

GENOTYPIC INFLUENCE ON CROSSABILITY OF WHEAT X HIMALAYAN RYE HYBRIDS

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A mong the various potential sources of resistance, rye (*Secale cereale*) has been identified as a potent reservoir of genes for resistance against biotic and abiotic stresses. However, in the hybridization of bread wheat and rye, there exists a significant barrier of cross incompatibility controlled by dominant alleles at four loci. These loci include *Kr1* and *Kr2*, located on chromosome 5B and 5A, respectively (Lein, 1943; Riley and Chapman, 1967; Lange and Riley, 1973; Sitch *et al.*, 1985), *Kr3* on chromosome 5D (Krolow, 1970) and *Kr4* on 1A (Luo *et al.*, 1989; Zheng *et al.*, 1992). Therefore, the ability of wheat to cross with rye is contingent upon the presence of dominant alleles of these four genes in the wheat genotypes, whereas the recessive form facilitates crossing with rye. Hence, for successful wide hybridization programmes in wheat, it is necessary to investigate the potential genotypes with higher crossability genes. Keeping in view the importance of genotype specificity in wide hybridization programme, present study was carried out to identify the crossability of local landraces, doubled haploids and high-yielding locally adapted wheat genotypes of Himachal Pradesh with rye. This study helped in identification of highly responsive wheat genotypes including local landraces and doubled haploids performing better in wheat x rye hybridization that can further be utilized in wheat improvement breeding programmes.

The current study initiated with the creation of rye introgressed wheat hybrids, employing Himalayan rye (*karoki* rye) as the source, along with various high-performing bread wheat genotypes selected based on their agronomic attributes, including local landraces and doubled haploids (Table 1). F₁ population was developed at Experimental Farm, Department of Crop Improvement, CSK HPKV, Palampur (32°5'46"N 76°32'43"E) representing North West Himalayas namely sub-humid mid hills (short photoperiods). The F₁ hybrids were further utilized to produce backcross

generations at Highland Agricultural Research and Extension Centre (HAREC), CSK HPKV, Kukumseri, Lahaul & Spiti (32°41'58"N 76°41'13"E).

The parental material including wheat genotypes and Himalayan rye was staggered sown with an interval of 15 days to ensure regular availability of pollen for a longer period of time. The wide hybridization programme was started from first week of March continued for 4-5 weeks. For F₁ generation, about 10-15 spikes from each wheat genotype were emasculated and next day early in the morning, at the suitable stage of stigma receptivity, spikes were pollinated with fresh rye pollen. As the crossability between wheat and rye can be identified from seed set (Fedak and Jui, 1982), therefore, data were recorded in terms of per cent seed set out of the florets pollinated in each cross and the different wheat genotypes were grouped into four categories following Lein (1943):

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

Data were subjected to simple *t*-test. The performance of wheat x rye F₁ hybrids was further analysed in backcross generation (BC₁F₁). So, for the aforesaid objective, BC₁F₁ population was developed by crossing wheat x rye F₁s with respective wheat genotypes during summer at Highland Agricultural Research and Extension Centre, Kukumseri, Lahaul & Spiti representing dry temperate zone (long photoperiods) in North West Himalayas. Again the data were recorded in terms of % seed set in BC₁F₁ generation and analysis was done using simple *t*-test.

The results of crossability and seed setting frequency (%) for various wheat x rye crosses are presented in table 2. The range of seed setting frequency was observed to be 0.00-30.67 per cent with an average of 5.09 per cent. The analysis of results revealed that

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Table 1. Parentage of various genotypes of wheat and Himalayan rye used in the present investigation

Sr. No.	Genotype	Parentage	Source
1.	HS 295	CQT//IAS55//ALDML'S'/3/ALDML'S'/NAFN/4/ PJM'S'/PEL1276.6	
2.	VL 829	IBWSN 149/CPAN 2099	Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora
3.	C 306	REGENT1974/3*CHZ//2C591/3/P 19/C281	ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal
4.	HPW 147	CPAN 1869 × Him 10	RWRC, Malan, CSK HPKV, Palampur
5.	HPW 89	INTERMEDIO RODI / HD 2248	
6.	HPW155	BT 2549/FATH	
7.	HPW 349	OASIS/SKUAZ114*BCN/3/PASTOR/4/KAUZ*2/ YACO//KAUZ CMSS98Y01808T-040M-0100M- 040Y-040M-030Y-1M-2Y-0M	
8.	HPW 360	WL 711/HPW 89S	
9.	HPW 368	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR	
10.	VL 892	WH 542/PBW 226	
11.	BW 215	RL 6059 x HS 295	HAREC, Bajaura, CSK HPKV
12.	C ₁ -1	-	
13.	VVFW	-	
14.	Saptdhara	Atou 'Sel' (Cappelle/Garnet)	Department of Crop Improvement, CSK HPKV, Palampur
Doubled haploids			
15.	DH 5	Saptdhara x HPW 184	Molecular Cytogenetics & Tissue Culture Lab, Department of Crop Improvement, CSK HPKV, Palampur
16.	DH 40	Saptdhara x HW 3024	
17.	DH 65	WW24 x PW 552	
18.	DH 84	VVFW 499 x HPW 147	
19.	DH 86	WW 24 x HW 3024	
20.	DH 100	WW 10 x WW 24	
21.	DH 109	PW 552 x UP 2418	
22.	DH 114	VVFW 452 x WW24	
23.	DH 120	PW 552 x HPW 42	
24.	DH 776	Pmfjoumee x HPW 143	
Local landraces			
25.	Kshun	Local Land race	North-West Himalayan Regions
26.	Badkanku 1	Local Land race	
27.	Badkanku 2	Local Land race	
28.	Badkanku 3	Local Land race	
29.	Badkanku 4	Local Land race	
30.	Badkanku 5	Local Land race	
31.	Badkanku 6	Local Land race	
32.	Tyari 1	Local Land race	
33.	Tyari 2	Local Land race	
34.	Tyari 3	Local Land race	
Rye			
1.	Himalayan rye	Collected from 'Koraki' village Lahaul & Spiti, Himachal Pradesh	Molecular Cytogenetics & Tissue Culture Lab, Department of Crop Improvement, CSK HPKV, Palampur

wheat parents viz., VL-829, HPW-360, HS-295, C-306, HPW-368, HPW-147, HPW-155, doubled haploids namely, DH-84, DH-5, DH-100 and landrace Tyari-3 were found to be significantly superior over other wheat genotypes in wheat x rye hybridization (Table 2). As per the results, C-306, VL-829 and Tyari-3 showed highest seed setting frequency of 30.67 per cent, 27.27 per cent and 17.41 per cent, respectively. On the other hand, DH-40, Badkanku 2, 3 and 5 gave negligible

results with rye. The results of present investigation showed better performance of pre Mexican wheats in wheat x rye hybridization. Our results are in agreement with studies conducted by Scoles (1983), Gordey and Gordey (1983), Chaubey and Khanna (1986) and Hills *et al.* (2007) who reported wide range of seed setting in wheat x rye hybridization.

In the present investigation, variation found in seed setting frequency can be explained on the genetic basis

Table 2. Crossability and seed set (%) of various wheat x rye crosses

Sr. No.	Crosses	No. of florets	F ₁ seeds	Seed Set (%)	Group
1.	VL-829 x KR	44	12	27.27**	II
2.	HPW-360 x KR	296	16	5.41*	I
3.	HS-295 x KR	488	46	9.43**	I
4.	HPW-89 x KR	638	8	1.25	I
5.	C-306 x KR	564	173	30.67**	III
6.	C ₁ -1 x KR	276	13	4.71	I
7.	VL-892 x KR	310	2	0.65	I
8.	HPW-368 x KR	322	26	8.07**	I
9.	VVFW x KR	526	17	3.23	I
10.	HPW-349 x KR	448	3	0.67	I
11.	BW-215 x KR	844	9	1.07	I
12.	HPW-147 x KR	586	29	4.95*	I
13.	HPW-155 x KR	762	50	6.56**	I
14.	DH-84 x KR	572	85	14.86**	II
15.	DH-5 x KR	596	31	5.20*	I
16.	DH-40 x KR	272	0	0	I
17.	DH-65 x KR	624	6	0.96	I
18.	DH-86 x KR	496	18	3.63	I
19.	DH-109 x KR	162	5	3.09	I
20.	DH-100 x KR	478	37	7.74**	I
21.	DH-114 x KR	594	23	3.87	I
22.	DH-120 x KR	372	7	1.88	I
23.	DH-776 x KR	556	4	0.72	I
24.	Saptdhara x KR	694	4	0.58	I
25.	Tyari-1 x KR	558	1	0.18	I
26.	Tyari-2 x KR	522	24	4.60	I
27.	Tyari-3 x KR	402	70	17.41**	II
28.	Badkanku-1 x KR	300	1	0.33	I
29.	Badkanku-2 x KR	314	0	0	I
30.	Badkanku-3 x KR	196	0	0	I
31.	Badkanku-4 x KR	230	1	0.43	I
32.	Badkanku-5 x KR	214	0	0	I
33.	Badkanku-6 x KR	344	3	0.87	I
34.	Kshun x KR	598	17	2.84	I
Mean				5.09	
SE±				1.26	

* Significant at $p \leq 0.05$ and ** Significant at $p \leq 0.01$; KR= Karoki Rye

as elucidated by earlier studies. Many researchers across the world worked on wheat and found that the crossability of wheat with different species is highly influenced by the genetic constitution of parents involved. As per earlier reports, it has been revealed that the extent of hybridization between wheat and different species depends on the presence of dominant crossability inhibitor alleles, namely *Kr1*, *Kr2* (Lein, 1943), *Kr3* (Krolow, 1970) and *Kr4* (Zheng *et al.*, 1992) located on the different chromosomes of wheat genome. These genes affect the crossability by inhibiting the insertion of pollen tubes into pistils and their growth in the style and ovary wall (Cameron and Rager, 1991) and disrupting the embryonic development (Behl *et al.*, 1981), thus limiting the production of inter generic hybrids (Riley and Chapman, 1967). Another possible reason for the poor performance of wheat x rye hybrids might be due to the fact that homoeologous pairing in wheat is suppressed by the *Ph1* locus (Riley *et al.*, 1959 and Sears, 1976). In wheat x rye F_1 hybrids, pairing is largely prevented by the pairing homoeologous1 (*Ph1*) gene and there are only rare instances of pairing in the presence of this gene. This results in univalent arrangement of most chromosomes and initiation of different pathways of the meiotic cycle at metaphase I (Silkova *et al.*, 2013). In meiotic-like pathway a combination of the reductional and the equational + reductional steps at AI is followed by the second division. As a result, the produced gametes are mostly non-functional (Silkova *et al.*, 2013).

As per the results given in table 2, a large proportion i.e. 30 bread wheat genotypes out of the 34, were found

to show low crossability (<10%) and so categorized as crossability group I. However, three genotypes namely VL-829, DH-84 and tyari-3 were found with 10-30% crossability i.e group II and only one genotype i.e. C-306 showed 30-50% crossability i.e. group III. Thus, as per Lein (1943), 30 wheat cultivars were assumed to have genotype *Kr1 Kr1 Kr2 Kr2*, 3 cultivars having the genotype *Kr1 Kr1 kr2 kr2* and only one with genotype *kr1 kr1 Kr2 Kr2*. No cultivar was found with genotype *kr1 kr1 kr2 kr2* i.e. >50% crossability. It is interesting that one landrace i.e tyari-3, one doubled haploid (DH-84) and one widely adapted cultivar VL-829 were found to show some crossability and only one wheat genotype C-306 was found with highest crossability with rye. It reveals wide distribution of crossing genes in the Himalayan wheat germplasm. The earlier workers also reported the high frequency distribution of crossability alleles (*kr*) in bread wheat landraces from Himalayan ranges (Singh and Sethi, 1991) and China, Japan, East Siberia and Iran (Zeven, 1987). The difference in crossability among the bread wheat genotypes with rye is also reported in earlier studies (Tanner and Falk, 1981; Chaudhary and Joshi, 1985; Zeven, 1986). However, on the basis of crossability of wheat x rye, present study reports that C-306 falls in group III of crossability as per Lein (1943) i.e. having genotype *kr1 kr1 Kr2 Kr2*. These results are in contrary to earlier studies by Chaudhary and Joshi (1985) and Singh and Sethi (1991) who reported >50% crossability in C-306 i.e. having genotype *kr1 kr1 kr2 kr2*. This difference could be explained on the basis of influence of environmental conditions and further need to be confirmed at multiple locations.

Table 3. Performance of various wheat x rye F_1 s in backcross generation (BC_1F_1)

Sr. No.		No. of florets	BC_1F_1 seeds	Seed set (%)
1.	(DH-5 x KR) x DH-5	76	0	0
2.	(DH-86 x KR) x DH-86	240	0	0
3.	(DH-100x KR) x DH-5	130	0	0
4.	(C-306 x KR) x C-306	154	0	0
5.	(VL-829 x KR)x VL-892	192	24	12.50*
6.	(VL-829 x KR)x HPW-349	202	28	13.86*
7.	(HPW-155 x KR)x HPW-155	304	0	0
8.	(HPW-147 x KR)x HPW-147	120	0	0
9.	(HPW-89 x KR)x HPW-89	194	85	43.81**
10.	(HPW-360 x KR)x HPW-360	84	0	0
11.	(HPW-368 x KR)x HPW-368	144	0	0
12.	(HS-295 x KR)x HS-295	438	240	54.79**
	Mean			10.41
	SE±			5.48

* Significant at $p \leq 0.05$ and ** Significant at $p \leq 0.01$; KR= Karoki Rye

Note :- Due to non-availability of pollen of the respective wheat genotypes, pollen of other related wheat genotype was used in backcrosses

To determine the performance of wheat x rye F_1 hybrids in backcross generations, BC_1F_1 was generated at HAREC, Kukumseri, Lahaul & Spiti. For the aforesaid purpose, 12 crosses were attempted and analysis of results revealed that only 4 crosses were found to exhibit significant positive response towards seed set (%) (Table 3). The range of seed setting frequency was observed to be 0.00-54.79 per cent with an average of 10.41 per cent. The average seed setting frequency was found to be higher than the F_1 generation which may be due to their higher wheat genetic content and genomic stability. This completely complies with the earlier study (Giacomin *et al.*, 2015) which revealed that to increase meiotic stability of triticale, backcrossing should be considered. As per the results, the cross (HS-295 x KR) x HS-295 showed highest seed setting frequency of 54.79 % followed by (HPW-89 x KR) x HPW-89, (VL-829 x KR) x HPW-349 and (VL-829 x KR) x VL-892. Due to non-availability of pollen of respective wheat genotypes, pollen of other related wheat genotype was used in backcrosses. However, no seed was found in all other crosses. In the present investigation, wheat genotype viz., C-306 which showed high seed setting frequency in wheat x rye hybridization (Table 2) was found with zero seed setting in backcross generation BC_1F_1 (Table 3). This might be due to the influence of environmental conditions, poor germination or other type of fertilization barrier that resulted in poor performance of the hybrids in backcross generation.

The present investigation reveals the wheat genotypic driven specificity for the differences in crossability with rye. This genotypic difference can be explained further on the basis of the crossing inhibitor genes present in the wheat genotypes. So the lines including local landraces and doubled haploid, performing better in this study can further be used as a parental source in wheat improvement breeding programmes. However, the genotypes found with low crossability can be neglected to use directly in wheat x rye hybridization and some other strategy like bridging species should be utilized in such genotypes to transfer the genes from rye. This study also encourages the use of backcross generations to overcome the meiotic instability of wheat x rye intergeneric hybridization.

Authors' contribution

Conceptualization and designing of the research work (HKC, VKS); Execution of field/lab experiments and data collection (PS, KS); Analysis of data and interpretation (PS, HKC, CK, NVM); Preparation of manuscript (PS, HKC).

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