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Research article

Morphology and karyology of *Thinopyrum elongatum* (Host) D.R. Dewey

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Abstract

Thinopyrum elongatum (Host) D.R. Dewey, one of the perennial species of Triticeae, is very important grass for the improvement of wheat owing to the presence of several useful genes in its genome. Present study attempted to characterize *Thinopyrum elongatum* (strain ID: TACBOW0120) collected by Dvorak and maintained in Kyoto University, Japan. The external morphology of plant was carried out taxonomically. Microscopic examination was done for pollen grain and mitotic chromosomes of root cells. Altogether, 14 chromosomes were observed and classified into five pair of more or less metacentric chromosomes and two pairs of sub-metacentric chromosomes with satellites (formula 2n=2x=10m+4SATsm=14). Pollen grains were spherical and measured about 38 micrometers. The morphological traits characterized will be useful to study wheat – *T. elongatum* disomic addition lines in which each pair of chromosomes of *T. elongatum* are maintained in 7 different types of common wheat cultivar "Chinese Spring".

Key-words: *Thinopyrum elongatum*, morphology, karyology, pollen.

Introduction

Thinopyrum elongatum (Host) D.R. Dewey syn. *Agropyron elongatum* (Host) Beauv; *Lophopyrum elongatum* (Host) Love*; Elytrigia elongata* (Host) Nevski; *Elymus elongatus* (Host) Runemark; is a perennial, diploid (2n=2x=14, Genome formula EE) weed belonging to the tribe Triticeae of Poaceae. As is evident from the classification of species belonging to Triticeae by several workers it is difficult tribe because of frequent hybridization and occurrence of polyploidy (Jarvie and Barkworth 1992). It is commonly known as wheatgrass and is distributed in eastern and southern Europe, Caucasus, Western Asia and Northern Africa. *Thinopyrum* differs from other Triticeae in its thick and stiff glumes as well as lemmas. These are several cells thick, even between the veins (Jarvie 1992).

According to Bajaj (1990), *Thinopyrum* consists of three species complexes grouped into three sections: *Thinopyrum* section (species of the *Thinopyrum junceum* complex, e.g., *T. bessarabicum, T. distichum, T. junceiforme, T. junceum* and *T. runemarkii*), *Lophopyrum* section (species of the *Thinopyrum elongatum* complex, e.g., *T. caespitosum, T. curvifolium, T. elongatum, T. ponticum,* and *T. scirpeum*) and *Trichophorae* section (species of the *Thinopyrum intermedium* complex, e.g., *T. gentryi, T. intermedium* and *h. podperae*). *Thinopyrum elongatum* belongs to the section *Lophopyrum*. According to the taxonomic treatment of Dewey (1984) most of the perennials used in the crossing with wheat for varietal improvement belongs to the genus *Thinopyrum* Love. because they contain genes for several useful agronomic traits, such as resistance to several biotic and abiotic stresses and increased protein content.

Being a member of the tertiary gene pool of wheat and carrying valuable genes for wheat improvement, researchers have extensively studied this plant. Karyotype analysis of *T. elongatum* has been carried out

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by Dvorak and Knott (1974), Wang (1985), Evans (1962) and others. The seven pair of chromosomes are classified on the basis of arm ratio as, metacentric or near metacentric (3 chromosome pairs; arm ratio 1.0 to 1.3), sub-metacentric (3 pairs; arm ratio 1.3 to 1.6), and heterobrachial (one pair; arm ratio 1.9 to 2.0). Among them two chromosome pairs have satellites; those on one pair being much shorter than those on the other. Dvorak (1980) classified *Thinopyrum elongatum* chromosomes into seven homoeologous groups, numbered from one to seven in relation to the seven homoeologous groups of common wheat. Again, Dvorak *et al*. (1984) classified *T. elongatum* chromosomes based on physical homoeology as 1E to 7E which showed differences with the previous classification of the same researcher. Because of this feature it is a potential plant to transfer genes of agronomic importance especially salt tolerance (Dvorak and Ross 1986) and disease resistance (Shen and Ohm 2007; Sepsi *et al*. 2008) genes to common wheat. Present study attempted to characterize *T. elongatum* for its morphological features along with chromosomes.

Materials and Methods

The seeds of *Thinopyrum elongatum* (Strain ID: TACBOW0120), collected by Dvorak and maintained in "National Bioresourse Project, Kyoto University, Japan were sown in pots at Central Department of Botany, Tribhuvan University (TU), Kathmandu, Nepal.

The mature plant was used for morphological characterization of different parts including culm, different parts of leaf, inflorescence, flower etc. Additionally, matured anther was taken for study of pollen morphology and viability. Pollen viability was carried out following the method adopted by Singh (2016). The pollen grains were firstly dusted on glass slide, stained in aceto-carmine, heated gently and observed under microscope.

The roots of the plant were used for karyotype analysis and characterization of chromosomes using standard protocol. Firstly, the root tips were cleaned with the help of a fine camel hair brush before pretreatment. The materials were pretreated in aqueous solution of 8-hydroxy-quinoline (0.002M) and kept in ice-water at 4 °C for 24 hours. The pretreated roots were fixed using glacial acetic acid and absolute ethyl alcohol in 1:3 ratios (Carnoy's solution) and kept for about one week at room temperature. Then roots were then transferred into vial containing aceto-carmine solution for about two days at room temperature for staining the chromosomes. The roots were then transferred into glacial acetic acid and absolute ethyl alcohol (1:3) for storage. For the chromosome observation the softening of root tip cells were made by boiling the roots in aceto-carmine solution for about 2-4 minutes. The root tips were cut off and placed on glass slide with a drop of 45% acetic acid. Tissue was spread gently by tapping with needle and allowed brief heat before squashed. The slide was then observed under the 100X magnification (LABOMED INC. Los Angeles CA. USA). Best metaphase spread slide was photographed. For the measurement of chromosome arms six cells were photographed and measured.

Results

MORPHOLOGY OF *THINOPYRUM ELONGATUM*

Plant is perennial, caespitose (Figure 1). Culms robust, 30 – 53 cm long, cylindrical, hollow, yellowish green with dark green lining, glabrous, comprising nodes and internodes (14.5 cm). Leaf sheath is closed, 5.5 - 10.5cm long, smooth, glabrous, dark green; margin entire, papery or transparent. Ligule: membraneous (2mm), auricle: claw-like auricle present. Leaf blade: lanceolate, 10.5 – 18 cm x 2.5 – 3.5 cm, upper surface smooth, lower surface ribbed, margin entire, acuminate. Inflorescence: Racemose, raceme, single, bilaterally arranged, 25.6 cm long, rachis flattened with green margin, rachis internodes 1.8 – 2.5 cm long. Flag leaf: lanceolate, 24.2 cm long. Spikelets: spikelets comprising of 5-6 fertile florets, Spikelets solitary, 1.2- 1.5 cm long, oblong or elliptic. Glume: glume persistent, smaller than spikelets. Lower glume: oblong, 7mm x 2mm, margin entire, papery or transparent, 12 veined, lateral veins ribbed, surface smooth, apex emarginate. Upper Glume: oblong, equal with lower glume, margin papery or transparent, 14 veined, 2 side veins are shorter than the middle veins, lateral veins ribbed, surface smooth, apex emarginated. Floret: consists of lemma, palea, anther and stigma. Lemma: fertile lemma lanceolate to oblong, 8mm x 3mm, 5 pairs of veins, margin papery or transparent, apex obtuse. Palea: elliptic, transparent, 7mm x 2mm, keeled, 2 veined. Flower: anther 3, 2mm long, yellow; stigma 2, whitish, hairy, ovary fleshy, membraneous (Figure 2). Pollen- spherical, and contains two crescent male nuclei (Figure 3).

Figure 1. *Thinopyrum elongatum* in pot.

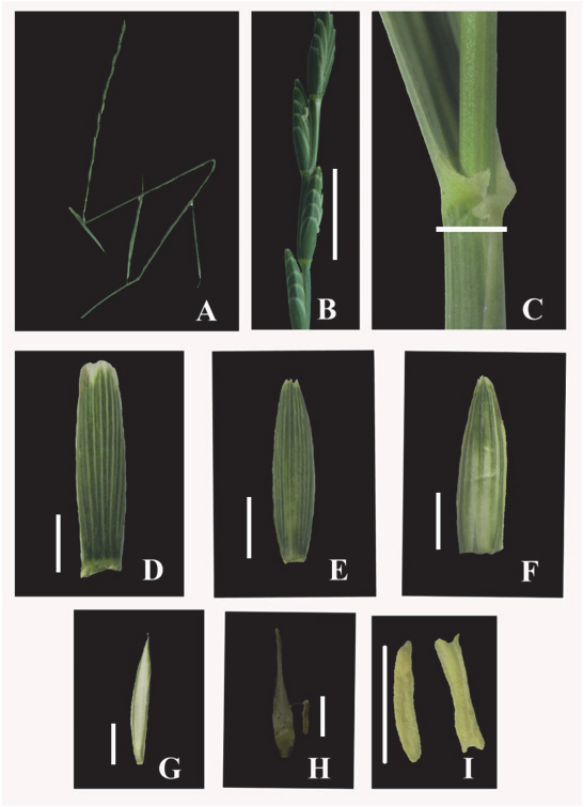

Figure 2. A. Habit of *Thinopyrum elongatum* B. Single spike C. Leaf blade showing auricle D. Lower glume E. Upper glume F. Lemma G. Palea H. Carpel and stamen I. Two anthers. White bars represent measurements (B = 2 cm and C-I = 2 mm).

KARYOMORPHOLOGY OF *THINOPYRUM ELONGATUM*

The root tip obtained from mature plant was used for karyotype analysis. The mitotic study of *T. elongatum* showed fourteen chromosomes. Among them ten chromosomes were metacentric and four chromosomes were sub-metacentric (Figure: 4, 5 and Table: 1). Furthermore, the arm ratio was ranged from 1.14 to 2.86. According to the classification based on Levan *et al.* (1964) chromosomes were classified into five pair of more or less metacentric chromosomes and two pairs of sub-metacentric chromosomes with formula 2n=2x=10m+4SATsm=14. Two pairs of chromosomes consist of satellites (SAT). The SAT chromosomes have one small pair and other large pair of satellite. The total length of *T. elongatum* chromosomes was about 96.6±0.99 micrometer. Chromosome length ranged from 5.82±0.17µm (shortest) to 8.6±0.13 µm (longest). Chromosomes were paired on the basis of arm length and centromere position, where maximum similarity was observed. SAT chromosomes were easily recognizable according to satellite size.

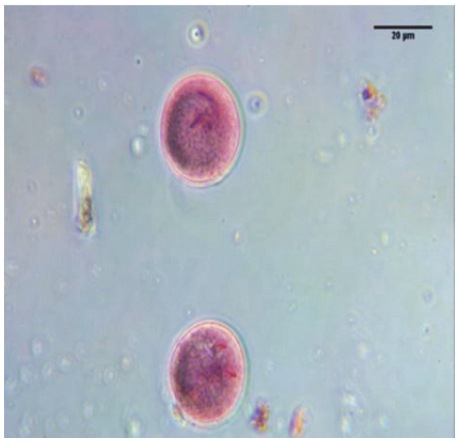

Figure 3. Pollen at 400x magnification with two nuclei

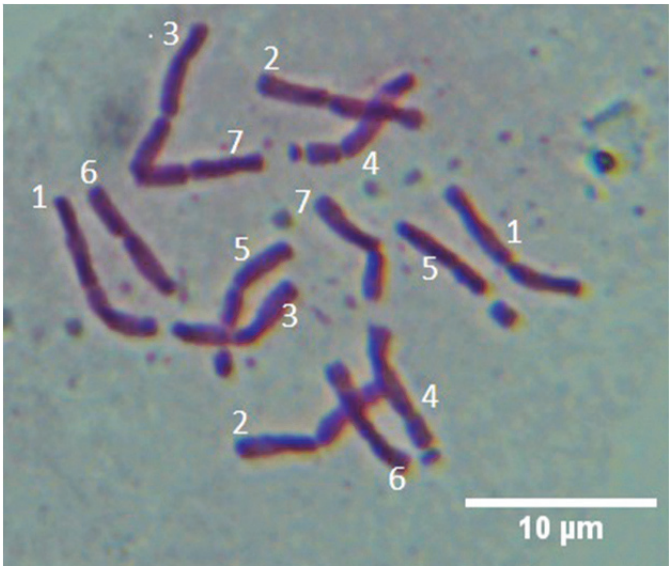

Figure 4. Karyotype of *Thinopyrum elongatum*.

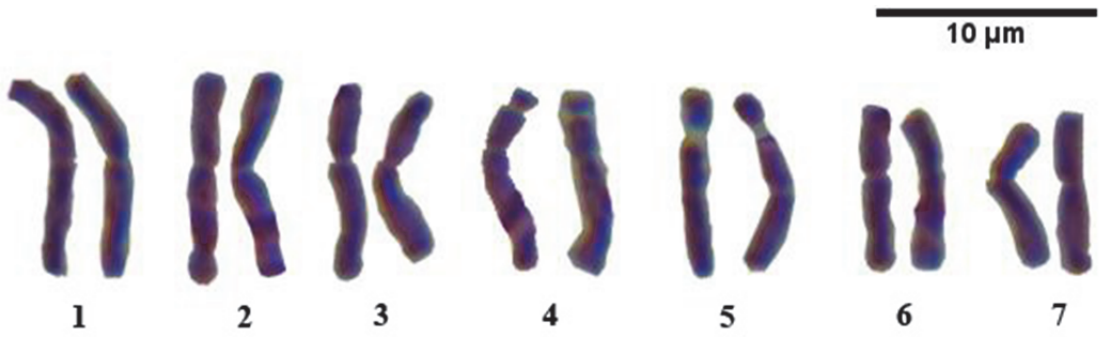

Figure 5. Karyogram of *Thinopyrum elongatum*.

Table 1. Measurement of chromosomes of *Thinopyrum elongatum*.

*Chr. No.	Long arm (µm)	Short arm (µm)	Satellite (µm)	Total Length (µm)	Arm ratio	*Chr. type
1	4.58 ± 0.14	3.92 ± 0.13		8.50 ± 0.18	1.19	M
2	4.45 ± 0.16	3.65 ± 0.11		8.11 ± 0.22	1.21	M
3	4.20 ± 0.17	2.68± 0.24		6.89± 0.22	1.5	M
4	3.09 ± 0.24	1.71 ± 0.14	1.12 ± 0.07	5.92 ± 0.18	1.8	SM
5	4.63 ± 0.14	1.66 ± 0.15	0.32 ± 0.02	6.61 ± 0.24	2.71	SM
6	3.72 ± 0.17	2.52 ± 0.09		6.24 ± 0.19	1.46	M
7	3.75 ± 0.16	2.27 ± 0.16		6.02± 0.24	1.64	M

*Chr = Chromosome, M = Metacentric, SM = Sub-metacentric

Discussion

Thinopyrum elongatum, commonly distributed in Africa, Asia-temperate and Europe, is useful genetic material for wheat improvement and has been well preserved in different gene banks. Seeds of *T. elongatum* (strain ID: TACBOW0120) donated by Dr. Dvorak and maintained in Kyoto University were grown in temperate climate of Kathmandu valley, Nepal. The seeds showed 100 percent germination and seedling formation. The seedlings were well grown in pot and natural habitat and developed seeds since last four years. Every year plant flowers during the month of May-June and produced fruits in June-July which is also similar to the range of month for flowering (January-June) suggested by Dewey (1984).

Similarly, the karyotype result obtained in this study was similar to the result suggested by Evans (1962) and Wang (1985). The size of chromosomes may vary due to the time and chemicals used in pretreatment of root tips. According to the length of chromosome and morphology they are assigned by number for pairing their homologue which forms the basis for preparing karyogram. The characterized accession of *Thinopyrum elongatum* will be useful to trace out morphological traits in wheat plants carrying *Thinopyrum elongatum* chomosomes.

Acknowledgements

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