

## Original Research Article

### Exploitation of wheat genome through triticales x wheat crossfor improvement and identification of large anther

#### Abstract

Triticale is a man-made cereal crop that combines the rye and wheat genome in a single crop. Rye is a cross pollinated cereal crop that has large anther size in comparison of wheat. To improve wheat anther size, cross with triticales is a noble practice to combine the A, B, D and R genome. In the current investigation wheat (AABBDD) were crossed with triticales (AABBRR) with the help of Ph suppressor Chinese Spring. Subsequently triticales wheat derivative lines populations were developed. The BC<sub>2</sub>F<sub>7</sub> generations of the derived population were planted in alpha lattice design to further evaluation. With the help of fixative, the fresh spikes were collected from individual derivative lines to measure anther the size. The anther size was measured in millimeter by the help of ocular micrometer. Twenty-two triticales wheat derivative lines were identified with greater anther size in comparison of triticales anther. The TWD26 (5.7mm) has larger anther size followed by TWD18 (5.4mm) and TWD36 (5.3mm). These derivative lines may use as a donor parent in hybrid wheat breeding programme.

**Key words:** Triticales, out-crossing, wheat, rye, anther size

#### Introduction

Wheat is considered as most important winter cereal in India. Scientifically it is known as *Triticum aestivum*. It contributes more than 40% calories and 20% proteins to the worldwide (Lawati et al., 2021). In terms of wheat productivity, India has historically made considerable strides in these days. Despite a constant area, the country's yearly wheat production has increased by around 5 million tons during the past ten years (Anonymous, 2021). Due to expanding population and rising per capita spending, this trend not only needs to be maintained, but further accelerated (Garcia et al., 2019). Because of climate change and depletion of natural resources, the task has become more difficult (Alietal., 2021). For hybrid development different

**Comment [F1]:** Some basics components of the research in lacking in this abstract such as statement of the problem, data collection, objective(s) and conclusion.

floral and yield features were considered, it is necessary to have understanding of their variability (Mathew et al., 2019). development of a hybrid variety and exploitation of sufficient heterosis is a different strategy to increase wheat production. Poor seed set on the male sterile lines is one of the main challenges in the hybrid seed production of wheat (Porter and Atkins 1963; Kharde et al. 1967). Wheat's floral components are very crucial for the productive development of hybrid seed. The floral structure and anther dehiscence patterns in wheat make this crop entirely autogamous(De Vries 1971). It was generally known that outcrossing occurred in wheat and that some cultivars tended to outcross more frequently than others. The size of the stigma and anther, anther extrusion, pollen viability are the significant floral features that affect outcrossing in wheat(P Plaha, 2000). According to Wilson (1968), cultivars with excellent anthers should be used as pollinators, while cultivars with open glumes and projecting stigma should be utilized as female parents to ensure successful open pollination. A large germplasm base is required for the response of hybrid development in wheat(De-zhen et al., 2023). Due to its evolutionary history, wheat among the major crops has a relatively small genetic basis.In order to accomplish cross-pollination, it is ideal for both parental plants used in the generation of hybrid seeds to have open blooming spikelets and the hairs on the stigma would be long, completely extruded, and open for an extended period of time(Whitford et al., 2015). Thus, introducing genetic variability from closely related species is a crucial way to enhance floral biology of wheat. Due to its significant genetic disparity from wheat, rye may be beneficial for enhancing the bread wheatkhare(2017). Because of its greater anther size, increased anther extrusion, and greater pollen load, rye can restore the pollen fertility (khareet al., 2018). By crossing triticale with wheat, these desired floral characteristics may be incorporated into wheat (Shokat et al., 2023). The number of pollen grains that a pollinator shed determines how much seed is set (Wilson, 1968). Although it is a very difficult to manage floral architecture. Triticale is more nutritiously desirable than wheat since it is not only more resistant to typical wheat illnesses but also contains higher levels of key amino acids (Horlein and Valentine 1995). Using enhanced triticale as a bridge species to transfer rye genes to bread wheat is an alternative strategy for doing so. By triggering homoeologous pairing in wheat, this obstacle is overcome. In addition to Ph gene knockouts, this may also be accomplished using stocks of *Aegilops speltoides* containing Ph suppressor genes (Chen et al. 1994). The floral traits like anther size, anther extrusion, pollen load and pollen viability are the most relevant trait for wheat hybrid development (khare et al., 2018). The researcher found

positive correlation between anther size and pollen load that avail more viable pollen grains to outcross. Natural out crossing in wheat varieties was discovered by Martin (1990), Hucl (1996), and Singh (2006) to be up to 6.05%, which has a positive link with anther size.

**Comment [F2]:** The paragraph is too lengthy. Make it at least three paragraphs in the introduction with the last paragraph for objective(s) and what is expected to be done as an addition to knowledge.

## Material and Methods

The present study focussed on anther size of rye x wheat derivative lines. The experiment was carried out at wheat field, department of plant breeding and genetics, collage of agriculture, Punjab Agricultural University, Ludhiana. The geographically PAU is situated at 30° 54' 14.886"N Latitude, 75° 49' 0.4836" E Longitude and 244 meters of elevation. The characterization was performed on a large collection of germplasm lines obtained from triticale x wheat crosses. Four improved triticale varieties TL2908, TL3048, TL3065, and TL3031 were used as donor parents while three commercially grown hexaploid wheat varieties PBW343, DBW17, and PBW550 were used as a recipient parent. The first filial generations of crosses were backcrosses with chine spring and hexaploid wheat varieties. Total six cross combinations viz., TL2908/2\*CS(S)//PBW343, TL3048/2\*CS(S)//PBW343, TL3065/2\*CS(S)//PBW343, TL3048/2\*CS(S)//DBW17, TL3048/2\*CS(S)//PBW550 and TL3021/2\*CS(S)//PBW550 were made by using various triticale and wheat varieties. Out of 246 lines 63, 73, 12, 25, 32, 41 lines were consisted by TL2908/2\*CS(S)//PBW343, TL3048/2\*CS(S)//PBW343, TL3065/2\*CS(S)//PBW343, TL3048/2\*CS(S)//DBW17 and TL3021/2\*CS(S)//PBW550 cross respectively (Table 1). Likewise including parents total 246 derivative lines were planted by using alpha lattice design with two replications. The trial was conducted in BC<sub>2</sub>F<sub>7</sub> generation. The whole population was examined with regard to several different features, with anther size being one of them. To record the anther size one week before anthesis three spikes were selected randomly from each lines including parent and checks. After collecting spikes, it was poured into fixative (Conroy's 2 solutions). Measurement of anther was taken by the help of glass slide, ocular micrometre and light compound microscope.

Table 1 List of crosses and their parentage

Cross	Numbers of derivative lines	Donor parents	Anther size(mm)	Recipient parents	Anther size(mm)
TL2908/2*CS(S)//PBW343	63	TL2908	4.7	PBW343	3

TL3048/2*CS(S)//PBW343	73	TL3048	5.1	PBW343	3
TL3065/2*CS(S)//PBW343	12	TL3065	4.9	PBW343	3
TL3048/2* CS(S)//DBW17	25	TL3048	5.1	DBW17	2.9
TL3048/2*CS(S)//PBW550	32	TL3048	5.1	PBW550	3.3
TL3021/2*CS(S)//PBW550	41	TL3021	4.9	PBW550	3.3

mm = millimeters

### Results and Discussion

The current study was focused on improvement of wheat anther size through R genome of triticale. Triticale is the best source to exploit the biotic, abiotic resistance and floral traits into wheat crop (Golebiowska et al., 2023). The range for anther size in the parents was recorded 2.9 mm (DBW17) to 5.1 mm (TL3048), in comparison the highest anther size of rye derivative lines was recorded 5.7 mm (table 1). The donor parents having large anther 4.7 mm to 5.1 mm in comparison of recipient parents (table 1). Whereas, the recipient parents having small anther 2.9 mm to 3.3 mm (table 1). High anther size for triticale in comparison to wheat was previously found by Yusuf (2015) and khare (2017). Out of 246 rye derivative lines, 51 were found higher anther sizes in comparison to recipient parent (Table 2). While 22 derivative lines achieved larger anther size than donor parent. The maximum number of derivative lines having larger anther size identified from cross TL2908/2\*CS (S)//PBW343 followed by TL3065/2\* CS(S)//PBW343 and TL 3048/2\* CS(S)//PBW550 (Table 2). From TL2908/2\*CS (S)//PBW343 cross four derivative lines TWD2, TWD8, TWD24 and TWD34 were identified with 9 % increase in anther size compare to donor parent. Similarly, TWD18, TWD26 and TWD32 belonging from TL2908/2\*CS (S)//PBW343 cross having 15%, 21% and 12% anther size increment respectively compare to donor parent TL2908. The TWD93 belonging from TL3065/2\*CS(S)//PBW343 cross reported 8 % increment in anther size comparison to donor parent. From various cross combinations three derivative lines having 4% anther size increment were identified. The anther size is directly correlated with the number pollen and number of pollen grains directly related with the ability to restore the fertility therefore for development of hybrid in wheat crop large anther is a prerequisite trait (Nguyen et al., 2015).

Table 2 List of derivative lines having significant large anther size along with percent increase

Line No.	AS	F	% IORP	% IODP	Cross
TWD1,TWD5,TWD14, TWD17,TW31,TWD71	4.5	6	50%	-4%	TL2908/2*CS (S)//PBW343

TWD12,TWD20	4.7	2	57%	0%	
TWD16,TWD36,TWD70	4.4	3	47%	-6%	
TWD21,TWD23	4.6	2	53%	-2%	
TWD2,TWD8,TWD24,TWD34	5.1	4	70%	9%	
TWD6,TWD7	4.9	2	63%	4%	
TWD9,TWD13,TWD15, TWD17,TWD27	4.8	5	60%	2%	
TWD18	5.4	1	80%	15%	
TWD26	5.7	1	90%	21%	
TWD55	5.0	1	68%	7%	
TWD32	5.3	1	76%	12%	
TWD28,TWD44	4.8	2	60%	-6%	TL3048/2*
TWD48	5.3	1	77%	4%	CS(S)//PBW343
TWD42	5.0	1	68%	-1%	
TWD89,TWD 94	5.0	2	68%	3%	TL3065/2*
TWD93	5.3	1	77%	8%	CS(S)//PBW343
TWD92	4.7	1	58%	-3%	
TWD58	5.2	1	79%	2%	TL3048/2*
TWD105	4.7	1	61%	-8%	CS(S)//DBW17
TWD107,TWD114	4.5	2	54%	-12%	
TWD69	5.1	1	55%	4%	
TWD67,TWD155	4.3	2	31%	-12%	
TWD156	4.4	1	34%	-10%	TL 3048/2*
TWD66,TWD163	4.7	2	43%	-3%	CS(S)//PBW550
TWD120	4.9	1	47%	4%	
TWD119,TWD122	4.5	2	36%	-4%	
TWD125	4.4	1	34%	-13%	TL3021/2*CS
TWD130	4.6	1	40%	-9%	(S)//PBW 550

AS = anther size, F = frequency, % IORP = percent increase over recipient parent, IODP = percent increase over donor parent.

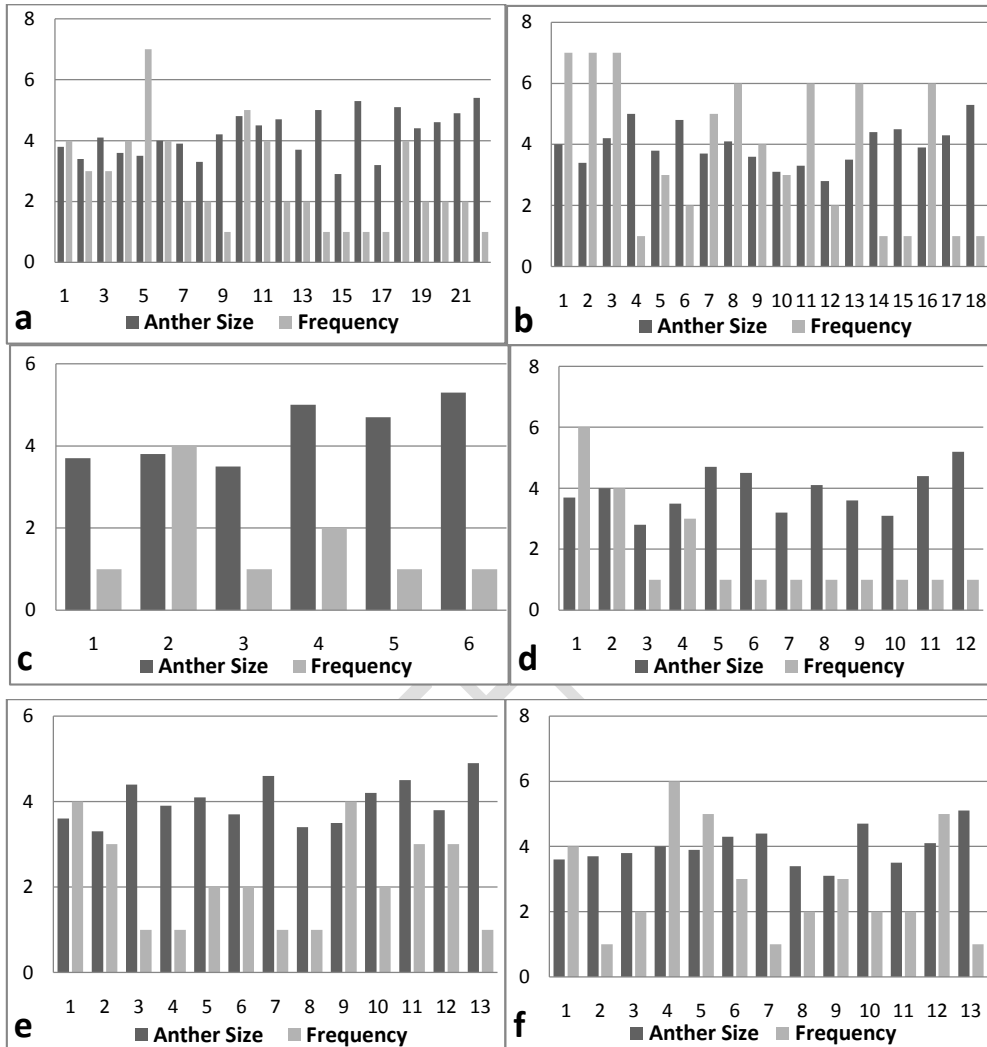


Fig: 1 Anther size frequency distribution Graph, a) for cross TL 2908/2\*CS (S)//PBW 343, b) for cross TL 3048/2\*CS (S)//PBW 343, c) for cross TL 3065/2\*CS (S)//PBW 343, d) for cross TL 3048/2\*CS (S)//DBW 17, e) for cross TL 3065/2\*CS (S)//PBW 550, f) for cross TL 3021/2\*CS(S)//PBW550.

**Comment [F3]:** What does the y-axis represent if the bars are anther size and frequency.



**Figure 2: Anther size variation shown by triticale x wheat derivative lines.**

Similar findings were obtained by many researchers (Song et al., 2018; Moskal et al., 2021; Tyson et al., 2019; Ren et al., 2017). The frequency distribution graphs (Figure 1) represent frequency of derivative lines according to anther size while figure 2 represents the variation of anther size shown by derivative lines. Chowdhary et al. (1994) and Singh et al. (2007) were suggested that the selection for long anthers may be effective in promoting natural cross pollination. The floral characters which enable sufficient cross fertilization like more open flowering habit, duration of flower opening, improved anther size in the male parent and stigma receptivity in the female parent more crucial for successful development of hybrids. Komaki and Tsunewaki (1981) find the significant association of anther size and it was suggested that the selection for high anther size promote outcrossing may result in the genotypes with more open pollination ability and these may be utilized as male parents to improve yielding ability. Singh et al., (2003) finds positive and significant correlation between yield and anther size. As the conclusion of all the reviews and study these derivative lines may be used as donor parents for provision of sufficient pollen availability in a hybridization programme.

### **Conclusion:**

In the present study triticale wheat derivative lines populations were developed by wheat x triticale cross. The  $BC_2F_7$  generations of the derived population were planted in alpha lattice design to further evaluation. With the help of fixative, the fresh spikes were collected from individual derivative lines to measure anther size. The anther size was measured in millimeter

by the help of ocular micrometer. Twenty-two triticale wheat derivative lines were identified with greater anther size in comparison of triticale anther. The TWD26 (5.7mm) has larger anther size followed by TWD18 (5.4mm) and TWD36 (5.3mm). These derivative lines may use as a donor parent in hybrid wheat breeding programme.

### References

Ali N, Hussain I, Ali S, Khan N.U., Hussain I. Multivariate analysis for various quantitative traits in wheat advanced lines. Saudi Journal of Biological Sciences. 2021; 28(1): 347–352.

Anonymous. ICAR-Indian Agricultural Statistics Research Institute (IASRI). New Delhi. 2021.

Chen P.D., Tsujimoto H, and Gill B.S. Transfer of *Ph* genes promoting homoeologous pairing from *Triticum speltoides* to common wheat. Theor Appl Genet. 1994; **88**: 97-101.

Chowdhary M.A., Mahmood N and Khaliq I. Pollen production studies in common bread wheat. Rchis. 1994; **11**: 68–72.

De-zhen K, Ying-bin N, Feng-juan C, Wei S, Hong-jun X, & Xiao-ming T. Research Status and Prospect of Hybrid Wheat Seed Production. Biotechnology Bulletin. 2023; 39(1): 95.

Devries A. Ph.. Flowering biology of wheat, particularly in view of hybrid seed production. Euphytica. 1971; **20**: 152-170

Feltaous Y. Molecular marker and morphological characterization of Triticale Wheat derivatives. Ph.D. Thesis, Punjab Agricultural University, Ludhiana, India. 2015.

Garcia M, Eckermann P, Haefele S, Satija S, Sznajder B, Timmins A, Baumann U, Wolters P, Mather D.E and Fleury D. Genome-wide association mapping of grain yield in a diverse collection of spring wheat (*Triticum aestivum* L.) evaluated in southern Australia. 2019; 14: 1-19

Golebiowska-Paluch G, and Dydá M. The genome regions associated with abiotic and biotic stress tolerance, as well as other important breeding traits in triticale. *Plants*. 2023; 12(3): 619.

Horlein A and Valentine J. Triticale (Triticosecale). In Williams J T (Ed) *Cereals and Pseudocereals*. Chapman and Hall, New York, 1995; pp: 187–221.

Hucl P. Out crossing rates for 10 Canadian spring wheat cultivars. *Canadian Journal of Plant Science*. 1996; **76**: 423–427.

Khare V. Morphological and molecular marker analysis of rye chromosome introgressions stocks in wheat. M.Sc. Thesis, Punjab Agricultural University, Ludhiana, India. 2017.

Khare V, Srivastava P, Sharma A, Bains NS and Sinha Kavita. Rye confers high anther extrusion to bread wheat via triticale wheat crosses. *J Pharmacogn Phytochem*. 2018;7(1):2167-2170.

Kherade M.K., Atkins I.M., Merkle O.G and Porter K.B. Cross pollination studies with male sterile wheat of the cytoplasm, Seed size of F<sub>1</sub> plants and seed and anther size of 45 pollinators. *Crop Sci*. 1967; **7**: 389-394.

Komaki M.K and Tsunewaki K. Genetical studies on the difference of anther length among common wheat cultivars, *Euphytica*. 1981; **30**: 45–53.

Lawati A.H.A., Nadaf S.K., Saady N.A.A., Hinai S.A.A., Almamari A., Maawali A.A. Multivariate analyses of indigenous bread wheat (*Triticum aestivum* L.) landraces of Oman. *Emirates Journal of Food and Agriculture*. 2021; 33(6): 483–500

Mathew I, Shimelis H., Mutema M., Clulow A., Zengeni R., Mbava N., Chaplot V. Genome-wide association study of drought tolerance and biomass allocation in wheat. *PLoS One* 2019;14(12): 1-21.

Martin T.J. Outcrossing in twelve hard red winter wheat cultivars. *Crop Science*. 1990; **30**: 59–62.

Porter K.B and Atkins I.M. Hybrid wheat problems potentials and progress. *Tex agric. Prog.* 1963; **9**: 19-23

Moskal K, Kowalik S, Podyma W, Łapiński B, Boczkowska M. The Pros and Cons of Rye Chromatin Introgression into Wheat Genome. *Agronomy.* 2021; 11(3):456.

Nguyen V, Fleury D, Timmins A, et al. Addition of rye chromosome 4R to wheat increases anther length and pollen grain number. *Theoretical and Applied genetics.* 2015; 128(5):953-964.

P Plaha G. S. Long anther trait of rye (*Secale cereale* L.)its chromosomal location and expression in bread wheat (*Triticum aestivum* L.). *Wheat Inf Serv.*2000; 90: 47–48.

Ren T, Tang Z, Fu S, Yan B, Tan F, Ren Z and Li Z. Molecular Cytogenetic Characterization of Novel Wheat-rye T1RS.1BL Translocation Lines with High Resistance to Diseases and Great Agronomic Traits. *Front. Plant Sci.* 2017; 8:799.

Shoka S, Großkinsky D. K., Singh S., & Liu F. The role of genetic diversity and pre-breeding traits to improve drought and heat tolerance of bread wheat at the reproductive stage. *Food and Energy Security,* 2023; 3: 478.

Singh S.K and Joshi A. K.. Variability and character association for various floral characters in wheat [*Triticumaestivum*(L.) emThell.]. *Indian Journal of Genetics and Plant Breeding.* 2003; **63**: 153–154.

Singh S. K. Evaluation of spring wheat [*Triticumaestivum*(L.) emThell] germplasm for variousfloral characteristics.*Journal of Agriculture.* 2006; **4**: 167–177.

Singh S.K., Joshi A.K. and Arun B. Comparative evaluation of exotic and adapted germplasm of spring wheat for floral characteristics in the Indo-Gangetic Plains of India. *Plant Breeding* 2007; **126**: 559–64.

Song X, Feng J, Cui ., Zhang C, Sun D. Genome-wide association study for anther length in some elite bread wheat germplasm. *Czech J. Genet. Plant Breed.* 2018; 54: 109–114.

Tyson H, Moriconi J. I., Zhao X, Hegarty J, Fahima T, Guillermo E, Maria S, Dubcovsky J. A wheat/rye polymorphism affects seminal root length and yield across different irrigation regimes, *Journal of Experimental Botany.* 2019; 70(15): 4027–4037.

Whitford R, Fleury D, Reif J.C., Garcia M, Okada T, Korzun V and Langridge P. Hybrid breeding in wheat: technologies to improve hybrid wheat seed production *Journal of Experimental Botany.* 2013; **64**(18): 5411–5428.

Wilson T.A. Problems in hybrid wheat breeding. *Euphytica.* 1968; **11**: 13–33