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Research Article

CYTOGENETICS AND DEVELOPMENT OF MUTATION INDUCING WHEAT-*Thinopyrum elongatum* DISOMIC ADDITION LINES POSSESSING A GAMETOCIDAL CHROMOSOME 2C

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ABSTRACT

Development of wheat-alien addition lines including gametocidal (Gc) chromosome in monosomic condition (a line with 2n = 45 chromosomes) is an established tool to induce chromosomal aberration. Gc induced chromosomal aberrations of alien chromosomes maintained in different wheat lines are valuable materials to construct cytological maps. In this study, the development of hybrid wheat addition lines containing 45 chromosomes has been attempted by crossing seven different types of Wheat-*Thinopyrum elongatum*-2C double monosomic addition lines (DMALs) and seven types of Wheat-*Th. elongatum* disomic addition lines (DALs), each with 2n=44 chromosomes. The cytological screening of the progenies showed three different types of plants (i.e. with 43, 44, and 45 chromosomes) among the hybrid wheat addition lines. The progeny plants containing 43, 44, and 45 chromosomes were segregated in a ratio of 2:1:1, respectively. These different lines differed from one another in various morphological and agronomic traits. Altogether 12 progeny seeds with 45 chromosomes were obtained. After sowing only 5 plants survived and developed seeds. These lines can be used as potential mutation-inducing plants.

Keywords: Addition lines, cytological map, gametocidal chromosome, mutation breeding, wheat

INTRODUCTION

Wheat (*Triticum aestivum*), one of the first domesticated food crops belongs to the tribe triticeae which comprises approximately 27 genera and 501 species (Soreng *et al*., 2017). It is believed to have originated by hybridization of *T. durum* or *T. turgidum* sub sp. *durum* (2n=4x=28, AABB) with *Aegilops tauschii* (syn. *A. squarrosa*; 2n=2x=14, DD) (Jauhar & Chibbar, 1999; Snape & Pankova, 2006; Shewry, 2009). Wide hybrids in the tribe triticeae have been attempted for over 100 years and several hybrids between wheat and rye, wheat and barley etc. have already been made.

Thinopyrum elongatum (Host.) D.R. Dewey (Syn. *Agropyron elongatum* (Host.) P. Beauv); is a perennial diploid (2n=2x=14, EE) and closely allied to wheat (Dewey, 1984). It is used to transfer beneficial genes like those for salt tolerance (Dvorak & Ross, 1986) and disease resistance (Shen & Ohm, 2007; Sepsi *et al*., 2008) to common wheat. In this regard, Dvorak & Knott (1974) developed a set of disomic addition lines (DALs) in which each of the chromosomes of *Th. elongatum* (2n=14) was added to chromosome complement of *Triticum aestivum* cv. Chinese spring (2n=6x=42, AABBDD).

Aegilops cylindrica (CCDD), also known as jointed goatgrass, is an annual grass of triticeae belonging to a secondary gene pool. *Ae. cylindrica* and wheat are genetically linked through D genome. Some alien chromosomes, especially of *Aegilops* species, secure their existence in the host (mainly wheat) by causing chromosomal breakage in the gametes lacking them. The chromosomes causing abortions of gamete in their absence were named gametocidal (Gc) Chromosomes (Endo, 1990). Such chromosomes can be used as biological mutagens to induce chromosomal deletion and translocation for different purposes.

Induced chromosomal rearrangements have been identified and established in wheat stocks carrying wheat and alien (rye and barley) deletions or wheat-alien translocations (Endo, 2007; Joshi *et al.,* 2011). Such deletion stocks are valuable for cytological mapping, transfer of genes from wild relatives such as *Aegilops*, *Thinopyrum* etc. to the wheat for varietal improvement. The 2C chromosome causes moderate breakage of the chromosomes in the gametes (and the offspring from those gametes) that lack them. This brings chromosomal rearrangements in the offspring and produces deletion stocks (Endo & Gill, 1996). These deletion stocks have given rise to a new form of genetic mapping in plants, the deletion mapping or cytological mapping (Endo & Gill, 1996; Endo, 2007). Likewise, the added alien chromosomes increase the productivity in wheat by improving the traits such as disease resistance, biotic and abiotic stress tolerance, and others (Chen *et al.,* 2004, Colmer *et al.,* 2006).

The present study aimed to establish the mutation inducing 45 chromosome wheat plants carrying monosomic addition of 2C and disomic addition of each of the *Th.*

elongatum chromosomes through breeding and cytological screening of the hybrid progeny. The progeny of these lines will be useful for the development of deletion stocks which may ultimately help in the cytological mapping of *Th. elongatum* chromosomes and gene transfer from *Th. elongatum* to the hexaploid common wheat for its improvement.

MATERIALS AND METHODS

Plant materials

Three alien addition lines of common wheat cv. Chinese Spring were used for the study. The Wheat-*Th. elongatum* disomic addition lines (DALs) and the Wheat-2C disomic addition line (DAL) were obtained from National Bio Resource Project-Wheat, (NBRP-Wheat) Japan (http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp). Corresponding author made a cross between Wheat-*Th. elongatum* DALs (CS+1E" to CS+7E") and Wheat-2C (CS+2C") DAL to produce the plants monosomic for both chromosome; CS+1E'+2C' to CS+7E'+2C' (except CS+2E'+2C' and CS+5E'+2C'; no seeds after crossing) (Fig. 1)

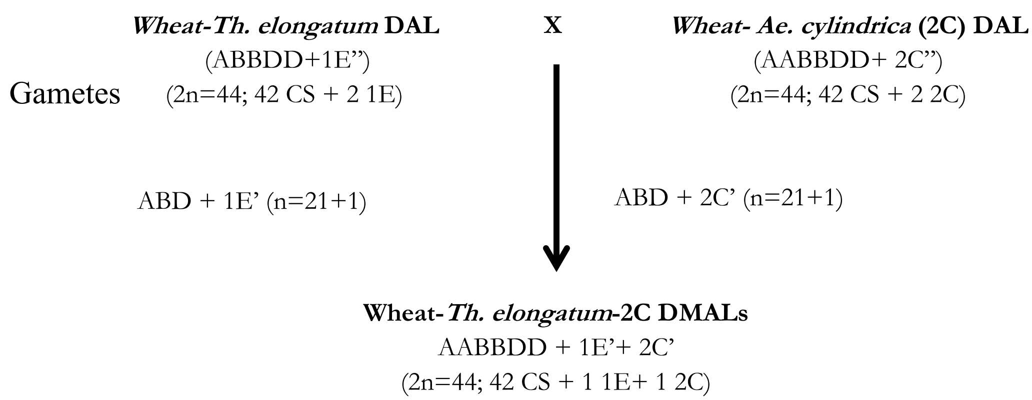

Figure 1. Schematic overview on production of Wheat-1E' -2C' double monosomic addition lines (DMALs). Source: Khatiwada, 2017. Alien chromosomes added to wheat in monosomic, and disomic condition is symbolically represented by (') and ("), respectively. The same process is repeated for the rest of the Wheat-*Th. elongatum* (DALs) e.g. 2E, 3E, 4E, 5E, 6E and 7E. Note: CS: Chinese Spring (a wheat cultivar) and the number before the letters CS and 1E to 7E representing the number of chromosomes of particular type.

Wheat-*Th. elongatum*-2C DMALs were then further backcrossed with Wheat-*Th. elongatum* DALs. The seeds obtained from backcross were used for cytological screening and establishment of mutation inducing 45 chromosome plants. The breeding scheme of producing 45 chromosome Wheat-*Th. elongatum*-2C addition lines is shown in Fig. 2.

Altogether, 92 seeds (1E = 15 seeds; 3E = 18; 4E = 22; 6E = 24; 7E = 13) of different hybrid wheat addition lines (HWALs) were developed from the above-mentioned backcross. The seeds were germinated in the petri-plates for further steps.

Karyology

Karyological study was carried out using root squash technique in which the counting of the somatic chromosomes was done on somatic cells obtained from the root tips (mitosis) of the germinating seeds (71 seeds out of 92) of different HWALs. The detailed karyological procedure has already been published by Khatiwada *et al.* (2019).

Fertility, spike length and seed set

The fully matured and harvest-ready (Feekes 11.4 stage) spikes were excised from each of the HWALs to measure fertility, spike length and seed set. The fertility of different lines was measured based on number of seeds produced per spikes.

Data Analysis

The data on frequency of the chromosomal segregation and other agronomic traits like spike morphology, seed set and fertility of different HWALs were analyzed by using Microsoft excel.

RESULTS

Status of hybrid wheat addition lines

Among 92 seeds developed from the cross, 71 seeds germinated and 57 of them sprouted. Out of 57 plants, 48 were able to develop spikes. The germination percentage was found to be highest (94 %) in the HWALs 3E and lowest (55 %) in 4E. Similarly, the survival percentage was also higher (93 %) in 1E, moderate (58%) in 6E and low (36%) in 4E line. The percentage of spikes with seeds was found highest (100%) in lines 4E, 6E, and lowest (63%) in 3E. There were no plants of HWALs 2E and 5E (Fig. 3).

Karyology

Altogether 71 germinated seeds were used for cytological screening to determine the segregation pattern based on chromosome numbers possessed by mitotic cells of HWALs (table 1). The percentage of lines possessing 43 chromosomes was found to be lowest in 3E (35%) and highest (55%) in 6E. Similarly, the percentage of lines with 44 chromosomes was lowest (25%) in 4E and 7E and highest (43%) in 1E. The percentage of lines with 45 chromosomes was found between 7% (in 1E) to 25% (in lines 4E and 7E). The segregation ratio of plants with 43, 44 and 45 chromosomes was 2:1:1 in lines 4E and 7E, while the ratio deviated from this value in rest of the lines. The photographs showing the karyotype of the different lines with 43, 44, and 45 chromosomes are shown in Fig. 4.

Wheat-1E-2C DMAL X **Wheat-1E DAL**

(2n=44; 42 CS + 1 1E+ 1 2C) (2n=44; 42 CS + 2 1E)

Possible gametes

1. n = 21 (21 CS)
2. n = 22 (21 CS +1 1E)
3. n = 22 (21 CS +1 2C)
4. n = 23 (21 CS +1 1E + 1 2C)

n = 22 (21 CS + 1 1E)

Possible offspring

2n= 43 (42 CS + 1 1E)
2n= 44 (42 CS + 2 1E)
2n= 44 (42 CS + 1 2C + 1 1E)
2n= 45 (42 CS+ 2 1E+ 1 2C) Mutation inducing line

Figure 2. Schematic overview on the production of mutation inducing 45 chromosome wheat plant disomic for *Th. elongatum* 1E and monosomic for *Ae. cylindrica* 2C chromosome. The same process is repeated for rest of the Wheat-*Th. elongatum* addition lines e.g. 3E, 4E, 6E and 7E. Note: CS: Chinese Spring (a wheat cultivar) and the number before the letters CS and 1E to 7E representing the number of chromosomes of particular type.

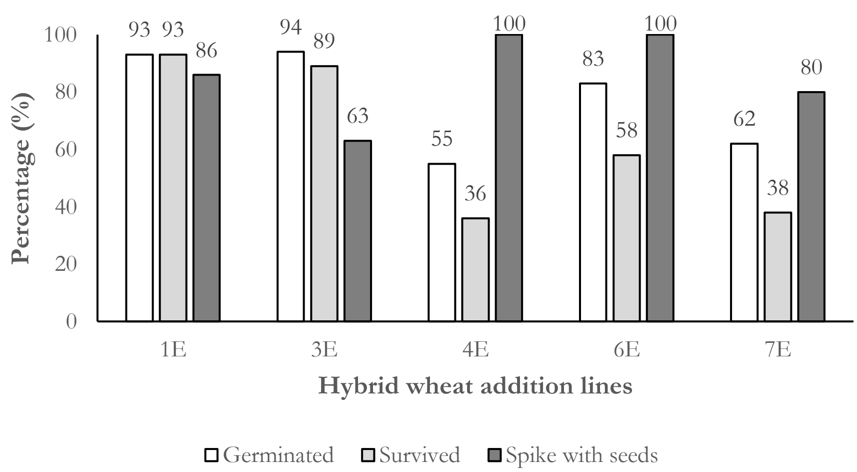

Figure 3. Status of the HWALs in terms of germination, survival and seed set in mature spikes.

Fertility

The fertility percentage of different HWALs is presented in table 2. Among the lines carrying 43 chromosomes in their somatic cells, the highest (100%) fertility was observed in 1E and the lowest (67%) was observed in 3E and 4E. In the lines with 44 chromosomes, 6E showed highest fertility (86%) and 4E showed the lowest (33%). In the case of the lines with 45 chromosomes, the highest fertility (67%) was observed in 4E and lowest (0%) was observed in 6E and 7E.

Table 1. Segregation percentage of HWALs with different chromosome numbers.

Hybrid wheat lines	% of plants with 2n number of chromosomes			Total seeds tested
	43	44	45	
1E	50	43	7	14
2E	NA	NA	NA	0*
3E	35	41	24	17
4E	50	25	25	12
5E	NA	NA	NA	0*
6E	55	35	10	20
7E	50	25	25	8
Total				71

*No seeds were obtained from the cross of CS+2E" and CS+5E" disomic addition lines with CS+2C" lines. NA: Not available

Table 2. Fertility of hybrid wheat addition lines.

Hybrid wheat lines	Fertility % of plants		
	43 Chromosome	44 Chromosome	45 Chromosome
1E	100	67	33
3E	67	57	50
4E	67	33	67
6E	73	86	0
7E	75	50	0

Spike length and seed set

The spike was found to be longest (10 cm) in 3E and the shortest (7.5 cm) in 4E and 7E lines. Likewise, the number of seeds per spike was found highest (25 seeds) in 7E and lowest (5 seeds) in 3E (Fig. 5).

DISCUSSION

The hybrid wheat addition lines of the present study, developed from backcrossing of Wheat-*Th. elongatum*-2C DMALs and Wheat-*Th. elongatum* DALs, showed failure in germination, survival and seed set in some lines. Among the lines, ca. 50% were able to attain maturity and bore seeds. Shi and Endo (1997) suggested that the reduction in fertility is due to chromosomal aberration caused by 2C which impacts on indehiscence of anthers during the self-pollination. Furthermore, Sears and Sears (1978) also suggested that wheat monosomic addition lines (MALs) tend to become less stable than DALs. The less percentage of germination and survival of plants in this study may be due to presence of gametocidal chromosome 2C in monosomic condition which reduces plant vigor and seed fertility.

The karyology of the HWALs showed three different types of chromosomal combinations i.e. 43, 44 and 45. As these plants were backcross product of Wheat-*Th. elongatum*-2C DMALs and Wheat-*Th. elongatum* DALs, the pattern of segregation was according to the chromosomal theory of inheritance. The possible explanation for the anomalous segregation pattern may be the meiotic behavior of alien chromosomes during gametogenesis. Theoretically, only one type of gametes is possible from each of the wheat-*Th. elongatum* DALs, while four different combinations are possible from each of the Wheat-*Th. elongatum*-2C DMALs, and their fusion result types of chromosomal combinations in progeny plants as shown in Fig. 2. This result is further supported by the evidence that disomic additions are meiotically more stable than monosomic additions (Shi & Endo, 1997; Sears & Sears, 1978).

Additionally, the segregation pattern and frequencies of progeny plants with 43 chromosomes was found highest (except in 3E) than that of plants with 44 and 45 chromosomes. The high frequency of lines with 43 chromosomes suggests that the segregation pattern of both alien chromosomes (1E and 2C) in gametes is either a rare event or their presence creates some abnormalities in the gametes. Tsujimoto and Tsunewaki (1984) reported that the gametocidal chromosome 3C of *Aegilops triuncialis* causes semi-sterility in monosomic state in common wheat cultivar. In addition to gametocidal action, presence of certain strong alleles in the alien genes tends to promote the homoeologous pairing in wheat to a greater extent by suppressing the activity of the Ph locus on chromosome

5B since their effects appear to be similar to that found in wheat-rye hybrids, *Triticum aesticum* with *Aegilops speltoides* and *Aegilops longissima* (Dvorak, 1972; Dover & Riley; 1972; Mello-Sampayo, 1971). Chen *et al.* (1989) even observed high pairing of wheat and *Agropyron cristatum* chromosomes in their hybrids which could have occurred when Ph1 was at least partially disabled. As one of parents used in the present study possessed *Thinopyrum* and 2C chromosome in double monosomic condition, it is likely to cause more meiotic abnormalities.

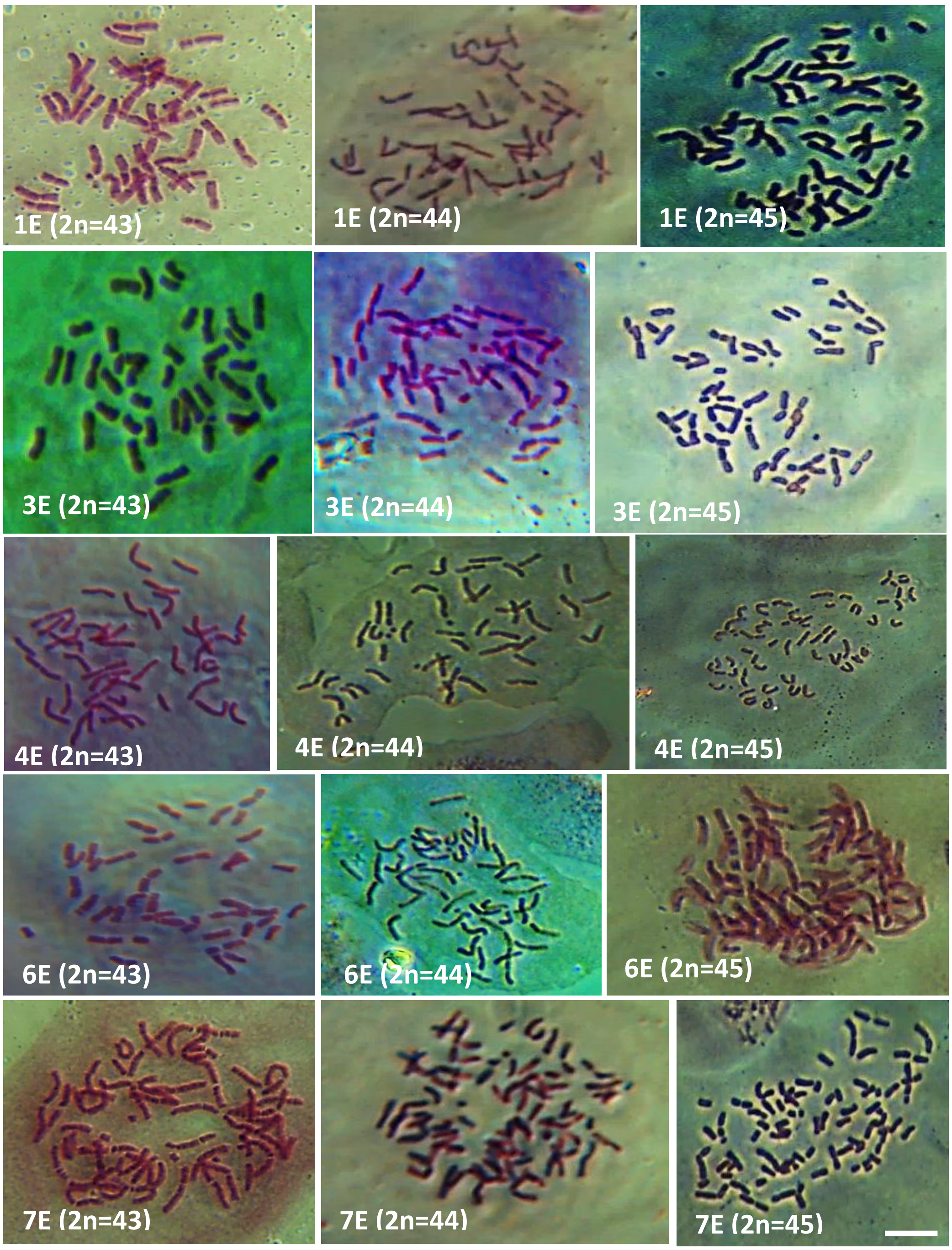

Figure 4. Karyotype of hybrid wheat addition lines with different chromosomes (1E to 7E). Bar (white) at the bottom right corner = 10 µm.

In this study, the percentage of progeny plants carrying 44 chromosomes ranged between 25-43%. However, the highest frequency of lines with 44 chromosomes was reported for wheat-barley disomic addition lines and wheat-rye disomic addition lines (Shi & Endo*,* 1997). These findings again suggested that the segregation pattern in Wheat-alien double monosomic addition lines differs from that in Wheat-alien disomic additions. Sears (1956) suggested that the addition of gametocidal chromosome, 2C on wheat tends to form the cells with full bivalent formation but with a lower stability. Similarly, Riley and Chapman (1957) and Zhang *et al.,* (2007) reported low and high percentages of lines with 44 chromosomes from monosomic and disomic additions, respectively. They suggested that instability of monosomic additions and stability of disomic additions might be behind such unequal pattern of segregations.

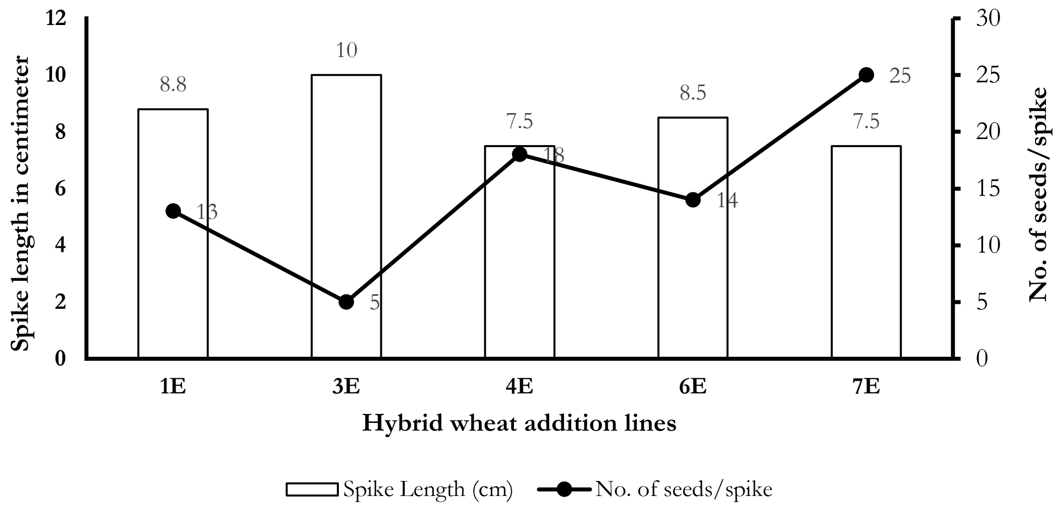

Figure 5. Spike length and seeds number in different hybrid wheat addition lines.

Although, the development of lines with 45 chromosomes from 44 chromosome disomic addition lines is rare phenomenon, the presence of 23 functional chromosomes within the same gamete may have happened by asynaptic pairing (Morrison, 1953; Person, 1955). Furthermore, the euploid condition in progenies of 44 chromosome disomic addition lines were supposed to bring some meiotic irregularities and failure of forming bivalents in pollen mother cells thus leading to the formation of 45 chromosome plants (Gerstel, 1945; Jauhar, 2007; Cai *et al.,* 2010). In this study, the percentage of the progeny plants carrying 45 chromosomes was smaller than lines with 43 and 44 chromosomes. These plants may be formed by the fusion of gametes carrying 22 chromosomes from Wheat-*Th. elongatum* DALs and 23 chromosomes from Wheat-*Th. elongatum* 2C DMALs. In the latter case the gamete should include both *Th. elongatum* and 2C chromosomes along with normal wheat chromosomes. As the double monosomic condition creates problems in meiosis, either the gametes with 23 chromosomes are formed in very small number or they may be non-functional in course of development which leads to form the lowest number of 45 chromosome plants.

The higher percentage of fertility in Wheat-*Th.elongatum*-2C lines possessing 43 chromosomes in comparison to those containing 44 and 45 chromosomes in this study suggests that the presence of the gametocidal chromosome, 2C from *Aegilops cylindrica* reduces plant vigor and seed fertility. Similar results were also obtained by Islam *et al.* (1975) while working with wheat x wheat hybrids. Shi and Endo (1997) deduced two possibilities regarding the low fertility in wheat alien addition lines especially carrying gametocidal chromosome; one is the chromosomal aberrations caused by gametocidal chromosome and another is indehiscence of anthers during self-pollination. In addition, the low fertility may also be related to the meiotic irregularities of the lines (Jena & Khush, 1989). Also, it is likely that genetic characters are more important in determining fertility than the degree of chromosomal pairing. When the low fertility of the line is not caused by the meiotic irregularities then it must be due to the genetic effects of the chromosome with low fertility in the monosomic addition lines (Makino, 1976). Furthermore, the low fertility in both the monosomic and disomic alien addition lines may be affected by the ploidy level of the recipient and donor species and the genetic constitution of added chromosomes (Riley & Chapman, 1958).

The present study showed the longest spike length in 3E (10 cm) and smallest in 4E and 7E lines (7.5 cm) which is comparatively more or less similar to the size of their parents (Khatiwada, 2017). Khatiwada, (2017) reported that length of the spikes of Wheat-*Th. elongatum*-2C

DMALs were higher than Wheat-*Th. elongatum* DALs which were again higher than the wheat cultivar "Chinese Spring". There was no definite pattern of spike length observed in the studied lines except that they all showed higher spike length than Chinese Spring. The increase in spike length suggests that there was no loss of spike controlling character when mixing the chromosomes of *Thinopyrum* and *Aegilops* into "Chinese Spring" (Dvorak, 1980).

In present study, the seed set of the HWALs were found very low compared to the common wheat cv. "Chinese Spring", Wheat- *Th. elongatum* DALs and Wheat- *Th. elongatum*-2C DMALs. The reduced seed set in most of the studies suggests that the gametocidal chromosome in monosomic condition might have direct effect on it. The decrease in the plant vigor and fertility may be partly because of the chromosomal aberrations caused by 2C as suggested by Shi and Endo (1997). Also, Robbelen and Smukkupt (1968) suggested that the relatively slow growth of wheat pollen tube in style of alien species with gametocidal chromosome in monosomic condition affects the seed set per spike. Blanco *et al.* (1987) also reported the lower seed setting in spikes of monosomic alien addition lines and proposed that the seed setting might be affected by the male and female sterility caused by addition of alien gametocidal chromosomes in presence of wheat chromosomes. The lowest frequency of segregation of 45 chromosomal plants in the present study suggests that both alien chromosomes, i.e., *Thinopyrum* and *Aegilops* hardly segregate in the same gamete. In most lines, the plants possessing 43 chromosomes showed the highest fertility percentage compared to 44 and 45 chromosome plants. The low seed set was observed in almost all progeny plants which may be probably due to double monosomic condition of one of the parental lines, in which 2C chromosome generates different level of abnormalities during gametogenesis and even at the time of fertilization.

CONCLUSIONS

In this study, different hybrid wheat addition lines showed lower percentage of germination, survival and even facing severe sterility problems Altogether five plants, one belonging to hybrid wheat addition line 1E and two each to 3E and 4E carrying 45 chromosomes were developed in this study. Cytological screening of progeny seeds from these plants may carry chromosomal aberrations. Stably maintained aberrations of *Th. elongatum* chromosomes in wheat plant can be useful in cytological mapping of *Th. elongatum* chromosomes.

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AUTHOR CONTRIBUTIONS

RM contributed to field work, lab experiments, data collection, data analysis and manuscript writing. SD contributed to the field work and data collection. DRP contributed to experimental design, data analysis and manuscript writing. GPJ performed experimental design, crossed the wheat lines to develop seeds for the study, karyotype analysis, data analysis and manuscript writing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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