Advances in Triticale *(X Triticosecale)* Improvement: Chromosome Manipulation and Biotechnological Approaches.

Abstract:

Triticale *(X Triticosecale)* is a hybrid cereal crop with great potential for enhancing food security. It is a synthetic cereal. Meanwhile, certain genetic instabilities arising from the merging of the rye and wheat genomes have impeded the advancement of triticale, chromosome engineering advancements along with biotechnological approaches might potentially unleash the full potential of triticale. This article provides a comprehensive summary of the historical development and current status of research on conventional and molecular breeding and manipulating triticale chromosomes in order to introduce beneficial traits, correct genetic abnormalities, and accelerate breeding. Among the major strategies covered are chromosomal doubling, addition, replacement, translocation, and deletion. Beneficial genes from rye for quality of grain, yield, and disease resistance were incorporated into triticale backgrounds using addition and replacement lines. In general, using chromosome-modifying technologies within an integrated breeding framework may help with genetic stabilization, the planned evolution of triticale for greater productivity and robustness, and strategic trait integration. This study looks at the advantages, disadvantages, and potential benefits of using chromosomal engineering to enhance triticales.

Keywords: Triticale, molecular marker, chromosomal translocation, chromosome addition, chromosome replacement, and triticale breeding.

INTRODUCTION:

Triticale, a hybrid cereal grain, is a testament to human agricultural innovation. It was developed by crossing wheat and rye parents, crossing 4x and 6x wheat as females and rye as males. Despite genetic barriers, pioneering research and plant breeding techniques successfully created a new cereal crop (McGoverin, 2011). There are two main classifications of Triticale based on ploidy levels: the hexaploid variety (2n=42), known as *X Triticosecale,* and the octoploid type (2n=56) (Shkutina, 1971). Triticale's 6x and 8x variants are compatible, and hybridization between the two forms is common in triticale breeding programs. The octoploid form should be used as the female in this hybrid for the best outcomes. Secondary Triticale are viable progenies that result from crossing 8x and 6x Triticale and can also be created by crossing primary hexaploid wheat with another hexaploid wheat.

Triticale, a versatile and resilient crop, has diverse agricultural applications beyond traditional grain production. Its nutritional profile offers higher protein content and increased lodging resistance, making it a valuable addition to animal feed, human nutrition, and bioenergy production (Mergoum, 2009). Its genetic makeup encompasses the yield potential and adaptability of wheat, coupled with the hardiness, disease resistance, and environmental tolerance of rye. This amalgamation of traits has positioned triticale as an attractive option for farmers facing challenges such as fluctuating climatic conditions, soil degradation, and pest pressures (Meale, 2015). Triticale has evolved from experimental hybrids to commercially viable varieties, grown globally in diverse agroecosystems, from temperate to marginal environments where wheat or rye cannot thrive optimally (Ayalew, 2018). Triticale's adaptability to a wide range of growing conditions has contributed to its adoption in various agricultural systems, including conventional cropping, organic farming, and conservation agriculture.

Due to its limited genetic foundation, short evolutionary time, and restricted genotypes of wheat and rye, the artificial crop is more prone to disease. (Mergoum, 2009). Modern crop breeding techniques have further reduced the genetic diversity of Triticale, leading to a small number of elite lines dominating breeding programs and seed production. This has resulted in a decline in the population's genetic heterogeneity (Reif *et al.*, 2005). However, crossing synthetic hexaploid wheat with rye can create several 6x triticale lines with the entire 28 intact A/B and 14 R chromosomes and different chromosome arrangements (Hao *et al.*, 2013).

Triticales breeding presents challenges like introducing quality traits, transferring biotic resistance genes, and exploiting heterosis. Fungal diseases like powdery mildew, rusts, and Fusarium head blight have been prevalent in certain cultivars (Arseniuk & Góral, 2015). However, recommendations for triticale use in biofuel production systems suggest increased genetic diversity, removal of negative linkage drags, and exploration of the current genepool (Meale & McAllister, 2015). Research and breeding efforts aim to enhance triticale's agronomic performance, nutritional quality, and stress tolerance through techniques like marker-assisted selection, hybridization, and C-banding analysis.

In this review, we delve into the historical development, genetic makeup, agronomic characteristics, and potential applications of triticale, highlighting its contributions to modern agriculture and its prospects for addressing future challenges.

Table 1. Major milestones in triticale improvement and wheat-rye hybridizations:

Year	**Research Findings**	**References**
1876	Early hybridization attempts produced a sterile hybrid.	Wilson *et al.*, 1876
1884	Characterization of wheat-rye hybrids	Carman *et al.*, 1884
1928	reported significantly less crossbreeding between rye and wheat varieties, indicating that natural wheat-rye hybrids are rare and typically sterile.	Leighty *et al.*, 1928
1983	Comparison of hybrid embryos of bread wheat and durum wheat with rye	Oettler *et al.*, 1983
1984	Causes of wheat-rye hybrid lethality	May and Appels,1984
1986	Role of Kr1Kr1Kr2Kr2 gene configuration for reduced crossability	Zeven and Waninge, 1986
1987	Role of Kr genes on control of crossability of wheat and rye	Zeven *et al.*, 1987
1990	*In vitro* cultivation of wheat-rye hybrids	Carman and Campbell, 1990
1998	wheat cultivars featuring 1BL.1RS translocation	Rabinovich *et al.*, 1998
2006	The barriers in the 6x wheat × rye cross were overcome by wheat polygenes, with Chromosome 1D playing a crucial role.	Ceoloni, 2006
2008	AFLP and RFLP profiling of triticales	Ma, & Gustafson (2008)
2010	The gene Gb2, transferred from rye to wheat	Rudd *et al.*, 2010
2012	Yr9, Lr26, Pm8, and Sr31, located on the 1R chromosome introgressed in wheat	Pretorius *et al.*, 2012

2015	Wheat-Rye' hybridizations for pest-resistant traits	Andersson *et al.*, 2015
2017	Wheat chromosomes 4 and 7 share partial reciprocal homology with rye chromosomes 4R and 7R, enabling the introduction of beneficial agronomic traits from rye into wheat.	Bauer *et al.*, 2017
2021	CRISPR/Cas9 gene editing used to improve triticale.	Michalski, 2021
2023	Crossing wheat and rye results in nucleolar dominance, inactivating rRNA genes from rye on chromosome 1R, thereby suppressing rye's genetic influence.	Pikaard, 2023

Conventional Triticale development:

Since Triticale is a self-pollinating plant with little out-crossing, pure line selection is the preferred breeding method for its improvement. A generalized method of performing it involves hybridizing two or more parents and isolating the lines based on specific objectives until they reach homozygosity (Lelley,2006). Due to the amphidiploid nature of Triticale, cytological stability is crucial to initiate any selection endeavours (Lelley,2006). When these breeding programmes began, the primary obstacles were shrivelled grains, delayed maturity, flower sterility, and excessive plant height and lodging (Mergoum *et al.*, 2009). The first significant advance in triticale breeding came from the unintentional discovery of a naturally occurring out-crossed triticale with a semi-dwarf bread wheat, improving grain yields up to 300% (Hede, 2000).

Triticale is a self-pollinating plant, exhibits minimal inbreeding depression and simple parent development due to its male fertility restorer gene and cytoplasmic male sterility (Goral *et al.,* 2015). Breeders are becoming more interested in creating hybrid triticale cultivars as a result. Previous research on triticale hybrids revealed rapid vegetative development and good grain production due to non-additive gene activities (Barker and Varughese,1992; Goral *et al.,* 2015; Warzecha *et al.*, 2014). According to Oettler *et al.* (1991), rye adds a significant amount of non-additive genetic variety to the triticale genome, making hybrid Triticale breeding both reasonable and hopeful. High levels of dominant gene actions (specific combining ability) govern grain and biomass yields, whereas additive gene effects (generic combining ability) govern other yield component qualities (Oettler *et al*., 2005). Triticale breeding differs from wheat and barley due to its fragile genetic makeup, which may be influenced by cross-breeding between wheat and rye accessions, causing phenotypic variance in progeny (Lelley 2006). Breeders attempting to enhance Triticale through traditional breeding should thus concentrate in the initial generations on reestablishing the balance between the genomes of rye and wheat (Lelley,2006). Traditional breeding methods have made significant progress in improving triticales, but they have disadvantages like labor intensity, longer time frames, limited genetic variation, and low genetic gain.(Zimny and Lorz 1996).

Biotechnology in Triticale Breeding:

The genetic base for Triticale is limited by the cross-incompatibility barrier between rye and wheat. Biotechnology in crop breeding employs techniques like genetic engineering, somatic embryogenesis, molecular markers, and androgenesis to address the challenge of acquiring triticale as in vivo crosses. Microspore and another culture are widely used in triticale breeding programs, as reported by Ya-Ying in 1973(Zimny and Loerz, 1996). After consuming 12-15 years, it is still not assured that the variety will be released since it highly depends on the parental combination, so breeders are moving towards molecular marker and DNA technology.

Molecular markers are used extensively in triticale breeding studies, with several applications such as evaluation of genetic diversity, germplasm collection characterization, prediction of the performance of the hybrids and in the facilitation of the assigning of certain genes and even the insertion of segments of chromosomal DNA from alien species.

Table-2: Achievement in Triticale breeding through biotechnological approaches.

Year	Methods applied	Achievement	Reference
1982	Cytoplasmic male sterility (CMS)	Development of commercial hybrid cultivar	Gupta and Priyadarsan, 1982
2005	Marker- assisted selection (MAS)	Improved grain yield, disease resistance, baking quality	Tams *et al.,* 2005

2005	Effectiveness of AFLP analyses in identifying optimal parents for hybrid winter triticale breeding, assessing phenotypic and genomic diversity.	The study confirms marker preselection's effectiveness in obtaining AFLP-GS better correlated with heterosis, and derived matrices' use is promising for reducing cross combinations tested for specific combining ability.	Góral *et al., 2005*
2006	Examined genomic changes in early generation triticale allopolyploids using AFLP markers, revealing significant DNA sequence eliminations and rearrangements, and observing reproducible patterns among triticale lines.	The study conducted a comprehensive molecular analysis of genomic changes in triticale allopolyploids, revealing rapid changes, greater genomic shock in polyploids compared to hybrids, and reproducible DNA sequence alterations.	Ma and Gustafson, 2006
2011	DArT linkage map	Improved marker density for QTL and genomic studies	Badea, 2011
2016	Study 232 inbred triticale lines from Poland's breeding program using Diversity Arrays Technology (DArT). It identified redundancy and duplicate accessions, suggesting a diverse association mapping group.	The research demonstrated the effectiveness of DArT markers in characterizing diversity and relationships among triticales, guiding germplasm use and breeding for genetic enhancement.	Niedziela *et al.*, 2016
2016	use of biolistic particle bombardment or Agrobacterium-mediated transformation	Triticale undergoes bacterial Mannitol-1-phosphate dehydrogenase gene transfer for salinity tolerance.	Hakeem *et al.*, 2016
2020	Genome editing (CRISPR Cas-9)	Reduced gluten epitopes linked to celiac disease	Sánchez-León *et al.*, 2020

2021	Site directed mutagenesis	Recent genome editing advancements show faster progress in triticale breeding for PHS and disease resistance, with cas endonuclease-mediated editing	Michalski *et al.*, 2021

Application of molecular breeding in Triticale

Molecular breeding uses molecular methods like MAS and QTL mapping to improve crop species. Previously, morphological markers and protein isozymes were used, but with advancements, DNA-based markers are now widely used for precision selection and analysis (Xu Y,2012). Molecular markers help identify and tag key genes for possible transfer or cloning, which contributes significantly to the genetic improvement of agricultural plants (Collard and Mackill, 2008). Marker technology, specifically marker-assisted selection (MAS), is the first application of this technology in plant breeding, focusing on widely distributed, polymorphic, repeatable, and automated markers throughout the genome (Varshney *et al.*, 2014). While genetic mapping and the use of molecular markers are standard procedures in wheat, molecular breeding in triticale is still in its infancy. A few studies on QTL mapping and genetic map creation in triticale have been carried out (Tyrka *et al.*, 2015; Wen *et al.*, 2018). Since triticale retains a significant amount of both parental genomes, it can benefit from marker advancements and genomics technologies in both wheat and rye (Ma *et al.*, 2004; Ma and Gustafson, 2008). Only 356 markers on 73 double haploid (DH) lines made up the initial triticale linkage map (Gonzalez *et al.*, 2005) which were provided inadequate marker density and uneven distribution within and between chromosomes. Tyrka et al. (2011) revealed a reasonably dense map, with one new locus per 4 cM, while most of the markers remained mostly on the R genome. The resolution was increased to one marker every three cM density by adding additional marker types (SSR, DArT, and DArTSeq markers) (Tyrka et al., 2015). The best resolution and genome coverage were found in a consensus map made up of 2,555 DArT markers spread throughout 2,309.9 cM, with an average marker density of one unique locus per 1.2 cM (Alheit *et al.*, 2011). Nevertheless, there was also an uneven marker distribution throughout the three genomes in this consensus map. According to Tyrka *et al.* (2015), marker saturation in triticale is dependent on the variety of the mapping population and the contrast between parental lines (Alheit *et al.*, 2011; Gonzalez *et al.*,2005; Tyrka *et al.*, 2011; Tyrka *et al.*,2015;). Triticale genetic maps have been utilized to investigate the relationship between many significant economic features and markers. Numerous studies have identified QTLs linked to biotic and abiotic stressors, including drought, waterlogged soils, and aluminium toxicity (Alheit *et al.*,2014; Ayalew *et al.*,2018; Niedziela *et al.*,2012; Sapkota *et al.*,2018; Wen *et al.*,2018). Moreover, the finding of QTLs for a number of agronomic parameters, including biomass yield, grain yield, thousand-kernel weight, and plant height, has been reported in a number of triticale investigations (Busemeyer *et al.*, 2013; Alheit *et al.*, 2014; Wurchum *et al.*, 2014; Liu *et al.*, 2017). However, an inherent flaw in many QTL mapping studies is that the majority did not verify markers and QTLs for MAS. Because bi-parental populations (DH and RIL) hardly represent the available variety in the germplasm, QTLs that were reported using these populations may not be valid on genetic backgrounds other than the mapping populations themselves (Alyalew *et al.*, 2018). Very few studies have been done on triticale to examine the marker-trait association (Liu *et al.*, 2016; Wen *et al.*, 2018). Since most QTL studies could not confirm these QTL related markers for MAS, the applicability of discovered QTLs in the MAS remains unclear (Ayalew *et al.*, 2018). Even though triticale has started to create high density linkage maps and identify SNP markers using next-generation sequencing technology (like GBS), more research is needed to pinpoint and validate key QTLs and genes associated with economically significant traits so that MAS can be used in the breeding programme.

Genetic instability in Triticale:

Triticale lines were created in the 1940s when rye chromosomes were combined with the entire A, B, and D genome complement of hexaploid wheat (Kiss, 1966). This was acquired by crossing 6x wheat with 8x Triticale (AABBDDRR) and choosing offspring with 42 chromosomes. These secondary triticales were more fertile and suited for breeding and development. However, Triticale still exhibits genetic instability due to the mismatch in rye and wheat genomes. Variations in the timing and pattern of chromosomal compression were the first signs of this incapacity (Florell 1936). Shkutina and Khvostova (1971) discovered that triticales existed in two varieties: those with two nucleoli and those with just one. They found that the rye genome arranged its own nucleolus independently when two nucleoli were present, while three wheat chromosome pairs were related with it when one nucleus was present. In Triticale, the rye genome is inactive, following a random distribution of rye chromosomes or their lysis by the cytoplasm. Undefined physiological disturbances cause interference with normal spindle organization in Triticale, leading to multipolar divisions, univalents, and ultimately aneuploids.

Overall, the Triticale's potential is limited in comparison to its mother species by chromosome pairing failures, aneuploidy, instability, and linkage drag. Such limitations demand the need for chromosomal engineering techniques.

Hexaploid Triticale: Induction of Chromosome Aberration.

Creating amphiploids as the first steps towards improving Triticale.

Triticale breeding involves the hybridization of two species, producing novel amphiploids through intergeneric hybrids. This process involves crossing several species of Secale with different varieties of *Triticum turgidum* and *Triticum aestivum*. The doubling of the F_1 hybrid chromosomal number is the second step in creating new amphiploids (Kaltsikes, 1973). Crossings between 6x wheats and rye typically do not need embryo culture, but there are challenges associated with creating distant hybrids, such as the Ph1 gene’s presence on chromosome 5B in Triticale and wheat, which controls how homologous chromosomes pair during meiosis. (Riley & Chapman, 1958).

Rimpau (1891) conducted the first creation of intergeneric allopolyploids through the combination of the genomes of rye and wheat, which was used to produce primary Triticale by utilizing diploid, tetraploid, and hexaploid wheat. These techniques can also be used for the production of genetic stocks of the Triticale. Current hexaploid triticale cultivars are referred to as secondary ones because they either spontaneously emerged in octoploid triticales or were developed through various forms of hybridizations between distinct primary Triticale (Randhawa, Bona, & Graf, 2015).

By introducing new genes, the parental forms of Triticale (wheat and rye), their progeny, or associated species may be utilized to amplify genetic variety in Triticale (Schneider, Molnár, & Molnar-Lang, 2008). By creating wheat-alien hybrids, chromosomal addition, and translocation lines, certain agronomic features have been incorporated into wheat from wild relatives, making it suitable for triticale breeding. For cross-hybridization with hexaploid Triticale, Aegilops crassa, juvenalis, squarrosa, and triaristata are diploid wheat species used in hexaploid wheat and triticale breeding by introgressing different resistance genes to rust disease (Kowalczyk, 2011).

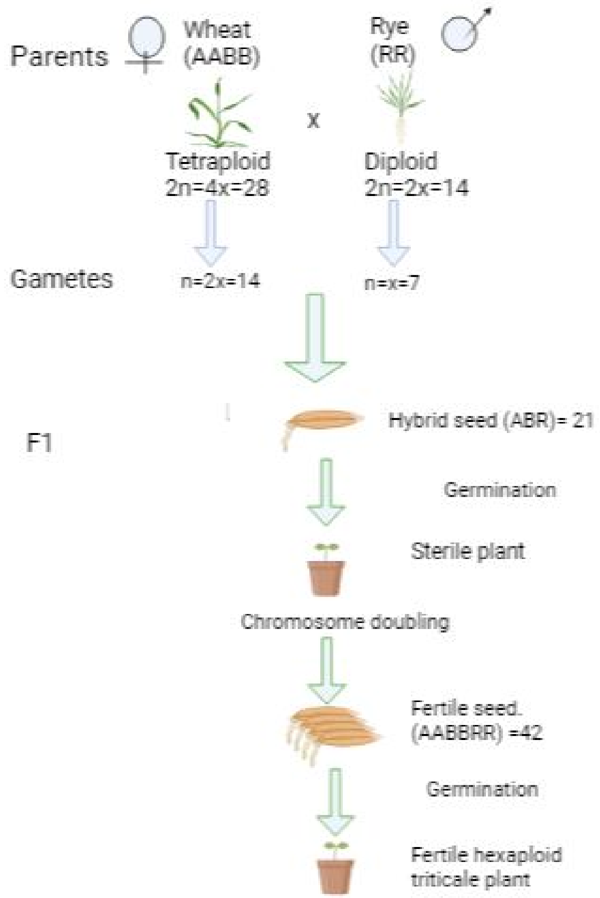

Fig 1. General illustration of creating amphiploids triticale.

Another approach put out by (Tsuchiya and Lambert 1968) involves doubling the number of chromosomes in each parent and then crossing those parents. By first crossing the double parents, then doubling the new hybrid, they were able to produce a greater seed set. Their primary issue was that they were not as good at doubling the parents as they were at doubling the hybrids. This method is not in use meanwhile.

Intergeneric polyploids can also be created through another method, that is including the application of “bridge forms” which hold a minimum of a set of common chromosomes for crossing with hexaploid Triticale. Bridge crosses are used when direct hybridization is challenging or impossible to transfer genetic material between various degrees of ploidy. For example, the gene Sr22 was transferred from 2x (AA) wheat to 4x (AABB) and subsequently to 6x (AABBDD) by Kerber and Dyck (1973). Another bridging technique is using natural amphiploids that share either the D genome with hexaploid wheat or the A genome with tetraploid wheat. After crossing the amphiploid intended for resistance with wheat the hybrid that is half fertile is repeatedly backcrossed to the wheat cultivar, resulting in meiotically stable and fertile plants. *T. timopheevii* (AAGG), *Ae. cylindrica* (CCDD), *Ae. ventricosa* (DDMvMv), and other naturally occurring amphiploids can be utilized (Feldman, 2012).

This form of genetic design can induce fusion among non-homologous chromosomes during meiosis of F_1 plants (Feldman & Levy, 2012). Examples include a tetraploid triticale (AB) for cross-hybridization with octoploid wheat/*Agropyron elongatum* form, and a Canadian hexaploid triticale T182 for reciprocal cross-hybridization with *Triticum turgidum* (L.) (AABB)/*Thinopyrum elongatum* (EE) amphiploid (AABBEE). Allopolyploid bridge forms were obtained through the cross-hybridization of rye and *Aegilops ventricosa*, and hybrids from *Aegilops biuncialis* using diploid rye (Bernard and Gay 1985).

Addition and substitutions of chromosome for triticale breeding:

When primary triticales are generated, unreduced gametes from modified meiosis, known as "meiotic restitution," may lead to spontaneous chromosome doubling in the progeny. Not reduced Somatic numbers of chromosomes are anticipated in gametes. But according to reports, newly synthesized hexaploid triticales frequently have odd chromosome constitutions including trisomies and monosomies, as well as translocations of homologues (Oleszczuk & Lukaszewski, 2014). Hao *et al.,* (2013) hybridized synthetic hexaploid wheat (SHW) with rye to create primary hexaploid lines. Meiotic restoration genes were present in synthetic hexaploid wheat strains. The researchers obtained hexaploid triticales with various chromosomal constitutions, including monosomic, substitution, and translocation lines, in addition to full hexaploid triticales with 28 intact A/B and 14 intact R chromosomes. Comparably, octoploid Triticale and partial amphiploids can be produced right away by the combination of unreduced gametes from F1 hybrids between synthetic hexaploid wheat and rye (Silkova, 2013). But the ultimate hybrids they produced over numerous generations, via fertility selection, were hexaploids. The preferential removal of D-genome chromosomes produced these hexaploids. (Gustafson and Zillinsky 1973) reported the first instance of chromosomal replacement.

One important method for introducing advantageous features from rye into wheat or triticale backgrounds was the insertion of single rye chromosomes. For every one of the seven rye chromosomes, disomic addition lines were created (Rabinovich 1998). These enabled identification and transfer of favorable rye chromosomes containing genes for greater protein content, disease resistance, and abiotic stress tolerance (Miedaner and Korzun 2012).

Individual rye chromosomes in wheat backgrounds were isolated and characterized using techniques such as cytogenetic analysis and molecular mapping (Evtushenko 2019). The rye chromosome was subsequently backcrossed into elite triticale cultivars using carefully chosen chromosomal addition lines. Homologous recombination and sub chromosomal segment introgression were made possible by monosomy adding lines. To include useful rye genes, homoeologous rye chromosomes were substituted for wheat chromosomes. To add rye disease resistance, grain quality, and yield qualities, replacement lines 1R-1A, 1R-1D, 6R-6D, and 5R-5A were produced (Kumlay *et al.*, 2003). Linkage drag was reduced by the compensating substitutions of the wheat-rye chromosome. Triticale's foliar disease resistance, grain filling, and stress tolerance were all enhanced by 1R and 6R introgressions (Niedziela 2014). Preharvest sprouting resistance was improved by the addition of 5R. Grain size and protein content increased with 2R introgression (Schneider *et al.*, 2008). The goal of ongoing research is to pinpoint particular rye genes and alleles for tactical integration into the genomes of elite Triticale.

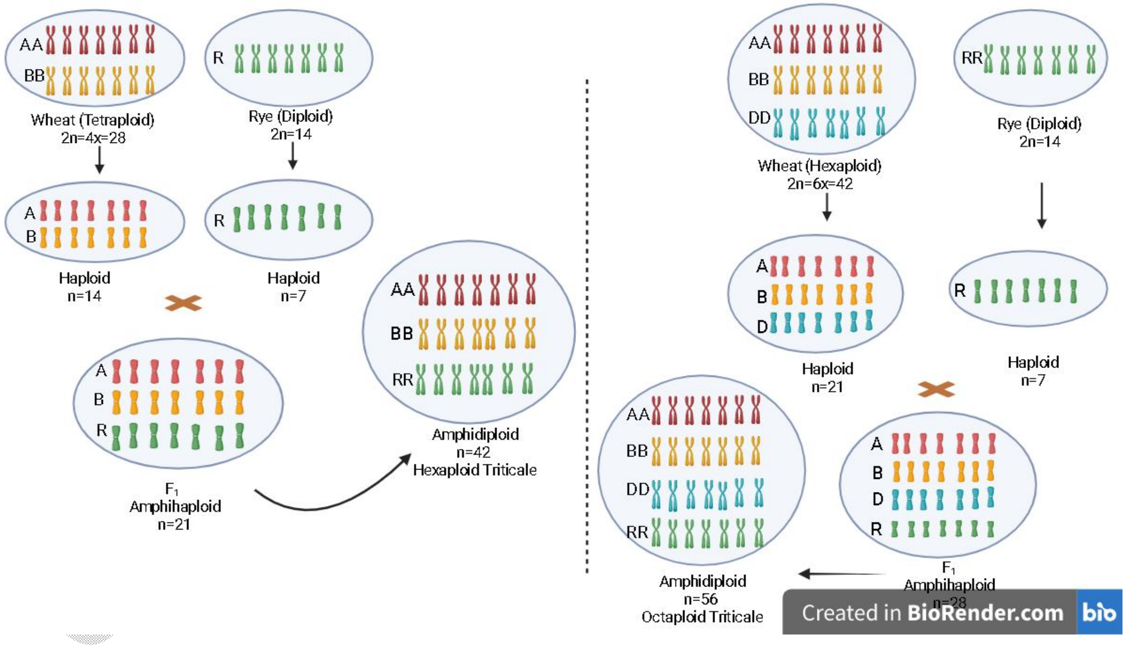

Fig. 2. Pictorial representation of development of Triticale on chromosome level

Translocation of chromosomes in Triticale

(Jiang *et al.*, 1993). Spontaneous translocation is the least effective of all techniques (Jiang *et al.* 1993). In order to use the ph1b mutant or gametocidal chromosomes in research, crosses between genetic stocks containing gametocidal chromosomes (Friebe *et al.* 2000) or lacking the Ph gene (Niu *et al.*, 2011) must be made, and unique genotypes must be created in order to induce chromosome variation. Chromosome translocations in Triticale can arise from distant cross-hybridization between Triticale and wheat or rye, which are frequent practices in traditional triticale hybridization.creating The R.D chromosome translocations are the most common in cultivated cultivars (Sreeja & Reddy, 2013).Univalent non-homologous chromosomes in distant hybrids have the potential to become stuck in the cytokinetic furrow and break during cytokinesis (Lukaszewski, 2016). For chromosome manipulation, a number of techniques have been devised. The first involves the translocation of the chosen alien chromosome fragment to the genome of a crop plant, and the second involves shrinking the transferred segment. The most important thing is that the alien chromatin fragment introduced shouldn't interfere with cell division's segregation process (Lukaszewski, 2016).In wheat chromosome engineering, the chromosome pairing regulator locus Ph1 is frequently manipulated. This component, the Ph1 gene, regulates diploid and is found on the long arm of the 5B chromosome similar to meiosis (Riley & Chapman, 1958). Lukaszewski (2006) transferred a segment of 1DL containing the GluD1 gene to chromosome 1R of triticale cv using substitution lines 1D(1A) and 5D(5B).In a central break-fusion translocation 1RS, homoeologous pairing was produced in a 5D(5B)-substituted line between the long arms of 1DL.1DL and the full 1R,and retrieved recombinant chromosomes (Lukaszewski, 2006).Three kinds of multi-breakpoint translocation chromosomes—called Valdy, FC, and RM—were produced as a result of this method. Gli-D1, Sec-1, and Glu-D1 loci are found on chromosome Valdy, which has three breakpoints; Gli-D1 and Glu-D1 loci introgressed on chromosomes FC, while Gli-B1 and Glu-D1 loci translocated on chromosomes RM. According to Lukaszewski (2006), a beginning examination of the impacts with altered chromosomes revealed that the recipient triticale Presto had an SDS-sedimentation value that was 230%–250% higher.

Chromosome aberrations can also be produced by gamma irradiation. The first person to employ irradiation to transfer the gene for leaf rust resistance from Aegilops umbellulata (Zhuk.) to the wheat genetic background was Sears (1956). Although triticale breeding has also used irradiation techniques, the effects are still unclear. Numerous chromosome abnormalities have been reported by several authors, including acentric fragments with or without translocations and wheat/rye, wheat/wheat, rye/rye, wheat/rye□wheat, and rye/wheat/rye translocations (Cermeno & Lacadena, 1983).A large variety of aberrations can be produced using radiation, although this process is more expensive because special equipment is needed so,if chemical agents are discovered, using them is the most practical and economical option because it is simple to modify the dosage and treatment duration for maximum effectiveness. DNA methylation or demethylation can result in chromosome abnormalities (Cho *et al.,* 2011) and alter the nuclear architecture (Espada and Esteller 2010). Several cytidine analogues, like 5-azacytidine, 5-aza-2=-deoxycytidine, and zebularine, can be used to deliberately de-methylate entire genomic DNA or specific DNA sequences (Espada and Esteller, 2010). Of them, zebularine is more secure and minimally lethal as compared to others (Cheng *et al.,* 2004). In particular, Cho *et al.,* (2011) found that in the sprouting seeds of a wheat – Leymus racemosus disomic addition line, zebularine caused many different kinds of chromosome abnormalities. Zebularine was utilized to cause chromosome abnormality in the 8x triticale cultivar Jinghui#1, which may be passed down through generations (Cermeno & Lacadena, 1983). This opened up a new avenue for the production of germplasms in Triticale by chromosome manipulation.

Table- 3 : Achievement in triticale breeding through chromosome manipulation.

Achievement	**Methods**	**References**
Leaf rust resistance	Through gamma irradiation	Sears, 1956
Increased grain number	Translocation fusing wheat 1D and 6D chromosomes.	Tyrka & Chełkowski 2004
Aluminium tolerance	Addition of 6RL chromosome arm from rye	Tyrka & Chełkowski 2004
Improved fertility and meiotic stability	Addition of wheat chromosome 5B carrying Ph1 locus which suppresses homologous pairing.	Mergoum *et al.,* 2009
Drought resistance	Addition of chromosome arm 4RL from rye with genes for osmotic adjustment	Mergoum *et al.,* 2009

Resistance to leaf rust	Addition of 6R chromosome arm from rye carrying Lr26 resistance gene.	Badea *et al.*, 2011
Enhanced grain filling and stress tolerance	Introgression of 1R and 6R in wheat from rye.	Niedziela, 2014
Resistance to fusarium head blight	Introducing rye chromatin containing resistance genes.	Ceoloni *et al.*, 2017
Improving baking quality	Substituting wheat chromosome 1B for rye 1R, resulting in increased dough strength and loaf volume.	Ceoloni *et al.*, 2017
Enhances nutritional quality.	Improvement of lysine content, an essential amino acid, through the intervarietal substitution of rye chromosomes 1R, 2R, and 5R carrying opaque-2 modifier genes	Marone, 2021

Conclusion:

Triticale, a highly productive hybrid cereal crop, has been significantly improved through various chromosomal engineering methodologies. Techniques like chromosome doubling, addition, replacement, translocation, and deletion have enabled targeted trait integration, genetic stabilization, and genome optimization. These modifications have led to higher protein quality, grain production, resilience to stress, and greater agronomic adaptability. Current approaches include chromosome fragmentation, fusion of pieces into chromosome structures, and induced recombination for Triticale prebreeding stocks. However, there are still issues to be addressed, and further research is needed to improve chromosome engineering techniques without disrupting the complex genetic system of Triticale. An integrated strategy combining gene editing technologies, genomics platforms, and conventional cytogenetic modification could lead to optimal triticale genomes for sustainable food production.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.
2.
3.

References:

Alheit, K. V., Busemeyer, L., Liu, W., Maurer, H. P., Gowda, M., Hahn, V., ... & Wurschum, T. (2014). Multiple-line cross QTL mapping for biomass yield and plant height in triticale (× Triticosecale Wittmack). *Theoretical and applied genetics*, *127*, 251-260.

Alheit, K. V., Reif, J. C., Maurer, H. P., Hahn, V., Weissmann, E. A., Miedaner, T., & Wurschum, T. (2011). Detection of segregation distortion loci in triticale (x Triticosecale

Wittmack) based on a high-density DArT marker consensus genetic linkage map. *BMC genomics*, *12*, 1-14.

Andersson, S. C., Johansson, E., Baum, M., Rihawi, F., & El-Bouhssini, M. (2015). New resistance sources to Russian wheat aphid (Diuraphis noxia) in Swedish wheat substitution and translocation lines with rye (Secale cereale) and Leymus mollis.

Arseniuk E., (2019). Recent Developments in Triticale Breeding Research and Production - An Overview. *Ekin Journal of Crop Breeding and Genetics*, 5(2), 68-73.

Arseniuk, E., & Góral, T. (2015). Triticale biotic stresses—known and novel foes. *Triticale*, 83-108.

Ayalew, H., Kumssa, T. T., Butler, T. J., & Ma, X. F. (2018). Triticale improvement for forage and cover crop uses in the southern great plains of the United States. *Frontiers in plant science*, *9*, 1130.

Ayalew, H., Kumssa, T. T., Butler, T. J., & Ma, X. F. (2018). Triticale improvement for forage and cover crop uses in the southern great plains of the United States. *Frontiers in plant science*, *9*, 1130.

Badea, A., Eudes, F., Salmon, D., Tuvesson, S., Vrolijk, A., Larsson, C. T., ... & Laroche, A. (2011). Development and assessment of DArT markers in Triticale. *Theoretical and Applied Genetics*, *122*, 1547-1560.

Barker, T. C., & Varughese, G. (1992). Combining ability and heterosis among eight complete spring hexaploid triticale lines. Crop science, 32(2), 340-344.

Bauer, E., Schmutzer, T., Barilar, I., Mascher, M., Gundlach, H., Martis, M. M., ... & Scholz, U. (2017). Towards a whole□genome sequence for rye (Secale cereale L.). The Plant Journal, 89(5), 853-869.

Bernard, S., & Gay, G. (1985). Introduction of Aegilops ventricosa germplasm into hexaploid triticale.

Busemeyer, L., Ruckelshausen, A., Möller, K., Melchinger, A. E., Alheit, K. V., Maurer, H. P., ... & Würschum, T. (2013). Precision phenotyping of biomass accumulation in triticale reveals temporal genetic patterns of regulation. *Scientific reports*, *3*(1), 2442.

Carman, J. G., & Campbell, W. F. (1990). Factors affecting somatic embryogenesis in wheat. *Wheat*, 68-87.

CarmanE. 1884. Rural topics. *Rural New Yorker*, 30 August.

Ceoloni, C. A. J. P. P., & Jauhar, P. P. (2006). Chromosome engineering of the durum wheat genome: strategies and applications of potential breeding value. *Genetic resources, chromosome engineering, and crop improvement, 2*, 27-59.

Ceoloni, C., Forte, P., Kuzmanovic, L., Tundo, S., Moscetti, I., De Vita, P., ... & D'ovidio, R. (2017). Cytogenetic mapping of a major locus for resistance to Fusarium head blight and crown rot of wheat on Thinopyrum elongatum 7EL and its pyramiding with valuable genes from a Th. ponticum homoeologous arm onto bread wheat 7DL. *Theoretical and Applied Genetics*, *130*, 2005-2024.

Cermeno, M. C., & Lacadena, J. R. (1983). Spatial arrangement analysis of wheat and rye genomes in Triticale interphase nuclei by gamma-radiation induced chromosomal interchanges. *Heredity*, *51*(1), 377-381.

Cheng, J. C., Yoo, C. B., Weisenberger, D. J., Chuang, J., Wozniak, C., Liang, G., ... & Jones, P. A. (2004). Preferential response of cancer cells to zebularine. *Cancer cell*, *6*(2), 151-158.

Cho, S. W., Ishii, T., Matsumoto, N., Tanaka, H., Eltayeb, A. E., & Tsujimoto, H. (2011). Effects of the cytidine analogue zebularine on wheat mitotic chromosomes. *Chromosome Science*, *14*(1+ 2), 23-28.

Collard, B. C., & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), 557-572.

Espada, J., & Esteller, M. (2010, April). DNA methylation and the functional organization of the nuclear compartment. In *Seminars in cell & developmental biology* (Vol. 21, No. 2, pp. 238-246). Academic Press.

Evtushenko, E. V., Lipikhina, Y. A., Stepochkin, P. I., & Vershinin, A. V. (2019). Cytogenetic and molecular characteristics of rye genome in octoploid Triticale (× Triticosecale Wittmack). *Comparative Cytogenetics*, *13*(4), 423.

Feldman, M., & Levy, A. A. (2012). Genome evolution due to allopolyploidization in wheat. *Genetics*, *192*(3), 763-774.

Florell, V. H. 1936, Chromosome differences in a wheat-rye amphiploid. J. Agr. Res. 52: 199-204.

Friebe, B., Kynast, R. G., & Gill, B. S. (2000). Gametocidal factor-induced structural rearrangements in rye chromosomes added to common wheat. *Chromosome Research*, *8*, 501-511.

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

Gonzalez, J. M., Muñiz, L. M., & Jouve, N. (2005). Mapping of QTLs for androgenetic response based on a molecular genetic map of× Triticosecale Wittmack. *Genome*, *48*(6), 999-1009.

Goral, H., Stojałowski, S., Warzecha, T., and Larsen, J. (2015). "The development of hybrid triticale," in Triticale, ed. E. François (Berlin: Springer), 33–66

Goral, H., Tyrka, M., & Spiss, L. (2005). Assessing genetic variation to predict the breeding value of winter triticale cultivars and lines. *J Appl Genet*, *46*(2), 125-131.

Gupta, P. K., & Priyadarshan, P. M. (1982). Triticale: present status and future prospects. *Advances in Genetics*, *21*, 255-345.

Gustafson, J. P., & Zillinsky, F. J. (1973). Identification of D-genome chromosomes from hexaploid wheat in a 42-chromosome triticale. In *Proceedings of the fourth international wheat genetics symposium*. Columbia, MO: University of Missouri.

Hakeem K. R., Tombuloglu H., Tombuloglu G., (2016). Plant Omics Trends and Applications. Springer, Switzerland. https://www.springer.com/gp/ book/9783319317014

Hao, M., Luo, J., Zhang, L., Yuan, Z., Yang, Y., Wu, M., ... & Liu, D. (2013). Production of hexaploid Triticale by a synthetic hexaploid wheat-rye hybrid method. *Euphytica*, *193*, 347-357.

Hede, A. (2000). A New Approach to Triticale Improvement. Mexico: CIMMYT, 21.

Jiang, J., Friebe, B., & Gill, B. S. (1993). Recent advances in alien gene transfer in wheat. *Euphytica*, *73*, 199-212.

Kaltsikes, P. J. 1973. Methods for triticale production. Pflanzenzficht. (In press)

Kerber, E. R., & Dyck, P. L. (1973). Inheritance of stem rust resistance transferred from diploid wheat (Triticum monococcum) to tetraploid and hexaploid wheat and chromosome location of the gene involved. *Canadian Journal of Genetics and Cytology*, *15*(3), 397-409.

Kiss, A. (1966). Improvement of the fertility of Triticale.

Kiss, A. (1975). Hexaploid triticale breeding in Hungary. Proc. Iat. Symp., 1973, Leningrad pp. 38-46.

Kowalczyk, K., Gruszecka, D., Nowak, M. I. C. H. A. Ł., & Lesniowska-Nowak, J. (2011). Resistance of triticale hybrids with Pm4b and Pm6 genes to powdery mildew. *Acta Biologica Cracoviensia*. *Series Botanica*, *53*(1).

Kumlay, A. M., Baenziger, P. S., Gill, K. S., Shelton, D. R., Graybosch, R. A., Lukaszewski, A. J., & Wesenberg, D. M. (2003). Understanding the effect of rye chromatin in bread wheat. *Crop science*, *43*(5), 1643-1651.

Kwiatek, M. T., & Nawracała, J. (2018). Chromosome manipulations for progress of Triticale *(× Triticosecale)* breeding. *Plant Breeding*, *137*(6), 823-831.

Leighty, C. E., & Sando, W. J. (1928). Natural and artificial hybrids of a Chinese wheat and rye. *Journal of Heredity*, *19*(1), 23-27.

Lelley, T. (2006). *Triticale: A Low-Input Cereal with Untapped Potential. Genetic Resources, Chromosome Engineering, and Crop Improvement: Cereals*, eds R. J. Singh and P. P. Jauhar. London: CRC Press, 395–430.

Liu, W., Leiser, W. L., Reif, J. C., Tucker, M. R., Losert, D., Weissmann, S., ... & Würschum, T. (2016). Multiple□line cross QTL mapping for grain yield and thousand kernel weight in triticale. Plant Breeding, 135(5), 567-573.

Liu, W., Maurer, H. P., Leiser, W. L., Tucker, M. R., Weissmann, S., Hahn, V., & Würschum, T. (2017). Potential for marker-assisted simultaneous improvement of grain and biomass yield in triticale. *Bioenergy Research*, *10*, 449-455.

Lukaszewski, A. J. (2006). Cytogenetically engineered rye chromosomes 1R to improve bread□making quality of hexaploid Triticale. *Crop science*, *46*(5), 2183-2194.

Lukaszewski, A. J. (2016). Manipulation of homologous and homoeologous chromosome recombination in wheat. *Plant Cytogenetics: Methods and Protocols*, 77-89.

Ma X. F., and Gustafson J. P., (2006). Timing and rate of genome variation in Triticale following allopolyploidization.Genome 49: 950-958.

Ma, X. F., & Gustafson, J. P. (2008). Allopolyploidization-accommodated genomic sequence changes in Triticale. *Annals of botany*, *101*(6), 825-832.

Ma, X. F., & Gustafson, J. P. (2008). Allopolyploidization-accommodated genomic sequence changes in triticale. *Annals of botany*, *101*(6), 825-832.

Ma, X. F., Fang, P., & Gustafson, J. P. (2004). Polyploidization-induced genome variation in triticale. *Genome*, *47*(5), 839-848.

Marone, D., Russo, M. A., Mores, A., Ficco, D. B., Laidò, G., Mastrangelo, A. M., & Borrelli, G. M. (2021). Importance of landraces in cereal breeding for stress tolerance. *Plants*, *10*(7), 1267.

May, C. E., & Appels, R. (1984). Seedling lethality in wheat: a novel phenotype associated with a 2RS/2BL translocation chromosome. *Theoretical and applied genetics*, *68*, 163-168.

Meale, S. J., & McAllister, T. A. (2015). Grain for feed and energy. *Triticale*, 167-187.

Mergoum, M., Singh, P. K., Pena, R. J., Lozano-del Río, A. J., Cooper, K. V., Salmon, D. F., & Gómez Macpherson, H. (2009). Triticale: a “new” crop with old challenges. *Cereals*, 267-287.

Michalski, K., Hertig, C., Mańkowski, D. R., Kumlehn, J., Zimny, J., & Linkiewicz, A. M. (2021). Functional validation of cas9/guideRNA constructs for site-directed mutagenesis of triticale ABA8′ OH1 loci. *International Journal of Molecular Sciences*, *22*(13), 7038.

Miedaner, T., Hübner, M., Korzun, V., Schmiedchen, B., Bauer, E., Haseneyer, G., ... & Reif, J. C. (2012). Genetic architecture of complex agronomic traits examined in two testcross populations of rye (Secale cereale L.). *BMC genomics*, *13*, 1-13.

Muntzing, A. (1972). Experience from work with octoploid and hexaploid rye-wheat (Triticale). Biol. Zentralbl. 91, 69-80.

Niedziela, A., Bednarek, P. T., Cichy, H., Budzianowski, G., Kilian, A., & Anioł, A. (2012). Aluminum tolerance association mapping in triticale. *BMC genomics*, *13*(1), 1-16.

Niedziela, A., Bednarek, P. T., Labudda, M., Mańkowski, D. R., & Anioł, A. (2014). Genetic mapping of a 7R Al tolerance QTL in Triticale (x Triticosecale Wittmack). *Journal of applied genetics*, *55*, 1-14.

Niedziela, A., Orłowska, R., Machczyńska, J., & Bednarek, P. T. (2016). The genetic diversity of triticale genotypes involved in Polish breeding programs. *Springerplus*, *5*, 1-7.

Niu, Z., Klindworth, D. L., Friesen, T. L., Chao, S., Jin, Y., Cai, X., & Xu, S. S. (2011). Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics, 187(4), 1011-1021.

Oettler, G. (1983). Crossability and embryo development in wheat-rye hybrids. Euphytica, 32, 593-600.

Oettler, G., Tams, S. H., Utz, H. F., Bauer, E., & Melchinger, A. E. (2005). Prospects for hybrid breeding in winter triticale: I. Heterosis and combining ability for agronomic traits in European elite germplasm. *Crop science*, *45*(4), 1476-1482.

Oettler, G., Wehmann, F., & Utz, H. F. (1991). Influence of wheat and rye parents on agronomic characters in primary hexaploid and octoploid triticale. *Theoretical and applied genetics*, *81*, 401-405.

Oleszczuk, S., & Lukaszewski, A. J. (2014). The origin of unusual chromosome constitutions among newly formed allopolyploids. American journal of botany, 101(2), 318-326.

Pikaard, C. S., Chandrasekhara, C., McKinlay, A., Enganti, R., & Fultz, D. (2023). Reaching for the off switch in nucleolar dominance. The Plant Journal.

Pretorius, Z. A., Bender, C. M., & Visser, B. (2015). The rusts of wild rye in South Africa. South African Journal of Botany, 96, 94-98.

Rabinovich, S. V. (1998). Importance of wheat-rye translocations for breeding modern cultivar of Triticum aestivum L. *Euphytica*, *100*(1-3), 323-340.

Randhawa, H. S., Bona, L., & Graf, R. J. (2015). Triticale breeding—Progress and prospect. *Triticale*, 15-32.

Reif, J. C., Zhang, P., Dreisigacker, S., Warburton, M. L., van Ginkel, M., Hoisington, D., & Melchinger, A. E. (2005). Wheat genetic diversity trends during domestication and breeding. *Theoretical and Applied Genetics*, *110*, 859-864.

Riley, R., & Chapman, V. (1958). Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature*, *182*(4637), 713-715.

Rimpau, W. (1891). Kreurungsproducte landvirtschaft licher. Kulturpflanzen, Landw. Jahsi., 21.

Ruud, A. K., Windju, S., Belova, T., Friesen, T. L., & Lillemo, M. (2017). Mapping of SnTox3–Snn3 as a major determinant of field susceptibility to Septoria nodorum leaf blotch in the SHA3/CBRD× Naxos population. *Theoretical and Applied Genetics*, *130*, 1361-1374.

Sanchez Leon, S. (2020). Identification and Development of Cereals Suitable for People suffering from Gluten Allergies and Intolerances: Directed Mutagenesis by Specific Nucleases (CRISPR/Cas9) of Immunodominant Genes in Relation to Celiac Disease.

Sapkota, S., Zhang, Q., Chittem, K., Mergoum, M., Xu, S. S., & Liu, Z. (2018). Evaluation of triticale accessions for resistance to wheat bacterial leaf streak caused by Xanthomonas translucens pv. undulosa. *Plant Pathology*, *67*(3), 595-602.

Schneider, A., Molnár, I., & Molnár-Láng, M. (2008). Utilization of Aegilops (goatgrass) species to widen the genetic diversity of cultivated wheat. *Euphytica*, *163*, 1-19.

Sears, E. R. (1956). The transfer of leaf-rust resistance from Aegilops umbellulata to wheat. *The transfer of leaf-rust resistance from Aegilops umbellulata to wheat.*

SHKUTINA, F. M., AND V. V. KHVOSTOVA, 1971. Cytological investigation of Triticale. *Theoret. Appi. Genet.* 41: 109-119.

Silkova, O. G., Adonina, I. G., Krivosheina, E. A., Shchapova, A. I., & Shumny, V. K. (2013). Chromosome pairing in meiosis of partially fertile wheat/rye hybrids. *Plant Reproduction*, *26*, 33-41.

Sreeja, P. S., & Reddy, V. R. K. (2013). Identification of rye chromosome substitutions in Triticale and its relation with kernel characters and seed setting through Giemsa C-banding technique. *Asian Pacific Journal of Reproduction*, *2*(4), 289-296.

Tams, S. H., Melchinger, A. E., & Bauer, E. (2005). Genetic similarity among European winter triticale elite germplasms assessed with AFLP and comparisons with SSR and pedigree data. *Plant breeding*, *124*(2), 154-160.

Tsuchiya, T., & Larter, E. N. (1968). Direct synthesis of triticale from colchicine-doubled parents.

Tyrka, M., & Chelkowski, J. (2004). Enhancing the resistance of Triticale by using genes from wheat and rye. *Journal of Applied Genetics*, *45*(3), 283-296.

Tyrka, M., Bednarek, P. T., Kilian, A., Wędzony, M., Hura, T., & Bauer, E. (2011). Genetic map of triticale compiling DArT, SSR, and AFLP markers. *Genome*, *54*(5), 391-401.

Tyrka, M., Tyrka, D., & Wędzony, M. (2015). Genetic map of triticale integrating microsatellite, DArT and SNP markers. *PLoS One*, *10*(12), e0145714.

Tyrka, M., Tyrka, D., & Wędzony, M. (2015). Genetic map of triticale integrating microsatellite, DArT and SNP markers. *PLoS One*, *10*(12), e0145714.

Varshney, R. K., Terauchi, R., & McCouch, S. R. (2014). Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. *PLoS biology*, *12*(6), e1001883.

Warzecha, T., Sutkowska, A., & Góral, H. (2014). Male sterility of triticale lines generated through recombination of triticale and rye maintainers. *Spanish Journal of Agricultural Research*, *12*(4), 1124-1130.

Wen, A., Jayawardana, M., Fiedler, J., Sapkota, S., Shi, G., Peng, Z., ... & Liu, Z. (2018). Genetic mapping of a major gene in triticale conferring resistance to bacterial leaf streak. *Theoretical and applied genetics*, *131*, 649-658.

Wen, A., Jayawardana, M., Fiedler, J., Sapkota, S., Shi, G., Peng, Z., ... & Liu, Z. (2018). Genetic mapping of a major gene in triticale conferring resistance to bacterial leaf streak. Theoretical and applied genetics, 131, 649-658.

Wilson, A. S. (1875). 1875. On wheat and rye hybrids. *Trans, and Proc. Bot. Soc. 12i*, 286-288.

Wurschum, T., Liu, W., Alheit, K. V., Tucker, M. R., Gowda, M., Weissmann, E. A., ... & Maurer, H. P. (2014). Adult plant development in triticale (× Triticosecale Wittmack) is controlled by dynamic genetic patterns of regulation. *G3: Genes, Genomes, Genetics*, *4*(9), 1585-1591.

Xu, Y., Li, Z. K., & Thomson, M. J. (2012). Molecular breeding in plants: moving into the mainstream. *Molecular Breeding*, *29*, 831-832.

Zeven, A. C. (1987). Crossability percentages of some 1400 bread wheat varieties and lines with rye. *Euphytica*, *36*, 299-319.

Zeven, A. C., & Waninge, J. (1986). The degree of similarity of backcross lines of Triticum aestivum cultivars Manitou and Neepawa with Aegilops speltoides accessions as donors. *Euphytica*, *35*, 677-685.

Zimny J., and Loerz H., (1996). Biotechnology for basic studies and breeding of Triticale. *In: Triticale today and tomorrow.* Kluwer Academic Publishers, (Eds. H. Guedes-Pinto, N. Darvey, V. P. Carnide), p. 327-337.