Chromosoma (2001) 110:335–344
DOI 10.1007/s004120100159

ORIGINAL ARTICLE

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The centromere structure in Robertsonian wheat-rye translocation chromosomes indicates that centric breakage-fusion can occur at different positions within the primary constriction

Received: 16 January 2001 / In revised form: 22 May 2001 / Accepted: 8 June 2001 / Published online: 27 July 2001

Abstract Univalent chromosomes at meiotic metaphase I have a tendency to misdivide at the centromeres. Fusion of the misdivision products may produce Robertsonian translocations. The fine structure of the centromeres in Robertsonian wheat-rye translocation chromosomes was analyzed by fluorescence in situ hybridization (FISH) using two centromere-specific DNA clones: pRCS1, derived from rice, and pAWRC1, derived from rye. Clone pRCS1 hybridizes to the centromeres of all grasses including wheat and rye, whereas clone pAWRC1 is rye specific and hybridizes only to the centromeres of rye. Four of the six wheat-rye translocations derived from a single centric misdivision event (1st generation translocations) had hybrid centromeres, with approximately half of the centromere derived from rye and half from wheat. In the two other 1st generation translocations, the entire centromere was derived from rye. Among eight reconstructed wheat and rye chromosomes that originated from two consecutive centric misdivision-fusion events (2nd generation translocations), T1BS·1BL (derived from T1BS·1RL and T1RS·1BL) and one of three T2BS·2BL (derived from T2RS·2BL and T2BS·2RL) had hybrid centromeres. T1RS·1RL (derived from T1BS·1RL and T1RS·1BL), two of three T2BS·2BL, and all three T2RS·2RL (derived from T2RS·2BL and T2BS·2RL) had rye centromeres. All three 3rd generation translocations had hybrid centromeres with approximately half of the centromere derived from rye. There were no indications that the composite structure of the centromere in these chromosomes affected their behavior in mitosis or meiosis. These observations support the notion of a compound structure of the centromere in higher organisms, and indicate that during the centric breakage-fusion event, centromere breakage may occur in different positions along the segment of the chromosome that interacts with the spindle fibers. Normal behavior of the 1st, 2nd, and 3rd generation centric translocations in mitosis and meiosis indicates that, at least in wheat and rye, centromeres are not chromosome specific.

Edited by: J.B. Rattner

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Introduction

The centromere is a cytologically visible component of chromosomes that appears as a primary constriction in metaphase chromosomes. It plays an essential role in the accurate segregation of chromosomes during mitosis and meiosis. The kinetochore is a highly differentiated structure located at the centromere and serves as an attachment site for spindle microtubules. Kinetochores also participate in the generation of forces required for chromosome movement in mitosis and meiosis (Nicklas 1989; Rieder et al. 1990).

Univalents at meiotic meta-/anaphase I have a tendency to misdivide at the centromeres. Based on misdivision studies in *Fritillaria*, Darlington (1939) suggested that the centromere was a compound structure, which can produce misdivision products where both arms have kinetic activity. Similar observations were made by Sears (1952) in wheat (*Triticum aestivum* L.). Centric misdivision followed by the fusion of broken arms from different chromosomes leads to the formation of Robertsonian translocations (Robertson 1916). Such translocations are very common among plants (Jones 1978), animals (White 1973) and humans (Hamerton 1975), and are believed to have played a significant role in karyotype evolution (Jones 1978). Robertsonian translocations can arise from the fusion of two telocentrics forming a metacentric chromosome (Holmquist and Dancis 1979; Schubert et al. 1995). Alternatively, recombination in the short arms of two acrocentric chromosomes followed by

the loss of the acentric fragment may lead to a metacentric chromosome that also results in a change of the basic chromosome number of an organism. Such chromosomes actually are dicentric with one inactive centromere (Holmquist and Dancis 1979; Schubert et al. 1995). Complete fusion-fission cycles have been reported by Schubert and Rieger (1985) and Schubert et al. (1995) for faba bean (*Vicia faba*), and by Lukaszewski (1993) for wheat-rye translocations. Recent observations on humans showed that most of the Robertsonian translocations arose by recombination events and are actually dicentrics. At least 90% of Robertsonian translocations occur between homologous sequences on nonhomologous acrocentric chromosomes (Niebuhr 1972; Daniel and Lam-Po-Tang 1976; Mattei et al. 1979; Cheung et al. 1990; Gravholt et al. 1992; Wolff and Schwartz 1992). Sullivan et al. (1996) and Page et al. (1996) showed that various regions of the acrocentric short arm, particularly, satellite III DNA sequences, are involved in the formation of Robertsonian translocations.

Compared with mammals and yeast, there have been relatively few molecular and structural analyses of centromeric DNA in higher plants. In recent years, several families of tandem and nontandem centromere-associated repetitive sequences have been characterized and localized to the centromeric regions of plant chromosomes by in situ hybridization analysis (Alfenito and Birchler 1993; Kamm et al. 1994; Murata et al. 1994; Chalmers et al. 1995; Harrison and Heslop-Harrison 1995; Leach et al. 1995; Aragon-Alcaide et al. 1996; Jiang et al. 1996; Thompson et al. 1996; Brandes et al. 1997; Round et al. 1997; Dong et al. 1998; Nagaki et al. 1998; Francki 2001). Although the function of these sequences remains unknown, it has been suggested that they may be related to centromere function because of their location (Maluszynska and Heslop-Harrison 1991), their generally conserved structure in distantly related plant species, and their high degree of repetition.

In this study, we analyzed the centromere structure of hexaploid wheat (*T. aestivum* L., 2*n*=6*x*=42, AABBDD), cultivated rye (*Secale cereale* L., 2*n*=2*x*=14, RR), and their centric translocation chromosomes. Two centromeric repetitive sequences, pRCS1 (grass centromeric sequence) (Dong et al. 1998) and pAWRC1 (rye-specific centromeric sequence) (Chalmers et al. 1995; Francki 2001), were hybridized simultaneously to several cytogenetic stocks, including centric and interstitial translocations of rye chromosome 1R with wheat chromosomes 1A, 1B or 1D, and centric translocation of rye chromosome 2R and 2B of wheat, as well as rye chromosomes 1R and 2R and wheat chromosomes 1B and 2B reconstructed from centric wheat-rye translocations (Lukaszewski 1997).

Materials and methods

Plant material

The material analyzed consisted of six wheat-rye translocation lines that were derived from a single centric misdivision event (1st generation translocations), ten reconstructed wheat, rye, or wheat-rye translocation lines that originated from two consecutive centric misdivision-fusion events (2nd generation translocations), and three lines that had translocations that were derived from three consecutive centric misdivision-fusion events (3rd generation translocations) (Table 1). The 1st generation translocations include $T1RS_{VEE}\cdot1BL_{VEE}$ [where 1RS and 1BL were from Genaro, a sister line of the Veery (VEE) series (Merker 1982) transferred to Pavon (PVN) spring wheat background], $T1RS_{AMI}\cdot1AL_{AMI}$ [where 1RS present in the germplasm Amigo (AMI) was derived from "Insave F. A." rye] (Sebesta et al. 1994), $T1BS_{PVN}\cdot1RL_{E}$ [with 1BS derived from Pavon and 1RL derived from a CIMMYT line E12165 (E)] (Lukaszewski 1993), $T1RS_{CIM}\cdot1BL_{CIM}$ (new translocation produced by CIMMYT derived from a wheat×triticale cross) (Lukaszewski, unpublished data), and two reciprocal translocations $T2RS_{B}\cdot2BL_{CS}$ and $T2BS_{CS}\cdot2RL_{B}$ produced by centric misdivision-fusion from 2R from Blanco rye and 2B from Chinese Spring wheat (Lukaszewski, unpublished data).

The 2nd generation chromosomes were wheat $T1BS_{PVN}\cdot1BL_{VEE}$ and rye $T1RS_{VEE}\cdot1RL_{E}$ reconstructed by centric misdivision-fusion from $T1RS_{VEE}\cdot1BL_{VEE}$ and $T1BS_{PVN}\cdot1RL_{E}$ (Lukaszewski 1993). Similarly, the six 2nd generation translocation chromosomes $T2BS_{CS}\cdot2BL_{CS}$ numbers 1 to 3 and $T2RS_{B}\cdot2RL_{B}$ numbers 1 to 3 were reconstructed from the 1st generation translocations $T2RS_{B}\cdot2BL_{CS}$ and $T2BS_{CS}\cdot2RL_{B}$, respectively (Brunell et al. 1999).

In the process of reconstructing complete chromosomes $T1RS_{VEE}\cdot1RL_{E}$ and $T1BS_{PVN}\cdot1BL_{E}$, two reciprocal translocation chromosomes were recovered that resulted from noncentromeric breaks in the short arms, $T1RS_{VEE}$-$1BS_{PVN}\cdot1RL_{E}$ and $T1BS_{PVN}$-$1RS_{VEE}\cdot1BL_{PVN}$. These chromosomes underwent a 2nd centric misdivision-fusion event that gave rise to chromosomes $T1RS_{VEE}$-$1BS_{PVN}\cdot1BL_{VEE}$ and $T1BS_{PVN}$-$1RS_{VEE}\cdot1RL_{E}$ (Lukaszewski 1997).

The 2nd generation reconstructed chromosome $T1RS_{VEE}\cdot1RL_{E}$ was used to create 3rd generation wheat-rye translocation chromosomes $T1RS_{VEE}\cdot1BL_{PVN}$, $T1RS_{VEE}\cdot1AL_{PVN}$, and $T1RS_{VEE}\cdot1DL_{PVN}$ (Lukaszewski 1997).

Fluorescence in situ hybridization (FISH) analysis

Root tips were pretreated with ice-water for 24 h and fixed in ethanol (100%):glacial acetic acid (3:1). Squash preparations were made in 45% acetic acid, coverslips were removed after freezing on dry ice, and the preparations were then dehydrated in ethanol for 5 min. The slides were pretreated with RNase A (100 µg/ml, Sigma) and denatured according to Jiang et al. (1995) immediately before hybridization.

Two centromere-specific repetitive DNA sequences were used for FISH analysis. Clone pAWRC1 was isolated from rye (Chalmers et al. 1995; Langridge et al. 1998; Francki 2001) and hybridizes to rye centromeres, but not to the centromeres of other grasses. Clone pAWRC1 contains a 3.5 kb repeat inserted into the *Sac*I restriction site of the plasmid pTZ19U. Clone pRCS1 was isolated from rice by Dong et al. (1998) and hybridizes to the centromeres of all grass species tested so far, including wheat and rye. It contains an 877 bp centromeric repeat inserted into the *Sau*3AI restriction site of the plasmid pUC18.

One microgram of pRCS1 and pAWRC1 plasmid DNA was labeled with digoxigenin-11-dUTP and biotin-16-dUTP (Boehringer Mannheim), respectively, using nick translation according to the manufacturer's protocols. Fluorescence in situ hybridization was carried out as described by Zhong et al. (1996) and Kynast et al. (2000) with a few modifications. The hybridization solution (with hybridization stringency of around 70%) containing 40% deionized formamide, 2×SSC (1×SSC is 0.15 M NaCl, 0.015 M sodium citrate.), 10% dextran sulfate, 0.3 mg/ml of sheared salmon testes

Table 1 Origin and centromere structure of wheat-rye Robertsonian translocation chromosomes. (PVN, Pavon; VEE, Veery (=Kavkaz); E, line E12165 from CIMMYT)

Chromosome	Origin	Generation of translocation	Structure of centromere (% rye centromere)
$1RS_{VEE}\cdot1BL_{VEE}$[a]	Original translocation from Aurora/Kavkaz. Here from Veery, after BC_8 to monosomic 1B of Pavon	1st	Hybrid, ~50%
$1RS_{AMI}\cdot1AL_{AMI}$[b]	Amigo translocation	1st	Hybrid, ~50%
$1BS_{PVN}\cdot1RL_{E}$[c]		1st	100%
$1RS_{CIM}\cdot1BL_{CIM}$[d]	Unknown, probably a new translocation from a wheat×triticale cross	1st	Hybrid, ~50%
$2RS_{B}\cdot2BL_{CS}$[d]	2RS from Blanco, and 2BL from Chinese Spring (CS)	1st	100%
$2BS_{CS}\cdot2RL_{B}$[d]	2BS from CS, and 2RL from Blanco	1st	Hybrid, ~50%
$1BS_{PVN}\cdot1BL_{VEE}$[c]	Reconstructed 1B	2nd	Hybrid, ~50%
$1RS_{VEE}\cdot1RL_{E}$[c]	Reconstructed 1R	2nd	100%
$1RS_{VEE}$-$1BS_{PVN}\cdot1BL_{VEE}$[e]		2nd	Hybrid, ~50%
$1BS_{PVN}$-$1RS_{VEE}\cdot1RL_{E}$[e]		2nd	100%
$2BS_{CS}\cdot2BL_{CS}$ no. 1[f]	One of three monosomic chromosomes 2B reconstructed from the centric translocations $2RS_{B}\cdot2BL_{CS}$ and $2BS_{CS}\cdot2RL_{B}$. Each is a different construction	2nd	Hybrid, ~75%
$2BS_{CS}\cdot2BL_{CS}$ no. 2[f]	One of three monosomic chromosomes 2B reconstructed from the centric translocations $2RS_{B}\cdot2BL_{CS}$ and $2BS_{CS}\cdot2RL_{B}$. Each is a different construction	2nd	100%
$2BS_{CS}\cdot2BL_{CS}$ no. 3[f]	One of three monosomic chromosomes 2B reconstructed from the centric translocations $2RS_{B}\cdot2BL_{CS}$ and $2BS_{CS}\cdot2RL_{B}$. Each is a different construction	2nd	100%
$2RS_{B}\cdot2RL_{B}$ no. 1[f]	One of three monosomic chromosomes 2R reconstructed from the centric translocations $2RS_{B}\cdot2BL_{CS}$ and $2BS_{CS}\cdot2RL_{B}$. Each is a different construction	2nd	100%
$2RS_{B}\cdot2RL_{B}$ no. 2[f]	One of three monosomic chromosomes 2R reconstructed from the centric translocations $2RS_{B}\cdot2BL_{CS}$ and $2BS_{CS}\cdot2RL_{B}$. Each is a different construction	2nd	100%
$2RS_{B}\cdot2RL_{B}$ no. 3[f]	One of three monosomic chromosomes 2R reconstructed from the centric translocations $2RS_{B}\cdot2BL_{CS}$ and $2BS_{CS}\cdot2RL_{B}$. Each is a different construction	2nd	100%
$1RS_{VEE}\cdot1AL_{PVN}$[e]		3rd	Hybrid, ~50%
$1RS_{VEE}\cdot1BL_{PVN}$[e]		3rd	Hybrid, ~50%
$1RS_{VEE}\cdot1DL_{PVN}$[e]		3rd	Hybrid, ~50%

[a] Merker (1982); [b] Sebesta et al. (1994); [c] Lukaszewski (1993); [d] Lukaszewski (unpublished); [e] Lukaszewski (1997); [f] Brunell et al. (1999)

DNA, and about 1 µg/ml of both labeled probes was denatured by boiling for 5 min. Thirty microliters of denatured hybridization mixture was applied to each slide and hybridization was performed in a humid chamber at 37°C overnight. Post-hybridization washes were done in 2×SSC at 42°C for 10 min, 30% formamide in 2×SSC at 42°C for 10 min (with washing stringency of around 70%), and 2×SSC at 42°C for 10 min.

The digoxigenin- and biotin-labeled probes were detected with rhodamine anti-digoxigenin antibodies (Boehringer Mannheim) and fluorescein-avidin DN (Vector Laboratories), respectively (Jackson et al. 1998). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) in Vectashield (Vector Laboratories). Slides were analyzed with an epifluorescence Zeiss Axioplan 2 microscope. Images were captured using a SPOT CCD (charge-coupled device) camera operated with SPOT 2.1 software (Diagnostic Instruments) and processed with Photoshop v5.5 (Adobe Systems).

Results

Fluorescence in situ hybridization with pAWRC1 and pRCS1 to mitotic metaphase chromosomes confirmed that clone pAWRC1 was rye specific as it hybridized only to the centromeres of rye chromosomes (Francki 2001), whereas clone pRCS1 hybridized to the centromeres of both wheat and rye (Dong et al. 1998). In the present study the term centromere is used for the primary constriction and the flanking regions that are labeled by clone pRCS1. Because clone pAWRC1 hybridizes only to rye centromeres while clone pRCS1 hybridizes to the centromeres of both rye and wheat, the fine structure of the centromeres in the Robertsonian wheat-rye translocation chromosomes could be analyzed.

The centromeric regions in metaphase chromosomes are highly condensed. However, the fine structure of the centromeres was clearly visible in stretched-out chromosomes, where the primary constriction was flanked by two V-shaped regions. Metaphase FISH allowed us to distinguish between three different centromere structures: (1) centromeres that were only labeled by the clone pRCS1 and were derived from wheat; (2) centromeres where the pAWRC1 FISH signal covered about the entire length of the pRCS1 signal, indicating that the centromere was derived from rye; and (3) hybrid centromeres, where the pAWRC1 FISH signal spanned about half of the extent of the pRCS1 signal, indicating that about half of the centromere was derived from rye and the other half was derived from wheat.

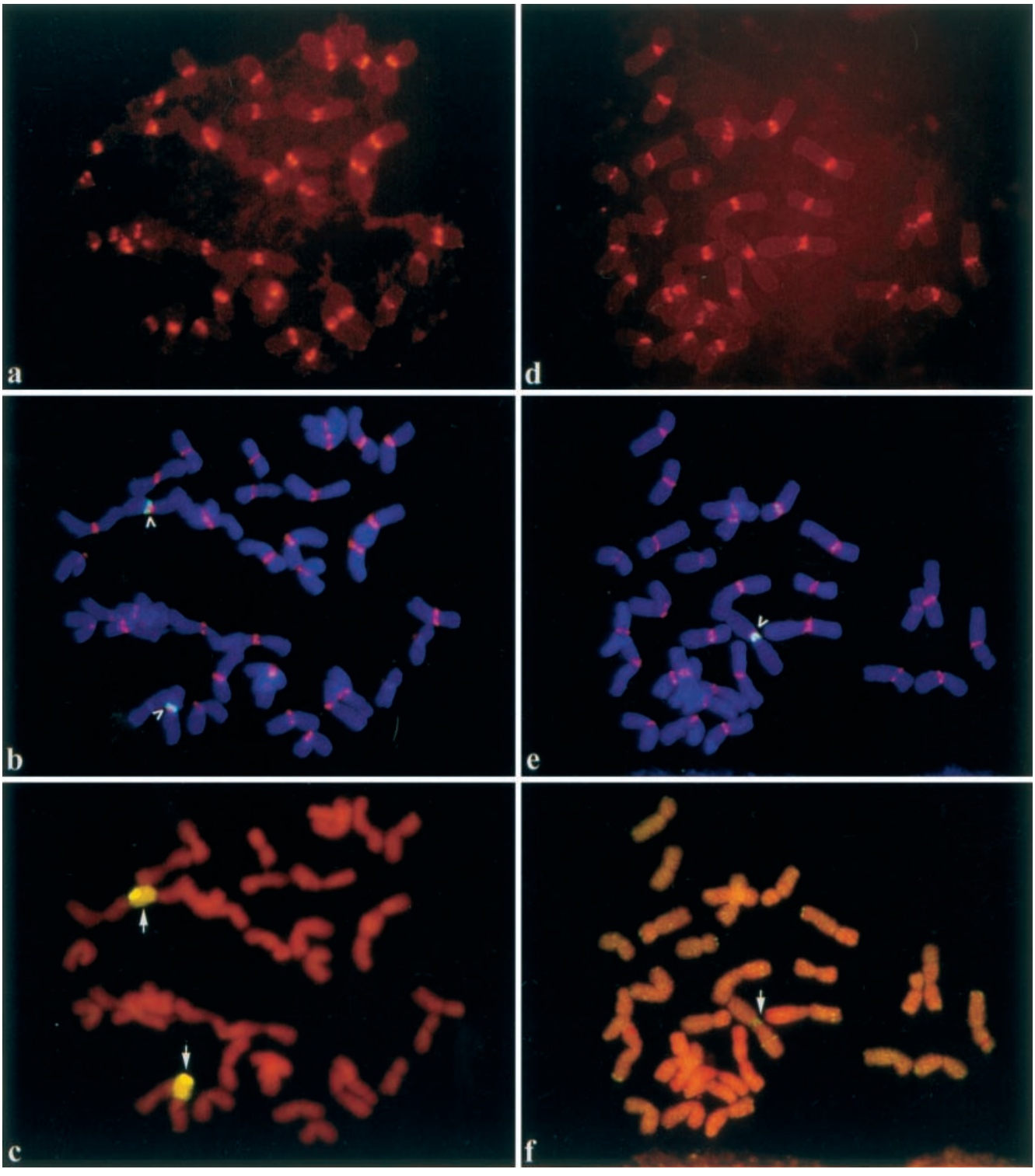

Fig. 1 Mitotic metaphase fluorescence in situ hybridization (FISH) (**a, b, d, e**) and sequential genomic in situ hybridization (GISH) (**c, f**) patterns of 1st ($T1RS_{CIM}{\cdot}1BL_{CIM}$) (**a, b, c**) and 2nd generation ($T2BS_{CS}{\cdot}2BL_{CS}$ no. 1) (**d, e, f**) Robertsonian wheat-rye translocation lines: **a, d** FISH patterns of clone pRCS1 detected by red rhodamine fluorescence; **b, e** merged images showing the hybridization of clone pRSC1 (visualized by red rhodamine fluorescence), and clone pAWRC1 [visualized by yellow-green fluorescein isothiocyanate (FITC) fluorescence]; chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue fluorescence); **c, f** sequential GISH pattern of the same cell using total genomic rye DNA as a probe visualized by yellow-green FITC fluorescence; chromosomes were counterstained with propidium iodide and fluoresce red. Note the hybrid centromere in $T1RS_{CIM}{\cdot}1BL_{CIM}$ and the rye centromere in the reconstructed 2B chromosome of wheat. *Arrowheads* point to the wheat-rye hybrid and rye centromeres

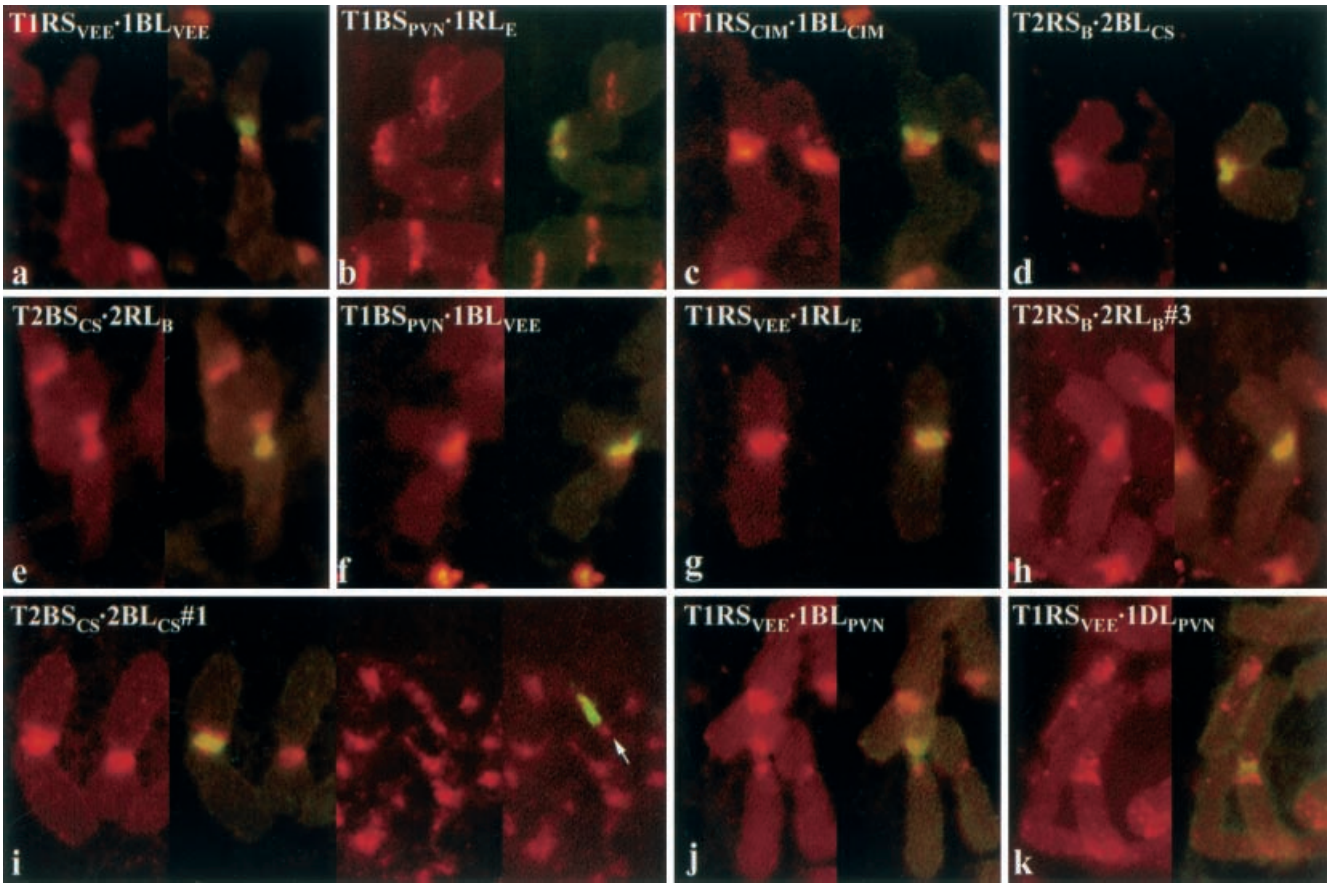

Fig. 2a–k Centromere structure of 1st, 2nd, and 3rd generation Robertsonian wheat-rye translocation chromosomes as revealed by FISH analysis. The rice clone pRCS1 is visualized by red rhodamine fluorescence and the rye-specific clone pAWRC1 is visualized by yellow-green FITC fluorescence

Four of the six 1st generation translocations (T1RS$_{VEE}$·1BL$_{VEE}$, T1RS$_{AMI}$·1AL$_{AMI}$, T1RS$_{CIM}$·1BL$_{CIM}$, T2BS$_{CS}$·2RL$_{B}$) had hybrid centromeres. In these chromosomes the pRCS1 FISH signal (visualized by red rhodamine fluorescence) spanned the entire length of the centromeres, whereas the pAWRC1 FISH signal [visualized by green fluorescein isothiocyanate fluorescence] only spanned the area derived from rye (Figs. 1a, b, 2a, c, e). The rye chromosome arm in these translocations usually could be identified by a bright fluorescent telomeric DAPI band. The size of the pAWRC1 FISH signal in these translocation chromosomes was approximately half of the entire length of the pRCS1 signal, indicating that about half of the hybrid centromeres was derived from rye and the other half originated from wheat. Sequential pAWRC1/pRCS1 FISH followed by genomic in situ hybridization (GISH) using total genomic rye DNA as a probe confirmed that clone pAWRC1 hybridized only to the rye arm in these translocation chromosomes (Fig. 1a–c).

A different centromere structure was observed in the 1st generation translocation chromosomes T1BS$_{PVN}$·1RL$_{E}$ and T2RS$_{B}$·2BL$_{cs.}$ In both instances, clone pAWRC1 hybridized to the entire length of the centromeres, indicating that they were completely derived from rye (Fig. 2b, d).

The pAWRC1/pRCS1 FISH patterns of the reconstructed 2nd generation translocation chromosomes T1BS$_{PVN}$·1BL$_{VEE}$ and T1RS$_{VEE}$·1RL$_{E}$ indicated that the former had a hybrid centromere (Fig. 2f), whereas the centromere of the later was completely derived from rye (Fig. 2g). Three different reconstructed 2nd generation translocation chromosomes 2B (T2BS$_{CS}$·2BL$_{CS}$ no. 1, T2BS$_{CS}$·2BL$_{CS}$ no. 2, T2BS$_{CS}$·2BL$_{CS}$ no. 3) and 2R (T2RS$_{B}$·2RL$_{B}$ no. 1, T2RS$_{B}$·2RL$_{B}$ no. 2, T2RS$_{B}$·2RL$_{B}$ no. 3) were derived from the 1st first generation translocation chromosomes T2RS$_{B}$·2BL$_{CS}$ and T2BS$_{CS}$·2RL$_{B}$. The metaphase pAWRC1/pRCS1 FISH patterns indicated that the centromeres in all reconstructed 2B and 2R were completely derived from rye (Fig. 2h, i). However, FISH on extended prometaphase and interphase chromosomes showed T2BS$_{CS}$·2BL$_{CS}$ no. 1 had in fact a hybrid centromere in which about 75% of the centromeric region was derived from rye and 25% was derived from wheat (Fig. 2i). Sequential pAWRC1/pRCS1 FISH followed by GISH analysis revealed that only the centromeres in the reconstructed 2B chromosomes of wheat were labeled and derived from rye (Fig. 1d–f). The additional small GISH sites in Fig. 1f represent regions that contain highly repetitive DNA families that are shared between rye and wheat (Dennis et al. 1980).

The pAWRC1/pRCS1 FISH patterns of the 2nd generation translocation chromosomes T1RS$_{VEE}$-1BS$_{PVN}$·1BL$_{VEE}$

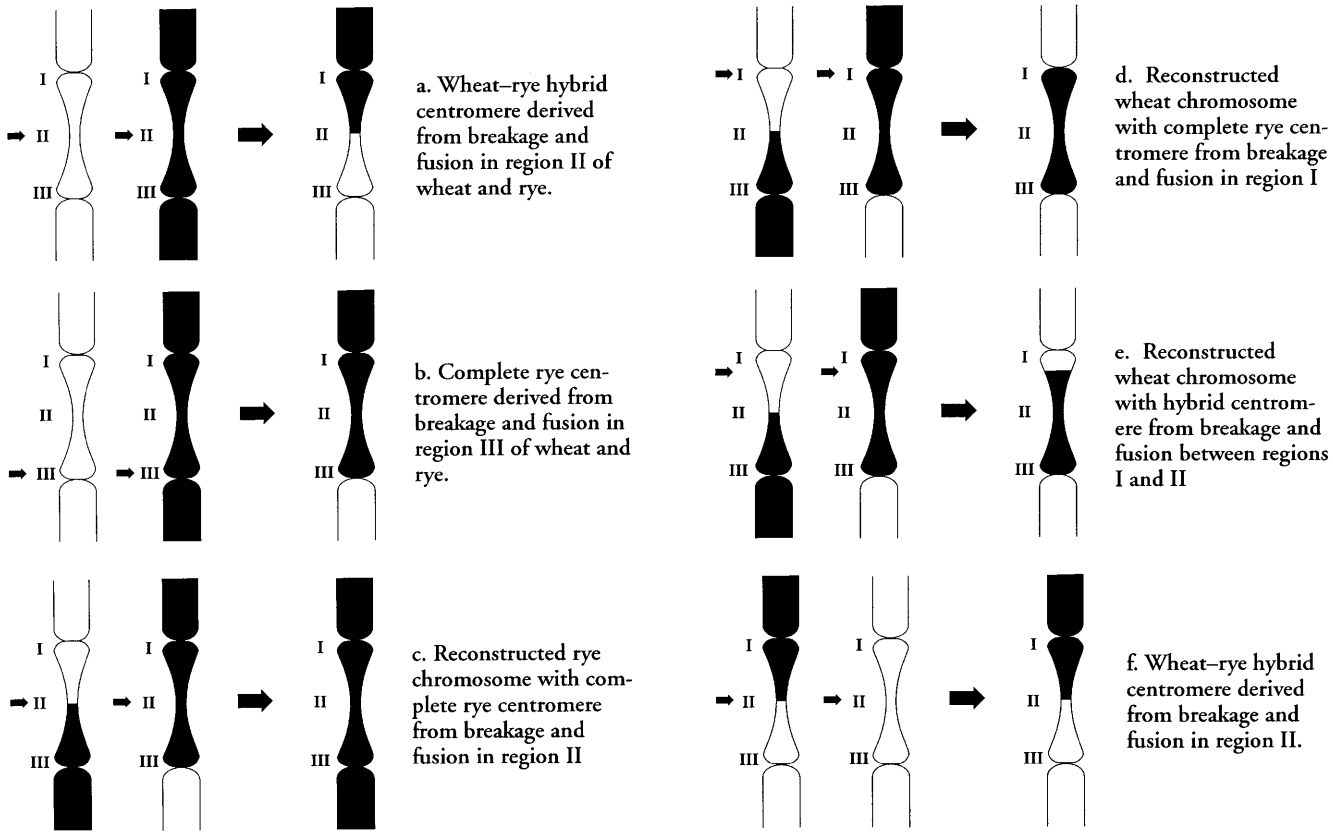

Fig. 3a–f Possible origins of compound centromeres in 1st, 2nd and 3rd generation Robertsonian wheat (*white*)-rye (*black*) translocation chromosomes. The primary constriction was arbitrarily divided into one central (*II*) and two adjacent pericentromeric (*I* and *III*) regions. Breakage and fusion in different regions can result in either wheat, rye, or wheat-rye hybrid centromeres

and $T1BS_{PVN}$-$1RS_{VEE}{\cdot}1RL_E$ indicated that the former translocation had a hybrid centromere, whereas the centromere in the latter translocation chromosome was derived from rye (data not shown).

The 3rd generation translocations $T1RS_{VEE}{\cdot}1AL_{PVN}$, $T1RS_{VEE}{\cdot}1BL_{PVN}$, and $T1RS_{VEE}{\cdot}1DL_{PVN}$ were derived from the 2nd generation translocation $T1RS_{VEE}{\cdot}1RL_E$, which, in turn, originated from centric misdivision of centric translocations $T1BS_{PVN}{\cdot}1RL_E$ and $T1RS_{VEE}{\cdot}1BL_{VEE}$. All three 3rd generation translocations had hybrid centromeres with about half of the centromere derived from rye (Fig. 2j, k).

Discussion

With the exception of the two 2nd generation terminal translocations $T1RS_{VEE}$-$1BS_{PVN}{\cdot}1BL_{VEE}$ and $T1BS_{PVN}$-$1RS_{VEE}{\cdot}1RL_E$, all wheat-rye translocations analyzed in the present study arose from centric breakage-fusion events. The centromeres in these translocations were either completely derived from rye or wheat or, as in the case of hybrid centromeres, were of hybrid wheat-rye nature. Our data suggest that centromeric breakage and fusion can occur at several positions within the primary constriction and that the position of breakage does not affect centromere function and chromosome segregation during mitosis and meiosis.

The possible origin of the centromere structure in wheat-rye translocations is given in Fig. 3. We divided the primary constriction into three regions: a central region (II), and the two flanking pericentromeric regions (I) and (III) of both arms that were labeled by the clones pAWRC1 and pRCS1.

Four of the six 1st generation translocations ($T1RS_{VEE}{\cdot}1BL_{VEE}$, $T1RS_{AMI}{\cdot}1AL_{AMI}$, $T1RS_{CIM}{\cdot}1BL_{CIM}$, $T2BS_{CS}{\cdot}2RL_B$) had hybrid centromeres. Breakage and fusion in region II of the original rye chromosome 2R ($2RS_B{\cdot}2RL_B$) and wheat chromosome 2B ($2BS_{CS}{\cdot}2BL_{CS}$) can produce a hybrid centromere that is composed of about half of rye and half of wheat chromatin (Fig. 3a). A similar mechanism of origin can account for the hybrid centromere structure of chromosomes $T1RS_{VEE}{\cdot}1BL_{VEE}$, $T1RS_{AMI}{\cdot}1AL_{AMI}$, and $T1RS_{CIM}{\cdot}1BL_{CIM}$. The centromeres of the remaining two 1st generation translocations were completely derived from rye. Presumably, breakage and fusion in regions III of chromosomes 2R and 2B resulted in the apparently complete rye centromere in $T2RS_B{\cdot}2BL_{CS}$ (Fig. 3b). Similarly, the complete rye centromere in $T1BS_{PVN}{\cdot}1RL_E$ can be explained by breakage and fusion in region I of chromosomes 1B ($1BS_{PVN}{\cdot}1BL_{PVN}$) and 1R ($1RS_E{\cdot}1RL_E$). However, the presence of small segments of wheat origin in $T2RS_B{\cdot}2BL_{CS}$ and $T1BS_{PVN}{\cdot}1RL_E$ cannot be ruled out because of the detection limitation of FISH to metaphase chromosomes.

Among the eight 2nd generation translocation chromosomes, reconstructed 1B ($T1BS_{PVN}{\cdot}1BL_{VEE}$) and reconstructed 2B no. 1 ($T2BS_{CS}{\cdot}2BL_{CS}$) had hybrid centromeres, whereas reconstructed 1R, 2B no. 2, 2B no. 3, and all three reconstructed 2R chromosomes had centromeres that were completely derived from rye (Fig. 3c, d). The origin of the hybrid centromeres of reconstructed 2B no. 1 can be explained by breakage and fusion between regions I and II of $T2BS_{CS}{\cdot}2RL_{B}$ and $T2RS_{B}{\cdot}2BL_{CS}$, respectively (Fig. 3e).

The complete rye centromeres in all reconstructed chromosomes 2R ($T2RS_{B}{\cdot}2RL_{B}$) could have originated from breakage and fusion in regions II of $T2BS_{CS}{\cdot}2RL_{B}$ and $T2RS_{B}{\cdot}2BL_{CS}$ (Fig. 3c). Similarly, breakage and fusion in region I of $T2BS_{CS}{\cdot}2RL_{B}$ and $T2RS_{B}{\cdot}2BL_{CS}$ produces a complete rye centromere in the reconstructed chromosomes 2B (Fig. 3d). The complete rye centromere in $T1RS_{VEE}{\cdot}1RL_{E}$ can be explained by breakage and fusion in regions II of $T1BS_{PVN}{\cdot}1RL_{E}$ and $T1RS_{VEE}{\cdot}1BL_{VEE}$, respectively.

Chromosomes $T1RS_{VEE}$-$1BS_{PVN}{\cdot}1RL_{E}$ and $T1BS_{PVN}$-$1RS_{VEE}{\cdot}1RL_{VEE}$ originated from noncentromeric translocations with breakage and fusion in the short arms of $T1BS_{PVN}{\cdot}1RL_{E}$ and $1RS_{VEE}{\cdot}1BL_{VEE}$. Chromosomes $T1BS_{PVN}$-$1RS_{VEE}{\cdot}1RL_{E}$ and $T1RS_{VEE}$-$1BS_{PVN}{\cdot}1BL_{VEE}$ arose after $T1RS_{VEE}$-$1BS_{PVN}{\cdot}1RL_{E}$ and $T1BS_{PVN}$-$1RS_{VEE}{\cdot}1RL_{VEE}$ underwent a 2nd centric misdivision-fusion event. In chromosome $T1BS_{PVN}$-$1RS_{VEE}{\cdot}1RL_{E}$, with the centromere derived completely from rye, breakage and fusion occurred between II and III of $T1RS_{VEE}$-$1BS_{PVN}{\cdot}1RL_{E}$ and between I and II of $T1BS_{PVN}$-$1RS_{VEE}{\cdot}1RL_{VEE}$; and in chromosome $T1RS_{VEE}$-$1BS_{PVN}{\cdot}1BL_{VEE}$, with a hybrid centromere, breakage and fusion occurred between I and II of $T1RS_{VEE}$-$1BS_{PVN}{\cdot}1RL_{E}$ and between II and III of $T1BS_{PVN}$-$1RS_{VEE}{\cdot}1RL_{VEE}$.

All three 3rd generation translocations, $T1RS_{VEE}{\cdot}1AL_{PVN}$, $T1RS_{VEE}{\cdot}1BL_{PVN}$, and $T1RS_{VEE}{\cdot}1DL_{PVN}$, had hybrid centromeres. The breakage and fusion occurred in regions II of $T1RS_{VEE}{\cdot}1RL_{E}$ and chromosomes $1A_{PVN}$, $1B_{PVN}$, and $1D_{PVN}$, respectively (Fig. 3f).

In the present study, centromere structure was analyzed mainly in condensed metaphase chromosomes, which limited the resolution of the FISH technique. Therefore, we cannot entirely rule out the possibility that some of the 2nd and 3rd generation translocation chromosomes have mosaic centromeres as implied by Fig. 3. Consecutive breaks need not occur in the same location and, consequently, transfer of small segments from rye to a wheat centromere or vice versa, seems plausible. Given the probe specificity, it would be impossible to detect small wheat inserts in rye, and the detection of inserts from rye to a wheat centromere primarily depends on the resolution of the FISH technique.

Eukaryotic centromeres can be grouped into three major types. The first type, represented by the centromeres of budding yeast (*Saccharomyces cerevisiae*) chromosomes, contains only about 125 bp of unique sequence, which have three types of relatively simple protein-binding DNA elements (Clarke 1990): CDEI (centromere DNA element I), CDEII, and CDEIII. The second type consists of a single class of repetitive DNA, e.g., mammalian centromeres are characterized by satellite DNA repeats of varying lengths and sequence composition. Among them are the 171 bp human α-satellite DNA repeat (Tyler-Smith et al. 1993) and the 120 bp mouse minor satellite DNA (Kipling et al. 1994; reviewed in Choo 1997). Despite the sequence divergence among centromeric satellite DNA repeats of different mammals, at least a subset of each contains a degenerate 17 bp long CENP-B (centromere protein B) protein-binding motif (Muro et al. 1992; Kipling et al. 1995; Yoda et al. 1996). Although the DNA-binding site is not highly conserved, the CENP-B protein itself is (Goldberg et al. 1996). The third major type of eukaryotic centromeres, such as those from fission yeast (*Schizosaccharomyces pombe*) (Clarke 1990) and *Drosophila melanogaster* chromosomes, consists of complex DNA including various classes of repetitive DNA elements. The centromeric regions of *S. pombe* chromosomes contain several classes of repetitive DNA elements and these elements are organized into patterns that are specific for each centromere (Clarke et al. 1993). For example, the putative 100 kb functional centromeric region of chromosome 2 contains a specific, nonhomologous 7 kb core that is surrounded by a unique 1.5 kb inverted element and four repetitive DNA elements that occur on all centromeres (Clarke 1990). The essential core of the centromere in *D. melanogaster* minichromosome *Dp1187* is contained within a 220 kb region called the *Bora Bora* island (Murphy and Karpen 1995). This essential core contains significant amounts of complex DNA, consisting of single-copy and middle repetitive sequences (Le et al. 1995). About 200 kb of DNA is required on either side of the essential core for normal chromosome stability.

Studies of centromeres from plants and animals have revealed no common sequence organization (Wiens and Sorger 1998). For instance, although the grass repetitive sequences, pSau3A9 (Jiang et al. 1996), CCS1 (Aragon-Alcaide et al. 1996), and pAWRC1 (Francki 2001), contain a CENP-B-like box, there is no homology between them. The basic requirement of a centromere sequence may be the presence of a sufficient number of centromere protein-binding motifs in a locus on a chromosome. Common to all centromere-specific sequences is that they contain retrotransposon-related DNA sequences (Miller et al. 1998; Presting et al. 1998). The *Ty1-copia* and *Ty3-gypsy* related retrotransposons in grass centromeres may be ancient insertions and are likely to have been amplified during centromere evolution. These retrotransposons in the centromeres are not intact elements and therefore, are most likely inactive. Centromeres are like “graveyards” for retrotransposons. Because the centromere-specific retrotransposons are remarkably conserved in the centromeres of distantly related plant species, one may speculate that these sequences might be part of the functional centromeres.

A centromeric repeated sequence has been recovered from a maize B chromosome (Alfenito and Birchler

1993). Pulsed-field gel electrophoresis analysis of misdivision derivatives from B chromosome in maize showed that following misdivision only a fraction of the B-specific cluster was present in the functional centromere. As all of these derivatives were different, the misdivision must have occurred at various places within the centromere. The molecular data of Kaszas and Birchler (1996) showed that centromeres were composed of repeat units, e.g., B repeat, and could be extensively subdivided, with various portions still retaining normal function. Several other studies also suggested that the centromere was a repeated structure and could be split into smaller fragments or significantly rearranged without affecting function. However, Kaszas and Birchler (1998) observed a strong correlation between the size of the centromere and meiotic transmission in maize. Therefore, the size of the centromere may not be reduced without limit.

Several studies have suggested that plant centromeres have a complex structure, and are composed of repeated units. They can be significantly reduced in size or rearranged without affecting function, which indicates considerable redundancy in the centromere regions (Steinitz-Sears 1966; Brown et al. 1994; Lukaszewski 1994; Kaszas and Birchler 1996; Platero et al. 1999). Zinkowski et al. (1991) observed that detached kinetochore fragments derived from mammalian and plant cells interacted with spindle microtubules and progressed through mitosis, which demonstrates modular organization within the kinetochore. The possibility of stretching or uncoiling of the metaphase centromere-kinetochore complex, further supports a repeat subunit model, i.e., the kinetochore is assembled from repetitive subunits tandemly arranged on a continuous DNA/protein fiber. Each subunit is a complex of constitutive and facultative proteins and DNAs that can function autonomously when detached from the chromosome. A highly redundant kinetochore organization involving one linear DNA molecule along the centromere could facilitate extensive breaks, exchanges, deletions, duplications, and inversions and still retain the capability to organize into a functional kinetochore.

This study demonstrates directly the compound structure of the centromere and the fact that individual subunits capable of interaction with the karyokinetic spindle are distributed along its entire length. For that reason, the centromeres can break at various positions within the primary constriction without affecting their function. All chromosomes described herein undergo normal chromatid and chromosome segregation during mitosis and meiosis, suggesting that the hybrid nature of their centromeres did not affect their function and behavior. However, there were indications of higher susceptibility of some of these hybrid centromeres to misdivision (Lukaszewski 1997).

Although many controversial questions about the centromere remain to be answered, this and other studies suggest that the reduction in size or the rearrangement of the centromere can occur without affecting its function. For instance, the study of Kaszas and Birchler (1996) indicated that centromeres reduced in size from several megabases to the 100–200 kb range still retained sufficient function. Therefore, artificial chromosomes for multicellular organisms could be constructed using only portions of the centromere present in natural chromosomes. Such artificial chromosomes would be very useful in studies of chromosome behavior and in genetic engineering, just like artificial chromosomes in unicellular organisms.

Acknowledgements This research was supported by the Kansas Wheat Commission and a special USDA grant to the Wheat Genetics Resource Center. We thank Dr. Peter J. Langridge, Waite Agricultural Research Institute, Glen Osmond, Australia for providing clone pAWRC1 and Dr. Jiming Jiang, Department of Horticulture, University of Wisconsin, Madison, USA for providing clone pRCS1. We also thank Dr. Ralf Kynast, Dr. Wanlong Li, John W. Raupp, and Duane Wilson for excellent assistance. This paper is contribution number 01-296-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506-5502, USA.

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