functional *Gc2* allele can be explained by non-Mendelian segregation of chromosomes 4B, 4S^{sh}#1, and T4BS·4BL-4S^{sh}#1L as the result of trivalent formation. In cross combinations of a disomic substitution line DS4S^{sh}#7(4B) having the group-4 *Ae. sharonensis* chromosome with a functional *Gc2* gene derived from a different accession and the *Gc2*^{mut}*#1* stock, no chromosome breakage was observed in 320 ana-/telophases analysed, whereas 79 out of 363 ana-/telophases (22%) suffered from chromosome breakage when the *Gc2*^{mut}*#2* mutant stock was used, showing that the second mutant is different and has a 'leaky' phenotype (Friebe et al., unpublished).

In cross combinations of T2B-2S (*Gc1*) with T4BS·4BL-4S^{sh}#1L having the functional *Gc2* allele, 60% of the ana-/telophases

suffered from chromosome breakage resulting in a reduced fertility of 1.1 seeds per spikelet, whereas in *Gc1/- Gc2^{mut}#1/-* hemizygotes 50% of the ana-/telophases had chromosome breaks leading to an improved fertility of 1.5 seeds per spikelet. Nasuda et al. (6) observed a higher percentage of aberrant ana-/telophases (73%) in *Gc1/- Gc2/-* genotypes with 33 gametophytes being normal, 55 having moderate chromosome breakage, and 25 suffering from extensive chromosome breakage and concluded that moderate chromosome breakage occurred in gametophytes lacking either *Gc1* or *Gc2*, whereas gametophytes lacking both had severe chromosome breakage. Our data do not support this assumption because we observed similar amounts of moderate and extensive gametophytic chromosome breakage in both *Gc1/-, Gc2/-* and *Gc1/- Gc2^{mut}#1/-* genotypes. The reduced level of chromosome breakage in *Gc1/- Gc2^{mut}#1/-* genotypes corresponds to that observed in *Gc1/-* hemizygotes (6) and is not the result of interaction between the *Gc1* and *Gc2^{mut}#1* alleles.

Table 1. Gametophytic Gc-gene induced chromosome breakage and plant fertility observed in different cross combinations with functional and mutant *Gc2* alleles.

Cross combination	Genotype	Ana/telophase		Seeds/ spikelet
		Normal	Aberrant (%)	
DA4S^{sh}#1 X T4BS·4BL-4S^{sh}#1L	*Gc2/Gc2*	190	22 (10)	1.9
DA4S^{sh}#1 X T4BS·4BL-4S^{sh}#1L	*Gc2/ Gc2^{mut}#1*	352	46 (12)	2.8
DA4S^{sh}#1 X T4BS·4BL-4S^{sh}#1L	*Gc2/ Gc2^{mut}#2*	286	47 (14)	1.9
T2B-2S X T4BS·4BL-4S^{sh}#1L	*Gc1/- Gc2/-*	123	188 (60)	1.1
T2B-2S / T4BS·4BL-4S^{sh}#1L	*Gc1/- Gc2^{mut}#1/-*	184	185 (50)	1.5
DtA3C^{t}L X T4BS·4BL-4S^{sh}#1L	*Gc3/- Gc2/-*	104	99 (49)	0.1
DtA3C^{t}L X T4BS·4BL-4S^{sh}#1L	*Gc3/- Gc2^{mut}#1/-*	211	3 (1)	0.3
DA2C^{c} X T4BS·4BL-4S^{sh}#1L	*GcAe.$^{cyl.}$/- Gc2/-*	141	115 (45)	0.4
DA2C^{c} X T4BS·4BL-4S^{sh}#1L	*GcAe.$^{cyl.}$/- Gc2^{mut}#1/-*	267	15 (5)	2.1
DA4M^{g} X CS	*GcAe.$^{genic.}$/-*	615	469 (43)	1.7
DA4M^{g} X T4BS·4BL-4S^{sh}#1L	*GcAe.$^{genic.}$/- Gc2/-*	249	295 (54)	1.0
DA4M^{g} X T4BS·4BL-4S^{sh}#1L	*GcAe.$^{genic.}$/- Gc2^{mut}#1/-*	280	179 (39)	1.7
DA4M^{g} X T4BS·4BL-4S^{sh}#1L	*GcAe.$^{genic.}$/- Gc2^{mut}#2/-*	216	166 (43)	1.5

In *Gc3/- Gc2/-* hemizygotes with functional Gc genes derived from *Ae. triuncialis* and *Ae,. sharonensis,* 49% of the ana-/telophases analysed had chromosome breaks and the plants were almost completely sterile and set 0.1 seeds per spikelet. In cross combinations of *Gc3* with the mutant *Gc2^{mut}#1,* allele only 1 % of the gametophytes suffered from chromosome breakage but the plants were still almost completely sterile with 0.3 seeds per spikelet. These data suggest that the majority of the chromosome breaks induced in *Gc3/- Gc2/-* hemizygotes are caused by the functional *Gc2* allele and shows that *Gc3* alone only induces a low level of chromosome breakage. The high level of sterility observed in both cross combinations is not the result of Gc gene-induced chromosome breakage but is likely caused by genetic interactions leading to physiological imbalance and plant sterility.

In *GcAe.$^{cyl.}$/- Gc2/-* hemizygotes with functional Gc genes derived from *Ae. cylindrica* and *Ae. sharonensis,* 45% of the gametophytes suffered from chromosome breakage and the plants were almost completely sterile with 0.4 seeds per spikelet. In cross combinations with the mutant *Gc2^{mut}#1* allele, only 5% of the gametophytes had aberrant ana-/telophases and the plants were completely fertile with 2.1 seeds per spikelet. The data show that the gametophytic chromosome breakage in *GcAe.$^{cyl.}$/- Gc2/-* genotypes is mainly caused by the action of the functional *Gc2* allele and confirms that *GcAe.$^{cyl.}$* alone causes only a low level of chromosome breakage. Plant fertility in these cross combinations is also likely to be affected by physiological imbalance in *GcAe.$^{cyl.}$/- Gc2/-* hemizygotes.

In plants hemizygous for *GcAe.$^{genic.}$* located on the *Ae. geniculata* chromosome 4M^{g}, 43% of the ana-telophases analysed were aberrant, which resulted in a fertility of 1.7 seeds per spikelet. In *GcAe.$^{genic.}$/-, Gc2/-* genotypes, 54% of the gametophytes suffered from chromosome breakage and their fertility was reduced to 1.0 seeds per spikelet, indicating that chromosome breakage occurred in gametophytes that were lacking either *GcAe.$^{genic.}$* or *Gc2* or both. In *GcAe.$^{genic.}$/-, Gc2^{mut}#1/-* and *GcAe.$^{genic.}$/-, Gc2^{mut}#2/-* genotypes, gametophytic chromosome breakage and plant fertility corresponded to those observed in hemizygous *GcAe.$^{genic.}$/-* plants. FISH using clone pGc1R-1 as a probe revealed that in *GcAe.$^{genic.}$/-, Gc2/-* genotypes three ana-/telophases with the *Gc2*-carrier chromosome T4BS·4BL-4S^{sh}#1L were normal (Fig. 1a), whereas the majority of them missing the pGc1R-1 FISH site were aberrant (7 out of 8, Fig. 1b). In cross combinations of *GcAe.$^{genic.}$* with *Gc2^{mut}#1* and *Gc2^{mut}#2* about half of the ana-/telophases with the pGc1R-1 FISH site were normal (3) and aberrant (3, Fig. 1c), and a similar ratio of normal (4, Fig. 1c) and aberrant (3) ana-/telophase were in gametophytes that were lacking the pGc1R-11 FISH site. These data suggest that the majority of the chromosome breaks observed in *GcAe.$^{genic.}$/- Gc2/-* hemizygotes resulted from the action of the functional *Gc2* allele, whereas in *GcAe.$^{genic.}$/- Gc2^{mut}#1/-* or *GcAe.$^{genic.}$/- Gc2^{mut}#2/-* hemizygotes the induction of chromosome breaks was independent from the presence of the mutant *Gc2* alleles and the result of *GcAe.$^{genic.}$* function. The introduction of mutant *Gc2* alleles that only encode for the protecting agent did not restore plant fertility in these genotypes,

although *GcAe*.$^{genic.}$ and *Gc2* are located on homoeologous group-4 chromosomes, indicating that they are functionally different.

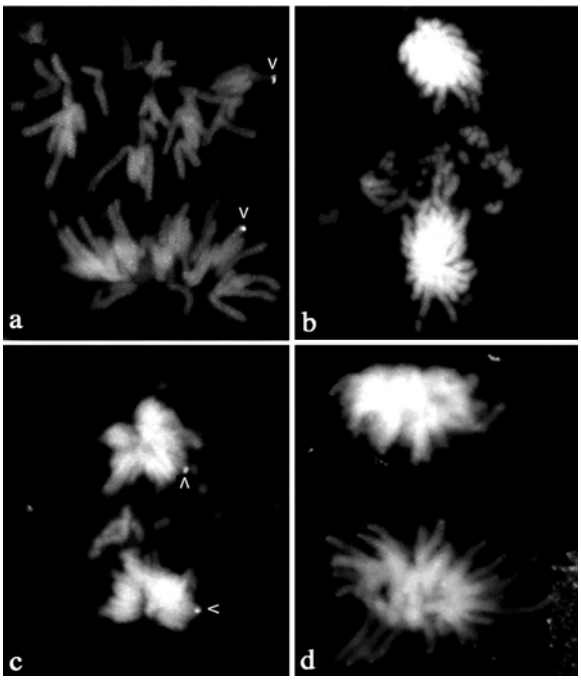

Fig. 1: Gc gene-induced chromosome breakage in *GcAe*.$^{genic.}$/- *Gc2*/- (a and b) and *GcAe*.$^{genic.}$/- *Gc2*^{mut}*#1*/- genotypes (c and d) after FISH using the clone pGc1R-1 as a probe. The presence of the *Gc2*-carrier chromosome T4BS·4BL-4S^{sh}#1L is visualized by bright fluorescence indicated by arrowheads.

DISCUSSION

Previous studies showed that Gc genes ensure their preferential transmission by inducing gametophytic chromosome breakage in gametophytes lacking them (2, 4, 6). Further evidence shows that Gc genes encode for two factors, one causing chromosome breakage in gametophytes lacking them, and another factor protecting those gametophytes that have the Gc-carrier chromosome, although the underlying molecular mechanism is unknown (3). Previous studies further showed that Gc genes located on homoeologous chromosomes have a similar mode of action (1, 8). The present study was initiated to analyze the functional relationships of Gc genes by analysing their effects on induced gametophytic chromosome breakage in genotypes with functional and mutant *Gc2* alleles. The presented data show that in none of the cross combinations, including those involving Gc genes located on homoeologous chromosomes, does the introduction of mutant *Gc2* alleles protect the gametophytes from chromosome breakage or restore plant fertility, suggesting that they are functionally different. In addition, we observed a high level of plant sterility in combination with Gc genes located on C-genome chromosomes, which is likely caused by a physiological imbalance. Work is in progress aimed at map-based cloning of the *Gc2* gene, which will unravel the molecular basis of Gc function.

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