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Crossability and Phenotypic Evaluation of a Himalayan Rye Landrace (*Secale cereale* L.)

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ABSTRACT

Wide hybridization helps to transfer the alien chromatin into the cultivated species, which is helpful for the development of pre-breeding material. The phenotypic characterization and growth stages of a Himalayan rye were recorded, which provided information regarding the adaptability in the north-west region of India. Wide hybridization was done among the bread wheat and Himalayan rye to check their crossability relationships and the effect of 2, 4-D applications on crossability. Three treatments i.e. control, spraying and injection were used to assess the effect of 2, 4-D. A successful seed set was observed in all six genotypes of bread wheat, and a maximum crossability percentage of 42.3 was observed in the HD 4672 × Himalayan rye cross. A significant difference was observed between the control and the treatments. A paired t-test showed significant difference between control and spray treatment (t = -3.8125) and control and injected treatment (t = -2.0296). In addition, it was observed that the Himalayan rye was completely self-incompatible and cross-pollinated in nature, as seeds were formed only in the spikes that were not covered with the butter paper bag. However, the seed set was not noticed in the spikes that were covered with butter paper bags because the spike covered with bag promoted self-pollination, which led to self-incompatibility in rye and resulted in no seed formation in the spike. The 2, 4-D treatment was found effective irrespective of the genotypes used for crossing which indicated thar it can be used to get a respectable seed set from a bread wheat x rye hybridization program.

Key words: Landrace, Himalayan rye, wheat, wide hybridization, 2, 4-D, self-incompatible

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is an important cereal crop worldwide, contributing approximately 20% of the calories of the human diet, with production reaching ~770 mt in 2020-21 (FAO, 2021). Multiple biotic and abiotic stresses constrain the yield for which breeders are in a constant search for novel resistance/ tolerant traits from the inherent variations present in gene pools of domesticated wheat and its close relatives (Crespo-Herrera *et al.,* 2017). Enhancing genetic variability of cultivated wheat gene pool by alien useful gene introgression can greatly contribute to genetic progress in wheat breeding (Giura, 2015). Wild relatives offer an immense reservoir for such important traits to provide allelic diversity to the existing high yielding cultivars. Allogamous grasses like rye (*Secale cereale* L.) developed a two-locus gametophytic self-incompatibility (SI) system that forces out-crossing and allows for maintaining a high level of diversity (Melonek *et al.,* 2021). Rye is one of the most utilized relatives of wheat (Chaudhary *et al.*, 2015). Wheat and rye hybridization can be traced back to Wilson's work as early as 1873. In 1878, Rimpau was able to obtain fertile amphiploids, invigorating efforts to produce wheat-rye hybrids (Mergoum *et al.,* 2019). 1BL.1RS translocations from "Petkus" rye developed in Germany accounts as an ancestor for majority of the wheat cultivars released in Western Europe, Russia, Mexico, Chile and other countries. At the International Maize and Wheat Improvement Center (CIMMYT), 60% of wheat descendants were 1BL.1RS genotypes in the 1990s, and in China, approximately 40% of wheat cultivars released between 1960 and 2000 were 1B/1R translocations with yield gains over the years, partly attributed to this characteristic.

Wild species and relatives, often called alien species, act as genetic reservoirs especially for tolerance against biotic and abiotic stresses (Rather *et al.*, 2017). Landraces are location-specific genetically variable populations that are sources of valuable genetic variation for utilization in present and future breeding (Crespo-Herrera *et al.,* 2017). There is renewed interest in using rye as a source of new resistance genes in wheat due to the challenges of stress under changing climate conditions. Cereal landraces possess genetic diversity, which is poorly documented and utilized, and their phenotypic assessment is valuable for the choice of parental lines for use in crosses for the development of cultivars or as a pre-breeding tool.

The crossability between wheat and related species is controlled by Kr1-Kr4 genes (crossability with rye, Hordeum and Aegilops spp.) and the SKr gene (Suppressor of crossability). SKr and Kr1 have the largest influence on the trait (Porotnikov *et al.*, 2020). The crossability of rye (*Secale cereale* L.) with wheat (*Triticum aestivum* L.) has been studied since the work of Backhouse in 1916. Crossing ability often depends on genetic background of the recipient varieties or of the donor, which hampers a larger use of wild resources in breeding programmes of many crops (Laugerotte *et al.*, 2022). This characteristic has attracted interest from various researchers owing to its genetic control and breeding applications. Considerable variation has been observed in the cross abilities of wheat and rye with respect to their area of adaptation. The present study investigated the crossability of a Himalayan rye landrace with adapted bread wheat cultivars of north-west region of India with the aim of recommending an easily crossable material to researchers engaged in crossing wheat with rye. Different rye sources used for introgression to wheat genotypes are shown in Table 1.

Table 1. Rye sources that have been used for research in wheat improvement

S. No.	Year	Rye source	Scientists
1.	1922	Rosen rye	Gaines and Stevenson
2.	1928	Abruzzes rye	Leighty and Sando
3.	1928	Jelissejev rye	Meister and Tjumjakoff
4.	1931	Champagner rye	Buchinger
5.	1935	Weihenstephaner rye	Katterman
6.	1951	Hungarian rye	Arpad kiss
7.	1970	Petkus rye	-

MATERIALS AND METHODS

Six bread wheat genotypes were used as female parents, and one Himalayan rye landrace genotype was used as the male parent (Table 2). Crosses were made during *rabi* 2021-22 at the Experimental Research Farm, Department of Genetics and Plant Breeding, Lovely Professional University, Phagwara.

Wheat is a bisexual crop that is self-pollinating (i.e. cleiostogamous). Emasculation was performed in the evening on selected spikes a day before anthesis, and pollinated the next day. Caution was taken to synchronize the flowering of both wheat and rye plants, as the crop durations were different. For assessing the effectiveness of 2, 4-D, three treatments (control, spray and injection) were used. For the spraying method of 2, 4-D application, the spike was sprayed with 2, 4-D after 24 h of pollination, whereas for the injection method, 2, 4-D solution was injected into the uppermost internode of the pollinated wheat spikelet after 24 h of pollination and control spikes were left untreated.

Table 2. List of genotypes

S. No.	Name of genotypes	Pedigree
1.	HD2329	HD 1962/E 4870/3/K 65/5/HD 1553/4/UP 262
2.	HD2643 (Ganga)	VEE'S'/HD2407//HD2329
3.	HD3086 (Pusa Gautami)	DBW 14/HD 2733// HUW 468
4.	HD4672 (Malva Ratna)	BIJAGA RED/PBW34//ALTAR84
5.	HS375 (Himgiri)	BB/G11/CJ71/3/TA EST// KAL/BB
6.	HW2004 (Amar)	C 306*7 /TR 380-14 # 7/3 AG14
7.	Himalayan Rye (Trilokinath)	Landrace

RESULTS AND DISCUSSION

The present investigation was carried out on the phenotypic characterization of Himalayan rye and wide hybridization between bread wheat and landrace of rye to check their crossability relationships and the effect of 2, 4-D applications on crossability.

The Himalayan rye population was heterogeneous and heterozygous. Phenotypic data were recorded for four different growth stages i.e. vegetative, tillering, inflorescence and maturity. At the vegetative stage, the plant growth habit was erect, hairs at nodes were present, and stem hair was present. The tillering was observed 40-45 days after sowing. At the inflorescence stage, the time of emergence of the ear was 85-90 days after sowing, days to 50% heading was observed 110-115 days after sowing, the number of productive tillers per plant was 7-9, the length of anthers was 50-60 m and the time of dehiscence was 9.30 -11.30 a.m. At the maturity stage, plant height was 167-185 cm, ear length was 22-30 cm, days to maturity were 175 days after sowing, number of spikelets per spike was 40-60, number of grains per spike was 50-60, awn length was 5-6 cm, number of nodes was 5-6, spike compactness was dense and 1000-grain weight was 34.5 g (Table 3). Similar results were reported in wheat by Rahaman *et al.* (2015) and Regmi *et al.* (2021). This information explains the adoptability of Himalayan rye in the north-western region of India and its use in future breeding programs. At the time of harvesting, the seeds were formed only in the spikes that were not covered with the butter paper bag, whereas the covered spikes did not produce any seeds (Fig. 2). Hence, it can be concluded that the Himalayan rye is self-incompatible.

The crossability was reported maximum between HW2004 × Himalayan rye (96 seeds) and minimum between HS375 × Himalayan rye (19 seeds); however, seeds were also formed in all other crosses (Table 4). The maximum crossability percentage was 42.3 (HD4672 × Himalayan rye), and the minimum percentage was 7.91 (HS375 × Himalayan rye). The results obtained in the present investigation showed that Himalayan rye had high cross-compactability with bread wheat, which can be used for future breeding programs.

The seed-setting percentage of the control ranged from 14 to 7.33 followed by spray range from 17 to 10 and in injection ranging from 17.33 to 11.66. There was a significant difference between control and spray and control and injected. In the 2, 4-D spray method, the seed set percentage ranged from 10 to 17, while in injection method of 2, 4-D application ranged from 11.66 to 17.33 and in control ranged from 7.33 to 10 (Table 5).

Analysis of variation showed highly significant variation between the control and treatment, between genotypes, and the interaction between genotype and treatment (Table 6). A paired t-test indicated that the effect of 2, 4-D on seed set was significant for control and spray treatment (t = -3.8125) and control and injected treatment (t= - 2.0296). Furthermore, the results also indicated that there was a significant difference between the spray and injected treatments (t = - 2.4579). The above results indicated that 2, 4-D would help in increasing the efficiency of pollination and also help the survival of the embryos and seed set, which can be used in wheat × rye hybridization programs.

Table 3. Phenotypic characterization of Himalayan rye

S. No.	Characteristics	States	Stage of observation
1.	Plant: growth habit	Erect	Vegetative stage
2.	Hair at nodes	Present	Vegetative stage
3.	Stem hair	Present	Vegetative stage
4.	Tillering (days)	40-45 DAS	Tillering stage
5.	Ear: time of emergence (first spikelet visible on 50% of ear)	85-90 DAS	Inflorescence stage
6.	Days to 50% heading	110-115 DAS	Inflorescence stage
7.	Number of productive tillers per plant	7-9 tillers/plant	Inflorescence stage
8.	Length of anthers (mm)	50-60 mm	Inflorescence stage
9.	Time of dehiscence (IST)	9.30-11.30 am	Inflorescence stage
10.	Plant height (cm) excluding awns	167-185	Maturity stage
11.	Ear length excluding awns	22-30 cm	Maturity stage
12.	Days to maturity	175 DAS	Maturity stage
13.	Number of spikelets/spike	40-60 spikelets/spike	Maturity stage
14.	Number of grains/spike	50-60 seeds	Maturity stage
15.	Awn length (cm)	5-6 cm	Maturity stage
16.	Number of nodes	5-6	Maturity stage
17.	Spike compactness	Tough	Maturity stage
18.	1000-grain weight (g)	34.5 g	Maturity stage

Fig. 1. Phenotypic characterization of Himalayan rye.

Table 4. Crossability percentage of Himalayan rye with wheat genotypes

S. No.	Name of genotype	No. of emasculated florets	No. of pollinated florets	No. of seeds obtained	Cross ability (%)
1.	HD2329	208	208	46	22.1
2.	HD2643	258	242	79	32.6
3.	HD3086	172	172	58	33.7
4.	HD4672	182	182	77	42.3
5.	HS375	240	240	19	7.91
6.	HW2004	284	284	96	33.8

Table 5. Mean performance of 2,4-D solutions for control, spray and injection

S. No.	Genotypes	Control	Spray	Injection
1.	HD2329	9.33	10.00	15.33
2.	HD2643	8.66	14.00	15.00
3.	HD3086	9.66	11.33	11.66
4.	HD4672	14.00	13.00	15.00
5.	HS375	10.00	12.66	15.00
6.	HW2004	7.33	17.00	17.33
	Lowest range	7.33	10.00	11.66
	Highest range	14.00	17.00	17.33
	C. V.	23.46	20.43	13.60
	S. E.	0.54	0.62	0.47

Table 6. Analysis of variance for control, spray and injection (ANOVA)

	d. f.	Seed set
Replication	2	0.12
2, 4-D treatment	2	117.46***
Genotypes	5	13.75***
Genotypes x 2, 4-D level	10	14.35***
Error	34	1.99
Total	53	9.72

CONCLUSION

The search for and incorporation of new alleles for stress tolerance in bread wheat are ongoing. Landraces are location-specific and genetically variable populations that are sources of valuable genetic variation. A primary phenotypic description and crossing ability of a rye population that has not been a part of the rye chromatin carried by the majority of modern wheat cultivators was developed. Himalayan (Trilokinath) rye thrived in the climate of the northern Indian plains and was highly crossable with bread wheat. Rye's crop duration is approximately 175 days. Himalayan rye is self-incompatible and highly cross-pollinating in nature. Himalayan (Trilokinath) rye pollen is available from 9:00 to 11:30 a. m. (IST) and was used to pollinate wheat. In Punjab's climate, when rye pollen was used to pollinate the bread wheat, a good seed set was obtained from 42.3 to 7.91%. In the 2, 4-D treatment, a significant difference was observed between the spray and injection treatments. These results indicated that 2, 4-D aided in the pollination and survival of embryos. Further studies on regeneration ability, genome stability, and trait expression are required to develop a potential breeding line for multiple stresses.

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