GISH Analysis Revealed New Aspect of Genomic Constitution of *Thinopyrum intermedium*

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Abstract: We conducted genomic *in situ* hybridization (GISH) analyses to clarify genomic constitutions of *Thinopyrum intermedium* (Host) Barkworth & D. R. The results revealed that *Th. intermedium* contains three kinds of genomes: St, E/J, and the third genome distinct from the previous two genomes which may be close to V genome. However, PCR analysis denied the presence of the present-day V genome in *Th. intermedium* but showed some similarity in R genome. Thus, the genomic formula of *Th. intermedium* can be tentatively re-designated as StStJ^{s}J^s(V-J-R)^s(V-J-R)^s. We also demonstrated that the probe of V genome would be useful to characterize wheat-*Th. intermedium* amphiploids and *Th. intermedium* chromosome addition lines of wheat.

Keywords: genomic *in situ* hybridization; *Thinopyrum intermedium;* genomic constitutions

Thinopyrum intermedium (Host) Barkworth and D. R. Dewey ($2n = 6x = 42$) has been utilized as alien genetic resources for wheat improvement of disease resistances (for review to see Fedak 1999). Despite of its importance of the species, its genomic constitution of the species seems to be not settled and unusual. It has been thought that *Th. intermedium* constitutes of two genomes closely related to E genome of *Th. elongatum* (Host) D. Dewey and of one distinct genome (Dewey 1962; Dvořák 1981). Liu and Wang (1993) identified that the distinct genome of *Th. intermedium* came from St genome of the genus *Pseudoregneria* (Nevski) Á. Löve and designated the species as E1E2St where E1 and E2 are related to E or J genomes of *Th. elongatum* or *Th. bessarabicum* (Savul. & Rayss) A. Love. More recently, Chen *et al*. (1998) analyzed this species in detail using genomic *in situ* hybridization (GISH) and reported the genome as JJJ^{s}J^{s}SS (S = St) where JJ is closely related to the genome of *Th. bessarabicum* and J^{s}J^s is a modified version of J genome with signal of St genome around the centromeric regions detected by GISH. However, the strange feature revealed in this analysis was that the numbers of chromosomes of J^s and J genomes were 6–8 and 20–22, respectively. It is obviously deviated from the basic chromosome number of the diploid genomes ($2n = 14$) in *Triticeae* species, and it calls for further analysis of *Th. intermedium* for the genomic constitution.

MATERIALS AND METHODS

Plant material

The plant species in this study were summarized in Table 1. The plant materials are maintained in USDA-ARS-FRRL in Utah State University (the United States) or the Tottori Alien Chromosome Bank of Wheat (TACBOW) in Tottori University (Japan).

Table 1. Plant materials used in this study

Species/Lines	Genome	Accession	Origin	Marinating site
Thinopyrum intermedium (Host) Barkworth & D. R. Dewey	StStJ^{s}J^s(V-R-J)^s(V-R-J)^s	PI 249144	Israel	USDA Utah
Thinopyrum intermedium	StStJ^{s}J^s(V-R-J)^s(V-R-J)^s	PI 547315	Former USSR	USDA Utah
Thinopyrum intermedium (Host)	StStJ^{s}J^s(V-R-J)^s(V-R-J)^s	MK10101–MK10103	China	TACBOW
Thinopyrum intermedium (Host)	StStJ^{s}J^s(V-R-J)^s(V-R-J)^s	MK10104–MK10113	U.S.A	TACBOW
Thinopyrum bessarabicum (Savul. & Rayss) Á. Löve	JJ	PI 531710	Former USSR	USDA Utah
Thinopyrum elongatum (Host) Nevski	EE		unknown	TACBOW
Aegilops squarrosa L.	DD	KT120-003		TACBOW
Agropyron cristatum (L.) Gaertn.	PP	PI 315357	Former USSR	USDA Utah
Dasypyrum villosum (L.) P. Candargy	VV		China	TACBOW
Hordeum chilense Roem & Schult	HH	KH16	Chile	TACBOW
Psathyrostachys huashanica Keng	NsNs	HT17585	China	TACBOW
Pseudoroegneria stipifolia (Czem. ex Nevski) Á. Löve	StSt		U.S.A	TACBOW
Secale cereale L.	RR	KS21		TACBOW
Triticum aestivum – *Th. intermedium* partical amphiploid (Yuan-5)	AABBDD + 14"	TACBOW0069	China	TACBOW
Th. intermedium chromosome addition line (Ai#E)	AABBDD + 1"[Ai#E]	TACBOW0137	Japan	TACBOW
Th. intermedium chromosome addition line (Ai#F)	AABBDD + 1"[Ai#F]	TACBOW0138	Japan	TACBOW

Genome symbols were adopted from Wang *et* al. (1994) except for *Th. bessarabicu*.

GISH and PCR analysis

Genomic *in situ* hybridization was carried out as described by Kishii *et al*. (2004). For each slide, 100 ng of probes was applied with or without 500 ng of blocking DNA. For V genome specific Sequence-tagged site (STS) analysis, the primers (Dv-F, 5'ggaacaatttcgcacttacagctc3' and Dv-R, 5'cc tcgatacctttccaacacctac3') designed according to the sequences AF472572 was employed with annealing temperature of 57°C for 20 cycles of PCR. Cleaved Amplified Polymorphic Sequence (CAPS) analysis was conducted by amplifying the STS fragment using primers (F03-F1, 5'tgatcacctggttgataagtca3' and F03-R1, 5'aaagtatttattcactcaaccggatct3') at 58°C for 20 cycles of PCR, followed by digestion of the 1277bp fragment with *Hin*dIII or *Eco*RI restriction enzyme.

RESULTS AND DISCUSSION

Since there was no report of double color GISH using E and St genomes as probes at same time on *Th. intermedium*, we firstly conducted this analysis using St (green) and E (red) genomic DNAs as probe. The result revealed that 14 chromosomes were not stained in both colors, though E genomic probe hybridized at some extent (Figure 1a), clearly indicating the presence of third genome in *Th. intermedium* which would be different from St and E/J genomes. Considering that these chromosomes could be easily recognized by the presence of centromeric St signals, we had tried to identify the third genome using many diploid species as probe. The hybridization patterns of genomic probe were species specific: scattered signals (*H. chilense* and *P. huanshanica*; Figure 1b), uniform signals in

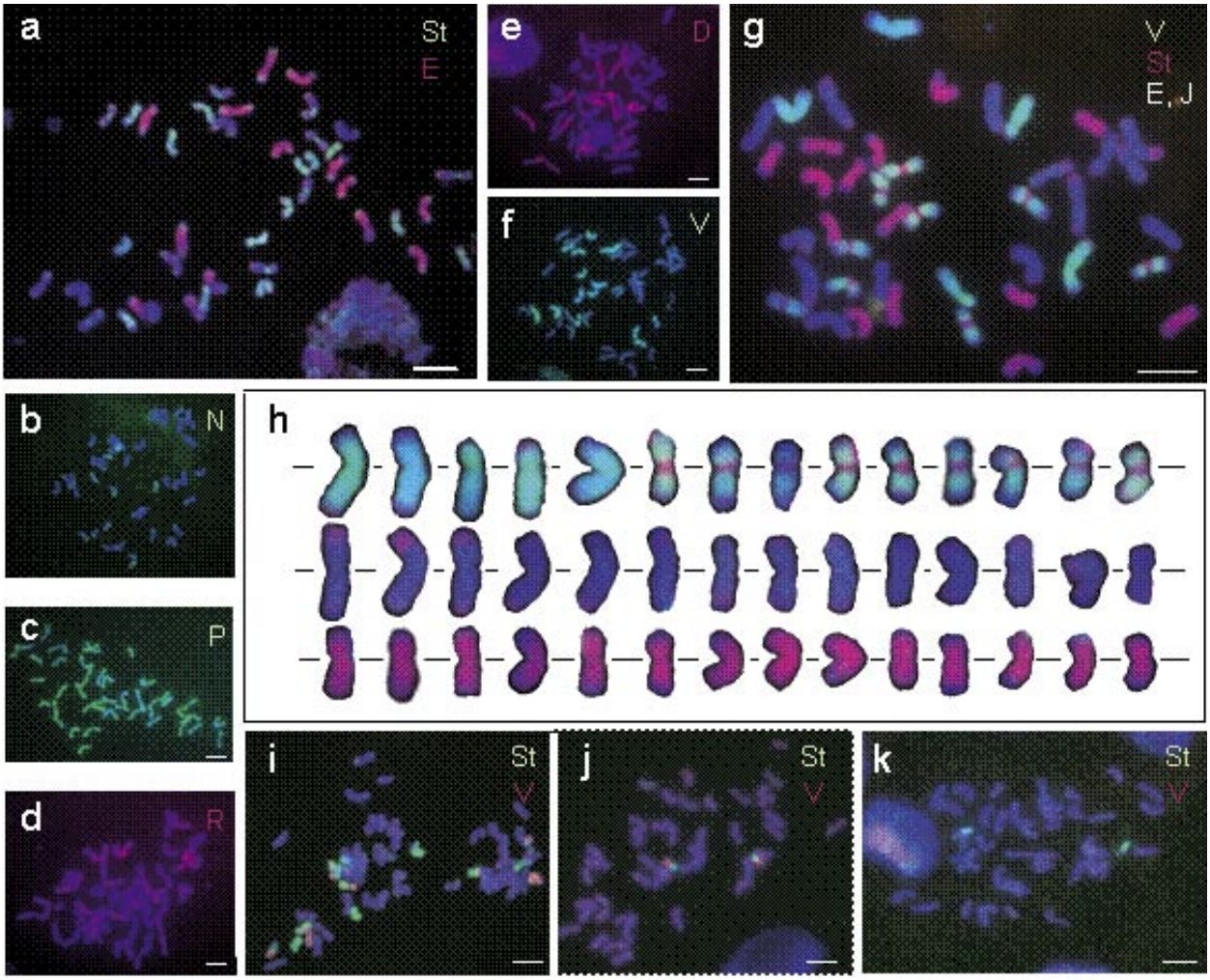

Fig. 1. GISH images of *Thinopyrum intermedium*, and its amphiploid and addition lines. (a)-(g) *Th. intermedium*, (h) cut-out picture of g, (i) *Triticum aestivum-Th. intermedium* amphiploid (Yuan-5), (j-k) *Th. intermedium* chromosome addition lines (j = Ai#E; k = Ai#F). The probes for each image were indicated in the upper right (green = green florescence labeled probe, red = red florescence probe, and white = blocking DNA). All bars equal 10 μm

all chromosomes (*A. cristatum*; Figure 1c); staining several chromosomes stronger than the rests (*S. cereale*; Figure 1d and *Ae. tauschii*; Figure 1e). When we used V genome as probe, the hybridization pattern was just same as that we had seen in Figure 1a (Figure 1f). The GISH using St and V genomes clearly showed that probe of V genome was hybridized to 14 chromosomes of a different genome rather than St and E genomes (Figure 1g and h). The nine chromosome of the genome had centromeric signals of St genome, and this number were various among accessions from eight to ten, indicating complex evolutional history of the V-like genome in the species. All chromosomes of J and V-like genomes showed faint signals of St genome in terminal regions at some extent. Some plants from the U.S.A origin were $2n = 43$ in chromosome numbers and also contained translocations among different genomes (data not shown).

We conducted PCR analysis using V genome specific STS markers to obtain additional evidences that V-genome is actually present in *Th. intermedium*. However, it did not give any amplification in *Th. intermedium* (Figure 2a), denying the presence of 'present-day' V genome in *Th. intermedium*. We found that one CAPS marker showing that *Secale cereale* and *Th. intermedium* shared the unique fragment (Figure 2b). These results and that V, J/E, and R genomes showed hybridization signals at varying intensities in GISH (Figure 1) may indicate that the third genome was a progenitor genome before the divergence of these three genomes. Therefore, we can tentatively re-designated the genomic formula of *Th. intermedium* as $StStJ^sJ^s(V\text{-}J\text{-}R)^s(V\text{-}J\text{-}R)^s$ (where

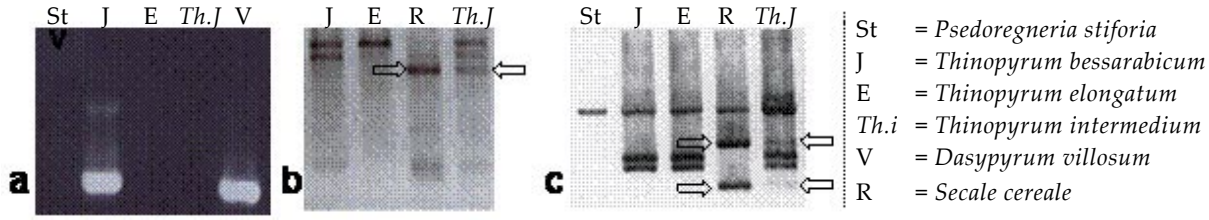

Fig. 2 Electrophoresis images of STS and CAPS analysis. (a) V genome specific STS marker. The *Dasypyrum villosum* and *Thinopyrum bessarabicum* showed the 239bp fragment but not in *Thinopyrum intermedium.* (b-c) CAPS marker. The gel images were inverted in black and white. *Th. intermedium* and *Secale cereale* share the CAPS fragments after *Hin*dIII (b) and *Eco*RI (c) digestions

superscripted s stands for St genome signals in E/J and V-J-R genomes, and V-J-R stands for a progenitor or derivative genome involved in the evolution of the three genomes). The cytogenetic analysis of meiotic pairing in hybrids would be necessary to lead to a conclusion.

When we did GISH in the amphiploid (Yuan-5) using V genome as probe, the *Th. intermedium* chromosomes could be not only distinguished from wheat chromosomes but also identified individually (Figure 1g). The V genome probe could also distinguish translocation between J^s and $(V\text{-}J\text{-}R)^s$ genomes (Figure 1i and j). The results show the usefulness of V genome as GISH probe. Considering that there are several subspecies in *Th. intermedium*, it may also need to evaluate those by GISH with V-genome probe to determine their genomic compositions.

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