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 derived from the crossbreeding of wheat (Triticum spp.) and rye (Secale cereale), stands as a testament to human ingenuity in agricultural innovation. Triticale is developed by crossing 4x and 6x wheat as female parents with rye as male parents. First conceptualized in the late 19th century, triticale's development required overcoming significant genetic barriers between its parent species. Through pioneering research efforts and advancements in plant breeding techniques, scientists successfully created a new cereal crop that combines desirable traits from both wheat and rye

(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, with its unique blend of characteristics inherited from its parent species, makes it a versatile and resilient crop with diverse agricultural applications. The utilization of triticale extends beyond traditional grain production, encompassing applications in animal feed, human nutrition, and bioenergy production. Its nutritional profile, although similar to wheat, offers unique advantages such as higher protein content and increased resistance to lodging (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). Over the decades, triticale has evolved from experimental hybrids to commercially viable varieties cultivated in regions spanning the globe. Its cultivation has expanded across diverse agroecosystems, ranging from temperate to marginal environments, where neither wheat or rye alone can 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 very short evolutionary time frame and limited wheat and rye genotypes involved in the making of this artificial crop's modern cultivars, it has a narrow genetic base. And a population with a narrow genetic base has less genetic variation, making the crop more susceptible to diseases (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). Techniques like marker-assisted selection, fluorescence and genomic in situ hybridization, and C-banding analysis can be used to identify advantageous genotypes. Approaches like distant hybridization, amphiploid generation, and chromosome additions and replacements can also be employed to achieve desired changes in chromosomal lnumber or structure. Continued research and breeding efforts aim to further enhance triticale's agronomic performance, nutritional quality, and stress tolerance through conventional breeding methods and emerging biotechnological approaches.

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	Ceoloni, 2006

	by wheat polygenes, with Chromosome 1D playing a crucial role.	
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 show partial reciprocal homology with rye chromosomes 4R and 7R. This homologous pairing allows for 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).

Because Triticale is a self-pollinating plant, inbreeding depression is minimal and inbred parent development is simple. Furthermore, Triticale has the male fertility restorer gene from its rye heritage and cytoplasmic male sterility (CMS) from its wheat progenitor, allowing for effective hybrid seed multiplication which is essential for hybrid cultivar development (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). There are several characteristics that set Triticale breeding apart from other major self-pollinated grain crops like wheat and barley. Firstly, it's quite likely that the fragile genetic makeup of triticale-which contains genomic material from both wheat and rye- will be impacted by a cross between two accessions, leading to phenotypic variance in the 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). Over the past few years, traditional breeding methods have been used to improve triticales, and a considerable amount of progress has been made. However, the disadvantages of traditional methods include labour intensity, longer time frames, limited genetic variation, and, most importantly, 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 has used techniques like genetic engineering, somatic embryogenesis, molecular markers, and androgenesis to address this issue. Triticale is arduous to acquire as in vivo crosses, apart from embryonic culture rescue. Microspore and another culture are the most broadly adopted methods in triticale breeding programs, as Ya-Ying first reported the creation of haploid triticale plants in 1973 through tissue culture (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

The use of molecular methods and tools through MAS and QTL mapping, to enhance crop species is known as molecular breeding. The most widely used techniques in plant breeding in the past were morphological markers and protein isozymes; but, with the technological advances, the use of DNA-based molecular markers for precision selection and analysis of crop species is now widely employed (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). The first use of marker technology in plant breeding is marker-assisted selection (MAS). In MAS, the most desirable markers are those that are widely distributed throughout the genome, exhibit polymorphism even among closely related genotypes, are repeatable, and can be automated (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).

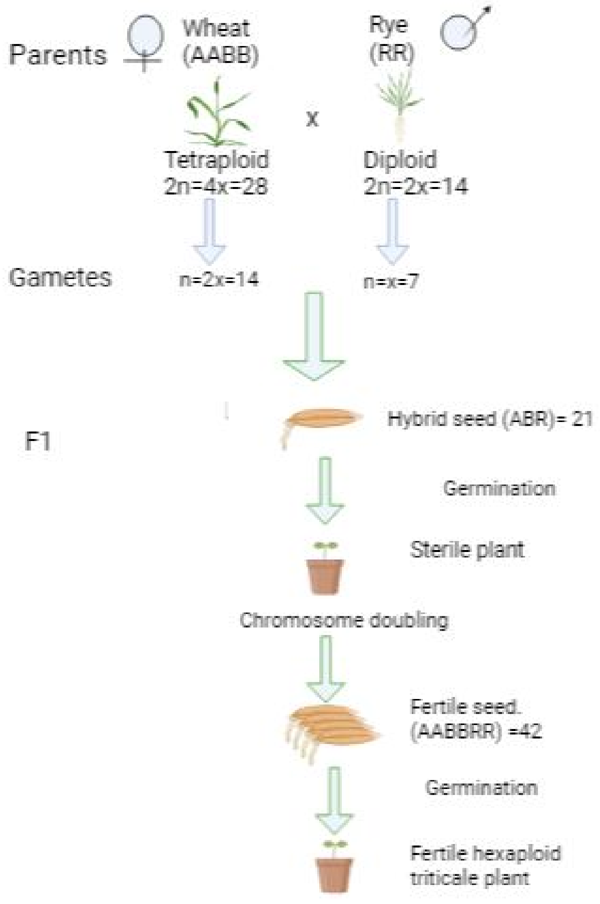

Fig 1. General illustration of creating amphiploids triticale.

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)/*Thinopyrumelongatum* (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 hexaploidtriticales 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 hexaploidtriticales with various chromosomal constitutions, including monosomic, substitution, and translocation lines, in addition to full hexaploidtriticales 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 *etal.*, 2008). The goal of ongoing research is to pinpoint particular rye genes and alleles for tactical integration into the genomes of elite Triticale.

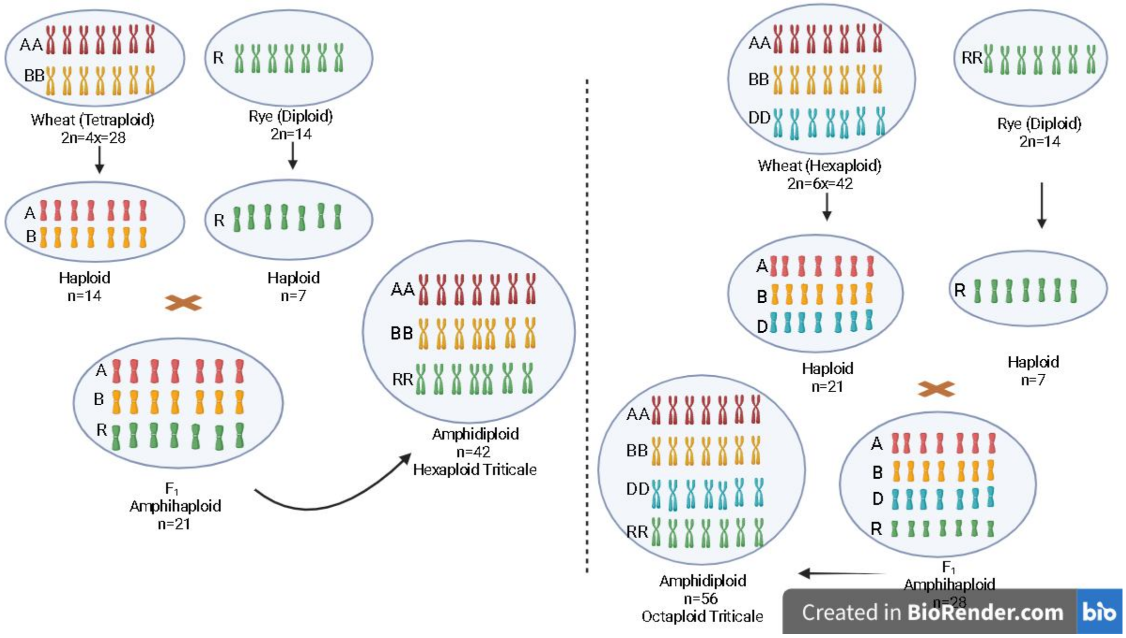

Fig. 2. Pictorial representation of development of Triticale on chromosome level

Translocation of chromosomes in Triticale

Chromosome translocations occur by genetic mechanisms. It has been reported that there are multiple techniques to generate the targeted chromosome translocations in Triticale, including spontaneous mutation, irradiation, somaclonal variation, and alteration of the mechanism of chromosomal pairing (Ph locus, on chromosome 5B) or the application of gametocidal (Gc) components (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 – Leymusracemosus 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:

The utilization of diverse chromosomal engineering methodologies has been important in facilitating Triticale's achievement as a highly productive hybrid cereal crop. Through the facilitation of targeted trait integration, genetic stabilization, and genome optimization, manipulation techniques such chromosome doubling, addition, replacement, translocation, and deletion have made considerable strides possible in the breeding of triticales. Fertility was restored in the original triticale lines by chromosome doubling and advantageous genes from rye could be effectively transferred into triticale backgrounds through addition and substitution..Crucial resources for genetic mapping across the chromosomes of wheat and rye were offered by translocations. The triticale genome's harmful sections were removed using deletions. When combined, these modifications have given breeders the opportunity to create genotypes that exhibit higher levels of protein quality, grain production, resilience to biotic and abiotic stress, and greater agronomic adaptability. At present time approaches, like chromosome fragmentation subsequently the fusion of pieces into chromosome structures, or induced recombination are being applied for the creation of the Triticale prebreedingstocks .

Nevertheless, several issues still exist, and further study is required to improve chromosome engineering techniques while causing the least amount of disturbance to the complex genetic system of Triticale. In the end, an integrated strategy integrating gene editing technologies, contemporary genomics platforms, and conventional cytogenetic modification will open up new avenues for creating optimal triticale genomes suited for sustainable food production.

References:

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