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Cytogenetic and agro-morphological study of Wheat addition lines possessing *Thinopyrum elongatum* and *Aegilops triuncialis* chromosomes

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ABSTRACT

Novel genetic variation can be induced by introgression of alien chromosomes in wheat for which wheat-alien addition lines carrying 45 chromosomes are instrumental. In the course of developing wheat addition lines with 45 chromosomes, two different parental lines Wheat-*Thinopyrum elongatum* disomic addition lines (Wheat-*Th*. DALs; 2n=44)) and Wheat-*Th. elongatum- Aegilops triuncialis* $3C^{SAT}$ double monosomic addition lines (Wheat-*Th*. DMALs; 2n=44) need to cross. The present study mainly focuses on the effects of chromosomal additions in wheat background by comparing the morphological and agronomic traits of the parental lines. Both the parental lines were reconfirmed 2n=44 cytologically by using squash technique. Pollen fertility was highest (75%) in Wheat-$3C^{SAT}$-6E DMAL and lowest (44.73%) in Wheat-5E DAL, whereas, spike length was longest (8.71 cm) in Wheat-$3C^{SAT}$-3E DMAL and shortest (5.92 cm) in Wheat-1E DAL. Plant height was highest (97.6 cm) in Wheat-$3C^{SAT}$-5E DMAL and lowest (55.33 cm) in Wheat-7E DAL. The plant height of DMALs always exceeded that of DALs. Furthermore, seed set was highest (31 seeds per spike) in Wheat-7E DALs and lowest (13 seeds per spike) in addition lines disomic for 4E chromosomes. The effect of different alien chromosomes in wheat is discussed in this paper.

Keywords: Wheat, addition lines, mutation, cytology, morphology, pollen fertility

सारांश

गहुँमा नयाँ आनुवांशक गुणहरुको विकास गर्ने एउटा माध्यम प्राकृतिक रुपमा उपलब्ध नातेदार प्रजातिको क्रोमोजोमलाई गहुँमा प्रत्यारोपण गर्नु पनि हो जसका लागि ४५ ओटा क्रोमोजोम भएका wheat-alien addition lines हरु महत्वपूर्ण साधन हुन । यस्ता विरुवाहरु तयार गर्नको लागि दुई थरिका माउ विरुवाहरु Wheat-*Thinopyrum elongatum* disomic addition lines (Wheat-*Th*. DALs; 2n=44) र Wheat-*Th. elongatum- Aegilops triuncialis* $3C^{SAT}$ double monosomic addition lines (Wheat-*Th*. DMALs; 2n=44) को बिचमा परपरागसेचन गराउनु पर्ने हुन्छ । यस अनुसन्धानको मुख्य उद्देश्य alien क्रोमोजोमहरुको उपस्थितिले गहुँको वाह्य आकार र agronomic traits हरुमा पर्ने प्रभाव र त्यसको तुलना परपरागसेचनका लागि छनौट गरिएका यिनका माउ विरुवासँग गर्नु रहेको छ । Squash विधिको प्रयोगबाट दुवै माउ विरुवाहरुको कोषलाई प्रयोग गरेर क्रोमोजोमहरुको संख्या 2n=44 भएको पुष्टि गरियो । Wheat-$3C^{SAT}$-6E DMAL मा उच्चतम पराग प्रजनन् क्षमता (७५%) र Wheat-5E DAL मा न्युनतम पराग प्रजनन् क्षमता (४४.७३%) थियो, जबकि Wheat-$3C^{SAT}$-3E DMAL को बाला सबैभन्दा लामो (८.७१ cm) र Wheat-1E DAL को सबैभन्दा सानो (५.९२ cm) थियो । Wheat-$3C^{SAT}$-5E DMAL को उचाई उच्चतम (९७.६ cm) थियो भने Wheat-7E DAL को उचाई न्युनतम (५५.३३ cm) । DMALs विरुवाहरुको उचाई जहिले पनि DALs हरु भन्दा बढि भएको पाइयो । यस बाहेक, Wheat-7E DALs मा उच्चतम दाना लागेको (३१ दाना/बाला) भेटियो भने Wheat-4E DALs मा न्युनतम (१३ दाना/बाला) । यस अध्ययनमा गहुँमा प्रत्यारोपित विभिन्न alien क्रोमोजोमहरुको प्रभाव बारे छलफल गरिएको छ ।

INTRODUCTION

In the course of wheat improvement, landraces derived through natural and farmer’s selection of diversity (Keller et al 1991, Vojdani and Meybodi 1993, Zou and Yang 1995) and bred with wild relatives are helpful in introducing novel genes into wheat (Hoyt and Brown 1988). Different traits of agronomic importance such as 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 and Sethi 2000) and high protein content (De Pace et al 2001) have been introduced by crossing wheat with species of its primary, secondary and tertiary gene pool. The genus *Thinopyrum*, related to the tertiary gene pool, has large source of desirable traits for wheat improvement (Dewey 1984). Bread wheat, being hexaploid, can accommodate alien chromosomes from its wild relatives and can tolerate chromosomal mutations up to certain limits. In this regard, the wheat alien disomic addition lines carrying normal wheat chromosome and a pair of chromosomes from desired diploid species are valuable materials to study the effects of alien chromosome in wheat genetic background. Dvorak and Knott (1974) were able to develop and morphologically characterize a set of lines in which each pair of diploid *Thinopyrum elongatum* chromosome was separately added to the chromosome complement of *Triticum aestivum* “Chinese Spring” (2n=42).

Mutagenesis are useful tools in plant breeding (Gaul 1964). The *Aegilops* chromosomes have been well recognized as mutagen for wheat chromosomes and alien chromosomes added to wheat (Endo 2007). The absence of *Aegilops* chromosomes makes gamete infertile and abortive, so they are called gametocidal chromosomes (Gc chromosomes). Because only the gametes with Gc chromosome are transmitted to next generation in such cases, they are also called ‘selfish’ chromosome (Tsujimoto 2005, Endo 2007, Su et al 2013) The mutants produced by the gametocidal action of Gc chromosomes are useful genetic materials in cytological mapping of alien chromosomes added to wheat.

The Gc induced mutations are only possible when Gc chromosomes are present in monosomic condition. Therefore, to develop 45- chromosome wheat plant with a pair of alien chromosome and one Gc chromosome, a 44- chromosome wheat-alien DAL and a 44- chromosome wheat-alien-Gc DMAL will be needed to cross. The seeds developed in this cross may possess variable number of chromosomes ranging from 43 to 45 which can be confirmed after cytological screening. In this study we aimed to find out the impact of chromosomal additions on different morphological and agronomic traits in wheat. The study in the long run supports to develop 45 chromosome containing mutation inducing lines which can be used for wheat improvement.

MATERIALS AND METHODS

Plant material

Present study used different types of wheat alien addition lines, of them seven were Wheat-*Th. elongatum* DALs (Wheat-1E to 7E) produced by Dvorak and Knott (1974) and Wheat-$3C^{SAT}$ DAL developed by Endo (1990). These lines were obtained from National BioResource Project-Wheat, (NBRP-Wheat) Japan (http://www.shigen.nig.ac.jp/wheat/komugi/). All seven types of Wheat-Th. elongatum DALs, and Wheat-$3C^{SAT}$ DAL were then crossed separately by the corresponding author at Central Department of Botany, T.U. to obtain Wheat-*Th. elongatum*-$3C^{SAT}$ double monosomic addition lines (DMALs) (**Figure** 1). Seeds of seven different types of Wheat-*Th. elongatum* DALs along with seven different types of Wheat-*Th. elongatum*-$3C^{SAT}$ DMALs were germinated and grown to conduct karyology, crossing and characterization of various agro-morphological traits.

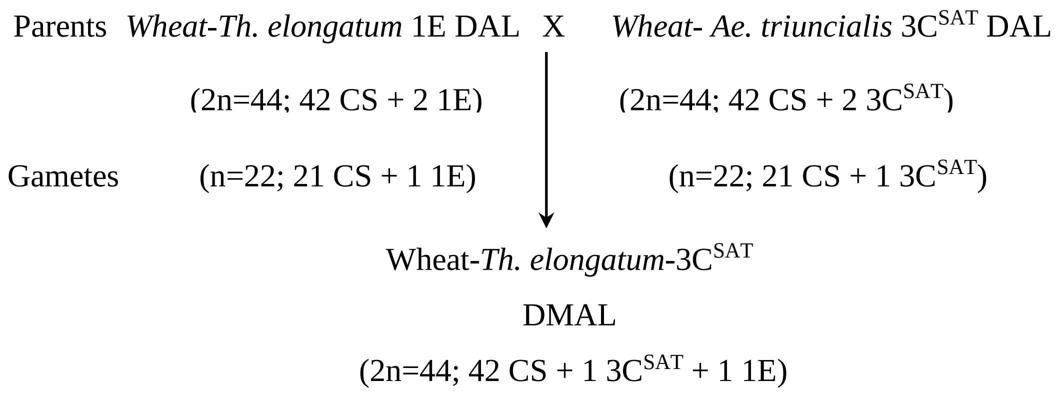


Figure 1 Schematic overview on production of Wheat-$3C^{SAT}$-1E DMAL. In the same way, other 6 lines carrying *Th. elongatum* chromosomes (Wheat-$3C^{SAT}$-2E to Wheat-$3C^{SAT}$-7E) DMALs were developed

Cytological study

The cytological screening of Wheat-*Th*. DALs and Wheat-*Th*.-3C^{SAT} DMALs (parental lines) was carried out by using root squash technique (Khatiwada et al 2019). Firstly, clean root tips were pre-treated in 0.002 M aqueous solution of 8-hydroxyquinoline and kept in ice-cold water for 24 hours. Then root tips were fixed in Carnoy's solution I (Ethanol 3: 1 Glacial Acetic Acid) at room temperature for 1 week. The root tips stained in 1% Aetocarmine solution were squashed and observed under the microscope. The cells showing best metaphase chromosomes were selected and photographed.

Pollen fertility screening

Pollen fertility of parental lines was checked microscopically following the method used by Singh (2003) with slight modifications. Firstly, mature anthers were sampled from all the 14 lines, transferred to eppendorf tubes and fixed in Carnoy's Solution I at room temperature for 24 hours. Then a drop of acetocarmine was put on a glass slide and fixed anther was smashed gently. The slide was gently heated and cooled two to three times until the pollen nuclei became clearly visible. The pollen with three intact nuclei were considered as fertile.

Crossing between DALs and DMALs

Altogether 98 plants, seven from each of 14 parental lines (Wheat-1E to 7E DALs and Wheat-3C^{SAT} -1E to7E DMALs) were grown in pots and kept in open place in October 2017. Crossing was performed between Wheat-*Th*. DALs and Wheat-*Th*.-3C^{SAT} DMALs plants (**Table** 1) during March-April 2018. Depending on the availability of florets at the time of crossing, the number of florets crossed ranged from 20 to 100 in different parental lines. However, due to unavailability of healthy florets, crossing couldn't be done between Wheat-3C^{SAT}-5E DMALs (mother plant) and Wheat-5E DAL (pollen donor).

Effect of alien chromosomal addition

The parental lines were assessed for the effect of chromosomal additions (disomic and double monosomic) by observing the plant height, spike length and seed set per spike in different lines. Height of the parental lines were measured above the ground to top of the spike. Three spikes of each lines were used to measure the spike length. The seed set (selfed) was counted from the first spike of three plants of each line and mean grain number/spike was determined.

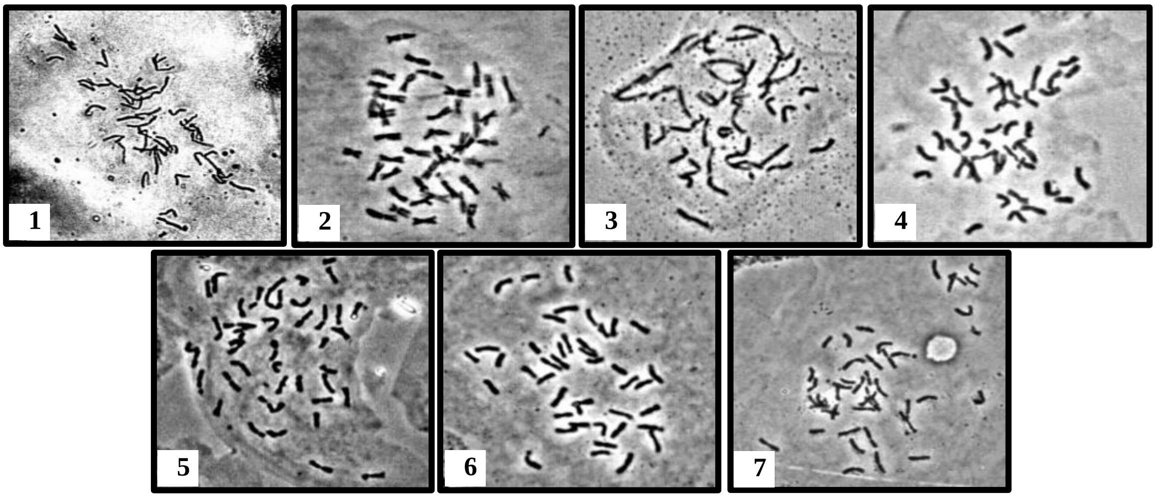
Statistical analysis

One-way analysis of variance (ANOVA) was carried out to find out the statistical significance of the difference of mean values of studied parameters among DALs and DMALs using Microsoft excel and SPSS v. 20.

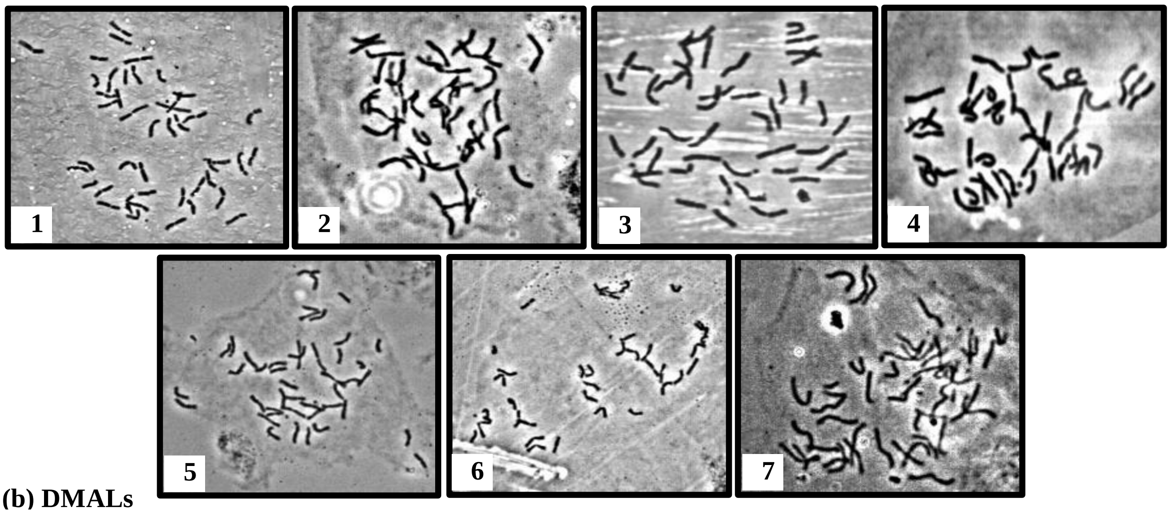
RESULTS

Cytogenetic study

The parental lines- DALs and DMALs, were confirmed for their chromosome number i.e., 2n=44 (**Figure** 2).



(a) DAL

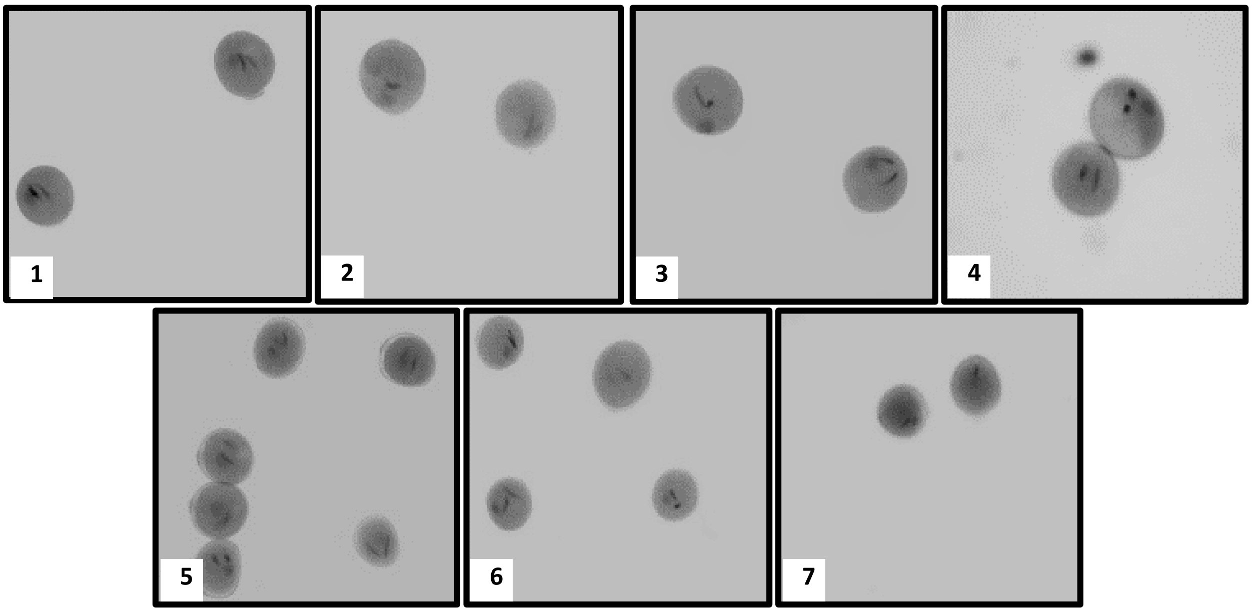


(b) DMALs

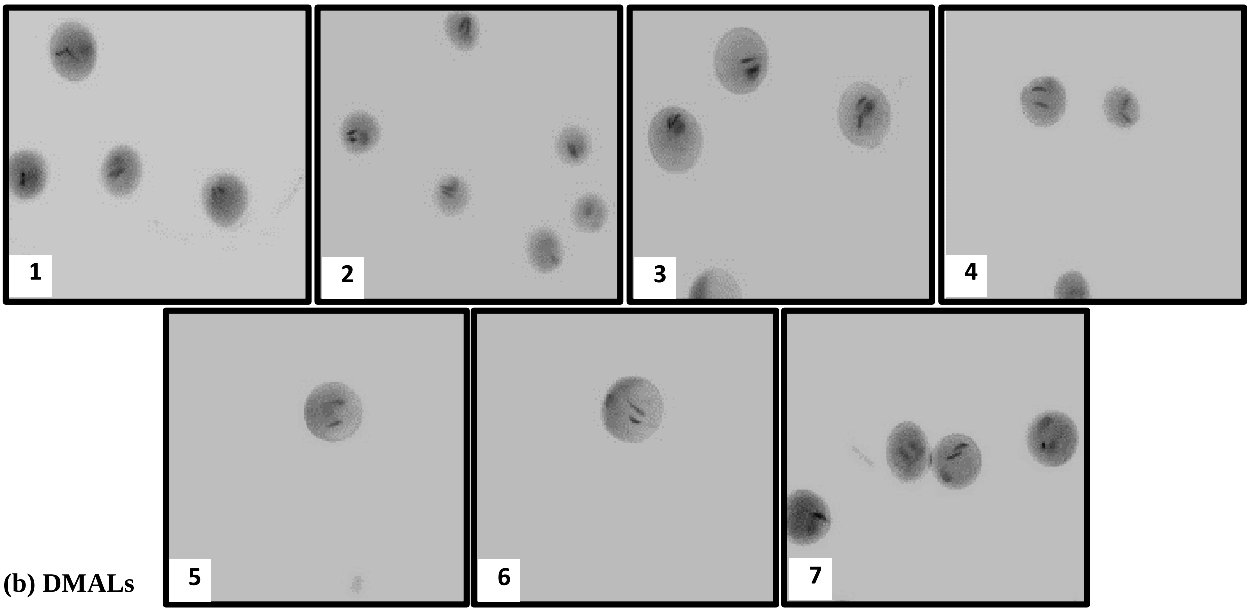
Figure 2. Karyology of parental lines; (a) Wheat-alien disomic addition lines (DALs, Wheat-1E to 7E) and (b) Wheat-alien double monosomic addition lines (DMALs, Wheat-3C^{SAT}-1E to 7E). The numbers 1, 2, 3, 4, 5, 6 and 7 in the figure represent the corresponding E chromosome from *Th. elongatum*

Pollen fertility

In this study fertility of pollen ranged from 44%-75% in wheat alien addition lines (DALs and DMALs). Overall, the fertility percentage was not very low as most of the lines having above 50% fertility (**Figures** 3 and 4).



(a) DALs



(b) DMALs

Figure 3. Pollen fertility screening of (a) Wheat-alien disomic addition lines (DALs, Wheat-1E to 7E) and (b) Wheat-alien double monosomic addition lines (DMALs, Wheat-3C^{SAT}-1E to 7E). The numbers 1, 2, 3, 4, 5, 6 and 7 in the figure represent the corresponding E chromosome from *Th. elongatum*

Agro-morphological variation

Plant height

The plant height was measured highest for the Wheat-6E (86.33±1.53cm) and lowest for Wheat-7E (55.33±5.03cm) among Wheat-*Th. elongatum* DALs. Similarly, in Wheat-*Th. elongatum*-3C^{SAT} DMALs, the highest plant height was recorded for Wheat-3C^{SAT}-5E (97.67±9.24cm) while lowest for Wheat-1E-3C^{SAT} (73.33±13.32cm). The plant height of the DMALs always exceeded that of the DALs (Figure 5).

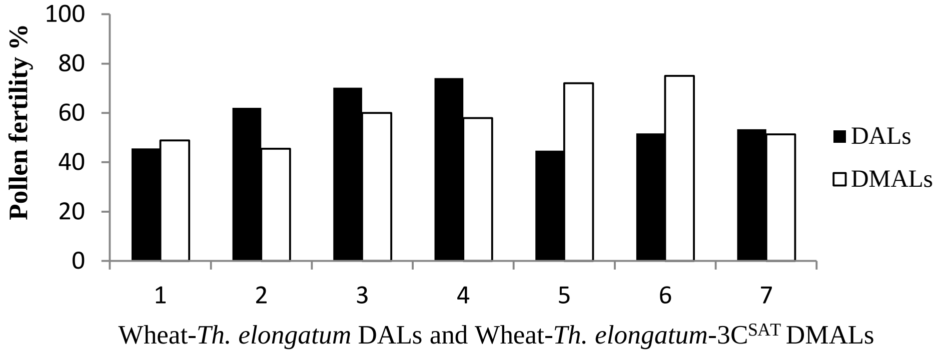


Figure 4. Pollen fertility (%) in Wheat-*Th. elongatum* DALs and Wheat-*Th. elongatum*-3C^{sat} (DMALs). The numbers 1, 2, 3, 4, 5, 6 and 7 in the figure represent the corresponding E chromosome from *Th. elongatum*

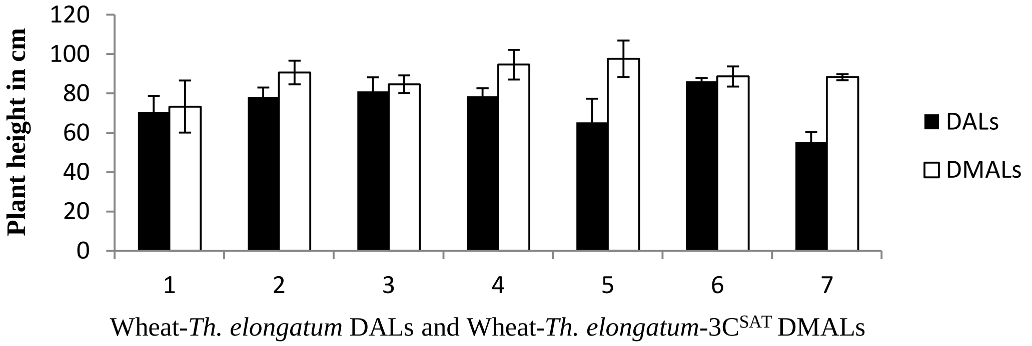


Figure 5. Plant height of Wheat-*Th. elongatum* DALs and Wheat-*Th. elongatum*-3C^{SAT} (DMALs). The numbers 1, 2, 3, 4, 5, 6 and 7 in the figure represent the corresponding E chromosomes from *Th. elongatum*

Spike length

The spike length was measured highest (7.85±0.44cm) for the Wheat-4E and lowest (5.92±0.37cm) for Wheat-1E line among the Wheat-*Th. elongatum* DALs. Similarly, among the Wheat-*Th. elongatum*-3C^{SAT} DMALs, the highest spike length (8.71±0.89cm) was recorded for Wheat-3C^{SAT} -3E and the lowest spike length (6.33±0.50cm) was recorded for Wheat-3C^{SAT}-1E. The spike length of DMALs always exceeded that of the DALs except for Wheat-6E. (Figures 6 and 7). The one way ANOVA showed significant difference among the mean values of spike length in DALs and DMALs.

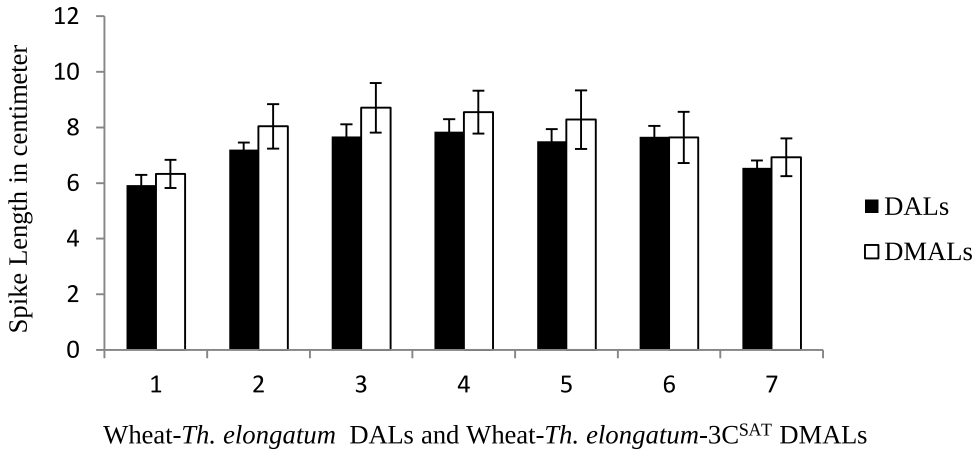


Figure 6. Spike length of Wheat-*Th. elongatum* DALs and Wheat-*Th. elongatum*-3C^{SAT} (DMALs). The numbers 1, 2, 3, 4, 5, 6 and 7 in the figure represent the corresponding E chromosomes (1-7) from *Th. elongatum*

Spikes of DALs

Spikes of DMALs

Figure 7. Spikes of parental lines; Wheat-*Th. elongatum* DAL and Wheat-*Th. elongtum*-3CSAT DMALs. The numbers 1, 2, 3, 4, 5, 6 and 7 in the figure represent the corresponding E chromosome from *Th. elongatum*

Table 1: Details of crossing scheme, the total number of florets crossed and percentage of seed set in different crosses between Wheat-*Th. elongatum* DALs and DMALs.

Crossed Lines		**No. of florets crossed**	**Seed set %**	**Total seeds**
Mother plant (2n=44)	Pollen donor (2n=44)			
Wheat-1E DAL*	Wheat-3C^{SAT}-1E DMAL**	100	54	130
Wheat-3C^{SAT}-1E DMAL	Wheat-1E DAL	100	76	
Wheat-2E DAL	Wheat-3C^{SAT}-2E DMAL	60	80	139
Wheat-3C^{SAT}-2E DMAL	Wheat-2E DAL	60	30	
Wheat-3E DAL	Wheat-3C^{SAT}-3E DMAL	50	30	33
Wheat-3C^{SAT}-3E DMAL	Wheat-3E DAL	60	30	
Wheat-4E DAL	Wheat-3C^{SAT}-4E DMAL	40	40	30
Wheat-3C^{SAT}-4E DMAL	Wheat-4E DAL	20	70	
Wheat-5E DAL	Wheat-3C^{SAT}-5E DMAL	20	30	6
Wheat-3C^{SAT}-5E DMAL	Wheat-5E DAL	0	0	
Wheat-6E DAL	Wheat-3C^{SAT}-6E DMAL	20	95	52
Wheat-3C^{SAT}-6E DMAL	Wheat-6E DAL	60	55	
Wheat-7E DAL	Wheat-3C^{SAT}-7E DMAL	40	73	77
Wheat-3C^{SAT}-7E DMAL	Wheat-7E DAL	60	80	

***** Hybrid plant carrying 42 wheat chromosomes and a pair of chromosome of *Th. elongatum* (1E to 7E; 2n= 44).

** Hybrid plant carrying 42 wheat chromosomes, a pair of chromosome of *Th. elongatum* (1E to 7E) and one gametocidal chromosome 3C^{SAT} of *Aegilops triuncialis* (2n= 44).

Seed set per spike (Selfing)

The mean value of seeds/spike ranged from 11-28 seeds/spike and was recorded highest (28±6.08) for Wheat-7E and lowest (11±3.51) was obtained for Wheat-4E among the Wheat-*Th. elongatum* DALs. Similarly, in case of DMALs the seed set/spike ranged from 20±3.61 (in Wheat-3C^{SAT}-1E) to 28.33±6.11 (in Wheat-3C^{SAT}-6E). The seed sets/spike was always higher in DMALs than DALs except in case of Wheat-3C^{SAT}-7E (**Figure** 8). The one way ANOVA showed significant difference in the mean values of seed set per spike among DALs and insignificant difference in the same among the DMALs.

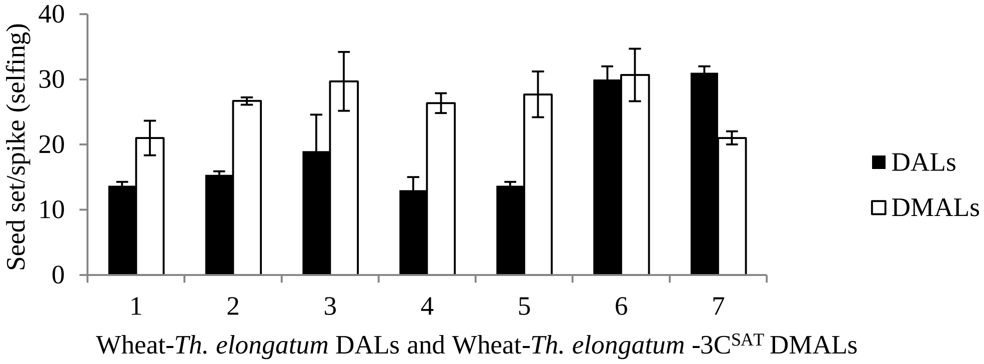


Figure 8 Seed set/spike of Wheat-*Th. elongatum* DALs and Wheat-*Th. elongatum*-3C^{SAT} (DMALs). The numbers 1, 2, 3, 4, 5, 6 and 7 in the figure represent the corresponding E chromosome from *Th. elongatum*

Seed set in crossed florets

Among the total number of florets crossed the seed set in different Wheat-*Th. elongatum* DALs and DMALs ranged from 30%-95%. When DALs were used as female, the seed set was highest in Wheat-6E (95%), and lowest in Wheat-3E and Wheat-5E (30% each). Similarly, when DMALs were used as female, the seed set was highest in Wheat-3C^{SAT}-7E (80%), and lowest in Wheat-3C^{SAT}-2E and Wheat-3C^{SAT}-3E (30% each) (no seed set in Wheat-3C^{SAT}-5E). Altogether 467 seeds were produced (**Table** 1).

DISCUSSION

Cytological screening of the parental lines with 2n= 44 chromosomes suggested that the chromosomes segregated stably during meiosis and there was no loss of chromosomes. Similar results were suggested in Wheat-barley disomic addition lines (Joshi et al 2011). Moreover, the rye chromosome was also found to be inherited independently in both double and triple substitution in Wheat-rye hybrids lines (Merker 1979). Khatiwada (2017) also reported similar segregation pattern of added chromosomes in all fifteen Wheat-alien addition lines i.e. seven wheat-*Th. elongatum* DALs (Wheat-1E to 7E), seven Wheat-2C-1E to 7E) DMALs, and one Wheat-2C DAL.

Tsujimoto et al (1984) suggested that the gametocidal chromosome 3C of *Aegilops triuncialis* causes the semi-sterility in monosomic state in common wheat cultivar. Also, this may be due to presence of gametocidal chromosomes that is 3C^{SAT} from *Aegilos triuncialis* which may be responsible for pollen infertility. Loegering and Sears (1963) also found that there may be presence of Ki locus (pollen killer) on gene of Chinese spring. Meiotic irregularities, genetic factor in hybrid lines also affect the fertility and cause low fertility in monosomic addition lines (Makino 1976). Some of these factors may be the reason behind relatively low fertility of some addition lines in the present study.

The shortening of plant height is possibly caused defects in production, transport or signaling of gibberellic acids in corresponding DALs. This type of response to gibberellin is conferred by mutant dwarfing alleles at one of two loci Rht-B1 and Rht-D1 for reduced height (Rht) gene (Peng et al 1999). Chen et al (2012) successfully introduced dwarfing genes from *Thinopyrum intermedium* and *Th. ponticum* to common wheat. As the height of all seven types of DMALs exceeded that of their respective DALs, it can be inferred that the 3C^{SAT} chromosome of *Ae. triuncialis* may be lacking dwarfing gene/s.

In wheat-alien addition lines the spike length is greatly influenced by the addition of chromosomes into wheat from their wild relatives. And the length of the spike depends on the type of species selected as chromosome donor. Blanco et al (1983) reported increment of spike length in hybrids of *Triticum durum* and *Haynaldia villosa*. Dvorak (1980) suggested the spike controlling character of *Thinopyrum* and *Aegilops* were not lost even after addition of their chromosomes into Chinese Spring. Similarly, Khatiwada (2017) also reported similar results in DALs of *Th. elongatum* and DMALs of *Th. elongatum* and *Aegilops cylindica* (2C) chromosomes to Chinese Spring. The findings of the present study and that of Khatiwada (2017) that the increase of spike length in both types of DMALs (wheat-*Th. elongatum*-3C^{SAT} and wheat-*Th. elongatum*-2C) suggest that the spike length trait has been transferred to wheat along with the addition of chromosomes from *Aegilops triuncialis* and *Ae. cylindrica*.

In hybridization of common wheat with its wild relatives, the lower seed set is attributed to various reasons including meiotic abnormalities and relative slow growth of pollen tubes in the styles (Roebbelen and Smutkupt 1968). Similarly, Blanco et al (1987) reported lower seed set condition in spikes of *Triticum durum-Dasypyrum villosum* monosomic addition lines. Furthermore, Khatiwada (2017) reported relatively higher number of seeds/spike in wheat-*Th. elongatum* DALs than in wheat-*Th. elongatum*-2C DMALs. The reason behind this difference in two similar type of studies is possibly due to the differences in the activity of two different types of Gc chromosomes. Some Gc chromosome like 4S^{1} (from *Aegilops sheronsis*) are also reported to cause high level of chromosomal aberration than 3C^{sat} (King 1991).

The percentage of seed set in selfed spikes of DMALs always exceeded that of the DALs except in Wheat-3C^{SAT}-7E. Similar trend was observed for the crossed seeds when Wheat-3C^{SAT}-1E, Wheat-3C^{SAT}-4E and Wheat-3C^{SAT}-7E were taken as female. The increment in seed set in DMALs than DALs may be due to presence of *Th. elongatum* chromosome in monosomic condition. This possibility has been suggested by Sears (1954) while producing many series of addition lines in common wheat. Similarly, Miller and Reader (1987) also suggested that addition of pairs of chromosomes to wheat complement produces changes in plant and seed morphology. The crossed seed set of Wheat-3C^{SAT}-2E and Wheat-3C^{SAT}-6E when taken as female was found to decrease. The reason behind it is hard to explain although it may be because of the presence of gametocidal chromosome 3C^{SAT}, low fertility of pollen grains and low incompatibility or all factors in combination. Niranjana (2017) also suggested that the Gc chromosomes can reduce fertility in wheat hybrids.

CONCLUSION

Cytogenetic study of the wheat alien addition lines revealed the consistency as well as stability in chromosomal number in offspring as expected according to the chromosomal theory of inheritance. However, the different alien chromosomes affects various agro-morphological traits of wheat addition lines differently. There were no specific pattern representing the effects of chromosomal additions on various traits observed. The lines developed in this study may serve as starting material to screen for mutation inducing lines (45 chromosome plants).

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