Molecular cytogenetic characterization of Thinopyrum genomes conferring perennial growth habit in wheat–Thinopyrum amphiploids

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Abstract

Seven wheat–Thinopyrum amphiploids, AT 3425, AgCs, PI 550710, PI 550711, PI 550712, PI 550713 and PI 550714, were evaluated for perennial growth habit in the field. Three of them, AgCs, AT 3425, and PI 550713, were identified as perennials. Fluorescent genomic in situ hybridization (FGISH) patterns of mitotic chromosomes indicated that AgCs had seven pairs of Thinopyrum chromosomes and 21 pairs of wheat chromosomes. PI 550713 and AT 3425 showed similar FGISH patterns of mitotic chromosomes with three pairs of wheat–Thinopyrum translocated chromosomes, seven pairs of Thinopyrum chromosomes, and 18 pairs of wheat chromosomes. Thinopyrum chromosome pairing in the F1 hybrid of AT 3425 with AgCs demonstrated differences between Thinopyrum genomes in these two amphiploids. Based on chromosome constitutions, pairing and reported pedigrees, AgCs and AT 3425 were identified as a wheat–Thinopyrum elongatum amphiploid and partial wheat–Thinopyrum ponticum amphiploid, respectively. Chromosome pairing in the F1 hybrid between AT 3425 and PI 550713 revealed that these two amphiploids contained the same Thinopyrum genome. Two different Thinopyrum genomes conferring perennial growth habit were identified from the perennial amphloids and characterized cytogenetically.

Key words: Thinopyrum — Triticum aestivum — chromosome pairing — cytogenetics — in situ hybridization — perennial growth habit

Production of annual wheat on highly erodible land has led to severe soil erosion and degradation of water resources (Wagoner 1990). Perennial wheat may be an alternative to annual wheat for soil erosion control on highly erodible land. Since perennial wheat development was initiated by scientists in the former Soviet Union during the 1920s, much effort has been invested to perennialize annual wheat by wide hybridization between annual wheat and its perennial relatives (Suneson and Pope 1946, Tsitsin 1960, Dewey 1984, Wagoner 1990, Cai et al. 1998b). Many perennial wheat lines have been produced from these crosses. However, little is known of the genomes conferring perennial growth habit and little work has been done on genetic characterization of perennial growth habit.

Many relatives of wheat have been used as donors of perennial growth habit and other desired agronomic genes to improve annual wheat (Tsitsin 1960, Dewey 1984, Shepherd and Islam 1988, Wagoner 1990). Among the perennial relatives of wheat, two Thinopyrum species, Th. ponticum (Podp.) Barkworth & D. R. Dewey (2n = 10x = 70) and Th. elongatum (Host) D. R. Dewey (2n = 2x = 14), have attracted the attention of wheat geneticists and breeders because of their desirable genes for wheat improvement and high cross-compatibility with wheat. Genes for resistance to wheat rusts, wheat streak mosaic virus, barley yellow dwarf virus, and Cephalosporium stripe have been successfully incorporated into wheat from these two Thinopyrum species by conventional hybridization (Knot 1968, Sears 1973, Yasumoto et al. 1981, Whelan and Hart 1988, Kim et al. 1992, Jiang et al. 1993, Friebel et al. 1994, Zhong et al. 1994, Cai et al. 1996, 1998b). Annual wheat has been perennialized by the production of wheat–Thinopyrum amphiploids and derivatives (Suneson and Pope 1946, Wagoner 1990, Cai et al. 1998b). Some of these perennial wheat–Thinopyrum amphiploids and derivatives are being considered as an alternative to annual wheat for soil erosion control in the US Pacific Northwest.

Th. elongatum and Th. ponticum traditionally have been placed under the same name, Agropyron elongatum (Host) Beauv. because of their close genetic relationship and morphological similarity (Shepherd and Islam 1988). This classification system caused confusion in the identification of these two species in previous studies. In fact, these two Thinopyrum species have different ploidy levels and no common genome, although their genomes are closely related (Dvorak 1975). The decaploidy of Th. ponticum makes it difficult to analyse its genome constitution and determine genome relationships with diploid Th. elongatum. However, the availability of partial wheat–Th. ponticum amphiploids provides an efficient approach to analyse genome relationships between these two Thinopyrum species. In addition, partial wheat–Th. ponticum amphiploids are desirable materials to dissect the complex genomes of Th. ponticum and characterize specific Th. ponticum chromosomes conferring desired agronomic traits.

Thinopyrum genomes conferring perennial growth habit in wheat–Thinopyrum amphiploids and relationships among the Thinopyrum genomes in the perennial amphiploids were characterized using fluorescent genomic in situ hybridization (FGISH) in the present study. Characterization of Thinopyrum genomes in the perennial amphiploids will aid in the genetic manipulation of perennial growth habit.

Materials and Methods

Plant materials and crosses: The wheat–Thinopyrum amphiploids, AgCs and AT 3425, were provided by Dr J. Dvorak, Department of Agronomy and Range Science, University of California, Davis, USA, and Dr D. E. Mathre, Department of Plant Sciences, Montana State University, Bozeman, USA, respectively. It was reported that AgCs...
originated from a cross between *Triticum aestivum* L. cv. 'Chinese Spring' (CS) and diploid *Agropyron elongatum* and AT 3425 from a cross between an unknown hexaploid wheat and diploid *A. elongatum* (Dvorak 1976, Mathre et al. 1985). The other wheat–*Thinopyrum* amphiploids, PI 550710, PI 550711, PI 550712, PI 550713, and PI 550714, were obtained from the National Small Grains Collection at Aberdeen, Idaho, USA. These amphiploids are listed as wheat–*Agropyron* amphiploids and their detailed pedigrees are not available (H. E. Bockelman, pers. comm.). Two annual winter cultivars, ‘Madsen’ and ‘Stephens’, were used as controls for determining annual vs. perennial growth habit. All crosses among the wheat–*Thinopyrum* amphiploids were carried out in a greenhouse, using conventional crossing techniques.

Perennial growth habit evaluation: The wheat–*Thinopyrum* amphiploids were evaluated for perennial growth habit in the field. The amphiploids and two annual wheat cultivars, ‘Madsen’ and ‘Stephens’, were sown in 1.2 x 2.5 m plots in September 1996 and harvested in August 1997. After harvesting, the stubble of the amphiploids and the annual wheat cultivars was left in the field for evaluation of regrowth. Volunteer wheat and weeds in the field were removed in early winter and spring. Plots were fertilized using urea in the spring. Amphiploids that regrew in the autumn of 1997 and spring of 1998 and set seed in the summer of 1998 were considered perennial.

Molecular cytogenetic analysis: Mitotic chromosomes at metaphase in root tip cells and meiotic chromosomes at metaphase I (MI) in pollen mother cells (PMCs) were prepared following the methods described by Cai et al. (1996). Mitotic chromosomes in the perennial wheat–*Thinopyrum* amphiploids and meiotic chromosomes in the F1 hybrids of AT 3425 with AgCs and PI 550713 were analysed using fluorescent genomic in situ hybridization (FGISH). Total genomic DNAs of *Th. ponticum* and *Th. elongatum* were used as probes for FGISH and labelled with biotin-16-dUTP by nick translation (Bioprobe Nick Translation System, Enzo Diagnostics Inc., New York, USA). Total genomic DNA of *T. aestivum* L. cv. ‘Chinese Spring’ was sheared as blocking DNA for FGISH by boiling in 0.4 M NaOH for 40-50 min. The hybridization and signal detection of FGISH were carried out as described by Cai et al. (1998a). *Thinopyrum* chromatin was detected with fluorescein isothiocyanate (FITC)-avidin (yellow-green fluorescence) and wheat chromatin was counterstained with propidium-iodide (red fluorescence).

Results

Perennial growth habit

All genotypes grown in the field were harvested in the autumn of 1997. Regrowth was observed from the crowns of AT 3425, AgCs, and PI 550713 during grain ripening and after harvest. Each plant of PI 550710, PI 550711, PI 550712, PI 550714, ‘Madsen’ and ‘Stephens’ died during ripening and no regrowth was found from the crowns or other tissues of these genotypes. Most of the autumn-regrown plants of AT 3425, and PI 550713 survived during the winter of 1997 and continued growing in the spring of 1998. However, most of the autumn-regrown plants of AgCs died during the winter because of lack of winter hardiness. In the spring of 1998, AT 3425, PI 550713 and AgCs initiated new regrowth from their crowns. All of the spring-regrown plants and the surviving autumn-regrown plants flowered and set seeds in the summer of 1998. After harvesting, the amphiploids AT 3425 and PI 550713 initiated the second regrowing season in the autumn of 1998 and spring of 1999. The regrowing plants flowered and formed seeds in the summer of 1999. The yield of the regrowing generations of these three amphiploids reached 60–70% of the yield of their first generation (data not shown).

FGISH patterns of mitotic chromosomes

FGISH was performed on mitotic chromosomes of the amphiploids AgCs, AT 3425 and PI 550713 (Fig. 1). In AgCs the hybridizations with *Th. elongatum* and *Th. ponticum* genomic DNAs gave similar results, showing seven pairs of *Thinopyrum* chromosomes and 21 pairs of wheat chromosomes (Fig. 1a,b). No wheat–*Thinopyrum* translocated chromosomes were detected in AgCs (Fig. 1a,b). Using *Th. elongatum* genomic DNA as a probe, AT 3425 showed a FGISH pattern with seven pairs of *Thinopyrum* chromosomes, three pairs of wheat–*Thinopyrum* translocated chromosomes, and 18 pairs of wheat chromosomes (Fig. 1c). A similar result was obtained after hybridizations with *Th. ponticum* genomic DNA in AT 3425 (Cai et al. 1998a). Seven pairs of *Thinopyrum* chromosomes, three pairs of wheat–*Thinopyrum* translocated chromosomes, and 18 pairs of wheat chromosomes were identified when PI 550713 chromosomes were hybridized with *Th. ponticum* genomic DNA (Fig. 1d). Wheat–*Thinopyrum* translocated chromosomes in PI 550713 and AT 3425 also showed similar FGISH patterns (Fig. 1c,d).

Chromosome pairing in the hybrids

AT 3425 was used as the female parent in crosses with AgCs and PI 550713. FGISH was performed on the meiotic chromosomes at MI to distinguish *Thinopyrum* chromatin from wheat chromatin in the hybrids. Among 123 pollen mother cells (PMCs) of the F1 hybrid between AgCs and AT 3425, 87 did not show any pairing between *Thinopyrum* chromosomes, 33 showed one rod bivalent formed by *Thino-

pyrum* chromosomes, and three had one ring bivalent formed by *Thinopyrum* chromosomes (Fig. 2a,b). Often, two of the three wheat–*Thinopyrum* translocated chromosomes from AT 3425 paired as bivalents with corresponding wheat chromosomes from AgCs. Most wheat chromosomes in the hybrid appeared as bivalents (Fig. 2a,b). *Th. elongatum* and *Th. ponticum* genomic DNAs hybridized with *Thinopyrum* chromatin derived from both parents in the hybrid (Fig. 2a,b). In the hybrid of AT 3425 with PI 550713, *Thinopyrum* chromosomes appeared as seven bivalents or six bivalents plus two univalents in all of the 97 PMCs analysed (Fig. 2c). The translocated chromosomes that showed similar FGISH patterns in both parents also paired with each other as bivalents in the hybrid. Most of the wheat chromosomes were present as bivalents (Fig. 2c).

Discussion

*Thinopyrum* genomes have been successfully introduced into annual wheat for perennializing annual wheat (Suneson and Pope 1946, Tsitsin 1960, Wagoner 1990, Cai et al. 1998b). In the present study, three perennial wheat–*Thinopyrum* amphiploids were identified. Among these three perennial amphiploids, AgCs and AT 3425 were reported to be produced from crosses between wheat and the diploid *Th. elongatum* (Mathre et al. 1985, Dvorak 1976). The third perennial amphiploid, PI 550713, was developed from crosses between wheat and an unknown *Thinopyrum* species by Dr C. A. Suneson (H. E. Bockelman, pers. comm.).

FGISH has proven to be a powerful molecular cytogenetic technique to distinguish chromatin of one genome from another genome (Mukai et al. 1993, Friebe et al. 1994, Zhong
et al. 1994, Cai et al. 1998a,b). In the present study, FGISH was used successfully to characterize Thinopyrum genomes in the perennial wheat–Thinopyrum amphiploids. Since Thinopyrum genomes in two of the three perennial amphiploids were reported to be derived from Th. elongatum, Th. elongatum genomic DNA was used as a probe to perform FGISH on mitotic chromosomes of the amphiploids. FGISH patterns indicated that AgCs had seven pairs of Thinopyrum chromosomes and 21 pairs of wheat chromosomes (Fig. 1a). However, AT 3425 appeared to have three pairs of wheat–Thinopyrum translocated chromosomes and 18 pairs of wheat chromosomes in addition to seven pairs of Thinopyrum chromosomes (Fig. 1c). A similar result was obtained using Th. ponticum genomic DNA as a probe (Cai et al. 1998a). According to chromosome constitutions of AgCs and AT 3425, it seems unlikely that these two amphiploids originated from the same source, i.e. hybridization between common wheat and diploid Th. elongatum.

Th. elongatum and Th. ponticum formerly shared the same name, Agropyron elongatum (Host) Beauv., because of their similar morphology. The Thinopyrum genomes in both AgCs and AT 3425 showed hybridization signal with Th. elongatum genomic DNA (Fig. 1a,c). Th. ponticum genomic DNA also showed hybridization signal with the Thinopyrum genomes in

Fig. 1: Fluorescent genomic in situ hybridization (FGISH) patterns of mitotic chromosomes. a. AgCs with Thinopyrum elongatum genomic DNA used as a probe; b. AgCs with Thinopyrum ponticum genomic DNA used as a probe; c. AT 3425 with Th. elongatum genomic DNA used as a probe; d. PI 550713 with Th. ponticum genomic DNA used as a probe. White, teal and blue arrows point to three pairs of wheat–Thinopyrum translocated chromosomes.
both AgCs and AT 3425 (Fig. 1b and Cai et al. 1998a). These results demonstrate that the genome in Th. elongatum is closely related to a genome in Th. ponticum, which is consistent with previous reports based on classical cytogenetic analysis (Dvorak 1975, 1981b). Hybridization of Th. ponticum genomic DNA with Thinopyrum chromatin in the amphiploids suggested that Th. ponticum might be involved in the production of the amphiploids.

AT 3425 was hybridized with AgCs and PI 550713 to analyse relationships among Thinopyrum genomes in the perennial amphiploids. FGISH patterns of meiotic chromosomes in the hybrid between AT 3425 and AgCs indicated that most Thinopyrum chromosomes appeared as univalents and only one bivalent was observed in 36 out of 123 PMCs analysed. This result revealed that the Thinopyrum genome in AT 3425 is different from that in AgCs. Therefore, the Thinopyrum genomes in both AT 3425 and AgCs cannot be derived from the same Thinopyrum species, i.e. Th. elongatum. At least one of these two amphiploids does not carry the Th. elongatum genome. Th. elongatum is a diploid species with 14 chromosomes. Hybridization between this species and hexaploid common wheat (2n = 6x = 42) allows production of

Fig. 2: Fluorescent genomic in situ hybridization (FGISH) patterns of meiotic chromosomes at MI. a. (AT 3425 × AgCs) F1 hybrid with Thinopyrum elongatum genomic DNA used as a probe; b. (AT 3425 × AgCs) F1 with Thinopyrum ponticum genomic DNA used as a probe; c. (AT 3425 × PI 550713) F1 with Th. ponticum genomic DNA used as a probe. White, teal, and blue arrows point to chromosome pairing configurations in which three wheat-Thinopyrum translocated chromosomes were involved.
an amphiploid with 14 Thinopyrum genomes and 42 wheat genomes. However, it is unlikely to produce a genetically stabilized amphiploid carrying wheat-Th. elongatum translocations in addition to all 14 Thinopyrum genomes. In a wheat-Th. elongatum amphiploid with all 14 Thinopyrum genomes, any wheat-Th. elongatum translocations result in duplication of translocated Thinopyrum chromosome segments. Chromosome duplication leads to abnormal meiosis and genetic instability. However, no abnormal meiosis was found in AT 3425 and all chromosomes in the amphiploid have been maintained for many generations (X. Cai and S. S. Jones, unpubl. data). Therefore, it is likely that AgCs is a wheat-Th. elongatum amphiploid, whereas AT 3425 is an amphiploid derived from a cross between wheat and a polyploid Thinopyrum species.

Th. ponticum is a decaploid species with 70 chromosomes. It is known that some of the genomes in Th. ponticum have a close homoeologous relationship with that of Th. elongatum, but no common genome has been identified in these two species (Dvorak 1975, 1981a,b). Many partial wheat-Th. ponticum amphiploids with 56 chromosomes have been produced from crosses between wheat and Th. ponticum. In addition, wheat-Th. ponticum chromosome translocations have been detected from these amphiploids (Dvorak 1976, Zhang et al. 1993, Zhang et al. 1996, Xu et al. 1994). The fact that Th. ponticum genomic DNA hybridized with Thinopyrum chromatin in AT 3425, and that AT 3425 carried three pairs of wheat-Th. ponticum translocated chromosomes in addition to seven pairs of Thinopyrum chromosomes, suggested that the Thinopyrum genome in AT 3425 was most likely to be derived from Th. ponticum and that AT 3425 might be a partial wheat-Th. ponticum amphiploid.

The perennial wheat-Thinopyrum amphiploid PI 550713 showed the same FGISH patterns of mitotic chromosomes as AT 3425 (Fig. 1c,d and Cai et al. 1998a). In the F1 hybrid between AT 3425 and PI 550713, all Thinopyrum and wheat-Thinopyrum chromosomes from both parents paired with each other in most of PMCs analysed (Fig. 2c). This result indicated that AT 3425 and PI 550713 had the same Thinopyrum genome. It is not known whether these two perennial amphiploids were derived from the same source.

The present study shows that the Thinopyrum genome conferring perennial growth habit in AgCs was derived from Th. elongatum and that the Thinopyrum genome conferring the perennial growth habit in AT 3425 was most likely derived from Th. ponticum. The other perennial wheat-Thinopyrum amphiploid, PI 550713, identified in the present study carried the same Thinopyrum genome as AT 3425. Thinopyrum genomes in AgCs and AT 3425 have different genetic sources for perennial growth habit and these different genetic sources may aid in more efficient development of perennial wheats.

References