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Identification of rye chromosome substitutions in Triticale and its relation with kernel characters and seed setting through Giemsa C-banding technique

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ABSTRACT

Objective: To study the individual characteristic features of chromosomes in the three varieties of diploid rye and the 54 hexaploid triticale strains and also to identify the replaced rye chromosome pair or pairs in triticale and the relation of rye chromosomal substitutions with kernel characters. **Methods:** The present study was conducted using Giemsa C-banding technique. **Results:** The results showed rye chromosome 2R was replaced in large number of cases followed by 4R/7R, 5R and 2R+5R. Triticale strains with 4R/7R substitution exhibited low degree of shriveling in grains with higher values for test weight, 100-grain weight and volume of water displaced by 100-grains whereas 4R/7R substitution along with another substitution for 2R or 5R or 6R showed medium shriveling and strains with substitutions either for 2R or 3R or 5R or 7R/4R exhibited high degree of shriveling and a cumulative effect was observed when two of these substitutions were presented together in one triticale. Triticale strains with 2R/2D substitution had higher seed set and it was medium in case of 4R/4D. But when both 2R/2D and 4R/4D substitution occur together the seed set was considerably high and was low when 3R and 5R substituted alone or in combination. **Conclusions:** It has shown evidence in the present study about the role of individual R/D substitutions on the quality of seeds it is necessary to introduce those substitutions which are of agronomic importance to the breeders.

1. Introduction

Triticale (X *Triticosecale* Wittmack Syn.) is an artificial cereal crop genus in the Family Poaceae, produced by doubling the chromosomes of the sterile hybrid that results when crossing wheat (*Triticum aestivum*) and rye (*Secale cereal* L.)[1] and the result of this doubling is a polyploid. The name triticale combines the scientific names of the two genera involved[2]. Triticale can be octaploids or tetraploids, however most triticale cultivars are hexaploids.

The aim of a triticale breeding programme mainly focuses on the improvement of quantitative traits such as grain yield, nutritional quality, plant height, as well as traits which are more difficult to improve such as earlier maturity and improved test weight (a measure of yield)[3]. These traits are controlled by more than one gene[4].

During crossing between wheat and triticale or wheat and rye, useful characters from rye genome where transferred to wheat either in the form of substitutions, additions and translocations[5,6]. Hence the objectives of this study are to study the individual characteristic features of chromosomes in three varieties of diploid rye and the chromosome constitution of different hexaploid triticale strains using Giemsa C-banding technique, to identify the replaced rye chromosome pair or pairs in triticale and to study the relation of rye chromosomal substitutions with kernel characters such as seed shriveling and seed setting in different hexaploid triticales.

Giemsa C-banding technique is widely used in cytogenetics to produce differently stained regions on condensed chromosomes. The metaphase chromosomes are treated with trypsin and stained with Giemsa. Dark bands that take up the stain are strongly A, T rich. Using this technique, detailed chromosome composition were analyzed in different hexaploid and octoploid triticales[7].

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2. Materials and methods

2.1. Seed materials

Three varieties of diploid rye namely Imperial rye, Assamese rye and Petkus rye and 54 varieties of hexaploid triticale (Table 1) were used in the present experiment for identification of individual chromosomes in rye and rye chromosomes in triticales. The seed materials of rye and triticale were obtained from Division of Genetics, IARI, New Delhi; Regional Research Station, IARI, Wellington; Department of Genetics and Plant Breeding, Punjab Agricultural University, Ludhiana and CIMMYT, Mexico.

Table 1
List of hexaploid triticale varieties used in the present study.

S.No.	Triticale variety	S.No.	Triticale variety
1.	AC Alta	28.	Marval
2.	AC Certa	29.	Northstar
3.	AC Copia	30.	OAC Decade
4.	AC Ultima	31.	OAC Trillium
5.	Banjo	32.	OAC Triwell
6.	Beavrarm	33.	OAC Wintir
7.	Binova	34.	Partout
8.	Blenvenue	35.	Pika
9.	Bobcat	36.	Pronghorn
10.	Cando	37.	Puma
11.	Carman	38.	Rose
12.	Claire	39.	Rosner
13.	Coorong	40.	Rymin
14.	Cylus	41.	Sandro
15.	DC-2	42.	Springfest
16.	Ego	43.	Taurus
17.	Era	44.	T 118
18.	Fidello	45.	TL 230
19.	Florico	46.	TL 1210
20.	Frank	47.	Tricolor
21.	Grace	48.	Trilogie
22.	IC 470	49.	Trinidad
23.	Karl	50.	Versus
24.	Kramer	51.	Vision
25.	Lamberto	52.	Wapiti
26.	Len	53.	Welsh
27.	Lupus	54.	Wheaton

2.2. Giemsa staining method

The following method was employed for identification of individual R-genome chromosomes in rye and possible R/D substitutions in different hexaploid triticales. The Giemsa banding technique was used following modification of techniques used by Gill and Kimber[8], Vosa[9] and Weimarck [10].

2.2.1. Giemsa solution

One gram Giemsa powder (BDH Ltd. England) was added to 66 mL of glycerin. The solution was boiled at 60 °C for 2 h, adding 66 mL of methanol and cooled at room temperature for one day. The filtered solution was the stock solution and was stored in refrigerator until use. 5 mL of Giemsa stock solution with 100 mL of phosphate buffer (2.279 g of KH_2PO_4 and 2.366 g of Na_2HPO_4 in 250 mL of distilled water; pH= 6.8) was used as 5% Giemsa solution for staining the rye chromosomes.

2.2.2. Preparation of slides

Triticale seeds were grown in moist filter paper. 1.5 to 2.0 cm long root tips along with seeds were pretreated in ice-cold water (1 °C) for 24 h. Root tips were excised and fixed in 1:3 aceto alcohol for one day and stored in 70% ethanol for short period (1-2 days).

Root tips were hydrolysed in 10 mL of 45% acetic acid containing 3-4 drops of N HCl, warmed at 60 °C for 10 minutes. Extreme root tips (1mm) were squashed on a slide with fresh 45% acetic acid and covered with glycerin coated cover slips. Cover slips were removed by dipping the slides in absolute alcohol for 10-15 minutes. Air-dried slides were pretreated with aqueous solution of barium hydroxide (5 g of $Ba(OH)_2.5H_2O$ in 100 mL of distilled water) at 20 °C for 9-10 minutes and were gently washed in distilled water with 3-4 changes. Slides were incubated for 1 h in 2 × SSC solutions (0.3 M Sodium chloride and 0.03 M trisodium citrate; 1:1) at 60 °C and then washed briefly in distilled water with two changes. Slides were stained in freshly prepared 5% Giemsa solution (5 mL of Giemsa stock solution in 10 mL of M/15 phosphate buffer; pH 6.8) for 3-5 minutes. Air dried slides were made permanent using Canada balsam.

Photomicrographs were taken on a Nikon microscope having automatic photo micrographic attachment.

2.2.3. Identification of rye chromosomes

For identification of Giemsa stained individual chromosomes in rye and in different hexaploid triticales, the classification given by Sybenga[11] and Naranjo *et al.*[12] were used.

2.2.4. Kernel characters in triticales

The following grain characters were recorded in the present experiment based on the classifications of Bennett [13] and Gill *et al.* [14]. 100 – grain weight: Mean data recorded on 5 plants from each of the triticale strain. Average seed set: mean data recorded on one spike from each five plants from each of the triticale strain. Seed set was represented as:

$$\frac{\text{Total number of seeds / spike}}{\text{Total number of florets / spike}} \times 100$$

Seed set was further classified as: Low-below 50%; Medium – 50% – 65%; High – 65% and above.

Volume (mL) of water displaced by 100 grains: Mean data recorded on 5 plants from each of the triticale strain.

Kernel shriveling: Based on visual observations, grain shriveling was classified into following four groups: (a) Very low; (b) Low; (c) Medium; (d) High.

Test weight – measured as weight per 100 litres of bulk volume.

3. Results

3.1. Identification of individual chromosomes of rye

Three varieties of diploid rye (*Secale cereale* L.) namely Imperial rye, Assamese rye and Petkus rye were studied through Giemsa C-banding technique to identify and characterize individual chromosomes of rye. All the three rye varieties showed similar banding pattern. The characteristic features of individual rye chromosomes in three rye genotypes are shown in Figure 1- 4.

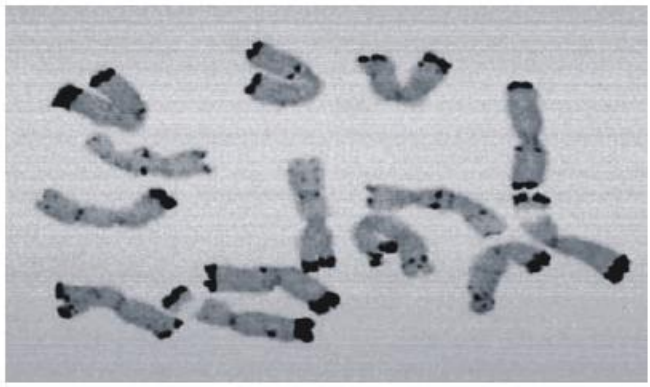

Figure 1. Imperial rye chromosomes.

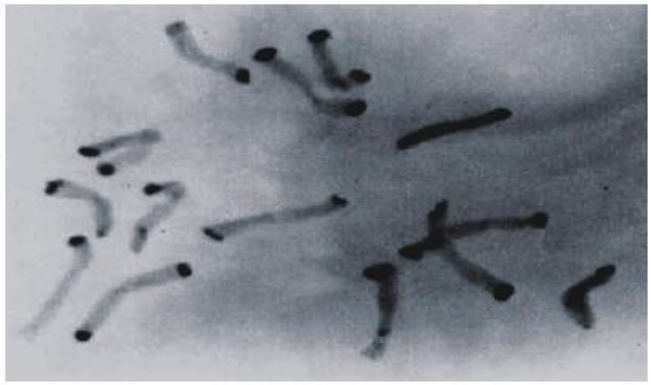

Figure 2. Assamese rye chromosomes.

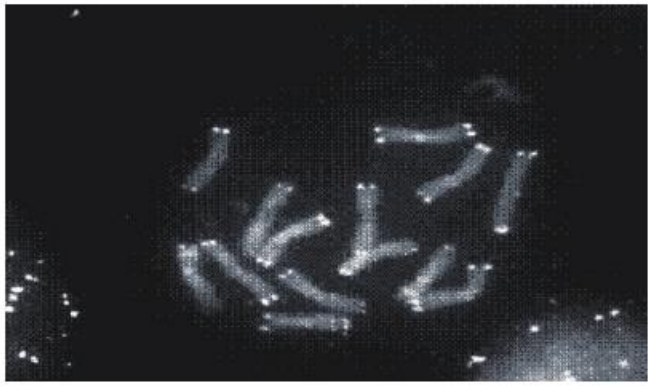

Figure 3. Petkus rye chromosomes (observed through phase contrast microscope).

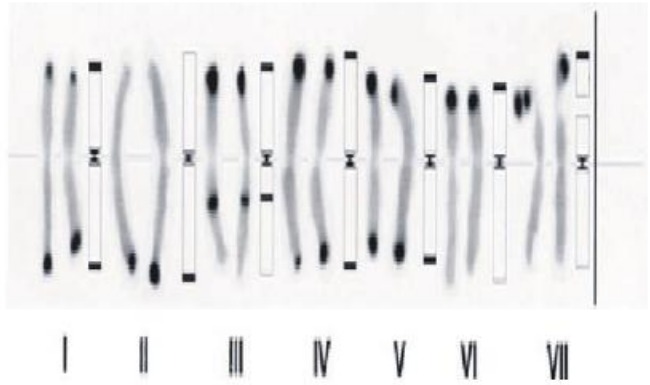

Figure 4. Ideogram of rye chromosomes showing characteristic features of individual chromosomes.

Chromosome 1R: It is nucleolar organizer chromosome; sub-metacentric and has a prominent band on both telomeres; short arm carries a second prominent band just adjacent to the satellite.

Chromosome 2R: It is nearly metacentric; characterized by a terminal band on each telomere, the prominent of the two bands being on the short arm.

Chromosome 3R: It is nearly metacentric, carries a prominent band at the end of long arm and a thin band at the end of the short arm.

Chromosome 4R/7R: It is a translocated chromosome and sub-metacentric; thin terminal band on the short arm long arm has three weak bands; an interstitial band located in the middle of the arm and two almost terminal bands.

Chromosome 5R: It is sub-metacentric; a terminal band on the short and two interstitial bands on the long arm.

Chromosome 6R: It is sub-metacentric; a prominent band on short arm; several weak bands on long arm.

Chromosome 7R/4R: It is a translocated chromosome and metacentric; terminal bands of different intensities at the two ends; the thinnest correspond to the telomere of the short arm.

3.2. Rye chromosome composition in different hexaploid triticales

Fifty four hexaploid triticales varieties were studied by Giemsa C-banding technique in order to identify individual rye chromosome composition (Table 2). From the data it is clear that out of 54 hexaploid triticale genotypes, 14 had full complement of rye chromosomes (seven pairs as revealed by 14 banded chromosomes), 24 had one pair of replaced rye chromosomes (12 banded chromosomes) and 16 had two pairs of replaced rye chromosomes (10 banded chromosomes). In triticales, where one pair is replaced, 2R was replaced in 13 triticale genotypes, 3R in 2 varieties, 4R/7R in 5 triticales, 5R in 4 varieties. In the 16 triticales, where two pairs were replaced, 2R and 4R/7R were replaced in two triticales, 2R and 5R in 4 varieties, 2R and 4R/7R in two varieties, 3R and 5R in 2 varieties, 4R/7R and 5R in 3 triticales and 4R/7R and 6R were replaced in three triticale genotypes.

Table 2
Rye chromosome composition of different hexaploid triticale varieties.

Replaced rye chromosome pair/pairs	Triticale varieties			
None	Beavrarm	Cando	Ego	Grace
	Lamberto	OAC Decade	Partout	Pronghorn
	Rosner	Sandro	Tricolor	Trinidad
	Wapiti	Wheaton		
2R	Claire	DC-2	Florico	Karl
	Lupus	OAC Triwell	Rose	Rymin
	Taurus	TL 230	Trilogie	Vision
	Welsh			
3R	Banjo	Pika	–	–
4R/7R	AC Ultima	Blenvenue	Coorong	Era
	T 118		–	–
5R	AC Certa	Fidello	Kramer	Marval
2R,4R/7R	Bobcat	OAC Trillium	–	–
2R, 5R	AC Alta	Cylus	OAC Wintir	Springfest
2R, 7R/4R	IC 470	Puma	–	–
3R, 5R	AC Copia	Frank	–	–
4R/7R, 5R	Carman	Len	Versus	–
4R/7R, 6R	Binova	Northstar	TL 1210	–

Overall, either individually or in combination with other substitutions, the frequency of replacement of rye chromosomes by wheat chromosomes are as follows: 2R in 21 triticales; 3R in 4 triticales, 4R/7R in 13 triticales, 5R in 13 triticales, 6R in 3 triticales; and 7R/4R in 2 triticales.

3.3. Kernel characters vs. rye chromosome composition in triticales.

The data on various kernel characters including kernel shriveling, 100-grain weight, volume of water displaced by 100-grains, test weight (grain weight per hundred liters of volume) and seed setting in all the 54 hexaploid triticales were recorded and are presented in Table 3.

From the data, it is evident that in five triticale varieties the kernel shriveling was 'very low'; in 15 triticale genotypes, it was 'medium'; it was 'Low' in nine triticales genotypes and in the remaining 25 triticale varieties, the shriveling was 'high'. The other parameters from which shriveling can be inferred include the test weight, 100-grain weight, and volume displaced by 100-grains. The results revealed that the values of these parameters were high in triticales with low shriveling and were low in genotypes with high shriveling.

From the data on average seed set it is evident that, six triticale varieties had 'low seed set' (below 50%), 20 triticale genotypes had a 'medium seed set' and the remaining 28 triticale varieties had 'high level of seed set' (above 65%).

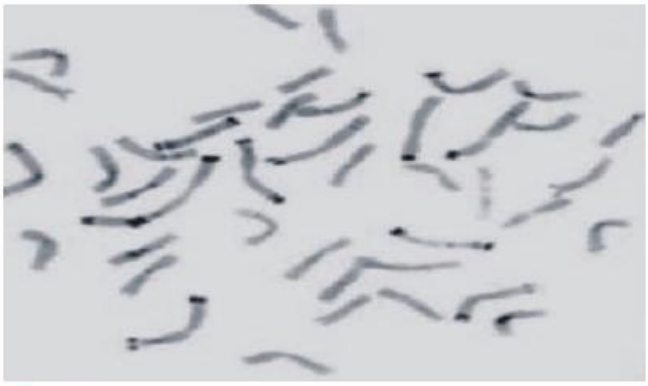

Figure 5. Triticale 'Cando'– full (14) complement of rye chromosomes. No R/D substitutions.

Figure 6. Triticale 'Bobcat'–2R, 4R/7R rye chromosomes are replaced by 2D and 4D wheat chromosomes.

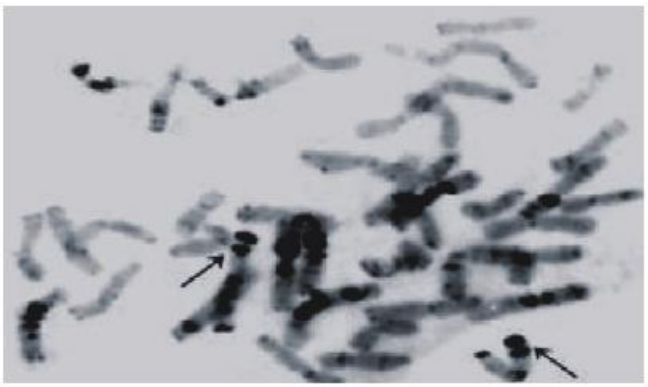

Figure 7. Triticale 'TL 1210'– 4R/7R and 6R rye chromosomes are replaced by 4D and 6D wheat chromosomes.

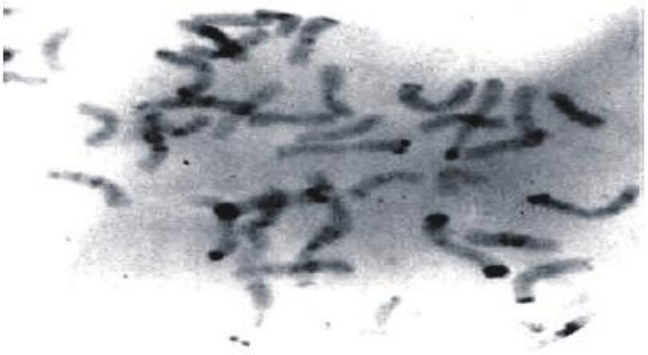

Figure 8. Triticale 'Cocorong'– 4R/7R was replaced by 4D of wheat chromosomes.

4. Discussion

Triticale is a man-made crop, created by combining the genomes of durum or bread-wheats and their close relative rye. The initial intent was to develop a new species that would combine the winter-hardiness and disease resistance of rye with the end-use quality of wheat. While triticale has been quite successful as a forage crop, its utilization as a grain crop has been limited. Along with useful genes from rye, deleterious genes effecting processing quality limit the use of triticale in traditional bread products. Most quality-related genes of wheat reside on the D-genome; a set of chromosomes absent in the most commonly used triticales, which contain the A and B genomes of durum wheat, and the R genomes of rye.

Using Giemsa C-banding technique, the characteristic features of individual chromosomes of the three rye varieties used in the present study were analyzed. This technique is found more reliable over simple karyotypic features where size of the chromosome, arms and position of centromere differs due to contraction of chromosomes.

The information obtained from this study was utilized to analyze rye chromosome composition in different hexaploid triticale varieties.

Rye chromosome composition in hexaploid triticales was analyzed with the help of Giemsa staining technique, it has been shown by several workers[15-17] that at least one to three D genome chromosomes were substituted in most of the secondary hexaploid triticales.

Table 3

The data on chromosome composition and kernel characters in different hexaploid triticale strains/varieties.

S.No.	Triticale strain / variety	R/D substitution(s)	Average seed set (%)	100-grain weight (g)	Volume (mL) of water/ displaced by 100 grains	Grain shriveling	Test weight (kg/hl)
1.	AC Alta	2R, 5R	84.2	2.65	2.89	High	51
2.	AC Certa	5R	49.1	2.89	3.01	High	49
3.	AC Copia	3R, 5R	46.2	3.00	3.02	High	51
4.	AC Ultima	4R/7R	57.4	3.41	3.51	Medium	62
5.	Banjo	3R	49.4	2.71	2.79	High	52
6.	Beavrarm	None	57.7	2.49	2.80	Medium	60
7.	Binova	4R/7R,6R	69.9	4.11	4.21	Very Low	71
8.	Blenvenue	4R/7R	65.4	3.74	3.83	Low	64
9.	Bobcat	2R,4R/7R	81.7	3.84	3.98	Very Low	70
10.	Cando	None	54.4	2.52	2.71	High	53
11.	Carman	4R/7R,5R	65.7	3.78	3.80	Low	67
12.	Claire	2R	71.2	2.51	2,78	High	53
13.	Coorong	4R/7R	57.8	3.78	3.84	Low	66
14.	Cylus	2R, 5R	87.6	2.47	2.76	High	52
15.	DC-2	2R	81.4	2.46	2.69	High	53
16.	Ego	None	51.4	2.63	2.84	High	57
17.	Era	4R/7R	59.5	3.77	3.81	Low	66
18.	Fidello	5R	61.9	2.98	3.01	High	52
19.	Florico	2R	69.4	2.34	2.63	High	54
21.	Grace	None	50.4	2.60	2.65	Medium	51
22.	IC 470	2R,7R/4R	69.6	2.69	2.72	High	53
23.	Karl	2R	74.5	2.69	2.76	High	56
24.	Kramer	5R	48.9	3.00	3.03	Medium	55
25.	Lamberto	None	52.3	2.49	2.54	Medium	52
26.	Len	4R/7R,5R	64.3	3.84	3.89	Low	68
27.	Lupus	2R	81.5	2.49	3.01	High	55
28.	Marval	5R	54.1	2.67	2.89	High	50
29.	Northstar	4R/7R,6R	69.7	4.13	4.20	Very Low	73
30.	OAC Decade	None	51.6	2.61	2.75	Medium	57
31.	OAC Trillium	2R,4R/7R	81.6	3.83	3.97	Very Low	72
32.	OAC Triwell	2R	68.2	2.41	2.76	High	54
33.	OAC Wintir	2R, 5R	74.9	2.72	2.77	High	51
34.	Partout	None	57.7	2.49	2.80	Medium	60
35.	Pika	3R	51.4	2.68	2.56	High	50
36.	Pronghorn	None	49.9	2.57	2.69	Medium	59
37.	Puma	2R,7R/4R	69.4	2.68	2.71	High	54
38.	Rose	2R	81.2	2.81	3.01	Medium	57
39.	Rosner	None	50.7	2.71	2.79	Medium	59
40.	Rymin	2R	78.5	2.89	2.98	High	56
41.	Sandro	None	54.4	2.52	2.71	High	53
43.	Taurus	2R	76.5	2.91	3.01	Medium	53
44.	T 118	4R/7R	59.3	3.83	3.85	Low	70
45.	TL 230	2R	75.6	2.68	2.78	High	53
46.	TL 1210	4R/7R,6R	69.8	4.12	4.22	Very Low	72
47.	Tricolor	None	53.7	2.38	2.69	High	56
48.	Trilogie	2R	86.8	2.71	3.22	Medium`	56
49.	Trinidad	None	62.4	2.49	3.04	Medium	57
50.	Versus	4R/7R,5R	64.3	3.84	3.89	Low	68
51.	Vision	2R	75.6	2.68	2.78	High	53
52.	Wapiti	None	73.6	3.01	3.08	Low	54
53.	Welsh	2R	76.5	2.91	3.01	Medium	53
54.	Wheaton	None	79.4	3.04	3.41	Low	68

In the present study fourteen triticale lines shows full complement of rye chromosomes. In an earlier study, Sapra and Stewart[18] analyzed fifteen lines of triticale using the C-banding staining method and found complete rye chromosomal complement in as many as 11 triticale lines, while in the remaining some variations were noticed. The results obtained in the present study also indicated that majority of the triticales lost one to two rye chromosomes as revealed by the presence of only ten to twelve banded rye chromosomes, thus confirming the earlier observations that in most of the spring triticales R/D chromosome substitutions are frequent and are common.

Out of 54 hexaploid triticales analyzed, rye chromosome 2R was replaced in maximum number of triticales i.e. 21 triticales (13 cases alone and 8 cases in combination with other chromosomes), followed by 5R in 13 triticales (4 individually and 9 in combination), 4R/7R replaced in 13 triticales (5 individually and 8 in combination). 3R in 4 triticales (2 individually and 2 in combination), 7R/4R were replaced in two triticales (in combination with 2R), and 6R was replaced in three triticales (in combination with 4R/7R). 1R chromosome was not replaced in any of the triticale varieties studied. According to Gustafson and Bennett [19] and Gustafson[20], variation in the DNA and heterochromatin contents between wheat and rye chromosomes are mainly responsible for preferential occurrence of R/D substitutions and therefore, in triticales, the largest rye chromosome 2R with highest DNA amount was the first to be replaced by D genome chromosome in triticales, followed by 4R/7R and 5R, which also have prominent telomeric heterochromatin bands presumed to be responsible for replacement of the chromosomes[21,22]. According to Lukaszewski *et al.*[23], Sandha *et al.*[7] substitution of 2R by 2D wheat chromosome was not because 2R is the largest rye chromosome with highest DNA content, but because 2D carries a potent gene for day-length insensitivity.

Genome characterization of 14 hexaploid lines that spontaneously appeared in octoploid Triticales was carried out by Dou *et al.*[24] by means of sequential genomic in situ hybridization and Fluorescence in situ hybridization, high molecular weight glutenin subunits and SSR marker analyses. All of the lines showed a chromosome constitution of complete A and B genomes and a composite genome consisting of the chromosomes of D and R genomes. Nakata *et al.*[25] also identified three of these lines and demonstrated that the lines carried a composite genome consisting of the chromosomes of genomes D and R together with the complete A and B genomes and also deficiency of the terminal heterochromatin in 3R and a translocation between wheat and rye chromosomes were reported. They concluded that C-banding and telosomic analysis were efficient methods to identify chromosome constitution of triticales.

The above results suggested that some rye chromosomes are more often replaced than others. Specific homoeologous relationships between wheat and rye chromosomes are responsible for frequent occurrence of some R/D substitutions [26]. In addition, due to gene interaction, absence or presence of specific genes on specific chromosomes of the D genome may also check the replacement of rye chromosomes or may inhibit substitution of a D chromosome for a specific rye chromosome[27]. In an earlier experiment, Merker[21] studied the effect of artificial selection on chromosome constitution in hexaploid triticales. It was shown that when selection was exercised for different agronomic traits, 2R followed by 5R and 4R/7R were replaced in more frequency, while 6R and 1R were not replaced in any of the 50 triticale strains analyzed by him. The results obtained in the present study confirmed these earlier observations.

Rye chromosome composition *vs.* seed characters results obtained in Giemsa staining technique, the presence or absence of substitutions and effect of individual R/D chromosome substitutions were correlated with various seed characters. In the present study, triticales with full complement of rye chromosomes exhibited either medium or high shriveling except in two triticales Beagle and Carman.

In the present study none of the triticale strains contain substitutions for 1R. However, there are few reports substitution of D-genome chromosomes with that of A or B-genome chromosomes without removing any R-genome chromosomes. Kazman[28] and Kazman and Lelley[29] studied the role of 1D/1A substitution on bread making quality in synthetic hexaploid triticale and found that this substitution improved the bread making quality of triticale.

Triticale strains with substitutions for 2R, 3R, 5R, 2R and 5R, 2R and 7R/4R, 3R and 5R showed high degree of shriveling in the kernels accompanied with lower values for 100-grain weight, volume displaced by 100-grains and test weight, suggesting that these chromosome substitutions are not favorable for improvement of kernel plumpness. However, shriveling and other parameters of these strains with 2R/2D substitutions when compared with the values in strains with 3R or 5R or 7R/4R substitutions suggested that the shriveling caused by 2R/2D was less severe than that caused by loss of 3R, 5R and 7R/4R.

The test weight of the strains with 2R/2D substitution was slightly higher than test weights of the strains with 3R, 5R, 7R/4R substitutions. On the other hand triticale strains with 4R/7R substitution either present alone or combined with 2R or 5R or 6R showed either low or medium shriveling with higher values for 100-grain weight, volume of water displaced by 100-grains and test weights. These values were particularly high in the triticale varieties 'Binova', 'Northstar', 'TL 1210 (the only three triticale with 4R/7R and 6R substitutions) suggesting that substitution of 6R has enhanced the value of kernel characters particularly test weight and displaced volume caused by 4R/7R substitution. Earlier, Varughese *et al.*[30] and Lukaszewski and Gustafson[31], had also shown that triticales with improved kernels having wheat like appearance had a substitution of 6R and or 4R/7R.

In an earlier study, Bennett[13], Singh and Robbelen[32] and Merker[21] studied the causes of grain shrivelling in triticales. According to them the kernel shriveling in different triticales is due to aberrant nuclei in the endosperm which in turn is caused by late replicating segments of heterochromatin on rye chromosomes and the occurrence of chromosome bridges at anaphase and of other aberrant nuclei in coenocytic endosperm. Therefore, loss of heterochromatin from rye chromosomes in the form of R/D substitutions naturally improved the quality of endosperm and hence no shriveling in triticale grains. It is concluded that the reduction or elimination of segments of late replicating DNA from rye chromosomes should be a major object in the breeding of economically useful triticales.

High shriveling accompanied with lower values for 100-grain weight, displaced volume and test weight for substitutions 2R and 5R, 2R and 7R/4R, 3R and 5R over

individual substitutions for 2R, 3R, 5R and 7R/4R indicated that there is cumulative effects for increasing kernel shriveling. Similarly, low shriveling with higher 100-weight, displaced volume, test weight of these triticales with 4R/7R substitution relative to the strains with substitutions for 4R/7R and 2R or 4R/7R and 5R suggested that, substitutions 2R or 5R might have reduced the effects caused by 4R/7R substitution. This can also be inferred from the fact that the strains with 2R and 5R substitutions, which showed high shriveling with lower values of 100-grain weight, displaced volume and test weight relative to the values of individual substitutions 2R and 5R.

Therefore, it is suggested that strains with full complement of rye chromosomes, although, do not have the required improvement in kernel shriveling but they did not belong to high shriveling group including triticales strains with 2R, 3R, 5R and 7R/4R substitutions. On the other hand triticales with 4R/7R and 6R substitutions had favorable effects reducing kernel shriveling, and substitution 4R/7R along with other chromosomes had an intermediate effect on kernel characters. Ortiz-Monasterio *et al.*[33] and Varughese *et al.*[34] also observed that 2R /2D substitution causes slight improvement in kernel shriveling in some hexaploid triticales.

Seed setting *vs.* rye chromosome composition in triticales can be seen from the data presented in the Table 3 that fifteen triticales with full complement of rye chromosomes had a low seed set except a few. On the other hand, in triticale strains with 2R/2D substitution alone or in combination with additional substitutions for 4R/7R or 5R or 7R/4R, the seed set was high, although the seed set was slightly higher when 2R/2D substitution was present alone. The effect of 3R and 5R substitutions present either alone or combined with other substitutions, the seed set was low and particularly it was considerably reduced when both chromosomes were replaced.

Earlier, Friebe and Larter[35] produced a complete set of isogenic wheat/rye D-genome substitutions by crossing an inbred line of spring rye *Secale cereale* L. cv. "Prolific" to tetraploid wheat, the A-and B-genomes of which had previously been extracted from hexaploid wheat, *Triticum aestivum* L. em Thell. cv. "Thatcher". After chromosome doubling, the derived hexaploid triticale (x *Triticosecale* Wittmack) was backcrossed to 6x "Thatcher" and selection for wheat/rye substitution lines was carried out in BCF3 to BCF6 families by using Giemsa C-banding. Five fertile disomic wheat/rye D-genome substitution lines were obtained and their chromosomal constitution was determined to be 1D/1R, 2D/2R, 7D/4R, 6D/6R, 7D/7R. Another 1D/7R substitution was detected but this plant was very weak and sterile, indicating that only substitutions between homoeologous chromosomes result in fertile, vigorous plants. Medium seed set was observed in triticale strains with substitution present either alone or combined with others. It can be further supported by the fact that the seed set was considerably high when 4R/7R substitution was associated with 2R/2D substitution, suggesting that the two substitutions might carry genes for good seed set. Since triticale strains with 6R substitution alone could not be identified, high seed set in triticales 'Binova', 'Northstar', 'TL 1210 having a pair of substitutions 4R/7R and 6R should be attributed to both the substitutions as in case of 2R/2D and 4R/4D together. In earlier studies [36] it has been shown that both substitutions and modifications of chromosomes were shown to play a role in agronomical and seed characters in triticales.

The results obtained in the present study however, suggests that 2R/2D substitution causes higher seed set and that 4R/7R substitution had an intermediate effect. The role of interaction between wheat and rye genes and chromosomes on various morphological, agronomical and yield characters in triticales were also emphasized by several studies [37].

A cereal grass plant Triticale obtained from hybridization of wheat with rye. In some triticale varieties, one or more rye chromosomes have been replaced by wheat chromosomes, giving secondary-substituted triticales, as contrasted to complete triticale having all seven rye chromosomes. Triticale tends to have a greater ability than wheat to grow in adverse environments such as saline or acid soils or under droughty conditions.

Triticale holds much promise as a commercial crop as it goes a long way toward addressing specific problems within the cereal industry. Conventional breeding has helped to establish triticale as a valuable commercial crop and more particularly where conditions are less favorable for wheat cultivation. Not withstanding the fact that triticale is a man-synthesized grain, many initial limitations such as an inability to reproduce due to infertility, and seed shriveling, low yield and poor nutritional value have greatly been eliminated.

The present study with 54 triticale varieties shows that 14 hexaploid triticales showed full complement of rye chromosome (as shown by 14 banded chromosomes), 24 hexaploid triticales had replacement of one pair of rye chromosome (12 banded chromosomes) and 16 hexaploid triticales had replacement of two pairs of rye chromosomes (10 banded chromosomes). Rye chromosome 2R was replaced in large number of cases (13 triticales) followed by 4R/7R (5 triticales), 5R (4 triticales) and 2R+5R (4 triticales). Triticales strains with 4R/7R substitution exhibited low degree of shriveling in grains with higher values for test weight, 100-grain weight and volume of water displaced by 100-grains. Triticale strains with 4R/7R substitution along with another substitution for 2R or 5R or 6R showed medium shriveling in grains. Triticale strains with substitutions either for 2R or 3R or 5R or 7R/4R exhibited high degree of shriveling and a cumulative effect was observed when two of these substitutions were present together in one triticale. Triticale strains with 2R/2D substitution had higher seed set and strains with 4R/4D substitution had a medium seed set. The seed set was considerably high when both 2R/2D and 4R/4D substitution occur together. The seed set was low when 3R and 5R substituted alone or in combination.

Notwithstanding the fact that triticale is a man-synthesized grain, many initial limitations such as an inability to reproduce due to infertility and seed shrivelling, low yield and poor nutritional value have greatly been eliminated. These R/D substitutions were also found to have profound effect on gluetenin quality of triticales [16, 38, 39].

As there was clear cut evidence in the present study about the role of individual R/D substitutions on the quality of seeds (shriveling and seed setting) it is necessary to introduce those substitutions which are of agronomic importance to the breeders.

Conflict of interest statement

We declare that we have no conflict of interest.

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