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Fixation of translocation 2A·4B infers the monophyletic origin of Ethiopian tetraploid wheat

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Abstract Analysis of structural chromosomal polymorphism revealed the presence of a previously reported 2A·4B translocation common to all 15 strains of Ethiopian tetraploid wheat examined. Using the C-banding technique, we found two new translocations,T1B·6B and T5B·6B, and a pericentric inversion of chromosome 5A. The C-banding pattern indicated that in all three translocations the breakpoint was located in the centromeric region. Sequential N-banding and genomic in situ hybridization (GISH) confirmed the location of the breakpoint of translocation 2A·4B, and revealed that the breakpoint of another known translocation, 2A-2B, was in the proximal region of 2BL. The fixation of the 2A·4B translocation indicates the monophyletic origin of Ethiopian tetraploid wheat and the presence of a very severe bottleneck effect during its dispersal.

Key words Tetraploid wheat, Landraces, Ethiopia, Translocation, Inversion

Introduction

Tetraploid emmer wheat (2n=4*x*=28; AABB genome) has been cultivated in Ethiopia since ancient times and this country is believed to be an important center of diversity (Harlan 1969, 1992; Zohary 1970). Many morphological variations have been recorded in Ethiopian landraces of tetraploid wheat, and a specific rank has been given to them such as *Triticum abyssinicum* Vav., *T. dicoccum* Schübl., *Triticum durum* Desf., *Triticum polonicum* L., *Triticum pyramidale* (Del.) Perc. and *Triticum turgidum* L. However, these morphotypes are now treated as one species, *T. turgidum* L. (Mac-Key 1966; van Slageren 1994) due to the lack of reproductive barriers. Typical characters of the Ethiopian tetraploid wheat are purple kernels, anthocyanin pigmentation in the vegetative organs and awnless forms. They are valuable genetic resources because of their rust resistance, long coleoptile, short culm, low tillering, early maturity and drought resistance (Mekbib and Haile Mariam 1990; Perrino and Porceddu 1990).

The effective use of the Ethiopian landraces of tetraploid wheat depends, to a large extent, on the general information not only on the amount of genetic variation but also on specific problems such as chromosomal translocations and inversions, since they drastically change the linkages of genes. Recent studies have shown that chromosomal polymorphism is common among Ethiopian tetraploid wheat (Belay et al. 1994). A preliminary screening of chromosome structures in tetraploid emmer wheat (Kawahara 1997) revealed not only the overall dominance of the standard type with regard to translocations but also the presence of a 2A·4B translocation in three strains of the ”abyssinicum” group, a unique endemic Ethiopian free-threshing spring emmer originally recognized as *T. durum* ssp. *abyssinicum* Vav. and *T. turgidum* ssp. *abyssinicum* Vav. (MacKey 1966).

In the present study, chromosomal rearrangements were examined in landraces of Ethiopian tetraploid wheat by crossing with tester lines and by C-banding to determine the degree of chromosomal polymorphism. Further, breakpoints in intergenomic translocations were precisely located by sequential N-banding and genomic in situ hybridization (GISH) (Jiang and Gill 1993).

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Materials and Methods

Genetic stocks

A total of 15 genotypes of Ethiopian tetraploid wheat were used (Table 1, Fig. 1). Among these genotypes, KU-186 and KU-188 have a 2A·4B translocation and KU-185 has two translocations, 4B·2A-2B, and they were used as translocation testers. Three additional strains, LD-222, KU-125 and KU-127, were used as the standard chromosome arrangement in the banding or crossing experiments. All these strains are maintained by controlled selfing at the Plant Germ-plasm Institute, School of Agriculture, Kyoto University.

Cytological methods

Chromosome pairing in the hybrids with testers was observed at first meiotic metaphase (MI) of the pollen mother cells (PMCs) by the aceto-orcein squash method. The C-banding procedure followed that of Giraldez et al. (1979) except that Wright's eosin methylene was used for staining. C-banding patterns and karyotypes were analyzed using LD-222 as the standard, based on the original cytogenetic identification of wheat chromosomes by Gill and Kimber (1974) and Gill et al. (1991). For sequential N-banding and genomic in situ hybridization (GISH) (Jiang and Gill 1993), slides were first stained for N-banding according to the method of Endo and Gill (1984). After recording the N-banding pattern, the slides were de-stained in 75, 95 and 100% ethanol each for 5 min, air-dried and processed for GISH according to the method reported by Murata et al. (1992). Biotin-labelled total genomic DNA from *Triticum monococcum* L. ssp. *monococcum* was used as a probe, and unlabeled genomic DNA from *Aegilops speltoides* Tausch was added as a block.

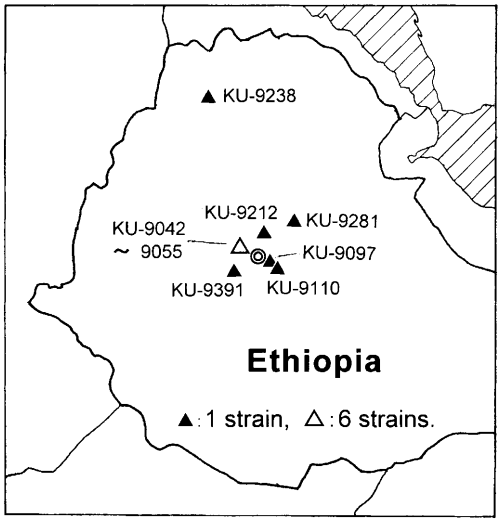

Fig 1 Collection sites of Ethiopian landraces of tetraploid wheat

Results and Discussion

Identification of chromosome structure

Five strains, KU-9043, 9047, 9048, 9054 and 9055, were crossed to both standard (KU-125 or 127) and 2A·4B (KU-186) testers and chromosome pairings at MI were observed in the hybrids (Table 2). The hybrids with the standard tester formed one quadrivalent in most of the PMCs, but hybrids with KU-186 showed regular meiosis with 14 bivalents. KU-9391 formed one quadrivalent in hybrids with KU-185 that carries translocations 4B·2A-2B but showed regular meiosis in hybrids with KU-186. From the present results, we conclude that these six strains have translocation 2A·4B of KU-186.

Chromosome structure was analyzed by C-banding in ten strains, LD-222, KU-186 (2A·4B), KU-185 (4B·2A-2B), KU-188, 9042, 9097, 9110, 9212, 9238 and 9281. The five strains KU-188, 9042, 9110, 9212 and 9238 had C-banding patterns almost identical to that of KU-186. This indicates that these five strains have the same 2A·4B translocation as KU-186. In strain KU-186, the short arm of 2A was replaced by the long arm of 4B and showed the arm combinations of 2AL·4BL and 2AS ·4BS (Figs. 2, 4). The breakpoints of this 2A·4B translocation were assumed to be in the centromeric region. The C-banding confirmed that strain KU-185 has a cyclic 4B·2A-2B translocation as

Table 1 *T. turgidum* strains used in the present study

Strain no.	Locality or source (altitude)	Donor name
LD-222	Prof. Nishikawa, Kifu University	–
KU-125	Collection of College of Agric., Hokkaido Univ., Japan	–
KU-127	Inner Mongolia Expedition, China	Hirayoshi
KU-185	Collected in Ethiopia by Dr. Furusato	Furusato
KU-186	Collected in Ethiopia by Dr. Furusato	Furusato
KU-188	Jenkins 4B-470	Jenkins
KU-9042	Mt. Entoto, Addis Ababa (2800 m)	KUSES[a]
KU-9043	Mt. Entoto, Addis Ababa (2800 m)	KUSES[a]
KU-9047	Mt. Entoto, Addis Ababa	KUSES[a]
KU-9048	Mt. Entoto, Addis Ababa	KUSES[a]
KU-9054	Mt. Entoto, Addis Ababa	KUSES[a]
KU-9055	Mt. Entoto, Addis Ababa	KUSES[a]
KU-9097	11 km SE of Debre Zeyit	KUSES[a]
KU-9110	12 km E of Debre Zeyit (2000 m)	KUSES[a]
KU-9212	65 km from Addis Ababa to Fiche (2600 m)	KUSES[a]
KU-9238	Approximately 40 km from Gonder to Debat (2890 m)	KUSES[a]
KU-9281	5 km from Molale to Debre Birhan (3180 m)	KUSES[a]
KU-9391	90 km from Addis Ababa to Giyon	KUSES[a]

[a] KUSES: Kyoto University Scientific Expedition to the Sahara and the surrounding areas (1967–1968)

Table 2 Metaphase-I pairing in F_1 hybrids between six Ethiopian landraces of tetraploid wheat and translocation testers

Cross combination[a]	No. of PMCs observed	Mean chromosome configuration			
		I	II	III	IV
KU-127×KU-9043	23	0.04	12.00	0.04	0.96
KU-186×KU-9043	18	–	14.00	–	–
KU-125×KU-9047	33	–	12.00	–	1.00
KU-186×KU-9047	11	0.18	13.91	–	–
KU-127×KU-9048	20	–	12.10	–	0.95
KU-186×KU-9048	33	–	14.00	–	–
KU-127×KU-9054	33	–	12.24	–	0.88
KU-186×KU-9054	33	–	14.00	–	–
KU-127×KU-9055	33	–	12.12	–	0.94
KU-186×KU-9055	33	–	14.00	–	–
KU-185×KU-9391	20	–	12.10	–	0.95
KU-186×KU-9391	22	0.09	13.95	–	–

[a] Tester strains. KU-125 and KU-127: standard; KU-186: 2A ·4B translocation; KU-185: 4B·2A-2B translocation

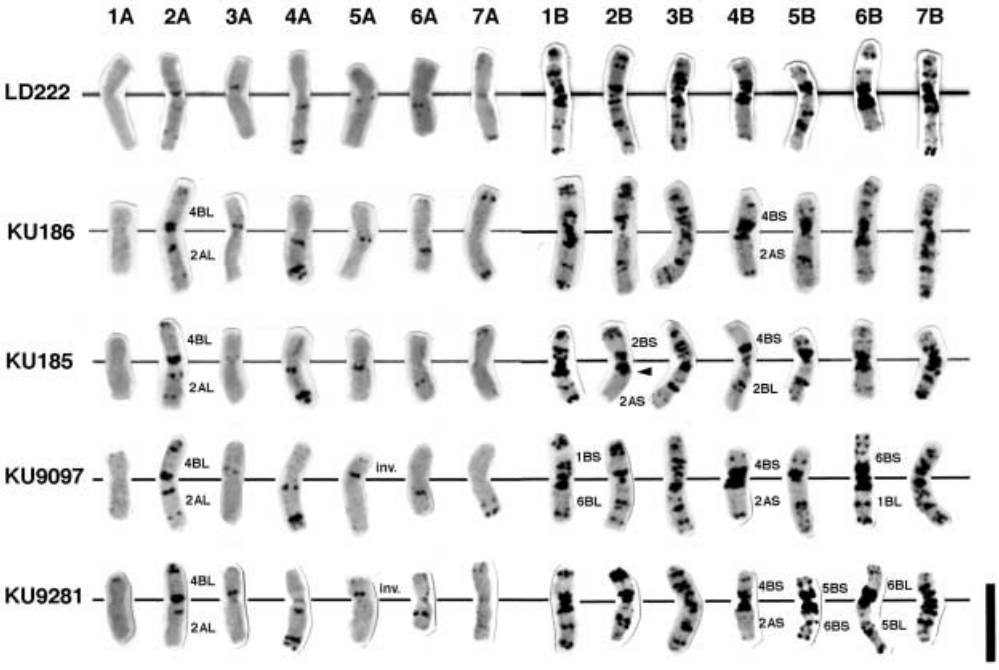

Fig 2 C-banding pattern of standard LD-222 (*top*) and four strains of Ethiopian tetraploid wheat. *Bar* represents 10 μm

found previously by pairing analysis (Nishikawa et al. 1986). In addition to this 2A·4B translocation, another translocation 1BS·6BL, 6BS·1BL was detected in KU-9097 and T5BS·6BS, T6BL·5BL was found in KU-9281, as shown in Fig. 2. The breakpoints of these two translocations are also located in or near the centromere.

LD-222 and KU-186 had a proximal C-band in 5AL. In seven strains, KU-188, 9042, 9097, 9110, 9212, 9238 and 9281, such a 5AL band was not observed while a proximal band was detected in 5AS (Fig. 2). This indicates the presence of a pericentric inversion in 5A but we could not determine its precise size because of the small number of C-bands on the 5A chromosome. Since this 5AS band has not been observed in the wild progenitor, *T. turgidum* ssp. *dicoccoides* (Körn. ex Asch. & Gräbn.) Thell. (Taketa and Kawahara 1996), this inversion probably arose within Ethiopian tetraploid wheat.

The position of the breakpoint in intergenomic translocations

The precise location of translocation breakpoints in KU-185 and KU-186 were established by sequential N-banding and the GISH method. Two pairs of chromosomes identified by N-banding as 2AL·4BL and 2AS·4BS in KU-186 were shown to be intergenomic by GISH (Fig. 3). The GISH pattern also revealed a small 7B segment translocated to 4A, which was fixed during the evolution of tetraploid wheat (Gill and Chen 1987; Naranjo et al. 1987, 1988). Two pairs of chromosomes with A-B arm combinations, T4BL·2AL and T2BS·4AS, were detected in KU-185 by GISH. The breakpoint was located in the centromeric region in one pair of chromosomes, T4BL·2AL, and near but not in the centromeric region in the other. We concluded from the banding pattern and the GISH data that T2A·4B occurred first and then 2AS arms and 2BL were further exchanged in KU-185. This differs from the estimation based on pairing data by Nishikawa et al. (1986) who

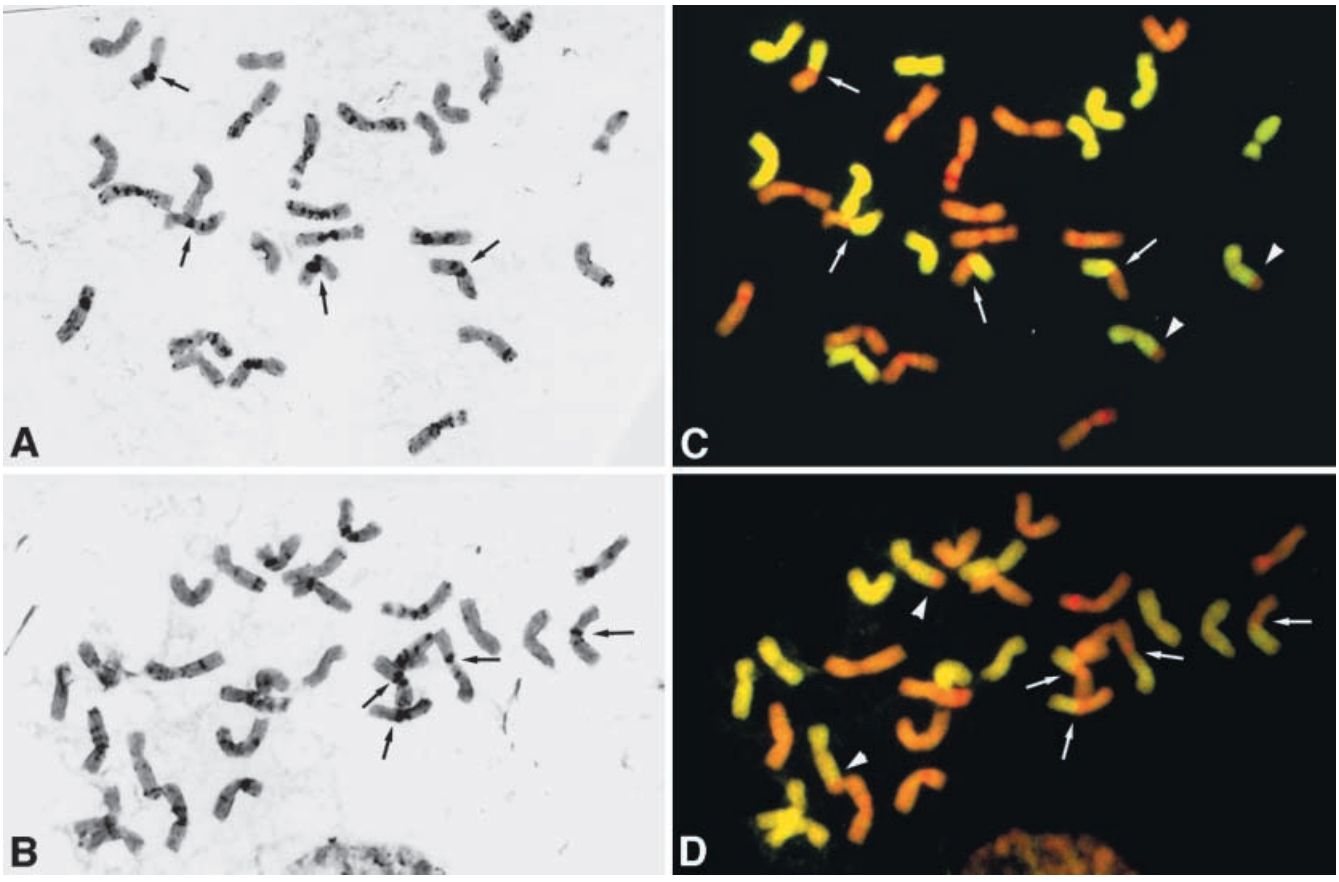

Fig 3A–D Sequential N-banding (**A**, **B**) and GISH (**C**, **D**) in KU-186 (**A**, **C**) and KU-185 (**B**, **D**). Chromosomes of the A genome were labeled with biotin (*yellow*) using total genomic DNA from *T. monococcum* ssp. *monococcum* as a probe. Proximal trans-location breakpoints, 4BL ·2AL and 4BS·2AS in KU-186 and 4BL·2AL and 2BS·2BL-2AS in KU-185, were detected by *yellow*/*red* colors (*arrows*). Small 7B terminal segments caused by species-specific 4A-5A-7B translocation were confirmed (*arrowheads*)

Table 3 Classification of Ethiopian landraces of tetraploid wheat based on chromosomal translocations

2A·4B: KU-186, 188, 9042, 9043, 9047, 9048, 9054, 9055, 9110, 9212, 9238, 9391
4B·2A-2B: KU-185
2A·4B, 1B·6B; KU-9097
2A·4B, 5B·6B: KU-9281

assumed that the two breakpoints on 2A were located on different arms.

Evolution of chromosome structure in Ethiopian landraces of tetraploid wheat

On the basis of the chromosomal translocations determined by chromosome-pairing analysis with the tester strains and by C-banding, the 15 Ethiopian strains were classified into four groups as shown in Table 3. Twelve strains had the same 2A·4B translocation as KU-186 and 188. KU-185 had a cyclic translocation, 4B·2A-2B. KU-9097 had 2A·4B and 1B·6B translocations and KU-9281 had 2A·4B and 5B·6B translocations. The present results showed that all the samples originating from Ethiopia had the same translocation with arm combinations of 2AL·4BL and 2AS·4BS. This is unexpected because earlier studies on translocations in wild and cultivated emmer wheat revealed the overall dominance of the standard type without translocation. Kawahara and Nevo (1996) reported that about one-fifth of the genotypes has a translocation in the Israeli population of *T. turgidum* ssp. *dicoccoides*. The remaining genotypes had the standard chromosomal arrangement with regard to translocation. Similarly, of 25 strains of cultivated emmer examined by Kawahara (1997), 20 had the standard chromosome structure.

Based on the structural rearrangements detected in the present study, a phylogenetic tree of Ethiopian landraces was constructed, as shown in Fig. 4. Strains in which the 2A·4B translocation was found by the analysis of pairing were not included in Fig. 4 because information on the 5A inversion was not obtained by pairing data. This tree apparently indicates that the 2A·4B translocation occurred in Ethiopian tetraploid wheat and was fixed sometime at an early stage in the dispersal of tetraploid wheat into Ethiopia. All the strains whose collection site is well documented, KU-9042, 9097, 9110, 9212, 9238 and 9281, had the 5A inversion in addition to the 2A·4B

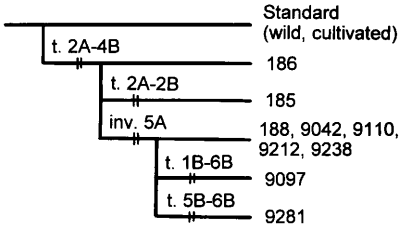

Fig 4 Phylogeny of Ethiopian landraces of tetraploid wheat inferred from chromosomal polymorphism (*t* translocation, *inv* inversion)

translocation (Fig. 1). KU-9097, that has an additional 1B·6B translocation, was found in the SE of Addis Ababa, and KU-9281, with an additional 5B·6B translocation, was collected in the NE of Addis Ababa. The fixation of several structural rearrangements during the evolution of polyploid wheats has been reported. Tetraploid emmer wheat and hexaploid common wheat have a species-specific 4A-5A-7B cyclic translocation (Naranjo et al. 1987, 1988), and tetraploid timopheevi wheat *T. timopheevi* (Zhuk.) Zhuk., has a $6A^t$–1G-4G cyclic translocation (Jiang and Gill 1994a) in addition to a $4A^t$–$5A^t$ translocation which was inherited from its ancestor, *T. urartu* Tum. ex Gandilyan (Jiang and Gill 1994b; Maestra and Naranjo 1999). Similarly, a very high frequency of reciprocal translocations has been reported in Japanese common wheat cultivars (Ali et al. 1992; Yasumuro et al. 1998). Yasumuro et al. (1998) reported that 11 of the 15 landraces, and 51 of the 102 bred cultivars, had one or two translocations relative to Chinese Spring which has the primitive chromosome structure of hexaploid common wheat (Kawahara 1988). Therefore, fixation of a particular chromosomal rearrangement is not uncommon in the wheat lineage.

Tetraploid emmer wheat was introduced into Ethiopia in ancient times from their place of origin, the Near East. Harlan (1969) suggested its very ancient and primary dispersal based on the resemblance of Ethiopian tetraploid wheat to the South Indian ”Khapli” wheat. Tetraploid wheat and barley are still important crops in Ethiopia and they were also the main crops of ancient Egypt around 4500 B.C. (Harlan 1969). It is highly probable that the 2A·4B translocation was fixed before or during the dispersal process, due to a very severe bottleneck effect, and that the additional chromosomal rearrangements, other translocations and the 5A inversion, occurred later. Further studies on the distribution of chromosomal rearrangements in Ethiopian tetraploid wheat are required to determine the detailed pattern of their dissemination into, and differentiation within, Ethiopia, as well as to facilitate the use of these novel genetic resources for wheat breeding.

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