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CHROMOSOMAL STABILITY AND ITS EFFECT ON MORPHOLOGY OF WHEAT-Thinopyrum

elongatum DISOMIC ADDITION LINES Homnath Khatiwada^{1,2}, Deepak Raj Pant¹, Giri Prasad Joshi^{1,*}

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ABSTRACT

The introduction of chromosomes from wild relatives is of great importance as it helps in wheat improvement programs by introducing many useful traits like those related to stress tolerance that lead to increased yield, thereby contributing to food security and safety. In this regard, seven Chinese spring wheat addition lines disomic to different chromosomes from *Thinopyrum elongatum* were compared with the normal Chinese spring in order to study the chromosome stability and effect of disomic addition lines on spike characteristics. All the wheat lines were obtained from NBRP-Wheat Japan. Chromosome numbers were reconfirmed by counting from the mitotic metaphase spread of root tip cells, and spike and seed characteristics were measured from fully matured spikes. All the disomic addition lines showed a stable number of chromosomes (i.e., 44). The spike lengths of disomic addition lines were significantly different (P<0.05) from that of Chinese Spring (control). The seed set was higher in CS+2E" disomic addition lines, while the seed size and seed weight of 100 seeds were lower than that of Chinese Spring.

Keywords: Chromosome, disomic, morphology, stability, Thinopyrum elongatum

INTRODUCTION

Wheat is one of the most important crops feeding the humanity. It is consumed by 75% of the world's population as a part of their daily diet (van Ginkel & Ogbonnaya, 2006). It is cultivated in an area larger than for any crop in the world and has a global trade greater than that for all other crops combined (Curtis, 2002).

The increase in human population has put the resources required to produce food at their limits, but there is a need to increase production to meet the needs of the growing population. It is estimated that global crop production needs to double by 2050 to meet the projected demands of the rising population (Ray et al., 2013). Development of superior wheat varieties with high yield, better quality, resistance to biotic and abiotic stresses, varieties for the new season, removal of toxic compounds, etc. is essential to meet global demand. One of the possible ways for achieving wheat improvements is to increase genetic diversity by introducing novel genes from wild relatives through breeding. It is a challenging task to introduce desirable genes from wild species to cultivated ones. Generally, the transfer of genes from the members of the primary gene pool may occur via direct hybridization, homologous recombination, backcrossing, and selection (McIntosh, 1991; Friebe et al., 1996).

Several efforts have been made to improve wheat using various methods. Some involved in generating synthetic allopolyploids such as Triticale (*T. turgidum* x *Secale cereale* L.), Tritordium (*T. turgidum* x *Hordeum chilense* Roem. & Schult.), and Secalotriticum (*T. aestirum* x *S. cereale*) while others focused on introgression of desired

genes via translocation of either whole chromosome arm or a small chromatin segment from wild relatives to wheat (Wulff & Moscou, 2014). One well-known example of chromosome arm introgression is the rye (S. cereale) 1RS.1BL or 1RS.1AL translocation in which the short arm of the rye 1R chromosome is translocated to the long arm of the 1B or 1A chromosome of common wheat. Such translocation led to resistance against multiple diseases (Mago et al., 2005), improved root structure (Sharma et al., 2011), and improved agronomic traits (Rajaram et al., 1983). Besides, there was several evidence of small chromatin introgressions from wild relatives to wheat. Those were generated to introduce desirable characteristics such as disease resistance, high yield (Reynolds et al., 2001), early maturity (Koba et al., 1997), drought tolerance (Molnar-Lang et al., 2014), salt tolerance (Hohmann et al., 1996), cold tolerance (Vaishnavi & Sethi, 2000) and high protein content (De Pace et al., 2001) from the species of primary, secondary and tertiary gene pool to wheat.

Thinopyrum elonagtum (Host.) D.R. Dewey (syn. Agropyron elongatum (Host.) P. Beauv.) is a perennial diploid (2n=2x=14, Genome formula EE) weed and closely related to wheat. Being a member of the tertiary gene pool and carrying valuable genes for wheat improvement, researchers were extensively studying this plant. Karyotype analysis of *Th. elongatum* has been carried out by Dvorak and Knott (1974), Dvorak *et al.* (1984) Wang (1985), and Khatiwada *et al.* (2019). Dvorak (1980) classified *Th. elongatum* chromosomes into seven homoeologous groups, numbered from one to seven in relation to the seven homoeologous groups of common wheat. Later on, Dvorak *et al.* (1984) classified *Th. elongatum* chromosomes based on physical homoeology as 1E to 7E.

Various studies on diploid Th. elongatum found that it consists of several desirable genes for wheat improvement and also it has high cross-compatibility with wheat. According to Jauhar and Peterson (1996) certain genes of Th. elongatum possess resistance to head scab diseases, tolerance to salinity (Dvorak et al., 1988), and capacity for increased yield (Mujeeb-Kazi et al., 2008). Therefore, it is an important source of genes for the improvement of wheat. In this regard, Dvorak and Knott (1974) developed a set of disomic addition lines in which each chromosome of Th. elongatum (2n=14) was added to the chromosome complement of Triticum aestivum cv. Chinese Spring (2n=6X=42, genome formula AABBDD). Each addition line contains the chromosome complement of wheat and a pair of chromosomes from Thinopyrum elongatum. Disomic addition lines have been instrumental in studying different traits like 4E disomic addition line for perenniality (Lammer et al., 2004), 7E disomic addition line for resistance to Fusarium head blight (Fu et al., 2012), and 4E and 5E addition lines for drought tolerance (Rahmani et al., 2013).

Wheat-*Th. elongatum* disomic addition lines (CS+1E", CS+2E", CS+3E", CS+4E", CS+5E", CS+6E", CS+7E") contain altogether 44 chromosomes of which 42 represent the normal wheat chromosomes, and while the remaining pair represents the respective chromosome pair of *Th. elongatum* chromosomes. The present study was focused on reconfirming all these addition lines with mitotic observation of 44 chromosomes from root tip cells and studying variation in different morphometric characters like those of spike, spikelet, and seeds in different addition lines under wheat genetic background.

MATERIALS AND METHODS Plant Materials

The addition lines of common wheat cv. 'Chinese Spring' (CS) were used. The Wheat-*Thinopyrum elongatum* disomic addition lines (CS+1E", CS+2E", CS+3E", CS+4E", CS+5E", CS+6E" and CS+7E") developed by Dvorak (1980) were obtained from National Bio Resource Project-Wheat, (NBRP-Wheat) Japan (KOMUGI, 2024).

Karyology and Morphology of the Addition Lines

The seeds of each Wheat-*Thinopyrum elongatum* disomic addition lines including control (Chinese Spring), were germinated in petri plates containing moistened tissue paper. The tips of seminal roots were excised and used for further karyological studies while seedlings were transferred to plastic pots containing the soil mix consisting of topsoil, sand, and compost in a ratio of 2:1:1. The plants were watered at regular intervals. The fully mature plants were used for morphological analysis. Segregation of chromosomes in disomic addition lines was carried out using six seeds from each line to observe their chromosome number. Spike morphology and grain characteristics were carried out from the mature potted plants. Both karyological and morphological characterization were carried out at the Central Department of Botany, Tribhuvan University (TU), Kathmandu, Nepal.

The karyological studies were carried out following the method described in Khatiwada et al. (2019). The excised root tips were cleaned with the help of a fine camel hairbrush before pretreatment. The materials were pretreated in 0.002 molar aqueous solutions of 8hydroxy-quinoline and kept in ice-water at 4 °C for 24 hours. The root tips were then fixed using Carnoy's solution and kept for about one week at room temperature. Roots were then transferred into vials containing aceto-carmine solution and allowed to stain at room temperature for about two days. The roots were then transferred back to Carnoy's solution for storage. The root tip cells were softened by treating them with 1N HCl for about 3 hours. The tips were then boiled in aceto-carmine solution for about 2-4 minutes. The stained roots were cut off and placed on a slide and a few drops of 45% acetic acid were poured on top of them. The root tips were then covered gently with the help of a cover slip and gently tapped with a needle to spread the cells. Spread tissue was then heated briefly and squashed. The slide was observed under 100X magnification of a compound microscope (LABOMED INC. Los Angeles CA. USA) to locate the position of best metaphase spreads which were later photographed at 400X.

Six fully matured spikes were excised from different Wheat-*Th. elongatum* disomic addition lines and control (Chinese spring) when the plant showed complete senescence. Different parameters like spike length, number of grains set per spike, number of grains per spikelet, dimensions (length and width) of the grain, and hundred grain weight were measured in different wheat lines.

Statistical Analysis

Statistical analysis was done by using Microsoft Excel and SPSS. The statistical significance of differences in various traits like seed set and spike morphology was analyzed using ANOVA in Microsoft Excel and SPSS Statistics v. 20.

RESULTS

Karyology of Wheat Lines

Triticum aestivum cv. Chinese Spring (control) and wheat-*Thinopyrum elongatum* disomic addition lines were cytologically examined and their chromosome number was reconfirmed. *Triticum aestivum* cv. Chinese Spring possessed 2n=42 and all the disomic addition lines of wheat consisted of 44 chromosomes (Table 1, Fig. 1).



CS+7E"

Figure 1. Mitotic metaphase of wheat lines. Microscopic observation of root tip cells of different Wheat-*Th. elongatum* disomic addition lines (CS+1E''~CS+7E'') with 44 chromosomes and *Triticum aestivum* cv. Chinese Spring (CS) with 2n= 42 chromosomes

CS

Table 1.	Chromosome	number in	control a	and addition	lines
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Wheat Lines	Chromosome number
T. aestivum cv. 'Chinese Spring'	42
Wheat-1E disomic addition	44
Wheat-2E disomic addition	44
Wheat-3E disomic addition	44
Wheat-4E disomic addition	44
Wheat-5E disomic addition	44
Wheat-6E disomic addition	44
Wheat-7E disomic addition	44

Spike Length, Seed Set, and Seed Weight

There was a significant difference in spike morphology and seed set in Wheat-*Th. elongatum* addition lines and Chinese Spring (P<0.05). Spike length was found to be longest in CS+4E" (9.53 \pm 0.52cm). The spikes in all the addition lines were longer than the spike of Chinese Spring (6.63 \pm 0.71cm) (Fig. 2). Seed set per spike was highest in CS+2E" (44 \pm 7.43).



Figure 2. a). Spikes of Wheat- *Th. elongatum* disomic addition lines (CS+1E", CS+2E", CS+3E", CS+4E", CS+5E", CS+6E", CS+6E", CS+7E") and *Th. elongatum* from left to right. b). Spike of Chinese Spring

Table 2.	Spike and seed	characteristics	of Wheat-Th.	elongatum	disomic	addition lines
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Wheat Line	Spike length (cm)	Number of seed/spike	Number of seed/spikelet	Seed length (mm)	Seed width (mm)	Weight of 100 Seeds (gm)
CS+1E"	7.2 ± 0.8 ^{ab}	22.1±15.1 ª	1.8±0.7 ª	5.4 ± 0.3 ab	2.46±0.3 ^b	1.83 ± 0.13
CS+2E"	7.5±0.7 ^{ab}	44.0±7.4 ^b	3.3±0.8 ^b	5.5 ± 0.2 ab	1.99±0.4 ^{ab}	1.09 ± 0.19
CS+3E"	$9.0\pm0.6^{\mathrm{bc}}$	36.1±5.3 ^{ab}	2.6±0.5 ^{ab}	5.2 ± 0.3 ab	1.96±0.1 ^{ab}	1.34 ± 0.06
CS+4E"	9.5±0.5 °	33.6±9.3 ^{ab}	2.3±0.5 ^{ab}	5.5 ± 0.2 ab	2.26±0.2 ^{ab}	1.46 ± 0.06
CS+5E"	$8.5 \pm 0.7^{\mathrm{bc}}$	28.6±1.9 ^{ab}	2.3±0.5 ^{ab}	4.9±0.0 ª	1.97±0.2 ^{ab}	1.11±0.01
CS+6E"	9.0±0.3 °	33.0±7.1 ^{ab}	2.5±0.8 ^{ab}	5.3±0.3 ^{ab}	1.85±0.2 ª	1.03 ± 0.04
CS+7E"	7.4±0.9 ^{ab}	19.1±9.7 ª	1.8±1.1 ^a	5.6±0.6 ^b	2.04±0.2 ^{ab}	1.12 ± 0.09
CS	6.6±0.7 ª	34.0±14.3 ^{ab}	3.0±0.8 ^{ab}	5.8±0.3 ^b	3.27±0.1 °	3.70±0.1

Note: In each column values with different letters are significantly different (P<0.05) within the group. Analysis was not performed in the case of seed weight due to a lack of sample replication.

None of the additional lines met the size of the Chinese spring seed. i.e., length = $(5.87\pm0.35 \text{ mm})$ and width = $(3.27\pm0.19 \text{ mm})$. The weight of 100 seeds was greater

in CS+1E" (1.83 ± 0.13 gm) addition line but none of the wheat-*Th. elongatum* addition lines met the weight of Chinese Spring (3.7 ± 0.1 gm) (Table 2, Fig. 3).



Figure 3. Seeds of Common Wheat cv. "Chinese Spring", Wheat-*Thinopyrum elongatum* disomic addition lines CS+1E" to CS+7E" and *Thinopyrum elongatum* (Scale in Centimeter)

DISCUSSION

The chromosome counting of *T. aestivum* cv. Chinese Spring (CS) and addition lines were used for confirming the status and segregation pattern of added chromosomes. *Triticum aestivum* cv. Chinese Spring possessed 2n=42 and all the disomic addition lines of

wheat consisted of 44 chromosomes confirming the stability in the segregation pattern during meiosis. Similar results were also suggested in wheat-barley disomic addition lines (Merker, 1979; Joshi *et al.*, 2012). Such a type of stability is of great importance to maintain the desired character in wheat (Zhao *et al.*,

2021), which may be due to the partial homology between *Th. elongatum* and wheat (Dvorak, 1980). Another factor for chromosome stability includes genes responsible for partial homology (Cai and Jones, 1997) found in *Thinopyrum* sp. The wheat Ph1 gene is an obvious factor that ensures correct pairing between homologous and homoeologous chromosomes (Griffiths *et al.*, 2006).

Longer spikes and more grain per spike were reported by Qi *et al.* (2010) in wheat-*Thinopyrum bessarabicum* translocation lines and Nie *et al.* (2019) in wheat *Thinopyrum intermedium* disomic addition lines. Similarly, seed per spikelet was highest in CS+2E'' (3.33±0.82).

The highest seed set in CS+2E" (44 ± 7.43) with compact spike and spikelet and the longer spike in all addition lines were due to the traits present in the chromosomes of *Th. elongatum* suggesting that there was no loss of spike controlling trait of Th. elongatum when mixed with CS. However, along with several traits that contribute to biotic and abiotic stress tolerance, the addition of chromosomes to Chinese Spring may increase yield attributes such as increased grain protein (Dvorak & Sosulski, 1974) and blue color in aleurone layer (Soliman et al., 1980) which causes 15% increase in total grain protein in addition lines. A low seed set was observed in the disomic addition line CS+1E" and CS+7E" which was a result similar as given by Dvorak, (1980). A compact spikelet with the highest seed per spike in CS+2E" may be suitable for developing a higher productivity line if we can improve the size and weight of seeds.

CONCLUSIONS

This study suggests that the wide crossing of *Triticum aestivum* with the tertiary gene pool producing disomic addition lines produces significant morphological variation in wheat with constant segregation of chromosomes. Some negative results were possibly due to the insertion of whole chromosomes. Therefore, further work should be carried out to develop deletion and translocation lines to know the effect of part of the chromosome which will be helpful in locating the particular gene conferring desired phenotypic traits.

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AUTHOR CONTRIBUTIONS

HK: Field work, lab experiments, data collection and analysis, manuscript writing; DRP: experimental design, data analysis, manuscript writing; GPJ: experimental design, karyotype and data analysis, manuscript writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of the present work are available from the corresponding author upon reasonable request.

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