DEVELOPMENT AND CHARACTERIZATION OF NOVEL WHEAT-RYE AMPHI-DIPLOID LINES

Thesis Submitted for the Award of the Degree of

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Genetics and Plant Breeding

By

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DECLARATION

I, hereby declared that the presented work in the thesis entitled **"Development and Characterization of Novel Wheat-Rye Amphi-diploid Lines"** fulfilment of the degree of **Doctor of Philosophy (Ph.D.)** is the outcome of research work carried out by me under the supervision of Dr. Sanjeet Singh Sandal, Assistant Professor, Department of Genetics and Plant Breeding School of Agriculture Lovely Professional University, Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whenever the work described here has been based on the findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)

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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **"Development and Characterization of Novel Wheat-Rye Amphi-diploid Lines"** submitted in fulfilment of the requirement for the reward of the degree of **Doctor of Philosophy (Ph.D.)** in the Department of Genetics and Plant Breeding, is a research work carried out by **Patil Kulbhushan Savindra (Registration No. 12020442),** is a bonafide record of his original work carried out under my supervision and that no part of the thesis has been submitted for any other degree, diploma or equivalent course.

(Signature of Supervisor)

Name of the Supervisor: **Dr. Sanjeet Singh Sandal**

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Department/school: **Genetics and Plant Breeding, School of Agriculture**

University: **Lovely Professional University, Punjab, India.**

Abstract

The present study, "Development and Characterization of Novel Wheat-Rye Amphidiploid Lines," was conducted at two locations. 1. Agriculture Farms, Department of Genetics and Plant Breeding, School of Agriculture, LPU, Punjab and 2. Village-Kawaring, Keylong, Himachal Pradesh, during *Rabi* 2021-22, *off-season* (May-June 2022), and *Rabi* 2022-23. The experimental material consisted of ten hexaploid wheat genotypes [*T. aestivum* (9) and *T. sphaerococcum* (1)], eight tetraploid wheat genotypes [*T. durum* (7) and one *T. dicoccum* (1)], and one diploid rye. This study aimed to explore the phenotypic characterization and adaptation of a high-altitude Himalayan rye landrace in the Northwest Indo-Gangetic plains as a pollen source. The crossability between hexaploid and tetraploid wheat genotypes with diploid rye was investigated to develop primary triticale at the octoploid and hexaploid levels. The study also focused on the trait expression of parents in their respective hybrids. Hexaploid wheat varieties exhibited variable crossability with Himalayan rye, ranging from 32.91% to 43.51%, with an overall mean of 38.02%, whereas in tetraploid wheat, crossability ranged from 34.24% to 48.04% with Himalayan rye, with an average crossability of 44.09%. Colchicine application influenced chromosome doubling, with better results obtained in the seed set using 0.1% colchicine and 3% DMSO, which is 20%. The number of embryos obtained from dissected seeds in tetraploid wheat genotypes varied from 5 to 10, with the highest 10 observed in two crosses, PDW 215 × H. rye and PDW 314 × H. rye. Expression studies revealed both complete and partial expression of rye traits in F_1 hybrids and various characteristics such as coleoptile colour, stem colour, shoot-base colour, auricle pubescence intensity, waxy leaf sheath, greenness of leaves, node colour, hairy peduncle (intensity) and early growth habit exhibited both complete and partial manifestation of rye traits in F_1 hybrids and amphidiploids. The hybrids did not show the absence of auricles of the rye. The present research suggests the crossability percentage of wheat with Himalayan rye and the methods of polyploidy induction and trait expression in wheat-rye hybrids, which are useful for developing novel wheat × rye amphidiploid lines.

***Keywords*:** Colchicine, Hexaploid wheat, Himalayan rye, Tetraploid wheat, Trait expressio

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Place: Phagwara, Punjab

Date: ……

Patil Kulbhushan Savindra

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Abbreviations

°C	Degree Celsius
cm	Centimeter
et al.	And others
F_1	First filial generation
g	Gram
kg	Kilo gram
Max.	Maximum
Min.	Minimum
ml	Milli liter
viz.	Namely
vs	Versus
h.	Hours
H. rye	Himalayan rye

Symbols

=	Is equal to
%	Per cent
x	Cross
:	Colon
( )	Bracket
;	Semi colon

CHAPTER 1

INTRODUCTION

Wheat, a cereal grain, has been grown for thousands of years and has been crucial in advancing agriculture, establishing settlements, and growing society worldwide (Bell, 1987). Belonging to the *Triticum* genus, wheat was one of the first cultivated crops, playing a pivotal role in the transition from nomadic hunter-gatherer societies to stable agricultural communities (Ghahremaninejad *et al*., 2021). Its origins are believed to be traced back to the Fertile Crescent region in the Middle East (Charmet, 2011).

Wheat, one of over 300,000 potentially edible plant species, along with maize and rice, provides about 60% of global human caloric intake. With the global population expected to reach 9 billion by 2050, addressing food demand is critical. Cereal crops, particularly wheat, are vital, contributing 55% of the world's carbohydrates, 21% of total protein, and 19-20% of caloric intake (Shiferaw *et al*., 2013). Current global wheat production is 785 million tonnes, while consumption is 803 million tonnes (FAO, 2023-24). To meet future food needs, wheat production must increase at an annual rate of 1.7%, with a target of 5 tonnes per hectare by 2050, up from the current 3 tonnes (Leegood *et al.*, 2010; Singh *et al.*, 2016). In India, feeding 1.73 billion people by 2050 will require over 140 million tonnes of wheat.

Breeding efforts face significant challenges, including limited arable land and the need for environmental sustainability. Breeders focus on mitigating biotic and abiotic stresses that reduce wheat yields by exploring resistance and tolerance traits within wheat's genetic diversity and related species. One promising approach is the introduction of beneficial genes from outside the wheat gene pool (Crespo-Herrera *et al.*, 2017; Giura, 2015). Although genetic resources for wheat breeding have diminished, opportunities remain to integrate valuable traits from wheat relatives. The feasibility of crossbreeding wheat with its relatives is crucial for leveraging this genetic diversity (Singh & Sethi, 1992).

Landraces, characterized by their adaptation to specific agro-climatic conditions and substantial diversity within and between populations (Brown and Munday, 1982), represent a valuable genetic resource for future breeding efforts and developing new agricultural systems and products. These landraces often carry untapped sources of resistance, which can help diversify the limited resistance spectrum seen in modern cultivars (Marone *et al*., 2021). Despite being generally less productive than commercial cultivars, landraces possess the crucial advantage of adaptability to diverse climatic conditions. They have evolved in various natural farming systems, resulting in abundant variation and a broader genetic foundation due to natural and human selection (Weissenbach *et al*., 1992). Landraces excel at adapting to local stresses prevalent in their cultivation areas, resulting in stable yields and a repository of variable alleles. Consequently, they play a vital role in expanding allelic diversity for desired traits and broadening the genetic foundation of cultivated wheat (Adhikari *et al*., 2022).

Plant breeders often use wide hybridization to introduce tolerance and resistance traits into wheat from alien sources like rye (*Secale cereale*), *Hordeum californicum*, *Leymus mollis*, *Agropyron elongatum*, *Haynaldia villosa*, *Thinopyrum*, and *Aegilops peregrine*. Rye is particularly valuable for improving hexaploid and tetraploid wheat due to its resilience to various biotic and abiotic stresses. It thrives in poor conditions such as infertile, sandy, acidic, and mildly saline soils and faces fewer pathogen attacks, leading to reduced yield loss. Its strong root system also boosts drought tolerance. (Schlegel, 2013).

Within wheat breeding, rye serves as a pivotal reservoir of genetic diversity and resistance, constituting a component of the tertiary gene pool of wheat. Numerous investigations have delineated methodologies for integrating rye chromatin into the wheat genome. Typically, this process involves the acquisition of addition or substitution lines, which are subsequently employed to generate translocation lines for breeding purposes (Sears, 1976; Jiang and Gill, 1994; Lukaszewski, 2015; Molnar *et al*., 2014; Endo, 2007; Rather *et al.*, 2017).

The wheat variety ‘Chinese Spring’ offers an advantage in crossbreeding with rye, achieving crossability rates ranging from 36.9% to 88% due to the absence of inhibitory factors (Zeven, 1987). Similarly, the Indian wheat variety ‘C 306’ has shown similar crossability with ‘Chinese Spring’ (Joshi *et al.*, 2007). Zeven (1987) compiled a list of 76 bread wheat varieties that exhibited crossability with rye above a threshold of 40%, underscoring the significance of such investigations in identifying compatible wheat genotypes. Furthermore, it has been observed that different rye genotypes vary in their ability to cross with wheat (Oettler, 1982, 1983 and 1984; Stafanowska *et al.*, 1984; Tarkowski *et al.*, 1984).

The hybridization of wheat and rye led to triticale, a man-made grain with notable practical uses. A. S. Wilson reported the initial occurrence of sterile triticale plants in 1876, but it was not until 1891 that W. Rimpau documented the first fertile triticale. Later, in 1936, Muntzing identified it as an amphiploid. Over more than a century, triticale has gained substantial importance in global food production (Skovmand *et al*., 1984).

In the past five decades, substantial advancements have been made in developing *Triticum-secale* amphidiploid, commonly known as triticale, with particularly notable progress in the last thirty years. For example, Cholick, introduced in Canada in 1977, exhibited promising yields and outperformed ‘Rosner’ by 16% in terms of yield (Larter *et al*., 1974). Additionally, ‘Coorong,’ released in Australia in 1930, yielded even better than wheat (Gupta and Priydarshan, 1982).

In 1937, the discovery of colchicine represented a breakthrough in triticale production, allowing for the duplication of chromosomes (Kostoff, 1933). Combined with a technique for nurturing hybrid embryos in a nutrient culture medium (O’Mara, 1948), this innovation enabled plant breeders to create hexaploid and octoploid triticale with reasonable fertility assurance. Over the past five decades, triticale has evolved from being a rare phenomenon in taxonomy to becoming a practical and viable crop.

However, producing primary hexaploid triticale can be challenging due to the intricate process involved. Crossbreeding *Triticum durum* with *Secale cereale* yields hybrid seeds that lack endosperm, necessitating the use of embryo rescue for successful germination. Nevertheless, some hexaploid triticale have been successfully developed without embryo culture by crossing (*durum* × *durum*) F_1 with rye (Joshi and Chaudhary, 1985).

In triticale development, more emphasis has historically been placed on selecting suitable wheat parents, with relatively less attention given to the choice of rye parents (Muntzing, 1979). Qualset *et al.*, (1976) brought attention to the fact that crossing wheat with rye and subsequently doubling the chromosomes with colchicine can lead to the undesirable expression of rye genes, as it results in homozygosity at all loci in both the parent chromosomes. They suggested using highly inbred or self-fertile rye lines to address this issue. Sanchez Monge (1974) succeeded by employing self-fertile rye lines, which led to primary triticale with enhanced fertility. Self-fertile rye can yield superior triticale and is particularly valuable for genetic research because the natural out-crossing behaviour of rye, which is suppressed in triticale, can result in sterility. Moreover, the genetic heterogeneity of rye further complicates the expression of rye traits when introduced into the wheat background.

Oettler (1983) suggested the presence of 'incompatibility factors' within specific wheat genes, potentially influencing the manifestation of rye characteristics in triticale. For instance, dwarfing genes do not manifest at the same level in triticale as in wheat. While rye possesses dwarfing genes transferable to triticale, as documented by Nalepa 1980 and Nalepa *et al*., 1980 these genetic resources remain underutilized. Additional investigations are imperative to enhance comprehension of the interactions between the two genomes within the same organism. By carefully selecting appropriate parental wheat and rye, it may be possible to achieve the complete expression of traits from both parents in triticale.

Originally, wheat-rye hybrids were created to transfer beneficial traits from rye to wheat, but these efforts had limited success in making it a commercially viable crop. In

contrast, modern triticales, whether octoploid or hexaploid, offers several advantages: (a) Enhanced drought tolerance inherited from rye; (b) Well-suited for light soils; (c) Octoploid triticales have longer kernels and higher protein content (18.41%), and increased lysine levels compared to hexaploid wheat (13.51%), making them suitable for baking; (d) Tolerance to copper deficiency and acidic soils, with room for further improvement. (e) Increased resistance to wheat diseases like powdery mildew (*Erysiphe graminis tritici*), yellow rust (*Puccinia striiformis*) and Karnal bunt (*Neovossia indica*) (Randhawa *et al.,* 2015). Furthermore, triticale has been a conduit for transferring favourable traits from rye into bread wheat through triticale-wheat hybridization, including drought and cold tolerance, soil acidity tolerance and disease resistance (Plaha and Sethi, 1989).

However, despite its advantages over wheat, recent research has been dedicated to addressing specific challenges associated with triticale: (a) issues related to meiotic instability, aneuploidy, and partial sterility; (b) the presence of shrivelled kernels, leading to lower test weights compared to wheat; (c) vulnerability to kernel sprouting before harvest, particularly during rainy conditions; (d) problems with lodging, which have been observed in many triticale's (Skovmand *et al.*, 1984).

In advancing wheat breeding, conducting crossability studies with wheat varieties that can be crossed with triticale is essential. One of the limitations faced in triticale development is its genetic diversity, which hampers its progress. To overcome this limitation, it becomes crucial to incorporate desirable grain traits and adaptability into Indian wheat cultivars. This can significantly expand the genetic variability of triticale, especially within the Indian triticale improvement programme (Chaudhary and Joshi, 1985).

However, limited research exists on the potential for crossbreeding between local Indian wheat cultivars and improved semi-dwarf, high-yielding wheat varieties, which are well suited to local environments and have been bred to incorporate rye traits. To bridge this knowledge gap and address challenges related to resistance against biotic and abiotic

stresses in wheat, it is imperative to investigate the crossbreeding potential of local cultivars, particularly those from Punjab. This research, “Development and Characterization of Novel Wheat-Rye Amphidiploid Lines,” will significantly contribute to our understanding of the genetic compatibility of wheat-rye F_1 hybrids and facilitate their enhancement through targeted breeding strategies. The current study was designed with the following objectives:

1. **Agro-morphological evaluation of Himalayan rye.**
2. **Estimation of crossability and regeneration in wheat × Himalayan rye hybridization.**
3. **Stabilization of wheat-rye hybrids using chromosome doubling techniques.**

CHAPTER 2

REVIEW OF LITERATURE

The relevant literature for the current study has been examined based on the following categories:

2.1 Wide Hybridization-

Sr. No.	Author & Year	Research Findings
1.	Farrer, 1904	An Australian wheat breeder was the pioneer in effecting a cross between wheat and barley. Numerous endeavours have been undertaken to hybridize wheat and barley, aiming to generate a novel crop plant amalgamating advantageous traits from both cereal types.
2.	Derzhavin, 1938	First, the bread triticale from the cross (*T. durum*) wheat × *Secale montanum* was reported.
3.	Kasha and Kao, 1970	They generated haploids through distant hybridization encompassing the maternal parent's haploid genome, a phenomenon denoted as gynogenesis. To elucidate this process, a cross was conducted between barley *(H. vulgare*) and Bulbous barley (*H. bulbosum*). In this instance, the hybrid seed development procedure led to the degradation of the genome of Bulbous barley, ultimately yielding haploid barley plants.
4.	Barclay, 1975	After specifically eliminating *H. bulbosum* chromosomes from hybrid zygotes, thorough interbreeding between wheat and bulbous wild barley (*H. bulbosum*) pollen creates immature haploid wheat embryos. Nevertheless, the genetic regulation of the compatibility between wheat and *H. bulbosum* is governed by the *Kr*1&*Kr*2 genes, situated on chromosomes 5B&5A, respectively.

5.	Cohen and Galinat, 1984	Wide crossings, sometimes called intergeneric and interspecific hybrids, are great choices for crop improvement because they enhance the genetic diversity from which plant breeders can select for positive characteristics.
6.	Zenkteler and Nitzsche, 1984	In plant breeding, wide hybridization is considered beneficial for the induction of haploids, gene transfer, and the emergence of new species.

2.2 Phenotyping in rye –

Sr. No.	Author & Year	Research Findings
1.	Becker *et al.*, 1982	1. In experiment 1st, the yield performance of 3-way crosses and single crosses were the same, and in experiment 2nd, the yield performance of 3-way crosseswas more (1.5q/ha) than any other cross performance. 2. Phenotypic stability: a) The means for phenotypic stability in all hybrids was the same except for top crosses in experiment 1. b) Variability in environmental factors escalated sequentially from top crosses to 3-way crosses, reaching its peak in double crosses and subsequently in 3-way crosses. c) Evaluating factors such as performance, stability, and efficiency in seed production, the most appropriate rye hybrid type is the double cross involving a synthetic pollinator parent from a 2-line system.
2.	Jung and Lelley, 1985	1. Non-additive gene action governs the yield and yield contributing traits in rye.

		2. Grain weight per spike is the most significant character in rye because of its close correlation with most characters in parent's hybrids. 3. Tillering capacity and grain weight per plant are mostly influenced by the environment so lesspreference for selection for these traits shouldbe given.
3.	Singhara, 2005	1. Path-coefficient analysis and correlation were conducted for 12 agro-morphological traits, revealing positive direct impacts of specific traits on yield in rain-fed conditions. In descending order, the influential factors include biological yield, harvest index, days to maturity, length-width ratio (LWR) and 1000 grain weight. 2. Under irrigated conditions, positive direct effects on yield were observed for biological yield, harvest index, days to heading, spike length and spike per plant. Therefore, the selection of traits such as biological yield, harvest index, days to maturity, and LWR is crucial for enhancing drought tolerance, given their favourable direct effects on yield.
4.	Persson *et al.,* 2006	1. Variation in the phenotype of the landrace was determined using the Shannon-weaver diversity index. 2. The genetic variation was high, H0=0.56. Norway, Sweden, and Finland's landraces showed high variation. 3. Cluster analysis was done for the landraces, andimproved varieties were grouped into 8 classes.
5.	Li *et al.,* 2011	1. In three environments, *i.e.* controlled, semi- controlled and under field conditions, phenotypic data analysis was done, and the result was significant variation among the experimental material.

		2. The Cbf gene is the gene that confers freezing tolerancein rye. 3. Several gene × gene interactions were present, resulting in epistasis.
6.	Miedaner and Korzun, 2012	1. Significant genotypic variation ($p<0.01$) was observed for all the traits in 2 populations. 2. Genotype × environment variation was also significant ($p<0.05$), *i.e*. greater than 0 except for starch content. The heritability estimates were high to moderate, when phenotyping was done at 10 environments except for soluble pentosan content and falling number.
7.	Forsberg, 2015	1. About 434 genotypes from 76 accessions were analyzed using 576 SNP, the result was that cultivated rye had the highest genetic diversity whencompared with *Secale strictum* and *Secale africanum.* 2. *Secale strictum and Secale africanum* differfrom other species of rye, and their cluster analysis result was based on their origin and geographical distribution. *Secale vavilovii* was found to be the ancestor of cultivated rye.
8.	Targonska-Karasek *et al.*, 2020	1. Genetic analysis was done in the 100 accessions using an SSR marker. The result was that the gene pool wasvery large in the 100 unused accessions. 2. All the accessions had some similar phenotypic characteristics even though there was no similarity in their genetic make-up which is the cause for not choosing these varieties as a source in breeding programmes.

2.3 Crossability of rye with hexaploid and tetraploid wheat -

Sr. No.	Author & Year	Research Findings
1.	Wilson *et al.*, 1876	He performed a cross between wheat and rye for the first time, resulting in a sterile hybrid. He did emasculations in some wheat florets and then did artificial pollination with rye pollen. The hybrid's spike was intermediate to wheat and rye plants. The hybrid seed, when grown, showed opened florets in its spike, there was no pollen formation, or the anthers were not dehiscent. The under-developed pollen grains remained intact within the anthers. From the above experiment, it was clear that there was no seed set in the wheat-rye hybrid.
2.	Carman, 1884	Carman crossed wheat and rye and developed a hybrid.He used the seed of the hybrid for sowing to continue the experiment. There was a difference in tiller number in some pants and the matured spikes of the plants.He also performed selection in the plants based on the maturation of spikes.
3.	Leighty *et al.*, 1928	To obtain the crossability percentage, he planted alternate rows of one rye variety with 8 wheat varieties. The result was that no crossing occurred, and they concluded that a natural wheat-rye hybrid is very rare, and the hybrids are sterile.
4.	Sasaki and Wada, 1966	In addition to *kr* genes other factors like chromosomes 4A, 1D, and 7D must also be responsible for crossability with rye.
5.	Taira *et al.*, 1978	Conducted research using 7-lines, 6-inbred lines, and 1 open-pollinated variety of rye were used as male parents in crosses with durum wheat species Jori and Langdon. All the rye plant species used were highly diverse. This study was done to determine the effect of different kinds of temperatures on the

		development of hybrid embryos. 17ºc of temperature was found to be a goodfactor for the growth and development of embryos during the day and night. A synthetic chemical substance ε- amino-n-caproic acid (EACA) was administered continuously for 10 days into the female parent during embryo development. Both the 17ºc optimal temperature and the chemical induction helped in embryo development.
6.	Sebesta, 1980	Stated that a wheat cultivar, Amigo, with 1AL.1RS translocation, was developed using the irradiation method.
7.	Lukaszewski *et al.*, 1983	The fusion of chromosome arms during meiotic metaphase-1 leads to an exchange of the chromosomal segments. The fusion happens because of the breaking of the centromere, which leads to the formation of the telocentric, and this is how rye-wheat translocation lines are formed.
8.	Oettler *et al.*, 1983	Experiments using one variety of *T. aestivum* along with 3 other varieties of *T. durum* wheat D30, D40, D50 varieties and 19 inbred lines of rye were used for crossing to study crossability and embryo development. The crosses of rye with hexaploid wheat were superior to those with durum wheat genotypes. Well-differentiated embryos and a greater number were formed from Gotz crosses (Hexaploid wheat variety), whereas the crosses of durum wheat with rye formed embryos with poor differentiation and less in number. This is because of the strong influence of the maternal parent over embryo development.
9.	May and Appels,1984	It has been documented that seedling lethality in hybrids between *T. aestivum* (wheat) and *Secale cereale* (rye) arises from the novel combination of chromatin from both rye and wheat. Specifically, wheat plants exhibiting disomy for a

		translocation chromosome 2RS/2BL, substituting for chromosome 2B, display lethality during the seedling stage. Notably, introducing the 2RS/2BL translocation into a distinct genetic background can surmount this seedling lethality.
10.	Zeven and Waninge, 1986	It is concluded that scalavatis wheat plants are the plants that originated because of contaminations that happened in durum wheat landraces. This wheat variety belongs to the common wheat genus and occurs in a few durum wheat fields. This scalavatis wheat has low crossability with rye. Only some of the plants of scalavatis wheat have moderate crossability with rye. The genotype of scalavatis wheat that shows less crossability with rye has *Kr1Kr1Kr2Kr2* type of genes, and the wheat that shows moderate crossability with rye has *Kr1Kr1kr2kr2* or *kr1kr1Kr2Kr2* genes.
11.	Zeven *et al.*, 1987	He has given 2 lists of wheat varieties with crossability with rye. This information was published to help people who perform crosses between wheat and rye and cross varieties other than rye. He also gave that the *Kr* gene with 2 loci *Kr1* / *kr1* and *Kr2* / *kr2* on wheat chromosome influence its crossibility with rye. The wheat lines thathave 10 % or more crossability have 1 pair of a recessive allele of *Kr* gene, and the wheat lines with 50 percent or more crossability have 2 *Kr* genes in homozygous recessive form.
12.	Oettler *et al.*,1988	The research focused on hybrid breeding in winter triticale, exploring heterosis and combining ability in triticale. The study assessed 201 F_1 hybrids of winter triticale generated using a chemical hybridizing agent. Additionally, the investigation incorporated 57 ♀parents and 5 testers.

13.	Carman and Campbell, 1990	Described a method for synthesizing translocation lines, *i.e.* the wheat-rye hybrid seeds are to be grown in a media containing auxins, which helps in the callusproduction. The plantlets obtained are treated with colchicine to double their chromosome number.Several lines produced through the tissue culture method have 2BS.2RL, 2BL.3R and 4DL.1RS translocations.
14.	Oettler *et al*., 1991	The investigation centered on quantifying heterosis levels in initial triticale crosses containing either a heterozygous wheat genome paired with a homozygous rye genome or a homozygous wheat genome paired with a heterozygous rye genome. The results indicated that F_1 triticale crosses with a heterozygous rye component exhibited notably higher and predominantly significant levels of heterosis than those with a heterozygous wheat component. The rye-heterozygous crosses demonstrated highly significant heterosis for six distinct traits.
15.	Rabinovich,1998	Plant breeders did the incorporation of rye genes in wheat. This incorporation of rye genesis is possible through the utilization of translocation lines and substitution lines. 1AL.1RS and 1BL.1RS are translocation lines and (1BL) 1R is a substitution line for incorporating rye genes into the wheat genome.
16.	Jauhar *et al.*, 1999	Translocation lines can also be obtained by randomly using the irradiation method, but it harms the lines.
17.	Haley *et al.*, 2004	*Dn7* gene was found in the Turkey77 rye variety, which conferred resistance against *Divraphis noxia*. *Dn7* wasfound in the 1R chromosome of Turkey77.

18.	Marais *et al.*, 2005	Concluded that diploid and tetraploid wheat, whencrossed with diploid rye, did not give any proper seed set, but when hexaploid wheat (Chinese Spring monosomic series) was crossed with diploid rye, the cross yielded seed set. The barrier presents in the case of 6x wheat × rye cross was taken care of by the polygenes present in the wheat. Chromosome 1D played a vital role in eliminating the barrier in the latter cross. Without Chromosome 1D, the grain obtained from the 6x wheat × rye cross was shrunkenand inviable.
19.	Zhang *et al.*, 2005 and Lukaszewski *et al*., 2006	Ph1 mutant utilization and centromere breakage fusion methods were discovered, which helped analyze and produce new translocation substitutional lines from different rye species. Presto and Panda - triticale species, of which 1RS or 1R lines have been found with seedling resistance against *Schizaphis avenae* and *Rhopalosiphum padi* aphids.
20.	Knight *et al.*, 2010	Okadaic acid also favours homeologous pairing between wheat and rye chromosomes. Okadaic acid, when introduced on to the tillers where the cells are not in the meiotic phase, helps induce homologous pairing.
21.	Rudd *et al.*, 2010	*Gb2* was the first gene that was transferred into wheatfrom rye. It showed resistance against B, C, J biotypes of *Schizaphis graminum*. This *Gb2* gene was found inthe 1Rs chromosome of rye. *Schizaphis graminum* is genetically diverse, so the *Gb2* gene was inefficient in wheat. Another gene, *Gb6*, which was also found in the 1RS chromosome of rye, conferred resistance against the F,G,I and K biotypes of *Schizaphis graminum.* This gene was present in the winter wheat cultivar Amigo.

22.	Pretorius *et al.,* 2012	Powdery mildew, yellow rust, stem rust and leaf rust are all disease-resistance genes present in rye, which was transferred into wheat. *Yr9, Lr26, Pm8,* and *Sr31* genes confer resistance against yellow & leaf rust, P. mildew and stem rust. All these genes are present in the 1R chromosome of Petkus rye.
23.	Hawkesford *et al.,* 2013	As the population is growing, there is so much demandfor wheat. Wheat production is much less, so an efficient production system should be developed through plant breeding to produce wheat adequately without harming the environment.
24.	Gowda *et al*.,2014	Molecular markers were integrated with a factorial mating design as part of the experiment. The results showed that characteristics such as plant height and heading time in hybrid triticale may be predicted by analyzing mid-parent data.
25.	Andersson *et al*., 2015	The rye genome is a good source of pest-resistant traits that can be incorporated into wheat. The1R chromosome of rye has many such traits, and the 5R chromosome has genes resistant to aphids. Rye is resistant to: a) *Divraphis noxia* (aphid) a pest of wheat. b) *Heterodera avenae* (wollen web weaver) - nematode.c) *Aceria tosichell keifer*- mite d) *Mayetiola destructor* - Cecidomyid.
26.	Goral *et al*., 2015	Research findings are available regarding male-sterile lines in winter triticale. Initial heterosis estimates were derived from small-plot experiments using F_1 seed obtained through manual emasculation in both spring and winter triticale.
27.	Rahmatov *et al.,* 2016	The 2R rye chromosome has *Lr25* and *Lr45* genes that show resistance against leaf rust. The *sr59* gene confers resistance only to stem rust. In Rosen rye, 2R chromosome has genes

		like *Pm7-Lr25* and *Pm7,* which confer resistance against powderymildew. A particular rye cultivar, 3R chromosome, has aresistant gene *SR27* that confers resistance against stem rust. 4R and 6r chromosomes of rye also have resistant genes against powdery mildew.
28.	Bauer *et al.,* 2017	A, B and D are the 3 genomes that are present in wheat,which have a diploid origin, and even rye also has a diploidorigin, so the chromosomes 1,2,3,5,6 of wheat havehomology with rye chromosomes 1R,2R,3R,5R and 6R. The 4 and 7 chromosomes of wheat have partial reciprocal homology with rye chromosomes 4R and 7R. This feature of homeologous pairing helps in the introduction of desirable agronomical traits from rye into wheat.
29.	Amin *et al*.,2023	Wheat cultivars developed with the doubled haploid technology have better salt tolerance. They reported a phenomenon known as nucleolar dominance occurs, wherein the rRNA genes originating from rye (*Secale cereale*), specifically located on chromosome 1R, undergo near-complete inactivation due to the prevailing dominance of wheat.

2.4 Comparison of the regeneration frequency in different wheat × rye F_1 hybrid combinations -

Sr. No.	Author & Year	Research Findings
1.	Levitsky and Benetzkaia, 1930	The researcher noted disruptions in meiosis in triticale that led to univalents observed at metaphase I. The researcher initially reported univalents in Meister's octoploid wheat-rye hybrid.
2.	Lebedeff,1934	The initial documentation of aneuploidy in wheat-rye amphidiploid hybrids revealed instances of octoploid plants possessing fewer than 56 chromosomes.
3.	Tjio *et al*.,1954	He mentioned triticale obtained from the crosses *Triticum durum* × *Secale cereale, Triticum dicoccum* × *Secale cereale, and Triticum Dicoccides* × *Secale cereale.*
4.	Riley and Chapman, 1958	The meiotic behaviour attributed in wheat × rye is due to a diploidizing system that restricts the pairing of homologous chromosomes; this is because the pH locus on chromosome 5B has a major effect on the suppression of homologous chromosome pairing.
5.	Kiss *et al*.,1965	The initial triticale initiatives primarily focused on creating and assessing newly synthesized octoploid hybrids, including hybrids formed from bread wheat and rye. The research also emphasized the development of primary triticale in Hungary.
6.	Keyworth and Larter,1979	He mentioned that embryo development in a cross made between bread wheat × Rye shows less development compared to Durum wheat × Rye as the embryo development in Durum wheat takes 10 days whereas Bread wheat takes 18 days.

7.	May and Apples, 1980	Favourable genes can be introduced into wheat by employing D and R-genome chromosome substitution.
8.	Zeven and Keijzer,1980	The cross ability of wheat × rye is affected by the number of B chromosomes in the rye pollen. He found 2B rye plants. They have a much higher cross ability than 0B and 4B rye plants.
9.	Foroughi-Wehr *et al*., 1982	It is stated that to determine the success of androgenesis, the ratio of albino and green plants plays an important role.
10.	Falk and Kasha, 1983	The study focused on the genetics of bread wheat's crossability with rye and *H. bulbosum*. The analysis showed that allelic differences at the *kr* locus are more likely responsible for the chromosomes impacting variations in *kr* genes.
11.	Laurie and Bennett, 1986	The hybrids have unstable karyotypes; they rapidly lose chromosomes from maize and produce haploid wheat embryos.
12.	Ghaemi-Ahmadi, 1992	They observed that androgenesis depends on important factors like genotype, culture conditions, pre-treatments, and their interaction.
13.	El-Maksoud *et al*.,1993	The generation of haploids in bread wheat through anther culture leads to the emergence of albino plants, with the frequency of albino plants varying between 20% and 50%.
14.	Sirkka and Immonen ,1993	He discovered that rescuing embryos from triticale plants 15-17 days after pollination led to the best outcomes for both callus and embryo culture. Building on this finding, they applied the same approach to rescuing embryos from wheat- rye hybrids, using two-time points 16 and 20 days after pollination. Their results showed that 77.9% to 95.1% of embryos rescued at 16 days and 75% to 100% of

		embryos rescued at 20 days produced shoots by the fourth day after being placed in the rescue medium. This suggests that embryo rescue timing can significantly affect subsequent cultivation efforts success.
15.	Inagaki and Mujeeb-kazi, 1995	The application of 2,4-dichlorophenoxyacetic acid (2,4-D) during wheat plant pollination has been found to positively influence the development of globular embryos, facilitating their transformation into immature embryos with the potential for regenerating polyhaploid wheat plants.
16.	Immonen, 1996	The research investigated the effectiveness of various plant growth regulators during somatic embryogenesis in immature wheat-rye hybrid embryos. The results indicated that, compared to both concentrations of 2,4-D, the application of Dicamba at a concentration of 18.1 M significantly increased rates of callus induction and the proliferation of embryogenic callus per plated embryo ($p<0.05$). However, when considering frequencies per plated embryo, there was no noticeable difference between the two dicamba concentrations in their effects. Furthermore, dicamba-containing media exhibited a notably faster formation of embryogenic callus. The intensity of embryogenesis on media containing 18.1 M dicamba reached nearly three times higher levels than on media containing 2,4-D. Ultimately, plant regeneration from the embryogenic callus developed on dicamba-containing media surpassed that on media containing 2,4-D.

17.	Gupta *et al*., 1999	A haploid wheat plant can be produced by anther culture, microspore culture, androgenesis, egg culture, megaspore culture, gynogenesis and distant hybridization (interspecific and intergeneric cross).
18.	Pratap *et al*.,2005	The investigator examined nine elite and genetically diverse triticale genotypes possessing AABBRR chromosomes. These genotypes were crossed with seven wheat genotypes carrying AABBDD chromosomes, resulting in various hybrid combinations. Due to the substantial sterility observed in the anthers, emasculation was deemed unnecessary for the triticale × wheat hybrids. Fresh pollen from various Gramineae genera was collected and delicately applied to the feathery stigma of the F_1 hybrids. Subsequently, a 2,4-D solution with a concentration of 250 ppm was injected into the uppermost internode's base to facilitate *in vivo* seed and embryo development. This injection process was repeated for two consecutive days. The crossed spikes were collected eighteen days after pollination, and the seeds were examined closely to check to produce embryos.
19.	Pratap *et al*., 2006	Anther culture is more cost-effective for producing DHs than intergeneric crossings in many cereal breeding operations.
20.	Cistue *et al.*, 2009	The detected interplay between genotype and microspore culture techniques is one aspect, while the other involves a notable prevalence of albino plants regenerated in tetraploid wheat.

21.	Khan *et al*., 2012	Many environmental factors influence the production of haploid embryos, including soil, humidity, temperature, and light intensity.

2.5 Colchicine treatment in wheat × rye F_1 hybrids-

Sr. No.	Author & Year	Research Findings
1.	Love and Craig, 1919	He backcrossed many F_1 wheat flowers × rye with wheat and rye. Moreover, found that a back cross with parents can restore fertility, but in very less cases.
2.	Blakeslee *et al*.,1937	Seed treatment with colchicine and induced chromosome doubling, experiment on plant growth treated with colchicine at various concentrations and believed chromosome doubling occurs in somatic tissue.
3.	Bell, 1950	He documented that among the highly effective techniques for inducing chromosome doubling in cereal is the "inverted vial technique". The root immersion technique was also employed as an alternative approach for inducing chromosome doubling in barley haploids.
4.	Neubauer and Thomas, 1966	He found that auto Durum seedlings were more common at an acidic pH of 4.0 than when colchicine was applied at pH values of 6.3 and 7.6.
5.	Ouyang *et al.*,1973	The first time an alternate method was reported to create haploid wheat plants was in anther culture. The main problem in anther culture was the poor plant regeneration and the high number of albino plants in haploid populations.
6.	Subrahmanyam and Kasha,1973	Male chromosome elimination is caused by the development of multipolar spindles, asynchronous cell cycle, and asynchrony in the production of nucleoprotein, which results in a delayed loss

		of chromosomes and spatial separation of genomes during interphase.
7.	Jensen, 1974	A 90% induced seed-set in Mono haploid barley was achieved through treatment with a combination of 0.05% colchicine and 1.5% dimethyl sulfoxide (DMSO) solution, mirroring the method employed for obtaining amphidiploid from F_1 hybrids in *Triticinae*. The current study encompasses the comprehensive application of these treatments within a growth chamber setting.
8.	Jensen,1975	Colchicine is the prevalent chemical agent employed for chromosome doubling, disrupting mitosis by impeding spindle fiber formation and interfering with the typical polar chromosomal migration. This disruption leads to the doubling of chromosomes.
9.	Kaltsikes and Gustafson, 1986	A significant obstacle encountered in the hybridization of Durum wheat with rye is insufficient endosperm development and embryo abortion. Although hybrid embryos originating from crosses between Bread wheat and rye exhibit greater viability, their occurrence is typically limited due to low seed sets. In both scenarios, embryo rescue and in vitro culture methods are beneficial. Particularly, embryo culture is employed with the specific aim of producing amphiploid plants.
10.	Taira *et al*., 1991	Colchicine solutions with pH levels ranging from 3.5 to 7.5, combined with the root immersion approach, produced the maximum number of viable hybrids. The efficacy of the root immersion technique surpassed that of the inverted vial method, leading to a 6% higher frequency of induced fertile plants when applied within a growth chamber.
11.	Inagaki and Tahir, 1992	Haploid plants possess a single set of genes at each locus. Consequently, following chromosome doubling, the doubled

		haploid (DH) lines exhibit complete homozygosity & uniformity. This facilitates the swift generation of recombinant inbred lines (RILs) from hybrid offspring. The approach expedites the attainment of complete homozygosity in a single step and accelerates the fixation of alien chromosomes.
12.	Inagaki,1997	His proposed method for developing amphidiploids with the colchicine method is extremely effective. According to his method, the roots of the haploid seedling are cut, leaving a 2 to 3-cm zone, and then immersed in a 0.1% colchicine solution mixed with 2% dimethyl sulfoxide and approximately 0.05% Tween 20 at a temperature of 20°C for 5 hours. Following this treatment, the roots are thoroughly rinsed to remove any remaining colchicine and then transplanted into peat soil.
13.	Cheng and Murata, 2002	The initial advancement of triticale involved the generation of octoploid hybrids, possessing a chromosome count of 2n = 8x = 56 (AABBDDRR), achieved through chromosome doubling in crosses between bread wheat (*T. aestivum* L.) & rye. However, applying the embryo culture method in the late 1940s facilitated the development of Bread triticale with a chromosome count of 2n = 6x = 42 (AABBRR) by crossing Durum wheat (*T. turgidum* L.) with rye. This primary Bread triticale's encompass all 28 chromosomes of wheat and 14 chromosomes of rye, rendering them more stable and fertile than octoploid triticale's.
14.	Inagaki, 2003	He recommended pruning the roots around 2 mm below the crown during the tillering stage. The plants are then submerged for five hours at room temperature in a mixture of 0.05-0.1% colchicine, 2% dimethyl sulfoxide (DMSO), and 15 drops per liter in a flask. Following treatment, the plants are thoroughly cleaned with tap water before being transplanted and grown in

		environments encouraging tillering. The wheat spikes from the colchicine-treated plants are covered with glassine bags to stop outcrossing. A 60-70% success percentage is achieved in harvesting wheat grains.
15.	Han and Niimi (2004), Molnar and Molnar-Lang (2009) and Zhang *et al.,* (2013)	The restoration of fertility and subsequent seed set in interspecific F_1 hybrid plants can be used to assess the efficacy of chromosomal doubling treatment. Apart from these phenotypic markers, genomic in situ hybridization (GISH) can be used to determine the exact genomic makeup of the newly generated amphidiploids. GISH uses genomic DNA as a tagged probe to identify parental chromosomes based on their genome, highlight translocations, and find chromosomal rearrangements. This method is sensitive to distinguish between the wheat genomes A, B, and D.
16.	Basu *et al*., 2011	The conventional breeding timeline for cultivar development, typically 12 to 13 years, is significantly reduced to 6 to 7 years through the implementation of doubled haploid technology. This approach also diminishes the necessary selection cycles to attain the required homozygosity, decreasing from 6 to 7 cycles yearly.
17.	Nemeth *et al*., 2015	The restoration of fertility and subsequent seed set are useful metrics to assess the efficacy of chromosomal doubling treatment in interspecific F_1 hybrid plants. Genomic in situ hybridization (GISH) can be used to determine the exact genomic content of the freshly produced amphidiploids in addition to these phenotypic indications. To detect chromosomal rearrangements, highlight translocations, and identify parental chromosomes based on their genomes, GISH uses genomic DNA as a tagged probe. The sensitivity of this method allows it to distinguish between the wheat genomes A, B, and D.

2.6 2,4-D Application-

Sr. No.	Author & Year	Research Findings
1.	Laurie and Bennett, 1987 and Laurie and Reymondie, 1991	Three methods were used to administer 2,4-D to spikes that had been pollinated. These techniques included introducing the 2,4-D solution into the higher internode, soaking pollinated spikes in a 2,4-D solution, and cultivating spikelets in a 2,4-D medium. Among these methods, injecting the 2,4-D solution into the topmost internode of the emasculated and pollinated spike yielded the most favourable outcomes in the wheat × maize system, surpassing the efficacy of the other two techniques.
2.	Suenaga and Nakajima, 1989	Suggested that the 2, 4-D treatment may cause enlargement in fertilized and unfertilized ovaries.
3.	Suenaga and Nakajima, 1989; Inagaki and Tahir, 1990	Suggested that 2, 4-D application improved the growth of hybrid zygotes into haploid embryos.
4.	Inagaki and Tahir, 1992	The frequency of embryo development in all the wheat genotypes ranged from 8.3 to 21.1% when 2,4-D 100 ppm was given, and in the absence of 2,4-D injection, no embryos were formed.
5.	Inagaki and Mujeeb- Kazi 1995, Hussain *et al.*, 2013	Used various concentrations and various hormones such as 2, 4-D, indole acetic acid (IAA), GA3, 6- benzyl aminopurine (BA), naphthalene acetic acid (NAA), zeatin and kinetin and reported in wheat × maize crossing system increased frequency of haploid embryo was formed at a concentration of 100 mg/L of 2, 4-D when compared to other hormones.

6.	Khan *et al.*, 2012	Reported that the treatment of 2, 4-D enhances the seed formation frequency and haploid embryo production frequency and improves cell growth and replication.

CHAPTER 3

MATERIALS AND METHODS

The "Development and Characterization of Novel Wheat-Rye Amphidiploid Lines" study was conducted during the *Rabi* 2021-22, off-season (May-June 2022) and *Rabi* 2022-23. The materials and methods employed in conducting the current investigation have been elucidated using the following categories:

3.1 Site of Experiment

3.2 Climate and Weather

3.3 Experimental Materials

3.4 Experimental Method

3.1 Site of Experiment-

The study was conducted at two sites: 1) Agricultural Research Field at the Lovely Professional University, Phagwara, Punjab, and 2) Kawaring, Keylong, Himachal Pradesh, India.

3.2 Climate and Weather-

1) Lovely Professional University is located at a geographic coordinate in a latitude of 31° 15' 29.4804" N and a longitude of 75° 42' 28.5696" E, with an elevation of 243 m., above sea level. Phagwara is a part of the Kapurthala District. This place features a humid subtropical climate with cold winters and long, scorching summers. It is in the middle plain region of Punjab. The winter season endures from November to February and the summer season is from April to September. Typical summer temperatures are between 25°C to 48°C, while typical winter temperatures are from 4°C to 19°C. This region's soil is sandy loam with a pH range of 5.6 to 6.0, giving it a slightly acidic character.

2) Kawaring village (Keylong, HP.), in Lahaul & Spiti District, is positioned at a latitude of 32°34'15.6"N and a longitude of 77°1'55.12"E, with an elevation of 3080 m., above sea

level. The typical temperature in Keylong varies, with lows reaching as far as -20 °C and highs reaching 6 °C. The soil in this region has a sandy loam texture and maintains a pH level ranging from 5.5 to 6.5.

Emasculation and pollination were attempted in February and March 2022. During the off-season of May- June 2022, colchicine treatment was given to the F_1 hybrid plants in the high-altitude area of Kawaring (Keylong, HP.). Phenotypic characterization of wheat × rye F_1 hybrids and their parents was recorded during *Rabi* 2022-23. The information in Table 3.1 presents the meteorological data that was documented throughout the experiment.

Table 3.1.a Meteorological data during a crossing between wheat and Himalayan rye at weekly intervals in February and March 2022.

Month and Year	Week	Temperature (^{0}C)		Relative Humidity (%)		Rainfall (mm)
		Maximum	Minimum	Maximum	Minimum	
February	I	15	9	75.83	62.33	0.5
	II	13.28	8.28	78.85	63.42	0
	III	17.28	8.42	73.57	58.57	0
	IV	19.42	10.42	65.71	50.14	16
March	I	19	9.66	58	47.5	0
	II	21.71	18.14	55.28	43.71	0
	III	31.57	20.85	55	41.14	0
	IV	31.14	20.57	50.85	42.71	0

Source: Agro-meteorological Observatory Unit, LPU, Phagwara, Punjab.

Table 3.1.b Meteorological data during phenotypic characterization of wheat × Himalayan rye F_1 hybrids along with its parents at weekly intervals from November 2022 to April 2023.

Month and Year	Week	Temperature (^{0}C)		Relative Humidity (%)		Rainfall (mm)
		Maximum	Minimum	Maximum	Minimum	
November	III	27.85	18.28	78	69.42	0
	IV	25.62	12.25	85.87	67.75	0
December	I	27	11.75	84.75	62	0
	II	27.28	11.42	88	63.57	0
	III	25	10	89.28	77.57	0
	IV	21.57	8.71	95	79	0
January	I	17	7.5	87	77	0
	II	14.14	5.42	94.85	80.85	0.3
	III	11.54	7.78	97.42	83.71	0
	IV	18.44	7.44	86.71	70.85	0
February	I	21.62	8.9	95.4	70.2	0
	II	23.94	11.4	78.14	56	0
	III	26.51	11.42	64.85	41.71	0
	IV	28.08	14.55	91.14	57	0
March	I	27.54	13.7	51.8	41	0
	II	29.42	14.4	52.28	38.42	0
	III	27.44	16.5	76.25	48	2.14
	IV	24.74	13.7	93.42	59.57	5.48
April	I	27.43	14.07	66.37	40.12	0.30
	II	35.97	16.50	78.5	23.75	0
	III	34.59	15.82	78.37	29	0
	IV	34.68	20.15	74.5	34.16	0

Source: Agro-meteorological Observatory Unit, LPU, Phagwara, Punjab.

3.3 Experimental Material

The material used for this experiment consisted of 10 genotypes of hexaploid wheat (*T. aestivum* L. and *T. sphaerococcum* L.), 7 genotypes of tetraploid wheat (*T. durum* L.), 1 genotype of tetraploid emmer wheat (*T. dicoccum* L.) and 1 genotype of Himalayan rye landrace (*Secale cereale* L.).

Table No. 3.2. a List of Genotypes of hexaploid wheat (*Triticum aestivum* L.)

Sr. No.	Genotype Name	Pedigree	Source
1.	HD 2851	DL 509-2/DL 377-9	Dept. of GPB, LPU.
2.	HD 3086	ALD/COC//URES/HD 2160M/HD2278	Dept. of GPB, LPU.
3.	HD 2967	DBW 14/HD2733//HUW 468	Dept. of GPB, LPU.
4.	PBW 502	W 485/PBW 343//RAJ 1482	Dept. of GPB, LPU.
5.	PBW 550	PBW 378//PBW 343	Dept. of GPB, LPU.
6.	PBW 677	PBW 378//PBW 379	Dept. of GPB, LPU.
7.	UNNAT -343	PBW 343/WH890//WL 711	Dept. of GPB, LPU.
8.	WH 1105	MILAN/S87230//BABAX	Dept. of GPB, LPU.
9.	WL 711	S 308/CHR//KAL	Dept. of GPB, LPU.

Table No. 3.2. b List of Genotypes of hexaploid wheat (*Triticum sphaerococcum* L.)

Sr. No.	Genotype Name	Pedigree	Source
1.	*Triticum sphaerococcum*	Landrace	Collected from Haryana.

Table No. 3.2.c List of Genotypes of tetraploid wheat (*Triticum durum* L.)

Sr. No.	Genotype Name	Pedigree	Source
1.	PDW 291	Boomer/Mojo 2	Dept. of GPB, LPU.
2.	PDW 233	Y AV'S'/ TEN'S'	Dept. of GPB, LPU.
3.	PDW 274	DWL6018/KARPASIA	Dept. of GPB, LPU.
4.	PBW 34	AA 'S'/FGO'S'	Dept. of GPB, LPU.
5.	PDW 314	AJAIA12/F3LOCAL(SEL.ETHIO.135.85)// PLATA13/3/SOMAT3/4/SOOTY/RSACON37	Dept. of GPB, LPU.
6.	PDW 215	RAJ911//AA 'S'/D#2E/3/DWL5002	Dept. of GPB, LPU.
7.	WHD 943	GLARE/PLATA-16//AJAIA-3/SLIVER-16	Dept. of GPB, LPU.

Table No. 3.2.d List of Genotype of tetraploid emmer wheat (*Triticum dicoccum* L.)

Sr. No.	Genotype Name	Pedigree	Source
1.	Khapli Wheat	Landrace	Collected from Maharashtra.

Table No. 3.2.e Lit of the genotype of pollen parent (*Secale cereal* L.)

Sr. No.	Genotype Name	Pedigree	Source
1.	Himalayan rye	Landrace	Collected from Koraki village Lahaul & Spiti, Himachal Pradesh.

3.4 Methods

3.4.1 Sowing Plan

3.4.1.1 Sowing Plan of wheat genotype for crossing (*Rabi* 2021-22)

During *Rabi* 2021-22, three staggered sowings of all hexaploid and tetraploid wheat were done at 10-day intervals from the last week of October to the last week of November on the LPU research farm. 22.5 cm is row-row spacing, and three rows of each wheat line were sown at 3 m length.

3.4.1.2 Sowing Plan of pollen parent

Rye (*Secale cereale*), which belongs to the grass family, can grow in harsher environments than many other cereal crops. It can tolerate poor soil conditions and cold temperatures and is available in high-altitude areas. During *Rabi* 2021-22, two staggered sowings of rye were done at 15-day intervals in September and October for continuous pollen availability.

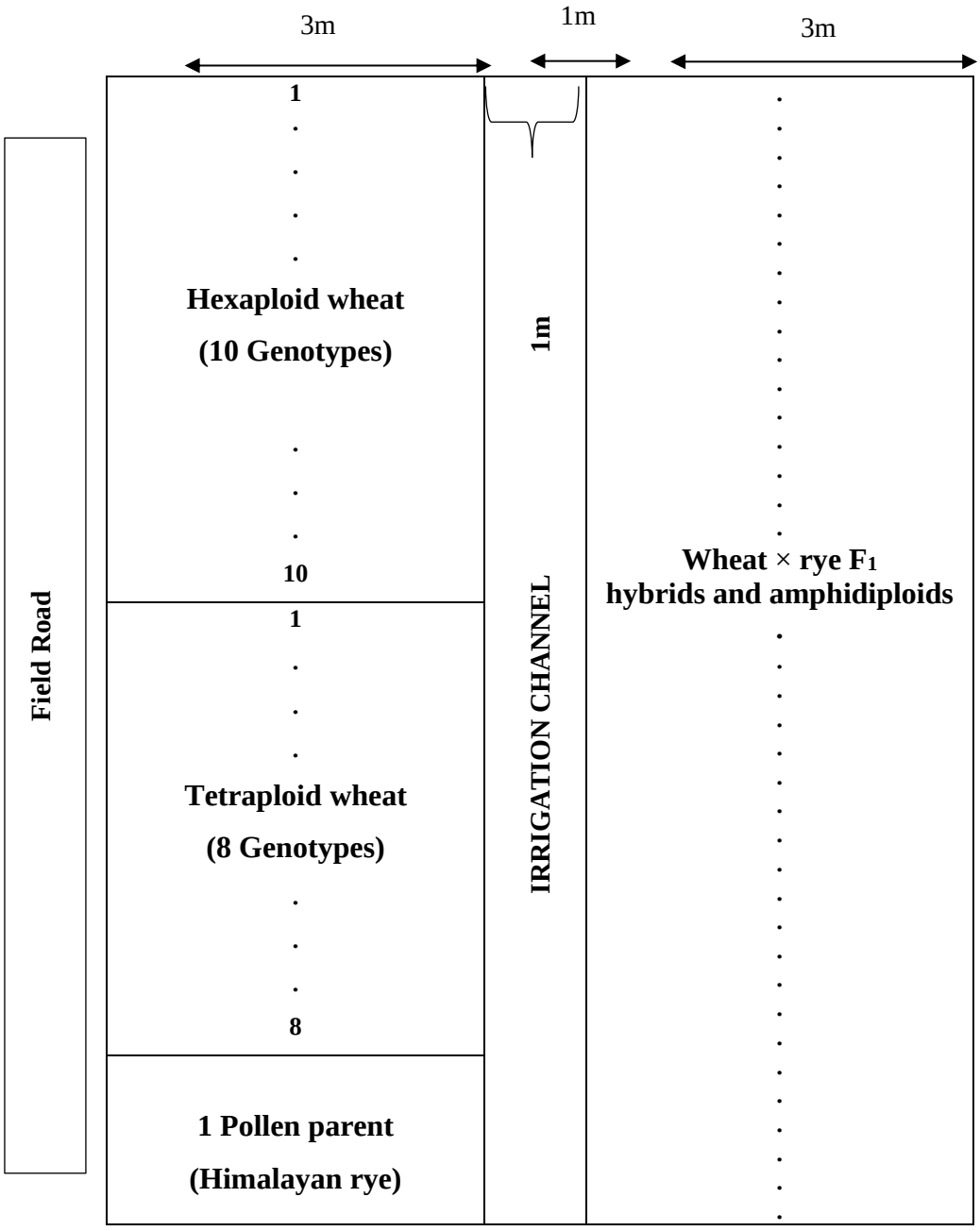
3.4.1.3 Sowing Plan and layout of wheat, rye and their F_1 hybrids (*Rabi* 2022-23)

During *Rabi* 2022-23, phenotypic data was recorded for parents, F_1 hybrids and amphidiplods. The data were recorded for 19 parental genotypes, 18 wheat × rye F_1 hybrids and 10 amphidiploid lines. Standard plant density was maintained, and experiments were conducted under the recommended package of practices.

Layout description:

Season:	*Rabi* 2022-23
Crop:	Wheat, rye, wheat × rye F_1 hybrids and amphidiploids
Number of Genotypes:	19
Size of Irrigation channel:	1m
Length of each line:	3m
No. of lines for each genotype:	Two lines
Net area:	73 m^2
Gross area:	76 m^2
Row to row distance:	22.5 cm
Date of sowing:	15/11/2022
The recommended dose of fertilizer:	@ 80: 40: 40 NPK kg/ha

Fig. 3. 1 Layout of Experimental plot (*Rabi* 2022-23)



3.4.2 Crossing method for wheat × rye

3.4.2.1 Emasculation

Removing immature anthers without damaging the stigma is known as emasculation. This process is carried out a couple of days before the anthesis stage. To begin, healthy wheat plants are chosen when their ears emerge. After selecting a healthy spike at the ear emergence stage, the awns were cut using scissors. Without causing harm to stigma, lemma and palea, the immature anthers were removed carefully using fine forceps. Emasculation is done in the evening time. The upper and basal spikelet's were removed to increase uniformity throughout the ear. To avoid contamination and to maintain humidity and moisture, the emasculated, healthy wheat spikes are covered using butter paper bags and paper clips. Details such as the emasculation date and genotype designation were inscribed on the tags to facilitate straightforward identification.

3.4.2.2 Pollination

In petri dishes, fresh pollen from Himalayan rye was collected. The petri dishes were sterilized in a 70% ethanol solution before collecting the pollen. The newly collected rye pollen was placed to the wheat stigma using a tiny camel hairbrush, ideally in the morning. The fresh Himalayan rye pollen was collected after every 15 to 20-minute interval. Petri plates and brushes are labelled and kept at a distance from each other for identification. After pollinating wheat, the spikes were covered with a butter paper bag and paper clips. Tags were tied with the date of pollination and the name of the pollen parent mentioned on them.

3.4.3 Crossability of tetraploid and hexaploid wheat cultivars with diploid Himalayan rye

After harvesting hybrid seeds, their quantity was measured, and each seed underwent examination to ascertain its likely origin. In the *Rabi* season of 2021-22, wheat cultivars were categorized into Group III. The level of crossability between wheat and Himalayan rye was expressed as a percentage, calculated from the total number of seeds obtained to the number of pollinated florets in each cross. The classification of different wheat genotypes adhered to the criteria outlined by Lein (1943) and was categorized accordingly.

Genotype	Crossability Percentage	Group
Kr1 Kr1 Kr2 Kr2	<5	I
Kr1 Kr1 kr2 kr2	10-30	II
kr1 kr1 Kr2 Kr2	30-50	III
kr1 kr1 kr2 kr2	>50	IV

Based on crossability percentage, the probable genotype of each cultivar was identified.

3.4.4 Application of 2, 4-D solution

A solution containing 2, 4-D at a concentration of 100 parts per million (ppm) was injected into the uppermost internode of the wheat spikelet that had already been pollinated. This injection process was carried out 24, 48, and 72 hours post-pollination. Three distinct treatments were employed to evaluate the effect of 100 ppm 2, 4-D on the crossability percentage of wheat with rye.

In the first treatment, a single dose of 2, 4-D at 100 ppm was injected 24 hours after pollination with rye. The second treatment involved administering two doses of 2, 4-D at 100 ppm after 24 and 48 hours post-pollination. The third treatment included three doses of 2, 4-D at 100 ppm injected at 24, 48, and 72 hours after pollination. In all cases, a freshly prepared 100 ppm 2, 4-D solution was stored in a black-labeled glass bottle under cool conditions. To prevent potential leakage, Vaseline was applied to cover the injection holes.

3.4.5 Synthesis of triticale's

3.4.5.1 Primary octoploid triticale's

The development of primary octoploid triticale is a hybridization between hexaploid wheat and diploid Himalayan rye. The seeds obtained after crossing hexaploid wheat with Himalayan rye are sterile.

3.4.5.1.1 Colchicine treatment

Bell (1950) protocol was followed for applying colchicine solution to hexaploid wheat × rye F_1 hybrid plants. The colchicine treatment is given to the plants in two different

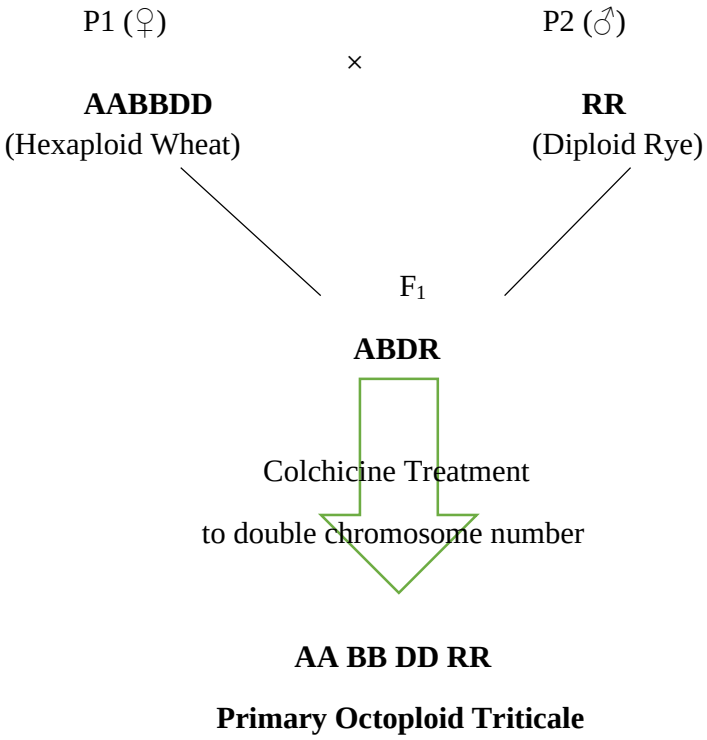
methods. The plants are uprooted and washed thoroughly with distilled water using root absorption. The roots are dipped in the solution for 6 hours. Special attention was given to ensure that the top part, specifically the crown, was immersed in the solution. Following this treatment, the plants were removed from the colchicine solution, left in flowing water overnight and then transplanted into pots filled with the potting mixture. In the injection method, one or more tillers (including the main axis) on the young plants were injected with a hypodermic needle. The injected holes were covered with Vaseline to prevent leakage. The method of application, growing stages of the plant, and concentration of the colchicine solution are detailed in Table 3.3.

Table no. 3.3 Concentration and method of application of colchicine solution

Concentration of solution	pH	Method of application	Duration of application
Colchicine (0.05%) + DMSO (3 %)	5.5-5.7	Root and Crown Immersion	6 hrs.
Colchicine (0.1%) + DMSO (3 %)	5.5-5.7	Root and Crown Immersion	6 hrs.
Colchicine (0.05%)	5.5-5.7	Injection	-
Colchicine (0.1%)	5.5-5.7	Injection	-

***DMSO- Dimethyl sulfoxide**

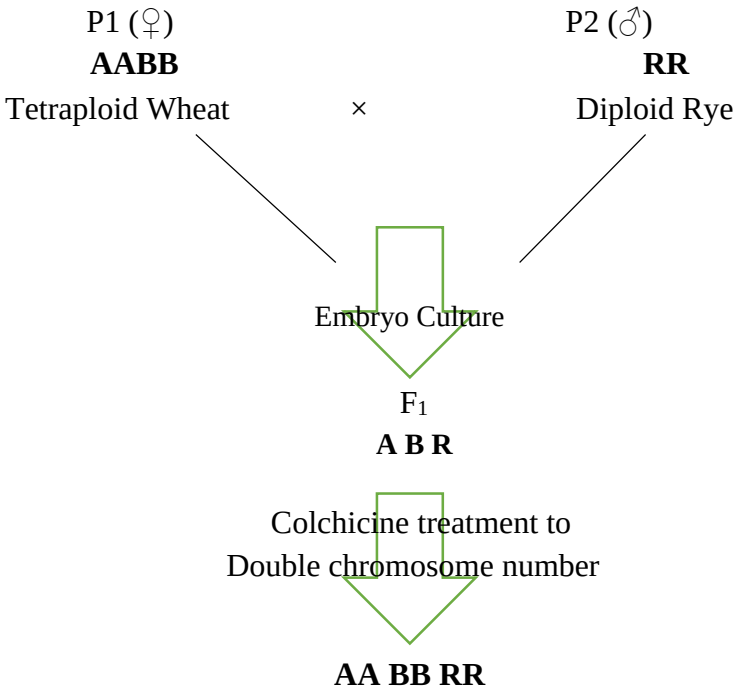
Fig. 3.2 Development of Primary Octoploid triticale



3.4.5.2 Primary hexaploid triticale

The development of primary hexaploid triticale involves the hybridization process between tetraploid wheat and diploid Himalayan rye. The requirement for embryo culturing arises from the lack of endosperm in the caryopsis. The colchicine treatment is not given to the tetraploid wheat × Himalayan rye F_1 hybrid because fewer plants were obtained after embryo culture.

Fig. 3.3 Development of primary hexaploid triticale



3.4.5.2.1 Media used and their preparation

During the culturing process, Murashige and Skoog's (1962) medium was employed and modified with varying concentrations and combinations of growth regulators, as outlined in Table 3.4.

Table 3.4 MS media composition for the culture of wheat × rye embryos

MACROELEMENTS	**mg/L**
NH4NO3	1650.000
$CaCl_2$	332.200
MgSO4	180.690
KNO_3	1900.000
KH_2PO_4	170.000
MICROELEMENTS	
H3BO3	6.200
Cl2CoH12O6	0.025
CuSO4. 5H2O	0.025
EDTA disodium salt dihydrate	37.300
FeSO4	27.800
MnSO4. H2O	16.900
Molybdic acid (sodium salt)	0.2
KI	0.830
ZnSO 4·7H2O.	8.600
VITAMINS	
Myo-Inositol	100.000
Nicotinic acid (free acid)	0.500
Pyridoxine HCl	0.500
Thiamine hydrochloride	0.100
AMINO ACID	

Glycine	2.000
OTHERS	
MES	500.000
Sucrose	30000.000
Agar-Agar	8000.000

In a 1000 ml flask, the stock solutions and sucrose were added, and a final solution of 1000 ml was made by adding sterilized water. The pH was adjusted between 5.5-6.0 and agar-agar was added and boiled until the agar-agar was dissolved. The liquid media were poured into 25 × 150 mm culture tubes at a rate of 15 ml per tube. The tubes were plugged with cotton plugs and autoclaved at 15 psi pressure for 20 min. The media were then allowed to set in the form of a slant.

Table 3.5 MS media supplemented with various growth regulators

Media	Growth Regulators	Quantity (mg/l)
MS Media	IAA	1
	Kinetin	0.5
MS Media	IAA	1
	Kinetin	0.5
	Casein Hydro lysate (CH)	500-1000
MS Media	2,4-D	1

3.4.5.2.2 Embryo culture

The fertilized spikes were removed from the field, specifically from the base of the plant, between 13 and 22 days after pollination. The age of the hybrid embryos significantly impacted their growth, and their developmental stage was a critical aspect during the culture process. Younger embryos (13-15 days post-pollination) tended to be weaker, thin

and abnormal-looking plantlets because they either stopped growing after experiencing an initial size rise or germinated too soon. On the other hand, because there was no endosperm present, the older embryos (20-22 days after pollination) had already started to degenerate and shrivel. These embryos produced callus with deformed plantlets or died in culture (Bajaj, 1990).

The caryopsis, detached from the spike, was properly cleaned with distilled water and surface sterilized with 0.1% mercuric chloride for two to three minutes to prevent contamination. One by one, the caryopses were examined under the microscope. An incision was made around the acutellum after the scalpel was cooled after being submerged in alcohol for a brief period to burn off the alcohol. Like how the scalpel was sterilized, forceps were used to hold the caryopsis in place. Afterwards, the forceps were used to peel back the testa and pericarp. Under aseptic conditions, the embryo was removed with the tip of a scalpel and deposited on the agar slant in the culture tube. Every time an instrument was used, it was flamed once more. To avoid contamination, the culture tube's entrance was flamed both before the embryo was placed inside and again right before it was sealed. After the embryos germinated and produced roots, the cultures were kept in darkness at 20-22 ° C. Afterward, they were exposed to 16 hours of light per day at 25 ± 2 °C. The developing plantlets were prepared for transplantation after reaching the culture tube's top.

3.4.5.2.3 Transfer of plantlets to soil less media

The young plants were relocated to small pots measuring 5 cm in diameter, containing a soil-less medium to facilitate root and shoot development. This soil-less medium was created by combining coco peat, vermiculite, and perlite in a ratio of 3:1:1. Ideal conditions were upheld to support healthy growth until the plants reached maturity.

3.4.6 Recording of observations on various traits

The observations were recorded at different growth stages on an individual plant basis on a randomly selected plant and tagged each plant separately. A brief description of the procedure adapted for recording observation for various traits is given as follows:

a. Germination (%):

$$\frac{Seed\ germinated}{Seeds\ sown} \times 100$$

b. Seedling survived (%):

$$\frac{Seedling\ survived}{Seeds\ germinated} \times 100$$

c. Survival to maturity after colchicine treatment:

$$\frac{Seedlings\ survived\ to\ maturity}{Seedlings\ treated\ with\ colchicine} \times 100$$

For some of the traits, visual observations were taken, and scores were mentioned against each trait, given:

d. Coleoptile colour:

0: green in colour; 1: light purple in colour; 2: medium purple in colour; 3: dark purple in colour

e. Stem colour (recorded at 3-4 tiller stage):

0: green; 1: light purple; 2: medium purple; 3: dark purple

f. Auricles:

Present / Absent

g. Auricle pubescence (intensity)

0: no pubescence; 1: less pubescence; 2: more pubescence

h. Shoot-base colour (recorded at the time of heading):

0: green; 1: light purple; 2: medium purple; 3: dark purple

i. Waxy leaf - sheath

0: non waxy; 1: less waxy; 2: medium waxy; 3: more waxy

j. Greenness of leaves:

1: light green; 2: medium green; 3: dark green.

k. Node colour:

0: green; 1: light purple; 2: medium purple; 3: dark purple

l. Hairy - peduncle (intensity):

0: non-hairy; 1: low intensity; 2: medium intensity; 3: high intensity; 4: very high intensity

m. Early Growth habit:

E: erect; SS: semi-spreading; S: spreading

n. Days to heading:

The number of days between the date of planting and the first heading.

o. Days to anthesis:

The number of days from the date of planting to the first anthesis.

p. Days to maturity:

The number of days it takes from the planting date to fully mature.

q. Productive tillers/plant:

The number of productive tillers per plant was recorded.

r. Plant Height (cm):

Excluding awns, measurements were taken from the plant's base to the tip of the main spike.

s. Internodes/plant:

Number of internodes recorded per plant in the main tiller.

t. Awn length (cm):

Recorded from the top of the terminal spikelet to the tip of the awn in the main spike of the plant.

u. Spike length (cm):

Excluding awns, measurements were taken from the base of the main spike to the top of the spike.

v. Spikelet's/Spike:

The number of spikelets recorded in the main spike of the plant.

w. No. of seeds/spike:

Average and range recorded for the number of seeds/spike in the hybrids.

x. Grain colour:

A: amber; LR: light red; R: red

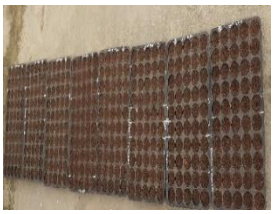
y. Grain length (mm):

The average length of 1-5 grains is recorded, depending upon the number of grains available.

z. Grain width:

Thick v/s thin, a visual observation.

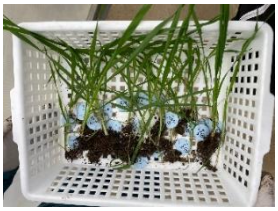
Preparation medium for seed sowing



Sowing of seeds in Port trays

Plants are in 3-4 leaf stage

Plants are in 2-3 tillering stage



Plants are uprooted for treatment

Plants are washed with distilled water

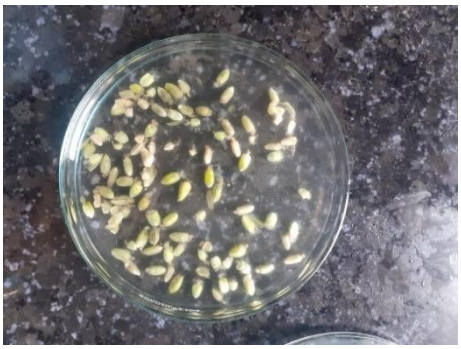
Plants are dipped in 0.05 & 0.1% colchicine solution for 6 hrs.

After treatment plants are transferred in to field

Plants are in maturity stage

Plate no. 3.1 Pictorial representation of colchicine treatment to the development of primary octoploid triticale

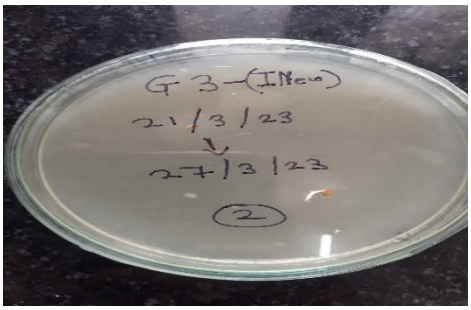
Spikes are harvested after 13-22 days after pollination



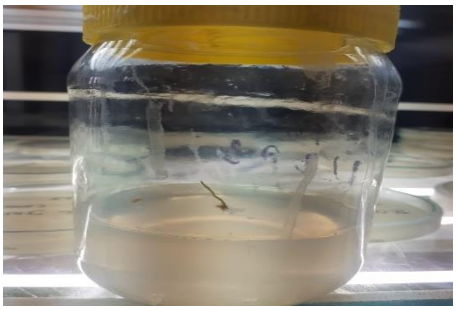
Crossed seeds of Durum wheat × Himalayan rye

Embryo rescue from crossed seeds

Embryo obtained from caryopsis



Culture is kept under light for 16 hrs. daily at 25 ± 2 ^{0}C



Plant is regenerated from embryo

Plate no. 3.2 Pictorial representation of embryo culture of tetraploid wheat

Plate no. 3.3 Pictorial representation of comparison between F_1 hybrid along its parent's Right side- Wheat (Female), Middle- F_1 hybrid, Himalayan Rye (Male)

Plate no. 3.4 Right side - F_1 hybrids of Hexaploid wheat × Himalayan rye, Left side- Amphidiploids (A_1) of Hexaploid wheat × Himalayan rye

Hexaploid Wheat × Himalayan rye

F_1 hybrid (Sterile)

A_1 Amphidiploids (Fertile)

Plate no. 3. 5 Pictorial representation of parents and their F_1 hybrids

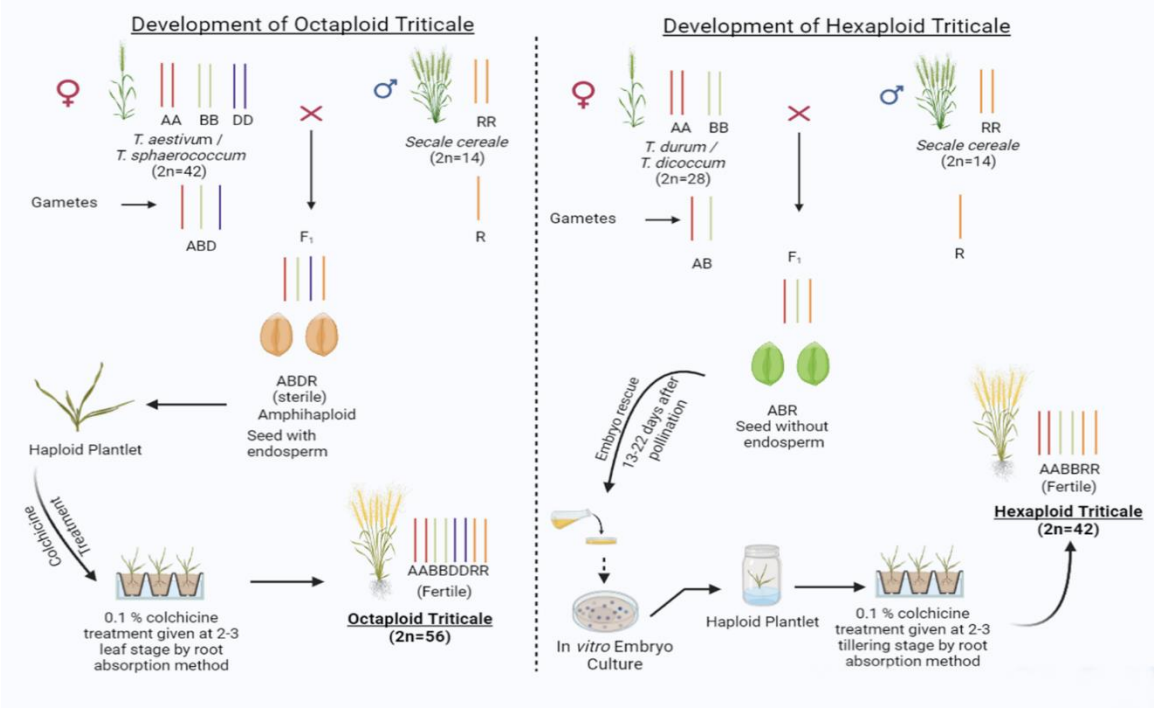


Plate no. 3. 6 Pictorial representation of the development of Octoploid and Hexaploid Triticale

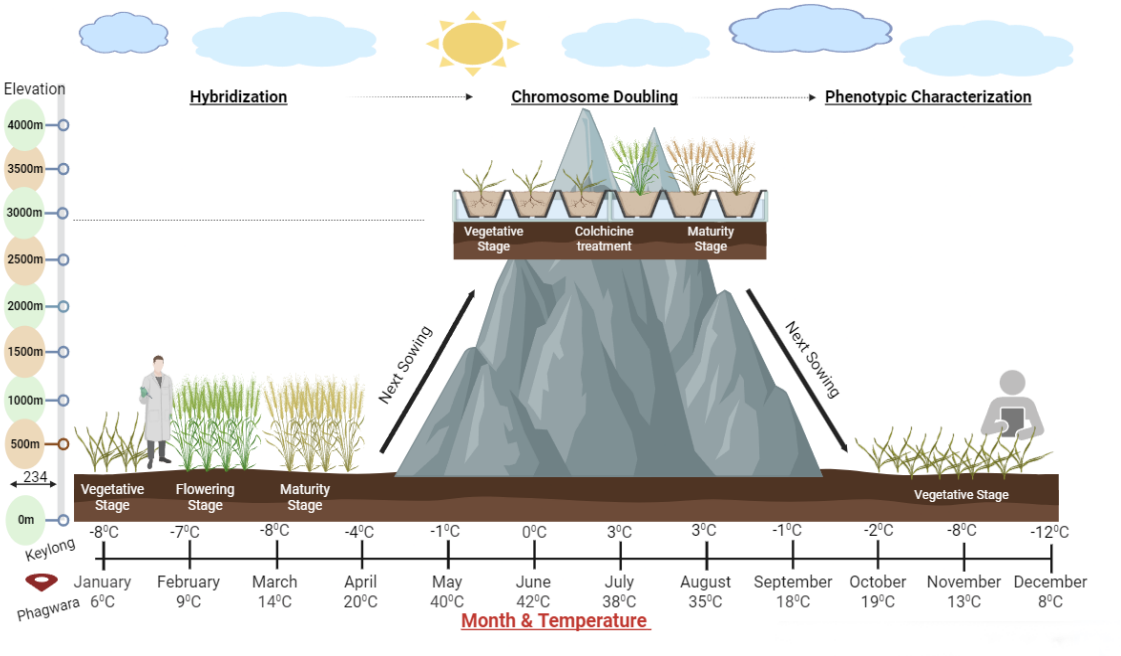


Plate no. 3. 7 Pictorial representation of summary of the experiment Hexaploid Triticale

CHAPTER 4

RESULTS AND DISCUSSION

Wheat is among the most valuable staple food crops, feeding almost 80 per cent of the population and providing 20 per cent (one-fifth) of the total food calories and protein in human nutrition (Shiferaw *et al*., 2013). Hence, it remains a central pillar of food security. In order to address the nutritional needs of the expanding global population and ensure socio-economic stability, the primary imperative is to enhance wheat production. Breeders around the globe have been working for decades to develop widely adaptable wheat cultivars with improved yield and resistance to various biotic and abiotic stresses resulted in the 'Green Revolution' during the sixties by developing semi-dwarf wheat cultivars through introgression of light and heat insensitivity genes. These elite cultivars have been the epicenter for wheat breeding for nearly four decades and have contributed to humongous growth in crop production; since the 1980s, a plateau has been observed in wheat production. This is a key concern for wheat breeders, as the population continues to grow logarithmically, and the statistical growth in the supply will not meet the demands in the coming years.

Conventional methods, such as selection, are used to improve wheat steadily. However, they lead to increased selection pressure and ultimately narrow down the genetic base of the crop, which will result in high-productivity cultivars and increased vulnerability to biotic and abiotic stresses (Hoisington *et al*., 1999). The common parentage of most high-yielding cultivars further complicates this situation by making them prone to genetic breakdown. Moreover, the steadily changing climate and continuously evolving pathogens have worsened this situation. Thus, the major focus of all crop improvement endeavours is to sustainably broaden the crop's genetic base. Wild relatives serve as a vast reservoir for crucial agronomic traits and contribute allelic diversity to enhance the genetic makeup of current high-yielding cultivars. Within the spectrum of wild relatives, rye has demonstrated exceptional capabilities as a contributor to disease resistance (specifically against powdery mildew, stem rust, stripe rust, and leaf

rust), stress tolerance (such as resistance to water and temperature stress), and favourable grain quality traits. These attributes can potentially augment the diversity and adaptability of commercially cultivated wheat varieties, as outlined by Friebe *et al*., (1996) and Kim *et al*., (2003).

Rye (*Secale cereale*) is a closely related wild relative to wheat. The introduction of genetic material into the wheat genome is made possible by the significant collinearity observed between the genomes of both species. This facilitates the creation of interspecific chromosomal translocation and substitution lines. Rye is a valuable alternative genetic resource in wheat, extensively utilized for its diverse and crucial traits, encompassing resistance genes against fungal diseases in wheat, various agronomic traits, and qualities pertinent to end-use applications.

Considering the above information, the present study entitled "Development of noval wheat-rye amphidiploid lines" focused on developing rye chromatin in wheat cultivars. The experimental results obtained during research work have been described under the following heads:

4.1 Crossability percentage

4.1.1 Crossability of Hexaploid wheat with Himalayan rye

4.1.2 Crossability of Tetraploid wheat with Himalayan rye

4.2 Effect of phytohormone application

4.2.1 Effect of 2, 4-D on Hexaploid wheat

4.2.2 Effect of 2, 4-D on Tetraploid wheat

4.3 Germination and Seedling survived percentage in Hexaploid wheat

4.4 Effect of concentration of colchicine solution on Hexaploid wheat

4.5 Response of Hexaploid wheat × Himalayan rye F_1 hybrids to colchicine solution

4.6 Percentage of embryos obtained in Tetraploid wheat

4.7 Effect of age of Embryo on different developmental stages in Tetraploid wheat

4.8 Effect of Tetraploid wheat on different developmental stages

4.9 Effect of media and growth hormone

4.10 Expression of Himalayan rye traits in F_1 hybrids and amphidiploids

4.10.1 Hexaploid wheat × Himalayan rye

4.10.2 Tetraploid wheat × Himalayan rye

4.1 Crossability percentage

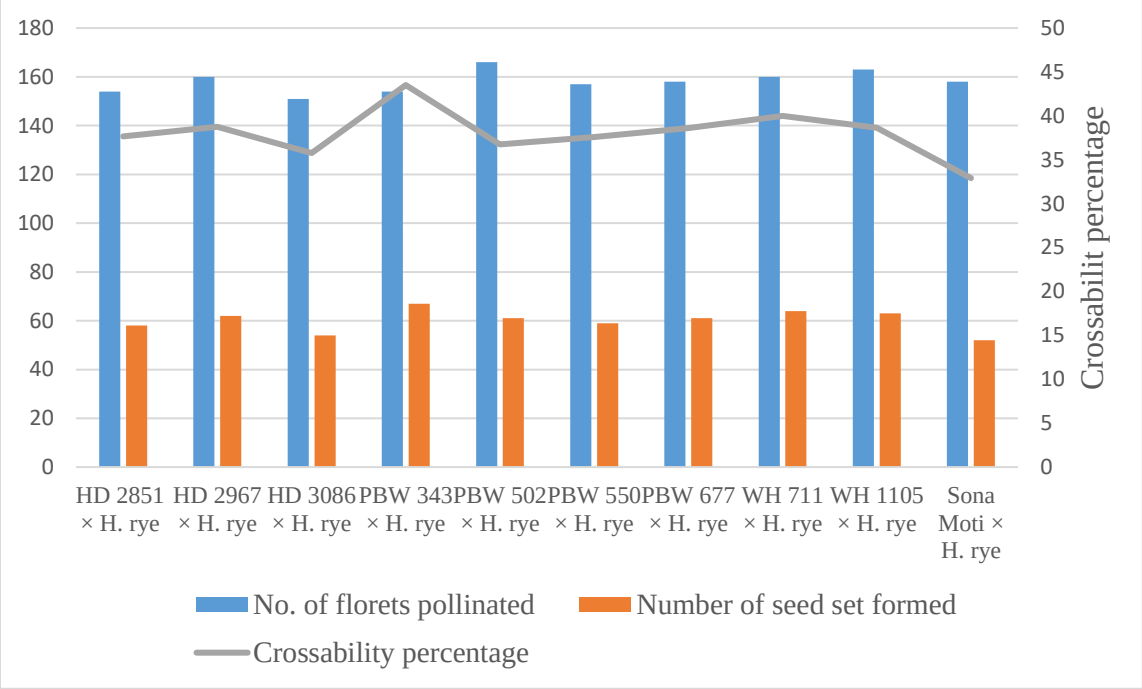
4.1.1 Crossability of Hexaploid wheat with Himalayan rye

The extent of crossability observed between wheat and rye is contingent upon the specific cultivars of both wheat and rye involved. The investigation into the crossability of diverse hexaploid wheat varieties with diploid rye revealed variability in crossability among the distinct wheat cultivars. The crossability dynamics between wheat and rye are influenced by two loci, *Kr1* and *Kr2*, where dominant alleles play a role in reducing crossability, as documented by Molnar-Lang *et al.*, (2015). Furthermore, various genetic factors, such as the *Ph1* gene, play a role in influencing the crossability between wheat and rye, as emphasized by the findings of Laugerotte *et al*., (2022).

The crossability percentage of hexaploid wheat with H. rye (Himalayan rye) ranges from 32.91 to 43.51. The general mean crossability percentage in hexaploid wheat and H. rye was 38.02%. The highest crossability percentage was observed in hexaploid wheat PBW 343 × H. rye (43.51%), followed by WH 711 × H. rye (40.00%), HD 2967 × H. rye (38.75%), WH 1105 × H. rye (38.65%), PBW 677 × H. rye (38.61%), HD 2851 × H. rye (37.66%), PBW 550 × H. rye (37.58%), PBW 502 × H. rye (36.75%), and HD 3086 × H. rye (35.76%) during the *Rabi* season 2021-22 (Graph 4.1).

As per Lein's (1943) crossability group, all hexaploid wheat cultivars belong to group III (*kr*1 *kr*1 *Kr*2 *Kr*2).

Genotype	Crossability percentage	Group
Kr1 Kr1 Kr2 Kr2	<5	I
Kr1 Kr1 kr2 kr2	10-30	II
kr1 kr1 Kr2 Kr2	30-50	III
kr1 kr1 kr2 kr2	>50	IV



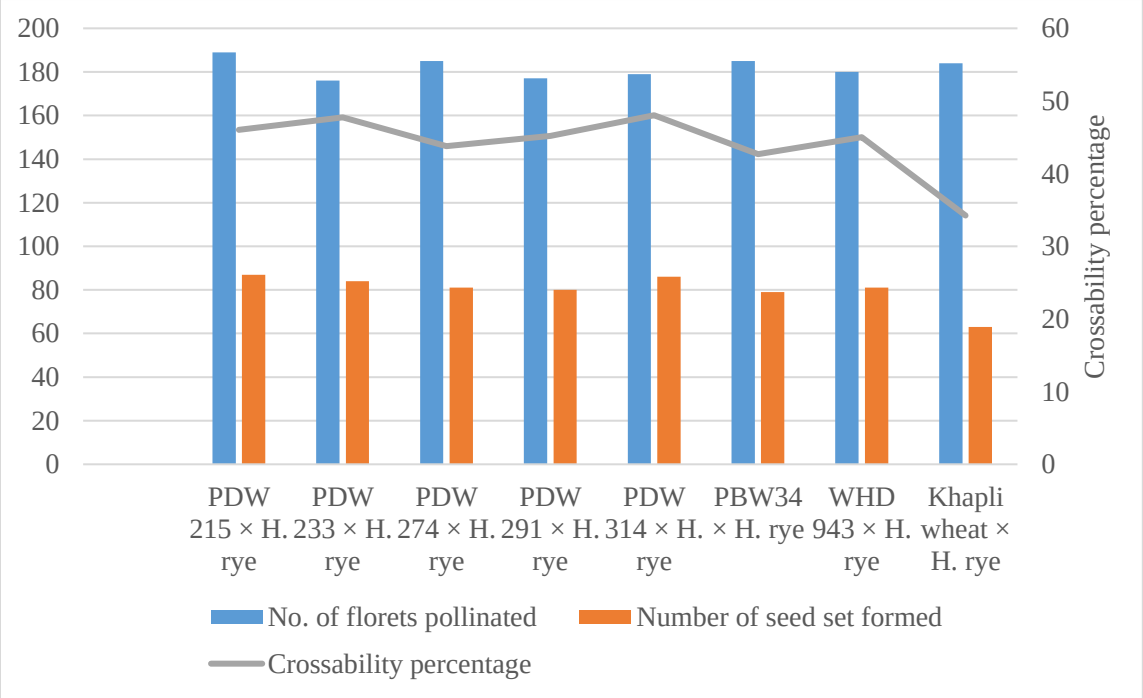
Graph 4. 1 Comparative cross ability of Hexaploid wheat (*T. aestivum / T. sphaerococcum*) cultivars with Himalayan rye landrace (*Secale cereale*)

4.1.2 Cross ability of Tetraploid wheat with Himalayan rye

An examination into the crossability of tetraploid wheat with Himalayan rye (H. rye) involved investigating the extent of crossability among tetraploid wheat varieties with H. rye. The findings revealed that the crossability of tetraploid wheat with H. rye is

comparatively modest, yet certain wheat genotypes displaying elevated seed sets have been identified.

The percentage of crossability of tetraploid wheat with H. rye ranged from 34.24 to 48.04. The general mean crossability percentage in tetraploid wheat and H. rye was 44.09%. In general, durum wheat showed better crossability than *T. aestivum* wheat did. However, the seeds obtained in the durum hybrids were markedly shriveled and invisible than those obtained in the hybrids with *T. aestivum*. The highest crossability percentage is obtained in tetraploid wheat PDW 314 × H. rye which is 48.04% followed by PDW 233 × H. rye (47.73%), PDW 215 × H. rye (46.03%), PDW 291 × H. rye (45.20%), WHD 943 × H. rye (45.00%), PDW 274 × H. rye (43.78%) and PBW 34 × H. rye (42.70%), during the *Rabi* season 2021-22. As per Lein's (1943) crossability group, all tetraploid wheat cultivars belonged to group III (*kr1 kr1 Kr2 Kr2*) (Graph 4.2).



Graph 4.2 Comparative cross ability of Tetraploid wheat (*T. durum / T. dicoccum*) cultivars with Himalayan rye landrace (*Secale cereale*)

4.2 Effect of phytohormone application

4.2.1 Effect of 2, 4-D on hexaploid wheat

The limited crossability observed in wheat cultivars carrying the dominant *Kr*1 and *Kr*2 alleles with rye, as indicated by Lange and Wojciechowska (1976), can be attributed to the hindrance and eventual inhibition of pollen tube growth at the base of the style and within the transmitting tissue of the ovary wall. This impediment results in the prevention of any pollen tubes from stretching out the embryo sac. The induction of parthenocarpy in wheat through 2, 4-D has a notable impact on ovule structure, akin to its effects in maize. It is reasonable to anticipate that this may similarly influence the cross-compatibility of wheat with other genera.

The application of 2, 4-D solution was given to pollinated hexaploid wheat spikes at three different periods at 24 h., 48 h. and 72 h. after pollination with H. rye. The percentage of hybrid seeds obtained in all three treatments ranged from 14.63 to 59.46. The mean for obtaining hybrid seeds without treatment was 19.57%. The mean value for the maximum percentage of hybrid seeds was obtained 72 h. after pollination (52.75 %), followed by 43.50% (48 h.), and 36.72 % in 24 h. after the pollination. The maximum percentage of hybrid seeds obtained was 59.46% in the WH 1105 × H. rye cross after 72 h. after pollination. The most effective treatment for the hexaploid wheat cultivar was observed at 72 h. after pollination (Table 4.1).

Table 4.1 Effect of 2, 4-D application at 24 h., 48 h. and 72 h. on pollinated hexaploid wheat (*Triticum aestivum / Triticum sphaerococcum*) spike

	Name of Genotype	No. of florets pollinated	No. of hybrid seeds obtained	Percentage of hybrid seeds obtained
Control (Without treatment)	***Triticum aestivum***			
	HD 2851	36	8	22.22
	HD 2967	40	9	22.50
	HD 3086	36	7	19.44

	PBW 343	42	9	21.43
	PBW 502	42	8	19.05
	PBW 550	41	8	19.51
	PBW 677	42	9	21.43
	WH 711	38	7	18.42
	WH 1105	41	6	14.63
	Triticum sphaerococcum			
	Sona Moti	35	6	17.14
24 hrs.	***Triticum aestivum***			
	HD 2851	42	13	30.95
	HD 2967	39	14	35.90
	HD 3086	39	13	33.33
	PBW 343	37	17	45.95
	PBW 502	38	14	36.84
	PBW 550	35	13	37.14
	PBW 677	37	14	37.84
	WH 711	41	15	36.59
	WH 1105	39	16	41.03
	Triticum sphaerococcum			
	Sona Moti	41	13	31.71
48 hrs.	***Triticum aestivum***			
	HD 2851	39	17	43.59
	HD 2967	38	18	47.37
	HD 3086	35	16	45.71
	PBW 343	35	19	54.29
	PBW 502	45	18	40.00

	PBW 550	39	17	43.59
	PBW 677	44	18	40.91
	WH 711	43	19	44.19
	WH 1105	46	19	41.30
	Triticum sphaerococcum			
	Sona Moti	44	15	34.09
72 hrs.	***Triticum aestivum***			
	HD 2851	37	20	54.05
	HD 2967	43	21	48.84
	HD 3086	41	18	43.90
	PBW 343	40	22	55.00
	PBW 502	41	21	51.22
	PBW 550	42	21	50.00
	PBW 677	35	20	57.14
	WH 711	38	23	60.53
	WH 1105	37	22	59.46
	Triticum sphaerococcum			
	Sona Moti	38	18	47.37

4.2.2 Effect of 2, 4-D on tetraploid wheat

Application of 2, 4-D solution for three different periods on pollinated tetraploid wheat spikes at 24 h. 48 h. and 72 h. after the pollination. The percentage of hybrid seeds obtained in all three treatments ranged from 22.00 to 71.05. The mean for obtaining hybrid seeds without treatment was 28.41%. The mean value for the maximum percentage of hybrid seed obtained in 72 h. after the pollination, *i.e.* 55.59% followed by 50.85% in 48 h. and

42.32 % in 24 h. The maximum percentage of hybrid seeds obtained was 71.05 in the cross between PDW 314 × H. rye after 72 h. after pollination. The tetraploid wheat cultivars observed the most effective treatment at 72 h. after pollination (Table 4.2).

Similar results were also reported in 1974 by Kruse in barley ears with 10-100 ppm 2,4-D two to three days prior to pollination, giving rise to approximately 80% seed set and 10-20% embryo formation after hybridization with a wide range of grasses including *Avena sativa, T. monococcum, Lolium perenne,* and *Festuca pratensis.*

Table 4.2 Effect of 2, 4-D application at 24 h., 48 h., and 72 h. on pollinated tetraploid wheat (*Triticum durum / Triticum dicoccum*) spike

	Name of Genotype	No. of florets pollinated	No. of hybrid seeds obtained	Percentage of hybrid seeds obtained
Control (Without)	***Triticum durum***			
	PDW 215	46	14	30.43
	PDW 233	42	13	30.95
	PDW 274	45	12	26.67
	PDW 291	43	13	30.23
	PDW 314	43	14	32.56
	PBW34	51	12	23.53
	WHD 943	42	13	30.95
	Triticum dicoccum			
	Khapli wheat	50	11	22.00
24 hrs.	***Triticum durum***			
	PDW 215	49	21	42.85
	PDW 233	46	21	45.65
	PDW 274	42	20	47.61
	PDW 291	47	19	40.42
	PDW 314	48	21	43.75

	PBW34	47	20	42.55
	WHD 943	48	20	41.66
	Triticum dicoccum			
	Khapli wheat	44	15	34.09
	Triticum durum			
	PDW 215	43	24	55.81
	PDW 233	40	23	57.5
	PDW 274	50	23	46
	PDW 291	41	22	53.65
	PDW 314	50	24	48
	PBW34	41	22	53.65
	WHD 943	41	23	56.09
	Triticum dicoccum			
	Khapli wheat	47	17	36.17
72 hrs.	***Triticum durum***			
	PDW 215	51	28	54.90
	PDW 233	48	27	56.25
	PDW 274	48	26	54.16
	PDW 291	46	26	56.52
	PDW 314	38	27	71.05
	PBW34	46	25	54.34
	WHD 943	49	25	51.02
	Triticum dicoccum			
	Khapli wheat	43	20	46.51

4.3 Germination and Seedling survived percentage in Hexaploid wheat

To identify the effect of parental genotype on the germination of hexaploid wheat × H. rye F_1 crosses, we separated 20 F_1 seeds from each cross. The germination percentage varied from 55 to 90. The highest germination is obtained in cross WH 1105 × H. rye, which is 90%. The general mean germination rate in hexaploid wheat was 66 %. The variation in the germination of F_1 hybrids is due to different ploidy levels in male and female parents and their genetic compatibility. The percentage of seedlings that survived to maturity ranged from 55 - 90%. The highest germination and seedling survived is obtained in the same cross WH 1105 × H. rye, which is 90% (Table 4.3).

Table 4.3 Germination and seedling survived percentage in hexaploid wheat × Himalayan rye crosses

Sr. No.	Name of F_1 hybrid	No. of seeds Sown	No. of seeds germinated	Germination Percentage	No. of Seedling survived	Seedling survived percentage
1.	**HD 2851 × H. rye**	20	14	70	13	65
2.	**HD 2967 × H. rye**	20	12	60	12	60
3.	**HD 3086 × H. rye**	20	15	75	15	75
4.	**PBW 343 × H. rye**	20	15	75	15	75
5.	**PBW 502 × H. rye**	20	17	85	16	80
6.	**PBW 550 × H. rye**	20	13	65	13	65

7.	**PBW 677 × H. rye**	20	11	55	11	55
8.	**WH 711 × H. rye**	20	17	85	17	85
9.	**WH 1105 × H. rye**	20	18	90	18	90
10.	**Sona Moti × H. rye**	20	13	65	11	55

4.4 Effect of concentration of colchicine solution in Hexaploid wheat

Colchicine is a widely used chemical to induce chromosome doubling in plants, which can lead to polyploidy. Colchicine prevents the segregation of chromosomes during meiosis, resulting in the production of gametes with double the number of chromosomes. The colchicine concentration used to induce polyploidy varies across plant species and has been successfully used in various crop plants. The success of chromosome doubling depends on several factors, such as the solution concentration, method of application, and plant part treatment. Colchicine solution with Dimethyl sulfoxide (DMSO) treatment showed better results than without Dimethyl sulfoxide (DMSO). The colchicine solution with DMSO increased the percentage of induced polyploid seedlings, indicating its potential role as an adjuvant for colchicine treatment.

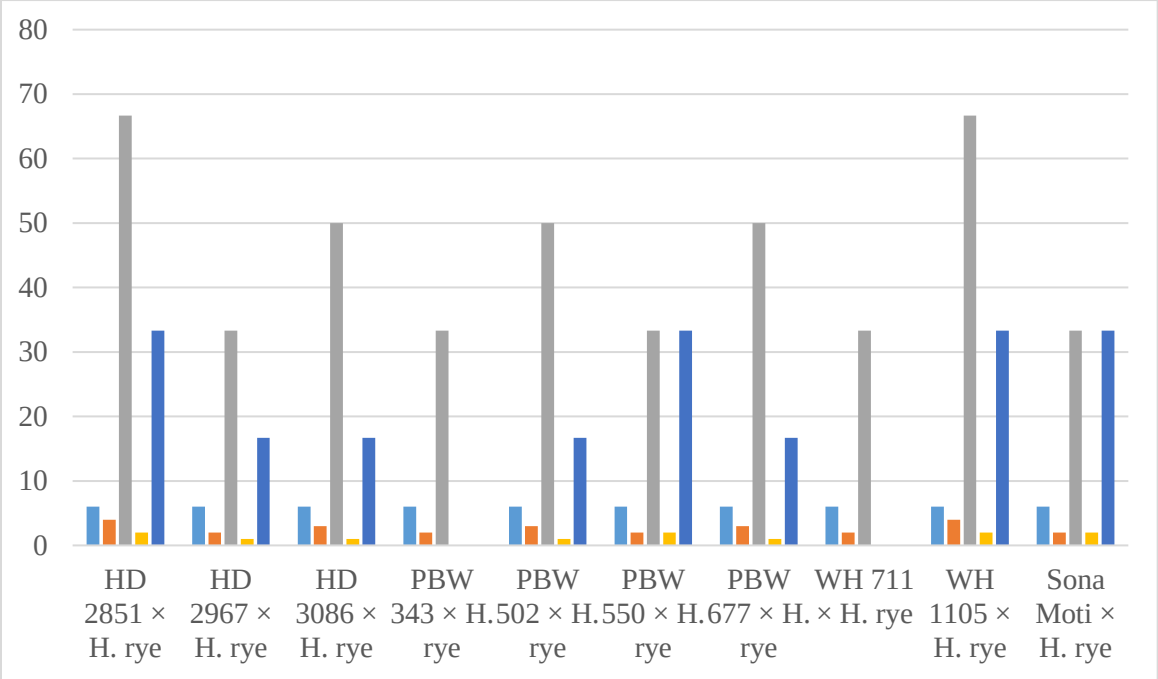
Sixty plants were treated with different solution concentrations using two different application methods. Based on these results, the root and crown immersion methods are more effective than the injection method. The number of plants that survived after the colchicine treatment was higher in 0.05% colchicine solution with 3 % DMSO, but number of plants with seed set was low (13.33). In the second treatment 0.1% colchicine with 3% DMSO), the number of plants that survived after treatment was low, but number of plants with seed set was higher (20 %) (Table 4.4). Bajaj (1990), Bell (1950), Chaikam *et al.*, (2019 and 2020), and Winkle and Kimber (1976) obtained similar results.

Table 4.4 Effect of various concentrations and methods of application of colchicine solution on the plant growth and seed set

Concentration of solution	pH	Method of application	No. of plants treated	No. of plants survived after treatment	Percentage of plant survival	No. of plants having seed set	Percentage of plants having seed set
Colchicine (0.05%) + DMSO (3 %)	**5.5-5.7**	Root and Crown Immersion	15	8	53.33	2	13.33
Colchicine (0.1%) + DMSO (3 %)	**5.5-5.7**	Root and Crown Immersion	15	7	46.67	3	20.00
Colchicine (0.05%)	**5.5-5.7**	Injection	15	6	40	1	6.67
Colchicine (0.1%)	**5.5-5.7**	Injection	15	6	40	2	13.33

4.5 Response of Hexaploid wheat × Himalayan rye F_1 hybrids to colchicine solution

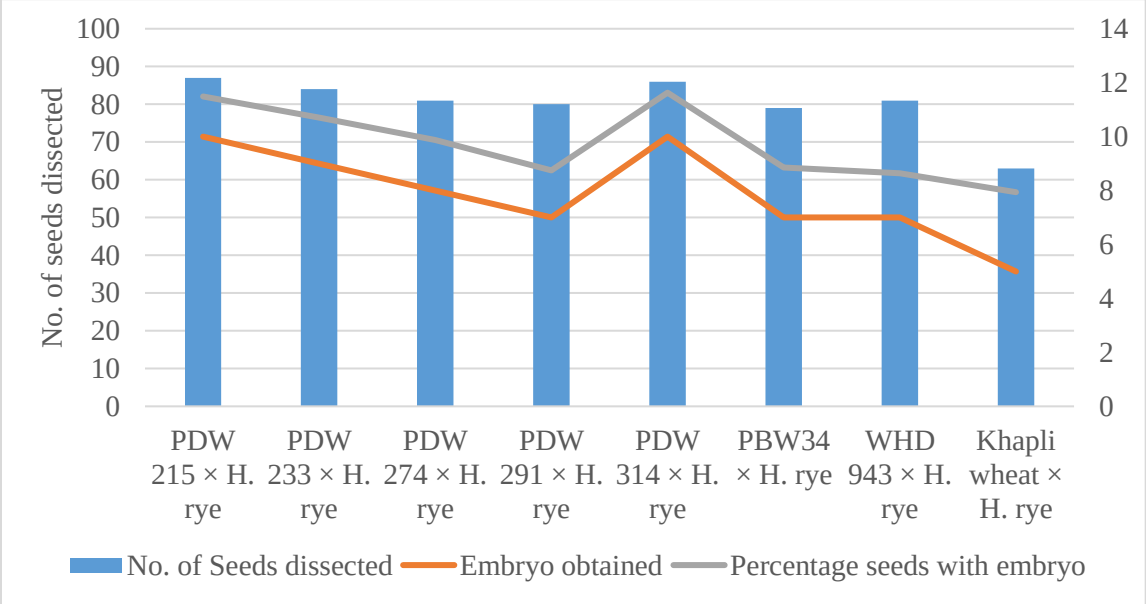
Colchicine solution was applied to sixty crosses between hexaploid wheat and H. Rye. The survival of plants following the treatment varied among different genotypes. Twenty-seven plants survived to maturity. The percentage of plants that survived until maturity is 33.33 to 66.67. A higher percentage of plants that survived until maturity was obtained in two crosses, HD 2851× H. Rye and WH 1105 × H. Rye (66.67%). Fertility restoration and seed set formation in the spikes ranged from 0 to 2. The number of spikes obtained with the seeds was 4. In four crosses, the percentage of spikes with seeds was high at 33.33% (Graph 4.3).



Graph 4.3 Response to colchicine for survival and seed set of Hexaploid wheat × Himalayan rye crosses

4.6 Percentage of embryos obtained in Tetraploid wheat

In tetraploid wheat, the number of seeds dissected and the embryos obtained differed in different genotypes. Embryos obtained from dissected seeds ranged from 5 to 10. The highest number of embryos was obtained in two crosses, PDW 215 × H. rye and PDW 314 × H. rye, which was 10 (Graph 4.4).



Graph 4.4 Percentage of seeds with embryo in tetraploid wheat (*T. durum* / *T.dicoccum*) × Himalayan rye cross

4.7 Effect of age of Embryo on different developmental stages in Tetraploid wheat

Embryos sourced from young seeds, spanning a developmental range of 13 to 22 days post-pollination, underwent cultivation on synthetic media. Notably, the developmental stage of the hybrid embryo at the initiation of the culture process emerged as a crucial factor, with their growth substantially impacted by their chronological age. A total of 63 embryos were cultured, of which 22 were germinated. Of the germinated embryos, only six developed calli. Seven were developed for minute shoots, and five were developed for minute shoots. More embryos germinated, and a percentage of plantlets with roots and shoots were obtained in 16-19 days after pollination (Table 4.5).

Three groups were formed based on the age of the embryos.

Group I	13-15 Days after pollination
Group II	16-18 Days after pollination
Group III	19-22 Days after pollination

Younger embryos, specifically those within the 13-15 days after pollination (DAP) range, exhibited a tendency to halt growth following an initial enlargement, or they proceeded to undergo premature germination, resulting in the development of feeble, slender, and atypically formed plantlets. On the other hand, the more mature embryos (20-22 DAP), where the abortion process had commenced due to the absence of endosperm, initiated a desiccation process, displaying indications of degeneration.

Table 4.5 Response of embryos of different ages to germination development of callus, minute shoots and plantlets with roots and shoot

Age of Embryo (DAP)	No. of Embryo cultured	No. of embryo germinated	Percentage of embryos germinated	No. of Callus developed	Percentage of Callus developed	No. of minute shoots	Percentage of minute shoots	No. of plantlets with roots and shoots	Percentage of plantlets with roots and shoots
13	4	0	0	0	0	0	0	0	0
14	4	1	25	0	0	0	0	0	0
15	5	2	40	0	0	1	20	0	0
16	7	3	42.86	1	14.29	1	14.29	0	0
17	8	3	37.5	1	12.50	1	12.5	1	12.5
18	8	4	50	2	25.00	1	12.5	1	12.5
19	8	4	50	1	12.50	1	12.5	2	25
20	8	3	37.50	1	0	1	12.50	1	12.50
21	5	1	20	0	0	0	0	0	0
22	6	1	16.67	0	0	1	16.67	0	0

4.8 Effect of Tetraploid wheat on different developmental stages

Of the 22 embryos, the highest number germinated in cross PDW 314 × H. rye (4). A higher percentage of callus (14.29 %) was obtained in two crosses, PDW 291 × H. rye and WHD 943 × H. rye. The percentage of plantlets with roots and shoots is observed in PDW 314 × H. rye, which is 20 (Table 4.6).

Table 4.6 Response of tetraploid wheat genotypes, in combination with rye, to embryo germination, development of callus, minute shoots and plantlets with roots and shoot

Name of F_1 hybrid	No. of Embryo cultured	No. of embryo germinated	Percentage of embryos germinated	No. of Callus developed	Percentage of Callus developed	No. of minute shoots	Percentage of minute shoots	No. of plantlets with roots and shoots	Percentage of plantlets with roots and shoots
PDW 215 × H. rye	10	3	30.00	0	0	2	20	1	10
PDW 233 × H. rye	9	3	33.33	1	11.11	0	0	0	0
PDW 274 × H. rye	8	3	37.50	0	0	1	12.5	1	12.5
PDW 291 × H. rye	7	2	28.57	1	14.29	1	14.29	0	0
PDW 314 × H. rye	10	4	40	2	20	0	0	2	20
PBW34 × H. rye	7	3	42.86	1	0	2	28.57	0	0
WHD 943 × H. rye	7	2	28.57	1	14.29	1	14.29	0	0
Khapli wheat × H. rye	5	2	40	0	0	0	0.00	1	20

4.9 Effect of media and growth hormones

The embryos obtained after dissection were kept on an MS medium supplemented with various growth hormones. Three different media were used for culturing: MS media with Indole-3- acetic acid (1 mg/l) + Kinetin (0.5 mg/l) + Casein Hydro lysate (500-1000 mg/l) had the highest percentage of callus development, the highest percentage of minute shoots and the highest percentage of plantlets with roots and shoots which was 14.29 (Table 4.7).

Table 4.7 Effect of media and growth hormones on embryo germination and development of callus, minute shoot and plantlets with roots and shoot

Medium	No. of Embryo cultured	No. of embryo germinated	Percentage of embryos germinated	No. of Callus developed	Percentage of Callus developed	No. of minute shoots	Percentage of minute shoots	No. of plantlets with roots and shoots	Percentage of plantlets with roots and shoots
MS + IAA (1 mg/l) + kinetin (0.5 mg/l)	21	7	33.33	2	9.52	2	9.52	2	9.52
MS + IAA (1mg/l) + kinetin (0.5 mg/l) + CH (500-1000 mg/l)	21	10	47.62	3	14.29	3	14.29	3	14.29
MS + 2,4-D (1 mg/l)	21	5	23.81	1	4.76	2	9.52	0	0

4.10 Expression of rye traits (characters) in F_1 hybrids and amphidiploids

For comparison, various characters were observed in hexaploid and tetraploid wheat, Himalayan rye and their corresponding F_1 hybrids and amphidiploids, as shown in Table 4.8.

The average of the parents, F_1 hybrids and amphidiploids for various traits was recorded. Moreover, the number of hybrids showing complete resemblance to wheat or H. rye parents or exceeding either of the parents for the expression of traits are given below in the table. Crosses that resembled H. rye fully or partially or were of the intermediate type, and the crosses, being exceptional cases that exceeded both parents for expressing a trait, have been highlighted.

4.10.1 Hexaploid wheat × Himalayan rye hybrids

Coleoptile Colour-

The coleoptile colour was green colour for four wheat parents (HD 2851, HD 2967, PBW 343, and WH 711), light purple for five wheat parents (HD 3086, PBW 502, PBW 550, PBW 677, and WH 1105) and medium purple for one wheat cultivar (Sona moti). Nevertheless, Himalayan rye (H. rye) exhibited a coleoptile with a dark purple and the expression of this trait, both complete and partial, was noted in seven specific F_1 hybrids and their amphidiploids (HD 3086) × H. rye, PBW 502 × H. rye, PBW 550 × H. rye, PBW 677 × H. rye, WH 711 × H. rye, WH 1105 × H. rye, Sona moti × H. rye). In the remaining three crosses H. rye trait was not expressed.

Stem Colour-

The stems were green in colour in five wheat parents (HD 2851, HD 2967, PBW 343, PBW 677, WH 711), light purple in three wheat parents (HD 3086, PBW 502, PBW 550), and medium purple in two wheat parents (WH 1105, Sona moti). Nevertheless, the stems of H. rye exhibited a dark purple colour. Seven F_1 hybrids and their amphidiploids displayed either complete or partial expression of this specific trait of H. rye, which is HD 3086 × H. rye, PBW 502 × H. rye, PBW 550 × H. rye, PBW 677 × H. rye, WH 711 × H. rye, WH

1105 × H. rye, Sona moti × H. rye. In the remaining three crosses H. rye trait was not expressed.

Auricles-

The auricles were present in all the wheat parents under study but were absent in the H. rye, and the H.rye characteristic did not express in any of the hybrid progeny.

Auricle pubescence intensity-

The four wheat parents had less pubescent (PBW 502, PBW 550, WH 711, WH 1105), and Six wheat parents were more pubescent (HD 2851, HD 2967, HD 3086, PBW 343, PBW 677, Sona moti). Nevertheless, Himalayan rye (H. rye) lacked pubescence, and partial expression of the rye trait was observed in three F_1 hybrids: HD 2967 × H. rye, HD 3086 × H. rye, PBW 677 × H. rye. In the remaining seven hybrids H. rye trait was not expressed.

Shoot-base colour-

The shoot base was light purple in three wheat parents (HD 3086, PBW 502, and WH 1105), medium-purple in six wheat parents (HD 2851, HD 2967, PBW 343, PBW 550, PBW 677, and WH 711) and dark purple had one wheat parent (Sona moti). On the contrary, Himalayan rye (H. rye) exhibited a shoot base colour of medium purple. The complete expression of H. rye traits was observed in six F_1 hybrids, namely HD 2851× H. rye, HD 3086× H. rye, PBW 343 × H. rye, PBW 502 × H. rye, PBW 550 × H. rye, WH 1105 × H. rye. In the remaining four hybrids, the H. rye trait was not expressed.

Waxy leaf sheath-

Each wheat parent demonstrated lower waxiness on the leaf sheath, unlike Himalayan rye (H. rye), which displayed markedly higher waxiness on the leaf sheath. Both complete and partial expression of this trait was noted in nine amphidiploids (HD 2851 × H. rye, HD 2967 × H. rye, HD 3086 × H. rye, PBW 343 × H. rye, PBW 502 × H. rye, PBW 677 × H. rye, WH 711 × H. rye, WH 1105 × H. rye, Sona moti × H. rye). In the remaining crosses, the H. rye trait was suppressed with less waxiness in wheat.

Greenness of leaves-

The leaves were light green in five wheat parents (HD 2851, HD 3086, PBW 502, PBW 550, Sona moti) and medium green in the remaining five wheat parents (HD 2967, PBW

343, PBW 677, WH 711, WH 1105). Nevertheless, Himalayan rye (H. rye) exhibited leaves with dark green, and this trait's complete and partial manifestation was evident in all the crosses.

Node colour-

The nodes were green in five wheat parents (PBW 502, PBW 550, PBW 677, WH 711, WH 1105) and medium purple in the remaining five parents (HD 2851, HD 2967, HD 3086, PBW 343, Sona moti). In contrast, the H. rye had dark purple nodes. Eight F_1 hybrids and amphidiploids had dark purple nodes, namely HD 2851 × H. rye, HD 2967 × H. rye, HD 3086 × H. rye, PBW 343 × H. rye, PBW 677 × H. rye, WH 711 × H. rye, WH 1105 × H. rye, Sona moti × H. rye. In the remaining two hybrids, the H. rye trait was expressed.

Hairy- peduncle (intensity)-

Wheat lacked hair on its peduncle, while Himalayan rye (H. rye) displayed a significantly high density of hair on the peduncle (neck), a trait replicated in all the F_1 hybrids and amphidiploids. However, the extent of expression varied distinctly among different amphidiploids, with four exhibiting a medium (HD 2851 × H. rye, HD 2967 × H. rye, PBW 550 × H. rye, WH 711 × H. rye), six had medium intensity of hairs (HD 3086 × H. rye, PBW 343 × H. rye, PBW 502 × H. rye, PBW 677 × H. rye, WH 1105 × H. rye, Sona moti × H. rye).

Early growth habit-

The growth habit was erect in all wheat parents. The H. rye had a spreading growth habit, and three F_1 hybrids and amphidiploids (WH 711 × H. rye, WH 1105 × H. rye, Sona moti × H. rye) partially resembled this trait. The remaining seven crosses H. rye trait, were not expressed.

Days to heading-

The eight wheat parents were early (90-100 days), namely HD 2851, HD 2967, HD 3086, PBW 343, PBW 502, PBW 550, PBW 677, Sona moti, and the two wheat parents were

medium (101–115 days) namely WH 711 and WH 1105 for heading. However, H. rye exhibited a late heading phenotype at 116 days. Partial similarity to this specific H. rye trait was identified in eight F_1 crosses (HD 2851 × H. rye, HD 2967 × H. rye, HD 3086 × H. rye, PBW 343 × H. rye, PBW 502 × H. rye, PBW 550 × H. rye, PBW 677 × H. rye, Sona moti × H. rye). The cross WH 1105 × H. rye notably showed an earlier heading than both parents.

Days to anthesis-

Of the wheat parents in the study, all ten were early (98-120 days) in anthesis. However, the H. rye was medium (121- 135) for 132 days for anthesis. Partial expressions of this H. rye character were found in nine F_1 hybrids, namely HD 2851 × H. rye, HD 2967 × H. rye, HD 3086 × H. rye, PBW 343 × H. rye, PBW 502 × H. rye, PBW 550 × H. rye, PBW 677 × H. rye, WH 711 × H. rye and Sona moti × H. rye. The cross WH 1105 × H. rye occurred earlier in anthesis than both parents.

Days to maturity-

All the ten wheat parents were early (141-170 days). The H. rye was medium (175 days) at maturity. Seven F_1 hybrids (HD 2851 × H. rye, HD 2967 × H. rye, HD 3086 × H. rye, PBW 343 × H. rye, WH 711 × H. rye, WH 1105× H. rye and Sona moti × H. rye) partially resembled H. rye in this trait. However, the two hybrids matured earlier than both parents.

Productive tillers/plant-

The average number of productive tillers per plant in wheat was 7.43 (6.0 - 8.7). In hybrids was 7.41 (6.1- 8.7) and in the H.rye was 15.8. Four F_1 hybrids showed complete and partial resemblance with rye trait for a greater number of tillers, namely HD 3086 × H. rye, PBW 550 × H. rye, WH 711 × H. rye, and Sona moti × H. rye. The H.rye character was not expressed in six crosses.

Plant height (cm)-

All the wheat parents were medium (86-100 cm) in height. However, the H. rye is tall (>100 cm). The highest plant height was observed in F_1 hybrids HD 2967 × H. rye, which is 110.11 cm, followed by WH 1105 × H. rye (109.02), HD 3086 × H. rye (106.72), PBW 343 × H. rye (103.23), WH 711× H. rye (102.57), PBW 677 × H. rye (101.78), HD 2851 ×

H. rye(101.60), PBW 550 × H. rye(99.27), PBW 502 × H. rye (98.89), Sona moti × H. rye (98.42). All hybrids had greater plant heights than the wheat parents.

Internodes/plant-

The internode/plant in the wheat parent lies between 7-11. Whereas in rye it is 14. In five F_1 hybrids, Internodes/plant is more than the wheat parent, namely HD 2967 × H. rye, PBW 550 × H. rye, PBW 677 × H. rye and Sona moti × H. rye. In the remaining five hybrids, the rye trait was not expressed.

Awn length-

The average awn length for all the wheat parents was 6.25 cm (5.6-6.9 cm), whereas in H. rye, it was 6.3. In F_1 hybrids, the average awn length was 6.64 cm (5.6-7.0 cm). The highest awn length was observed in cross PBW 502 × H. rye, which is 7.0 cm. Seven crosses showed complete resemblance with the H. rye for the awn length, namely HD 2851× H. rye, HD 3086 × H. rye, PBW 502× H. rye, PBW 550 × H. rye, PBW 677 × H. rye, WH 1105 × H. rye, Sona moti. In the remaining three hybrids H. rye trait was not expressed.

Spike length-

The average spike length in the wheat parents was 8.99 cm (8.1-9.9), whereas in H. rye, it was 10.6 cm. The average spike length in hybrids was 8.95 cm (8.2-9.7). The highest spike length was observed in cross WH 711 × H. rye, 9.7 cm. Five F_1 hybrids showed completely or partially resembled the rye in spike length (HD 2851× H. rye, HD 3086 × H. rye, PBW 343 × H. rye, WH 711 × H. rye and Sona moti × H. rye). The remaining five hybrids H. rye trait, was not expressed.

Spikelets/spike-

The average number of spikelets/spike in wheat was 17.2 (14-20), whereas in H.rye, it was 22. The average number of spikelets/spikes in hybrids was 17 (15-19). The highest number of spikelets/spikes was observed in two F_1 hybrids PBW 677 WH 711 × H. rye and WH 711 × H. rye, 19. Four hybrids had a larger number of spikelets /spikes than did the wheat parent.

Number of seeds/spike -

The average number of seeds found in a wheat parent was 44 (38-49), and in H. rye was the same 44. The average number of seeds/spike in F_1 hybrid was 8.2 (5-12). The highest number of seeds/spike was observed in cross PBW 343 × H. rye which is 12 followed by PBW 502 (10), HD 2967 (9), HD 3086 × H. rye (8), PBW 550 × H. rye (8), PBW 677 × H. rye (8), WH 1105 × H. rye (8), HD 2851× H. rye (7), WH 711 × H. rye (7) and Sona moti × H. rye (5).

Grain colour-

All wheat parents have an amber grain colour, and for H. rye, it is light red. All the crosses have the same colour, like a wheat parent. There was no expression of H. rye traits in any hybrids.

Grain length-

The average grain length in the wheat was 7.3 mm (4.76 - 7.95 mm), and in H. rye, it was 9.07 mm. In F_1 hybrids, the average grain length is 5.26 mm (4.13 - 5.75 mm). There was no expression of the H. rye trait in any hybrids.

Grain width-

The H. rye grains were very thin compared to those of wheat, whereas the hybrids and wheat were comparable for this trait.

4.8. a Coleoptile colour, Stem colour, Auricles, Auricle pubescence and Shoot-base colour of wheat/rye (P_1), F_1 hybrids (F_1) and amphidiploids (A_1)

Sr. No.	Name of Genotype	Coleoptile colour			Stem colour			Auricles			Auricle pubescence (intensity)			Shoot-base colour	
Hexaploid wheat		**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**	**P_1**	**F_1**
1.	**HD 2851**	0	0	0	0	0	0	P	P	P	2	2	2	2	3
2.	**HD 2967**	0	0	0	0	0	0	P	P	P	2	1	2	2	2
3.	**HD 3086**	1	3	3	1	3	3	P	P	P	2	1	2	1	3
4.	**PBW 343**	0	0	0	0	0	0	P	P	P	2	2	2	2	3
5.	**PBW 502**	1	2	2	1	2	2	P	P	P	1	1	1	1	3
6.	**PBW 550**	1	3	3	1	3	3	P	P	P	1	1	1	2	3
7.	**PBW 677**	1	2	2	0	2	2	P	P	P	2	1	2	2	2
8.	**WH 711**	0	1	2	0	1	1	P	P	P	1	1	1	2	2
9.	**WH 1105**	1	3	3	2	3	3	P	P	P	1	1	1	1	2
10.	**Sona Moti**	2	3	3	2	3	3	P	P	P	2	2	2	3	3
Tetraploid wheat															
1.	**PDW 215**	0	0	-	0	1	-	P	P	-	2	2	-	1	2
2.	**PDW 233**	0	0	-	1	0	-	P	P	-	2	2	-	1	1
3.	**PDW 274**	0	0	-	0	1	-	P	P	-	2	1	-	2	2
4.	**PDW 291**	0	1	-	1	2	-	P	P	-	2	2	-	1	1
5.	**PDW 314**	0	0	-	0	0	-	P	P	-	2	2	-	1	2
6.	**PBW34**	1	2	-	2	3	-	P	P	-	2	1	-	1	2
7.	**WHD 943**	1	1	-	2	2	-	P	P	-	2	1	-	2	2
8.	**Khapli wheat**	1	3	-	2	3	-	P	P	-	2	2	-	3	3
Diploid rye															
1.	**Himalayan Rye**	3	-	-	3	-	-	A	-	-	0	-	-	2	-

4.8 b. Waxy leaf sheath, Greenness of leaves, Node colour, hairy peduncle (intensity) and Early growth habit of wheat/rye (P_1), F_1 hybrids (F_1) and amphidiploids (A_1)

Sr. No.	Name of Genotype	Waxy leaf sheath			Greenness of leaves			Node colour			Hairy peduncle (intensity)			Early growth habit		
Hexaploid wheat		**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**
1.	**HD 2851**	1	3	3	1	2	2	2	3	3	0	2	2	E	E	E
2.	**HD 2967**	1	2	3	2	3	3	2	3	3	0	2	2	E	E	E
3.	**HD 3086**	1	3	3	1	2	2	2	3	3	0	3	3	E	E	E
4.	**PBW 343**	1	2	2	2	3	3	2	3	3	0	3	3	E	E	E
5.	**PBW 502**	1	1	2	1	2	2	0	0	0	0	3	3	E	E	E
6.	**PBW 550**	1	1	1	1	2	3	0	0	0	0	3	2	E	E	E
7.	**PBW 677**	1	2	2	2	3	3	0	2	2	0	3	3	E	E	E
8.	**WH 711**	1	2	2	2	3	3	0	2	2	0	2	2	E	SS	SS
9.	**WH 1105**	1	2	2	2	3	3	0	2	2	0	3	3	E	SS	SS
10.	**Sona Moti**	1	3	3	1	2	2	2	3	3	0	2	3	E	SS	SS
Tetraploid wheat																
1.	**PDW 215**	1	3	-	2	3	-	0	0	-	0	3	-	E	E	-
2.	**PDW 233**	2	2	-	2	3	-	0	0	-	0	3	-	E	E	-
3.	**PDW 274**	1	3	-	2	3	-	0	0	-	0	3	-	E	E	-
4.	**PDW 291**	2	2	-	2	3	-	0	0	-	0	2	-	E	E	-
5.	**PDW 314**	2	3	-	2	3	-	0	0	-	0	3	-	E	E	-
6.	**PBW34**	2	3	-	2	3	-	0	2	-	0	2	-	E	E	-
7.	**WHD 943**	1	3	-	2	3	-	0	2	-	0	2	-	E	SS	-
8.	**Khapli wheat**	3	3	-	1	2	-	0	2	-	0	3	-	E	SS	-
Diploid rye																
1.	**Himalayan Rye**	3	-	-	3	-	-	3	-	-	4	-	-	S	-	-

***E- Erect, SS- Semi Spreading, S- Spreading**

4.8 c. Days to heading, Days to anthesis, Days to maturity, Productive tillers/plant and Plant Height of wheat/rye (P_1) and F_1 hybrids (F_1)

Sr. No.	Name of Genotype	Days to heading		Days to anthesis		Days to maturity		Productive tillers/plant		Plant Height (cm)	
Hexaploid wheat		**P_1**	**F_1**	**P_1**	**F_1**	**P_1**	**F_1**	**P_1**	**F_1**	**P_1**	**F_1**
1.	**HD 2851**	92	94	101	105	143	146	8.7	7.9	96.53	101.60
2.	**HD 2967**	93	113	102	113	141	149	8.3	8.0	95.29	110.11
3.	**HD 3086**	93	98	99	109	149	152	8.3	8.7	99.32	106.72
4.	**PBW 343**	90	91	98	99	147	149	6.2	6.1	97.01	103.23
5.	**PBW 502**	96	99	105	111	159	155	8.1	8.0	93.23	98.89
6.	**PBW 550**	91	98	100	108	153	153	6.3	6.9	91.56	99.27
7.	**PBW 677**	99	103	108	114	161	159	7.3	6.9	94.81	101.78
8.	**WH 711**	108	108	115	121	157	164	7.2	7.6	99.87	102.57
9.	**WH 1105**	111	107	118	115	151	161	7.9	7.7	101.02	109.02
10.	**Sona Moti**	90	94	99	107	145	149	6.0	6.3	91.37	98.42
Tetraploid wheat											
1.	**PDW 215**	105	104	117	117	165	161	6.9	-	91.00	92.82
2.	**PDW 233**	112	111	124	122	169	167	6.3	-	92.07	93.80
3.	**PDW 274**	103	102	115	113	160	163	7.1	-	92.91	93.08
4.	**PDW 291**	107	106	116	118	162	161	6.1	-	91.56	91.67
5.	**PDW 314**	98	99	109	112	162	169	6.5	-	93.04	95.51
6.	**PBW34**	110	111	117	119	171	167	5.9	-	91.20	96.22
7.	**WHD 943**	96	99	104	113	169	171	6.3	-	91.66	93.38
8.	**Khapli wheat**	92	94	104	102	160	169	5.7	-	90.22	94.64
Diploid rye											
1.	**Himalayan Rye**	116	-	132	-	175	-	15.8	-	147.51	-

4.8 d. Internodes/plant, Awn length (cm), Spike length (cm) and Spikelets/spike of wheat/rye (P_1) and F_1 hybrids (F_1)

Sr. No.	Name of Genotype	Internodes/plant		Awn length (cm)		Spike length (cm)		Spikelets/spike	
Hexaploid wheat		**P_1**	**F_1**	**P_1**	**F_1**	**P_1**	**F_1**	**P_1**	**F_1**
1.	**HD 2851**	8	8	6.5	6.7	8.4	8.8	14	16
2.	**HD 2967**	8	8	5.8	5.6	9.5	9.1	19	17
3.	**HD 3086**	7	7	6.0	6.4	8.1	8.4	15	16
4.	**PBW 343**	7	7	6.8	6.6	8.7	8.9	17	18
5.	**PBW 502**	8	8	6.2	7.0	9.4	8.8	19	15
6.	**PBW 550**	9	9	6.1	6.5	9.1	9.0	17	17
7.	**PBW 677**	9	9	6.3	6.4	9.9	9.6	20	19
8.	**WH 711**	9	9	6.9	6.7	9.6	9.7	19	19
9.	**WH 1105**	8	8	6.3	6.9	9.3	9.0	18	17
10.	**Sona Moti**	7	7	5.6	5.9	7.9	8.2	14	16
Tetraploid wheat									
1.	**PDW 215**	7	7	7.5	7.1	6.9	6.8	14	13
2.	**PDW 233**	8	8	6.9	7.1	7.2	7.3	16	16
3.	**PDW 274**	7	7	7.4	7.0	7.6	7.1	18	16
4.	**PDW 291**	8	8	7.3	7.6	7.8	7.9	18	18
5.	**PDW 314**	8	8	7.6	7.4	7.3	7.8	15	18
6.	**PBW34**	7	7	6.7	6.9	7.0	7.4	14	16
7.	**WHD 943**	8	8	6.3	6.8	6.7	7.2	13	15
8.	**Khapli wheat**	7	7	6.2	6.7	6.6	6.9	12	13
Diploid rye									
1.	**Himalayan Rye**	14	-	6.3	-	9.4	-	22	-

4.8 e. Number of seeds/spike, Grain colour, Grain length (mm) and Grain width of wheat/rye (P_1), F_1 hybrids (F_1) and amphidiploids (A_1)

S. No.	Name of Genotype	Number of seeds/spike			Grain colour			Grain length (mm)			Grain width		
Hexaploid wheat		**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**	**P_1**	**F_1**	**A_1**
1.	**HD 2851**	46	7	3	A	A	A	7.95	5.75	6.84	Thick	Thin	Thick
2.	**HD 2967**	49	9	3	A	A	A	7.62	5.68	5.51	Thick	Thin	Thick
3.	**HD 3086**	46	8	2	A	A	A	7.83	5.32	5.72	Thick	Thin	Thick
4.	**PBW 343**	48	12	4	A	A	A	7.68	4.89	5.04	Thick	Thin	Thick
5.	**PBW 502**	44	10	2	A	A	A	7.58	5.74	5.11	Thick	Thin	Thick
6.	**PBW 550**	48	8	3	A	A	A	7.77	5.13	5.46	Thick	Thin	Thick
7.	**PBW 677**	46	8	4	A	A	A	7.82	5.63	6.03	Thick	Thin	Thick
8.	**WH 711**	39	7	2	A	A	A	7.31	5.56	6.26	Thick	Thin	Thick
9.	**WH 1105**	36	8	3	A	A	A	7.42	4.77	5.49	Thick	Thin	Thick
10.	**Sona Moti**	38	5	1	A	A	A	4.76	4.13	4.88	Thick	Thin	Thick
Tetraploid wheat													
1.	**PDW 215**	39	-	-	A	-	-	7.24	-	-	Thick	-	-
2.	**PDW 233**	41	-	-	A	-	-	7.13	-	-	Thick	-	-
3.	**PDW 274**	41	-	-	A	-	-	7.29	-	-	Thick	-	-
4.	**PDW 291**	43	-	-	A	-	-	7.22	-	-	Thick	-	-
5.	**PDW 314**	45	-	-	A	-	-	7.14	-	-	Thick	-	-
6.	**PBW34**	43	-	-	A	-	-	7.33	-	-	Thick	-	-
7.	**WHD 943**	41	-	-	A	-	-	7.30	-	-	Thick	-	-
8.	**Khapli wheat**	38	-	-	LR	-	-	7.65	-	-	Thick	-	-
Diploid rye													
1.	**Himalayan Rye**	44	-	-	LR	-	-	9.07	-	-	Thin	-	-

***A- Amber, LR- Light Red**

4.10.2 Tetraploid wheat × Himalayan rye

The data was recorded only for the parents and their F_1 hybrids. Due to fewer plants regenerating after the embryo culture, colchicine treatment was not given to tetraploid wheat × H. rye hybrids. The data of tetraploid wheat and H. rye parent along with F_1 hybrid are given Table 4.8.

Coleoptile Colour-

The coleoptile colour exhibited green tones in five wheat parents (PDW 215, PDW 233, PDW 274, PDW 291, and PDW 314) and light purple in three wheat parents (PBW 34, WHD 943, and Khapli wheat). In contrast, Himalayan rye (H. rye) displayed a coleoptile with a dark purple. Complete and partial manifestation of this trait was evident in three F_1 hybrids, namely PDW 291 × H. rye, PBW 34 × H. rye and Khapli wheat × H. rye. However, the H. rye trait did not manifest in the five hybrids.

Stem Colour-

The stem colour displayed green tones in three wheat parents (PDW 215, PDW 274, and PDW 314), light purple in two wheat parents (PDW 233, PDW 291), and medium purple in three wheat parents (PBW 34, WHD 943, and Khapli wheat). In contrast, Himalayan rye (H. rye) exhibited a stem with a dark purple. Complete and partial manifestation of this trait was noted in five F_1 hybrids, namely PDW 215 × H. rye, PDW 274 × H. rye, PDW 291 × H. rye, PBW 34 × H. rye and Khapli wheat × H. rye. However, the H. rye trait did not manifest in the remaining three F_1 hybrids.

Auricles-

The auricles were present in all the wheat parents under study but were absent in the H. rye, and the H. rye characteristic did not manifest in any of the hybrid progeny.

Auricle pubescence intensity-

Auricle pubescence intensity was higher in all the wheat parents, H. rye had no pubescence, and the partial manifestation of H. rye character was observed in three F_1 hybrids, namely

HD 2967 × H. rye, HD 3086 × H. rye, and PBW 677 × H. rye. In the remaining seven F_1 hybrids H. rye trait was not expressed.

Shoot-base colour-

The shoot base was light purple with five wheat parents (PDW 215, PDW 233, PDW 291, PDW 314 and PBW 34), medium-purple in two wheat parents (PDW 274 and WHD 943), and dark purple had one wheat parent (Khapli wheat). Nevertheless, the H. rye had a medium-purple shoot base colour. Three F_1 hybrids showed complete expression of H. rye traits, namely PDW 215 × H. rye, PDW 314 × H. rye and PBW 34 × H. rye. In the remaining five F_1 hybrids, the H. rye trait was not expressed.

Waxy leaf sheath-

Among the wheat parents, PDW 215, PDW 274, and WHD 943 exhibited lower waxiness on the leaf sheath compared to Himalayan rye (H. rye), which had a notably higher waxiness. This trait showed complete and partial expression in five F_1 hybrids (PDW 215 × H. rye, PDW 274 × H. rye, PDW 314 × H. rye, PBW 34 × H. rye and WHD 943 × H. rye).

Greenness of leaves-

All the wheat parents medium green colour leaves except khapli wheat. However, the H. rye had dark green leaves, and this character's full and partial manifestation was observed in all the F_1 hybrids.

Node colour-

All wheat parents exhibited a green node colour, while Himalayan rye (H. rye) displayed dark purple nodes. The expression of the H. rye character was observed completely and partially in three F_1 hybrids, PBW 34 × H. rye, WHD 943 × H. rye and Khapli wheat × H. rye. In the remaining five F_1 hybrids, the H. rye trait was not expressed.

Hairy- peduncle (intensity)-

Wheat lacked hair on its peduncle, while Himalayan rye (H. rye) exhibited a high intensity of hair on the peduncle (neck). All eight F_1 hybrids showed a resemblance to this trait.

However, the degree of expression varied among different hybrids, with three displaying a medium intensity (PDW 291× H. rye, PBW 34 × H. rye and WHD 943 × H. rye), and five had high intensity of hairs (PDW 215 × H. rye, PDW 233 × H. rye, PDW 274 × H. rye, PDW 314 × H. rye and Khapli wheat × H. rye).

Early growth habit-

The growth habit was erect in all wheat parents. The H. rye had a spreading growth habit, and two crosses (WHD 943 and Khapli wheat) partially resembled this trait. The remaining six crosse's rye trait was not expressed.

Days to heading-

The three wheat parents were early (90-100 days), namely PDW 314, WHD 943, and Khapli wheat. The five wheat parents were medium (101-115 days), namely PDW 215, PDW 233, PDW 274, PDW 291 and PBW 34 for heading. However, H. rye was late (116 days) for heading, and a complete and partial resemblance of this H. rye character was obtained in four F_1 hybrids (PDW 314 × H. rye, PBW 34 × H. rye, WHD 943 × H. rye, Khapli wheat × H. rye). The remaining four F_1 hybrids headed earlier than both the parents.

Days to anthesis-

Seven of the wheat parents in the study were early (98-120 days), and one wheat parent, PDW 233, was in medium (121-135 days) in anthesis. However, the H. rye was medium (121- 135) for 132 days for anthesis. Full and partial manifestations of this H. rye trait were observed in four F_1 hybrids, namely PDW 291 × H. rye, PDW 314 × H. rye, PBW 34 × H. rye and WHD 943 × H. rye. The remaining four F_1 hybrids have earlier anthesis than both the parents.

Days to maturity-

Seven wheat parents were early (141-170 days) for days to maturity (PDW 215, PDW 233, PDW 274, PDW 291, PDW 314, WHD 943 and Khapli wheat), and one wheat parent has in medium 171 days of maturity. The v H. rye was medium 175 days (171-190 days) at maturity. Four F_1 hybrids (PDW 274 × H. rye, PDW 314 × H. rye, WHD 943 × H. rye and

Khapli wheat × H. rye) complete and partially resembled H. rye in this trait. However, the four F_1 hybrids matured earlier than both the parents.

Productive tillers/plant-

The average number of productive tillers/plants in wheat was 6.44 (5.7 -7.1); in H.rye, it was 15.8.

Plant height (cm)-

All the wheat parents were medium (86-100 cm) in height. However, the rye is tall (>100 cm). The highest plant height was observed in F_1 hybrids PBW 34 × H. rye which is 96.22 cm followed by PDW 314 × H. rye (95.51), Khapli wheat × H. rye (94.64), PDW 233 × H. rye (93.80), WHD 943 × H. rye (93.38), PDW 274 × H. rye (93.08), PDW 215 × H. rye (92.82), PDW 291 × H. rye (91.67). All F_1 hybrids had greater plant heights than the wheat parents.

Internodes/plant-

The internode/plant in wheat parent is between 7-8. Whereas in H. rye, it is 14. The internode/plant in wheat and their hybrid were similar. There is no change in wheat parents and their hybrids.

Awn length-

The average awn length for all the wheat parents was 6.98 (6.2-7.6), whereas in the H. rye, it was 6.3. In F_1 hybrids, the average awn length was 7.07 cm (6.8-7.8cm). The highest awn length was observed in cross PDW 291 × H. rye, which is 7.6. Five crosses showed complete or partial resemblance with the H. rye for the awn length, which is PDW 233 × H. rye, PDW 291 × H. rye, PBW 34 × H. rye, WHD 943 × H. rye and Khapli wheat × H. rye. The remaining three F_1 hybrids, H. rye trait, were not expressed.

Spike length-

The average spike length in the wheat parents was 7.13 cm (6.6-7.8 cm), whereas in H. rye, it was 10.6 cm. In hybrids, the average spike length was 7.31 cm (6.8-7.9). The highest spike length was observed in cross PDW 291 × H. rye, which is 7.9 cm. Six F_1 hybrids showed completely or partially resembled the H. rye in spike length, namely PDW 233 ×

H. rye, PDW 291 × H. rye, PDW 314 × H. rye, PBW 34 × H. rye, WHD 943 × H. rye and Khapli wheat × H. rye. In the remaining two F_1 hybrids H. rye trait was not expressed.

Spikelets/spike-

The average number of spikelets/spikes in wheat was 15 (12-18), whereas in H. rye it was 22. The average number of spikelets/spikes in F_1 hybrids was 15.62 (13-18). The highest number of spikelets/spikes was observed in two F_1 hybrids, PDW 291 × H. rye and PDW 314 × H. rye, which is 18. Complete or partial manifestation of H. rye character was observed in six crosses, namely PDW 233, PDW 291 × H. rye, PDW 314 × H. rye, PBW 34 × H. rye, WHD 943 × H. rye, Khapli wheat × H. rye. The remaining two F_1 hybrids, H. rye trait, were not expressed.

Number of seeds/spike -

The average number of seeds obtained in a wheat parent was 41.37 (38-45), and in H. rye was the same 44.

Grain colour-

All wheat parents have amber grain color except khapli wheat it was Light red, and for H. rye, it is light red.

Grain length-

The average grain length in the wheat was 7.28 mm (7.13- 7.65 mm), and in H. rye, it was 9.07 mm.

Grain width-

The H. rye grains were very thin compared to those of wheat.

DISCUSSION

In recent years, the pool of potential genes available to wheat breeders has shrunk alarmingly, and it appears that much of the genetic variability in cultivated wheat has already been lost. However, cultivated wheat has many relatives possessing desirable genes that can be introgressed into wheat. The first and foremost requirement for utilizing such variability is information on the extent to which these relatives can be successfully crossed with wheat.

Rye emerges as the most desirable relative of wheat, given its status as a cultivated crop endowed with valuable characteristics, including disease resistance, resilience to environmental challenges such as drought and cold, adaptability to acidic soil, high lysine content, and efficient phosphorus uptake. Research endeavours focusing on the crossbreeding of wheat with other species have primarily emphasized its compatibility with rye. Recently, there has been a heightened emphasis on investigating crossbreeding due to the growing utilization of alien species for enhancing wheat improvement traits.

A robust barrier of incompatibility exists between wheat and rye, governed by the genes *Kr1* and *Kr2*, situated on chromosomes 5B and 5A, respectively (Riley and Chapman, 1967; Lange and Riley, 1973), with *Kr1* exhibiting greater strength than *Kr2*. Additionally, *Kr3* on chromosome 5D, identified by Krolow (1970) and Fedak and Jui (1982), has a comparatively weaker influence. The positioning of *Kr1* and *Kr2* on the long arm of chromosomes 5B and 5A was determined by Sitch *et al.*, (1985). Studies by Falk and Kasha (1983), Sitch *et al.*, (1985), and Sitch and Snape (1986) have elucidated that *Kr* genes not only impact the crossability of wheat with rye but extend to other species like *H. bulbosum*. Therefore, a crossability study is an aid in selecting genotypes of wheat that are likely to give better success in hybridizing wheat with the alien species of interest. Furthermore, the applicability of the bulbosum technique for haploid production in wheat is confined to crossable wheat genotypes.

Such a crossability study will be particularly useful for the development of triticale involving crossable wheat to expand the genetic base of triticale, whose narrow genetic base has been the main constraint to increasing the pace of its improvement (Chaudhary

and Joshi, 1985). Few studies have investigated the crossability of improved semi-dwarf high-yielding locally adapted wheat and local Indian cultivars with rye, particularly those from the northwest region of India.

A study on the crossability of such wheat cultivars with rye is expected to enhance triticale improvement by enlarging genetic variability among triticale and subsequent development of high-yielding triticale lines suited for Indian conditions.

a. Crossability percentage

In this investigation, the examination of crossability levels among various genotypes of hexaploid wheat, tetraploid wheat, and diploid rye was conducted. Significant disparities were noted among different hexaploid and tetraploid wheat cultivars in their crossability with rye, aligning with the observations of previous researchers (Rigin, 1968 and 1976; Prabhakar Rao, 1968; Falk and Kasha, 1981; Jalani and Moss, 1981; Tanner and Falk, 1981; Oettler, 1983; Scoles, 1983; Surikov and Kissel, 1985; Rekhmetulin, 1987). This underscores that wheat cultivars play a pivotal role as the primary determinant of crossability variations. The study's outcomes align with existing research, emphasizing the genetic influence on the crossability of wheat with rye, primarily attributed to two loci, *Kr1* and *Kr2*. These loci possess dominant alleles that diminish crossability, indicating a complex genetic foundation for this trait. Furthermore, as indicated in prior studies, the presence of the Ph1 gene contributes to the observed variability in crossability across diverse wheat and rye cultivars.

The crossability percentage of hexaploid wheat with H. rye (Himalayan rye) ranged from 32.91 to 43.51; tetraploid wheat was 34.24 to 48.04. As also reported by Krolow (1970), Bhupal Rao and Srinivasan (1978), and Shchevchenko and Karpachev (1985), durum wheat, in general, showed better crossability than *aestivum* wheat, which might be due to a gene (*Kr3*) on the D genome, which also acts as an inhibitor of crossability (Krolow, 1970). However, Priadcencu *et al.,* (1964) and Ramazanov (1977) reported just the reverse of these results.

The differences in the crossability of tetraploid and hexaploid wheat with rye might also be due to the ploidy level of wheat (Oettler, 1984), as the expression of *Kr* genes in

Triticum species appears to be enhanced by increasing ploidy levels, thereby diminishing crossability between *Secale* and polyploid *Triticum* species (Jalani and Moss, 1981).

The seeds obtained from hybrids with tetraploid wheat were markedly shrivelled and inviable. In contrast, those of the hybrids with hexaploid wheat were generally normal and viable, as also reported by Sulyndin and Naumova (1960), indicating that the gene(s) for the normal and viable grain development might be present on the D genome, as Pienaar and Marais (1977) reported that the combined presence of A, B, and D genomes in the hexaploids resulted in significant increase germination in hybrid kernels. The germinability of hybrid grains increased in the presence of the D genome.

All the hexaploid and tetraploid wheat cultivars investigated in this study were categorized into crossability group III. However, it is important to note that the grouping of wheat cultivars based on crossability is provisional and cannot be considered definitive. This is due to the potential influence of environmental and experimental factors during the hybridization process and the effects of crossability genes.

b. Effect of phytohormone application

The limited success in hybridization between wheat cultivars carrying the dominant *Kr1* and *Kr2* alleles and rye can be attributed to the hindrance and eventual inhibition of pollen tube growth. This inhibition occurs at the style base and the transmitting tissue of the ovary wall, preventing the penetration of pollen tubes into the embryo sac. Previous studies have highlighted this phenomenon, emphasizing the pivotal role of genetic factors in determining the cross-compatibility between wheat and rye.

To overcome these barriers in hybridization, the plant growth regulator 2, 4-D (2, 4-dichlorophenoxyacetic acid) was applied to pollinated wheat spikes. The 2, 4-D solution was applied at three distinct time intervals: 24 hours, 48 hours, and 72 hours after pollination. Remarkably, the highest percentage of hybrid seeds was achieved 72 hours after pollination. These results confirm that using 2, 4-D significantly increases the proportion of hybrid seeds in wheat-rye crosses.

c. Germination and Seedling survived percentage of Hexaploid wheat × Himalayan rye F_1 hybrids

The observed variation in germination percentages among the hexaploid wheat × H. rye F_1 crosses highlights the influence of the parental genotype on this critical developmental stage. The highest germination rate in the WH 1105 × H. rye cross suggests strong genetic compatibility between the parental genotypes, leading to favourable germination conditions. The range of germination rates underscores the diversity of genetic backgrounds and the potential for optimizing hybrid performance through parental selection. The cross WH 1105 × H. rye exhibited the highest germination rate and the highest percentage of seedlings that survived to maturity, ranging from 55% to 90%. The consistency in performance across germination and seedling survival reinforces the significance of the genetic makeup of the parental genotypes in determining the success of the F_1 crosses.

d. Effect of concentration of colchicine solution on Hexaploid wheat × Himalayan rye F_1 hybrids

This study explored the efficacy of colchicine in inducing polyploidy, a process crucial for enhancing the genetic diversity of F_1 hybrids. These results indicated that colchicine significantly increased the percentage of induced polyploid seedlings, particularly when supplemented with DMSO. Furthermore, comparing the application methods, root and crown immersion proved more effective than injection.

Notably, the concentrations of colchicine and DMSO affected both survival and reproductive success. While a lower concentration (0.05% colchicine with 3% DMSO) enhanced survival rates, it was correlated with a lower seed set percentage. Conversely, a higher concentration (0.1% colchicine with 3% DMSO) led to a higher seed set percentage despite lower survival rates.

e. Response of Hexaploid wheat × Himalayan rye F_1 hybrids to colchicine solution

Kostoff (1938) first used colchicine to induce chromosome doubling in triticale production. However, variable success in doubling has been observed in different cases (Kaltsikes 1974).

In the present study, the survival rates of seedlings ranged from 33.33% to 66.67% after treatment with colchicine up to maturity showed varying responses to the solution, indicating that colchicine treatment was quite effective, and survival of the seedlings was markedly affected in different hexaploid wheat cultivars.

f. Percentage of embryo obtained in Tetraploid wheat × Himalayan rye F_1 hybrids

The observed variation in the number of seeds dissected and embryos obtained from tetraploid wheat highlights the genotype-specific differences in reproductive success. Across different genotypes, the number of embryos obtained from dissected seeds ranged from 5 to 10, indicating fertility and seed development variability.

Notably, two crosses, PDW 215 × H. rye and PDW 314 × H. rye, yielded the highest numbers of embryos at 10. This suggests a potential genetic predisposition for enhanced seed development and embryo formation in these crosses, which could be attributed to favourable genetic interactions between parental genotypes.

g. Effect of age of Embryo on different developmental stages in Tetraploid wheat × Himalayan rye F_1 hybrids

The results underscore the importance of the age of embryos in the success of in vitro culture for hybrids between tetraploid wheat and H. rye. The stage of development at the time of culture significantly impacted embryo growth, with embryos harvested between 16-19 days after pollination exhibiting higher rates of germination and subsequent development, as also reported by Raina (1986). Other workers reported different results in this respect, *i.e.* 16-18 DAP (Bajaj *et al*., 1978), 12-14 DAP (Taira and Larter, 1978), under different conditions of plant growth prior to embryo excision.

Interestingly, the embryos harvested 16-19 days after pollination showed the highest germination rates and subsequent development of plantlets with roots and shoots. This underscores the importance of precise timing of embryo harvesting for successful in vitro culture, as embryos at this developmental stage appear more responsive to culture conditions and exhibit enhanced growth potential.

h. Effect of Tetraploid wheat × Himalayan rye F_1 hybrids on different developmental stages

These results highlight notable genotype-specific variations in the success of in vitro culture among tetraploid wheat × H. rye hybrids. In particular, the PDW 314 × H. rye cross demonstrated the highest number of germinated embryos, suggesting favourable genetic compatibility or developmental predisposition in this specific cross.

Although the overall percentage of callus induction was modest, two crosses, PDW 291 × H. rye and WHD 943 × H. rye exhibited higher percentages of callus formation (14.29 %). This indicated a potential genetic basis for enhanced callus induction in these crosses. This is also supported by the findings of Bajaj *et al.,* (1978) and Oettler (1984), indicating that embryo growth in vitro is markedly affected by the maternal wheat parent.

Moreover, the PDW 314 × H. rye cross had the highest percentage of plantlets with roots and shoots, reaching 20%. This suggests that this cross possesses genetic factors or developmental characteristics that promote plantlets' successful regeneration and development from cultured embryos.

i. Effect of media and growth hormone

Various media have been used by different researchers for embryo culture, such as modified Blaydes's medium, modified MS medium (Bajaj *et al*., 1978), modified Norstog's medium (Taira and Larter, 1978), modified B5 medium (Fedak and Armstrong, 1980), and Taira and Larter's modified Norstog's medium (Raina, 1984), with varying success for in vitro results. Given these earlier reports, MS media supplemented with various growth hormones were used for embryo culture to determine their effect in embryo growth in vitro.

Among the three media formulations tested, MS medium supplemented with Indole -3-acetic acid (1 mg/l), Kinetin (0.5 mg/l), and Casein Hydro lysate (500-1000 mg/l) demonstrated superior performance across multiple parameters. The highest percentage of callus development, minute shoots, and plantlets with roots and shoots, all at 14.29%, indicated the effectiveness of this hormone combination in promoting tissue proliferation, differentiation, and subsequent regeneration. The synergistic effects of IAA, Kinetin, and

CH likely stimulate cell division, shoot proliferation, and root formation, respectively, thereby facilitating the overall regeneration success.

j. Expression of rye traits in F_1 hybrids

Concerning the manifestation of rye traits, Oettler (1984) suggests that certain wheat cultivars possess incompatibility factors influencing the expression of specific rye traits. Kerber and Green (1930) have elucidated mechanisms that hinder the manifestation of rust-resistance genes in amphidiploids. The expression of dwarfing genes in triticale is not as pronounced as in wheat (Skovmand *et al*., 1984). An investigation into the manifestation of rye traits in wheat × rye hybrids, especially those involving self-fertile homozygous rye, would be valuable in discerning the degree of expression and inhibition of rye traits attributed to incompatibility factors or suppressors in diverse wheat cultivars.

Regarding quantitative traits, such as plant height, peduncle length, spike length, awn length, spikelets/spike, tillers/plant, and maturity duration, the interpretations could not be confirmed without statistical analysis because of the small number of hybrids in most of the crosses. For such traits, comparing hybrids with their respective parents is just an indication of their expression.

Characteristics like coleoptile colour, stem colour, auricle pubescence intensity, shoot-based colour, waxy leaf sheath, leaf greenness, node colour, hairy peduncle intensity and early growth habit exhibited complete and partial expression of rye traits in F_1 hybrids. The absence of auricles in rye was not apparent in any hybrid, indicating that rye traits were hypostatic or recessive to wheat for these specific attributes.

For other quantitative traits, distinctions were not discernible, and interpretation required statistical analysis due to the limited number of hybrids. Nevertheless, some wide hybrids exhibited full or partial resemblance to either rye or wheat for traits such as days to heading, days to anthesis, days to maturity, productive tillers per plant, plant height, internodes per plant, awn length, spike length, number of spikelet’s per plant, and number of seeds per spike, among others.

CHAPTER 5
SUMMARY AND CONCLUSION

The present investigation was undertaken to study the crossability of hexaploidy and tetraploid wheat with diploid rye, to develop primary triticale at the hexaploid and octoploid levels with self-compatible rye and to study the expression of some rye traits in the wheat × rye F_1 hybrids.

In recent years, genetic variability within cultivated wheat has diminished significantly, raising concerns among breeders about the limited gene pool available for improvement. However, genetic variability from wheat relatives, particularly rye, holds promise for enhancing wheat traits, such as disease resistance, drought tolerance, and nutritional content. Understanding the crossability of wheat and rye is crucial for effective hybridization.

The experimental material consisted of eight tetraploid wheat, ten hexaploid wheat genotypes selected for wide crossability, and one rye landrace. Emasculated spikes were pollinated with fresh rye pollen from each wheat genotype, and the crossability between wheat and rye was quantified by the number of seed sets formed relative to the number of florets pollinated in each cross. The various wheat genotypes were provisionally categorized according to Lein (1943), and groupings were established based on crossability percentages. Accordingly, the probable genotypes assigned to wheat falling in groups I (<10%), II (10-30%), III (30-50%), and IV (>50%) are *Kr1 Kr1 Kr2 Kr2, Kr1 Kr1 kr2 kr2, kr1 kr1 Kr2 Kr2,* and *kr1 kr1 kr2 kr2,* respectively. The crossability of different tetraploid wheat cultivars ranged from 34.24 to 48.04%. The highest crossability percentage was obtained for the cross PDW 314 × H. rye (48.04%). In hexaploid wheat, it ranged from 32.91 to 43.51%. The highest crossability percentage was obtained in the cross PBW 343 × H. rye (43.51%), indicating that the wide crossability was determined predominantly by the wheat genotypes.

Research has identified genetic barriers controlled by *Kr* genes located on specific chromosomes that influence the crossability of wheat with rye. Moreover, the presence of

Ph1 further complicates the genetic basis of this trait. Studies have shown significant variability in crossability among different wheat cultivars, with durum wheat generally exhibiting better crossability than hexaploid wheat.

Efforts to overcome barriers to hybridization, such as applying the plant growth regulator 2,4-D, have shown promise for increasing the percentage of hybrid seeds. The highest seed set percentage was obtained for the tetraploid wheat after 72 h. The application of 2,4-D solution was 71.05% in cross PDW 314 × H. rye. The highest seed set percentage was obtained in hexaploid wheat after 72 h. Application of 2,4-D solution was 59.46% in cross WH 1105 × H. rye.

This study explored the effect of parental genotypes on wheat-rye hybrid success. Germination rates varied significantly across crosses, demonstrating the influence of the parental background. The WH 1105 × H. rye (90%) cross achieved the highest germination and seedling survival percentage, suggesting strong compatibility with optimal conditions. This diversity emphasizes the potential of parental selection to optimize hybrid performance.

The effectiveness of colchicine in inducing polyploidy enhances the genetic diversity of F_1 hybrids. The results demonstrated that adding DMSO significantly increased the percentage of induced polyploid seedlings, with root and crown immersion proving more effective than injection. The concentrations of colchicine and DMSO played crucial roles in both survival and reproductive success. A lower concentration (0.05% colchicine with 3% DMSO) was associated with higher survival rates but a lower seed set percentage. In contrast, a higher concentration (0.1% colchicine with 3% DMSO) resulted in a higher seed set percentage despite lower survival rates. These findings emphasize the delicate balance required when selecting colchicine concentrations, considering both survival and reproductive outcomes, to optimize the induction of polyploidy in F_1 hybrids.

The effect of colchicine treatment on the survival rates of hexaploid wheat seedlings ranged from 33.33% to 66.67%. A higher percentage of plants that survived until maturity was obtained in two crosses, HD 2851× H. Rye (66.67%) and WH 1105 × H. Rye

(66.67%). This variability in survival rates among different hexaploid wheat cultivars indicates that colchicine treatment was effective, significantly influencing seedling's ability to reach maturity.

In tetraploid wheat, genotype-specific differences in reproductive success are evidenced by variations in the number of seeds dissected and embryos obtained. The observed range of 5–10 embryos from dissected seeds underscores the diversity in fertility and seed development among different genotypes. The crosses PDW 215 × H. rye and PDW 314 × H. rye stood out, with the highest number of embryos at 10. This indicates a potential genetic predisposition for enhanced seed development and embryo formation in these specific crosses, indicating the influence of favourable genetic interactions between parental genotypes on reproductive outcomes.

The success of in vitro culture in tetraploid wheat × H. rye hybrids was strongly influenced by embryo age. Findings reveal that Embryos harvested between 16-19 days after pollination exhibited higher germination rates and subsequent plantlet development. Embryos at this specific developmental stage demonstrate enhanced responsiveness to culture conditions and show optimal growth potential.

Genotype-specific variations have been observed in the success of in vitro culture and the regeneration of hybrids, highlighting the importance of parental selection. The cross PDW 314 × H. rye exhibited the highest germination rate, implying favourable genetic compatibility or developmental predisposition. While the overall callus induction percentages were modest, two crosses, PDW 291 × H. rye and WHD 943 × H. rye displayed higher callus formation rates, suggesting a genetic basis for enhanced induction in these specific crosses. Furthermore, the PDW 314 × H. rye cross demonstrated the highest percentage of plantlets with roots and shoots (20%), indicating that genetic factors or developmental characteristics favour successful regeneration and plantlet development from cultured embryos.

Among the three tested formulations, MS medium supplemented with Indole-3-acetic acid (1 mg/l), Kinetin (0.5 mg/l), and Casein Hydro lysate (500-1000 mg/l) exhibited

superior performance. This combination resulted in the highest percentages of callus development, minute shoots, and plantlets with roots and shoots (14.29 %). The effectiveness of this hormone combination suggests its role in promoting tissue proliferation, differentiation, and overall regeneration. The synergistic effects of IAA, Kinetin, and CH likely stimulated cell division, shoot proliferation, and root formation, emphasizing the potential for optimizing in vitro culture conditions in tetraploid wheat × H. rye hybrids.

Expression studies revealed complete and partial expression of rye traits in F_1 hybrids, with implications for trait inheritance and breeding. Further investigations are required into the expression and inhibition of rye traits in different wheat varieties.

Understanding crossability, induced polyploidy, in vitro culture, and trait expression in wheat-rye hybrids provides valuable insights for wheat improvement strategies, particularly for developing wheat × rye amphidiploid lines.

Thus, all results of the present investigation entitled "**Development of noval wheat rye amphidiploid lines**" can be concluded as under:

Crossability

- The crossability of tetraploid and hexaploid wheat with diploid rye revealed genotype-specific variation.
- The highest crossability percentage was observed in the tetraploid wheat cross PDW 314 × H. rye (48.04%) and in hexaploid wheat, which was 43.51% in the cross PBW 343 × H. rye.

***In vitro* culture**

- The success of in vitro culture of tetraploid wheat × H. rye hybrids was influenced by various factors, including embryo age and media formulations. Optimizing conditions, such as MS medium supplemented with IAA, Kinetin, and CH, enhances tissue proliferation and differentiation, contributing to successful regeneration.

Colchicine Treatment

- The effectiveness of colchicine in inducing polyploidy was explored, emphasizing the delicate balance required to select the concentrations for optimal survival and reproductive outcomes.
- The colchicine solution (0.1%) with dimethyl sulfoxide (DMSO) (3%) showed better results than without Dimethyl sulfoxide (DMSO).

Expression of Rye traits

- Expression studies demonstrated complete and partial expression of rye traits in the F_1 hybrids. This has implications for trait inheritance and breeding and offers valuable insights into the potential transfer of beneficial traits from rye to wheat.

CHAPTER 6

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List of Publications

Title of the Paper	Web link of journal indexing
Crossability and Phenotypic Evaluation of a Himalayan Rye Landrace (*Secale Cereale* L.)	https://agribiop.com/category/annals-of-agri-bio-research/vol-282-2-december-2023/
Effect of 2,4-D Dosage on Haploid embryo induction in Bread Wheat Following Wide Hybridization with maize	https://agribiop.com/category/annals-of-agri-bio-research/vol-282-2-december-2023/

List of Conferences

SYMPOSIUM IN THE HONOUR AND MEMORY OF Dr DARSHAN SINGH BRAR

TRANSFORMING THE GREEN REVOLUTION HUB OF INDIA:
INNOVATIONS IN CROP BREEDING, RESOURCE MANAGEMENT AND POLICY

AUGUST 17-18, 2022

Certificate

OF PARTICIPATION

This is to certify that Dr./Mr./Ms. KULBHUSHAN PATIL *has attended and presented the poster in the symposium entitled "Transforming the green revolution hub of India: Innovations in crop breeding, resource management and policy" held at Punjab Agricultural University, Ludhiana from August 17-18, 2022.*

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International Conference
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VAKSANA-2022

Certificate

This is to certify that **Mr. Patil Kulbhushan Savindra** has participated and presented paper in online poster presentation titled **The Development of Hybrid Triticale** in the international conference on **'Precision Agriculture'** organized by SVIAg, SVVV, Indore (M.P.) during September 26-27, 2022.

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