PHENOTYPING IN RYE LANDRACE AND REGENERATION FREQUENCY STUDY IN WHEAT × RYE F_1 HYBRID SEEDS

ABSTRACT

The present research aimed to study the phenotyping characterization of a high-altitude Himalayan rye landrace and its crossability with wheat genotypes and investigate the regeneration frequency of wheat × rye F1 hybrid seeds. The study was conducted during the rabiseason of 2022-2023 at the Agriculture Research Farm, Department of Plant Breeding and Genetics, Lovely Professional University, Phagwara, Punjab. The phenotypic characterization of the Himalayan rye landrace revealedseveral adaptive traits that were found suitable for cultivation in the northwest Indo-Gangetic plains. Furthermore, the study investigated the crossability of the Himalayan rye landrace with various wheat genotypes, including 4 genotypes of bread wheat HD2967, UNNAT 343, WH1105, and PBW502 and 4 genotypes of durum wheat PDW291, PDW274, PBW34, and WHD943. Among the germination percentage of all 8 genotypes, 4 in Bread wheat and 4 in Durum wheat was observed. Due to its self-incompatibility, Rye cannot be used to develop inbred lines directly. However, the doubled haploid approach can produce inbred lines in the rye, facilitating the extension of genetic bases and introducing alien chromatin to identify stress tolerances.

Keywords –Wide hybridization, wheat×Rye, Embryorescue,Crossbility percentage, Regeneration Frequency

INTRODUCTION

Bread wheat (*Triticumaestivum*L.) is an important cereal crop of the world contributing around 20% of the calories of the human diet with its production reaching ~770 Mt in 2020-2021 (Hawkesford, 2013, FAO 2021). Durum wheat (*Triticumturgidum*ssp. Durum). (2n=4x=28, AABB) is a significant crop globally, ranked tenth in importance. It is mainly cultivated in West Asia, North and East Africa, the Great Plains of North America, India, and Eastern and Mediterranean Europe, covering approximately 10% of the total wheat area. Typically, durum wheat production constitutes 5% of the total wheat production, with a worldwide cultivation area of 16 million hectares (Beres*et.al*., 2020). Rye is a cereal crop primarily grown in temperate areas (Sun *et.al*., 2022). Its genome 2n=14 places it in the Poaceae family. Rye's genetic material is 8100 Mb in size. (Li *et.al*., 2011). History believes

that rye originated in southwest Asia (Gyulai*et.al.* 2014). Recent studies revealed that rye underwent a particular evolution, changing from a wild plant to a weed and then from a weed plant to an agricultural plant (Gyulai*et.al*., 2014). Rye is one of the most utilized relatives of wheat in this process (Kim *et.al*., 2004). In 1873 Alexander Wilson first managed to manually fertilize the female stigma of wheat flowers with rye pollen (male gametes) but found that the resulting plants were sterile [12,13]. One of the first published reports on the use of triticale as a grain feed is (Meister, 1921). The first variety of wheat x rye F_1 was developed in laboratories during the late 19th century in Scotland and Germany by Meister in 1921. It was developed for the yield potential and grain quality of wheat with disease and environmental tolerance including soil conditions of rye. Wheat has been enhanced by introducing rye's genes into wheat with the help of substitution lines and translocation lines. When introduced into wheat, rye has various resistant genes that give wheat protection against several diseases. The hybrid plants were developed by crossing wheat x of rye and it has a lot of importance as they withstand the cold winter season better than Bread wheat (Meister, 1921). The major importance of triticale lies in its protein. Reported protein values range from 12 to 22%. triticale hybrids were all amphidiploid (Levitzky and Benetzkaja, 1930) which means having a complete diploid chromosome set from each parent. It is an allotetraploid possessing four times the chromosomes in haploid organisms (Neubauer, 1966).

Objectives:

1. Phenotypic characterization of a high-altitude Himalayan rye landrace in northwest Indo-Gangetic plains.
2. Crossability percentage in the (Bread 6x) and (Durum 4x) wheat.
3. Comparison of the regeneration frequency in different wheat × rye F_1 hybrid combinations.

MATERIAL AND METHODS

The present research entitled Phenotyping of rye landrace and Regeneration frequency study in wheat × rye F_1 hybrid seedswas conducted in the experimental fields of the Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara, Kapurthala, Punjab, India at coordinates latitude 31019’32’’N, a longitude 75034’45’’E, and an altitude of 243 meters above sea level. The research is done in the Rabiseason of 2022-2023. Punjab is in the western part of India which is the subtropical region. The climate in this region is humid subtropical, with cool winters and long hot

summers. The summer season lasts from April to June, and the winter season lasts from November to February. Summer temperatures range from 25°C to 48°C on average. Winter temperature ranges from 1 °C to 18 °C. The soil is slightly acidic, with a pH range of 5.6-6.4, and the soil texture is sandy loam. The regeneration frequency was done under controlled conditions at the Plant Breeding Laboratory, Department of Genetics. The Experimental materials that were used were Four Bread wheat genotypes namely WH1105, PBW502, HD2967, and Unnat343. Four Durum wheat genotypes namely PDW291, PDW274, PBW34, and WHD943 Rye Genotype Himalayan rye landrace, M.S media are collected from the Department of Agriculture, Punjab. The sowing of genotypes was done at three different intervals i.e. (5^{th} November 2022). Each genotype was sown in 2 lines with a total of 8 lines, and 4 genotypes of Durum wheat (4x) in 2 lines with a total of 8 lines, with a length of 2 meters. 2^{nd} and the 3^{rd} block sowing were done exactly in the same pattern as in the first block. On 28^{th} November 2022 and 14^{th} December 2022. Rye genotypes (Himalayan rye) are sown in 3 × 3meter lengths in two blocks of 10 lines. The removal of immature anthers without causing stigma damage is known as emasculation. Wheat plants are emasculated in the evening two to three days before anthesis it was done in the evening time. The rye pollen is available from 9.00 a.m. to 11.00 a.m. For calculating the crossability percentage of crosses the formula was used.

$$Seed\ set\ \% = \frac{no.\ of\ seeds\ formed}{no.of\ florets\ pollineted} \times 100$$

$$Crossabilty\ \% = \frac{no.\ of\ plants\ obtained}{no.of\ florets\ pollineted} \times 100$$

$$Regenration\ frequency\ \% = \frac{no.\ of seeds\ germinated}{no.of\ seeds\ sown} \times 100$$

The composition was used for preparing MS. Media for the embryo rescue process.

MS. media + IAA(1mg/l) + kinetin(0.5mg/l). MS. media + 2,4- D(1mg/l). Agar = 7gm per 100ml. 70gm per 1000ml. total of 63 gm agar is added in MS. media. After the preparation of MS media, the crossed spike of durum wheat is collected 15 to 20 days after the crossing and the pseudo seeds formed were collected with the help of forceps and transferred into the Petri plate filled with distilled water and embryo rescue is done.

RESULTS AND DISCUSSION.

Crossability percentage of Bread Wheat × rye

The crossability percentage between four types of bread wheat and Himalayan rye was assessed, resulting in the formation of 150 seeds out of 1262 pollinated florets, with an average crossability of 14.67% across all bread genotypes. This finding is consistent with the results obtained by GittaOettler in 1983, who also observed crossability percentages ranging from 20.7% to 37.5% when crossing bread wheat with rye. Among the four genotypes (HD2967, WH1105, PBW502, Unnat 343), crossability percentages varied from 6.21% to 31.97%. PBW502 showed the highest crossability with 197 florets producing 63 seeds, followed by WH1105 with 250 florets yielding 35 seeds. HD2967 produced 29 seeds from 445 florets, while Unnat 343 had the lowest seed formation with 23 seeds from 370 florets. These observations suggest that PBW502 has the highest seed formation potential when crossed with Himalayan rye, whereas Unnat 343 exhibits comparatively lower seed formation. Similarly, Oettler (1983) observed seed formation when crossing bread wheat with rye.

S.no	Bread wheat × Himalayan rye	No of the Spikes pollinated	No of the florets pollinated	No seeds formed after crossing	Crossability percentage
1	HD2967	13	445	29	6.51%
2	WH1105	9	250	35	14.00%
3	PBW502	7	197	63	31.97%
4	Unnat 343	10	370	23	6.21%

Table 1. Crossability percentage of Bread Wheat × rye

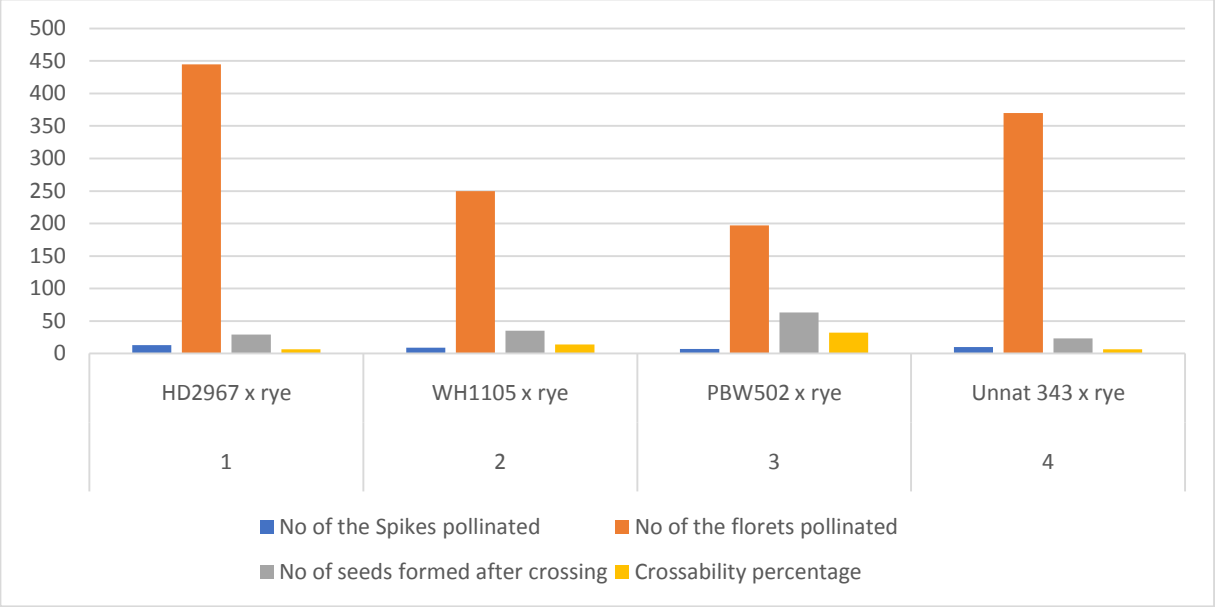

Figure 1. Bar graph representation of Bread wheat × rye

Crossability percentage of Durum Wheat × rye

The crossability percentage between four types of durum wheat and Himalayan rye was determined, revealing that unlike bread wheat, no seed set was observed in durum wheat varieties. Hybrid plants had to be obtained through embryo rescue techniques. Similar findings were reported by Bajaj et al. in 1978, suggesting that the optimal approach to produce bread triticale from an otherwise incompatible cross between Triticum durum and Secalecereale involved removing hybrid embryos 16 to 18 days after pollination and cultivating them carefully on a layer of macerated, immature endosperm dispersed over an agar nutritional medium containing IAA, kinetin, and casein hydrolysate.

A total of 908 florets were pollinated according to the specified procedure, resulting in 281 embryos. The average percentage of embryo formation frequency was 30.93%. The frequency of embryo formation ranged from 17.02% to 50.47% among the four genotypes (PDW291, PDW34, PDW274, and WHD943). PDW291 exhibited the highest percentage, with 210 pollinated florets yielding 106 embryos (50.47% formation rate), followed by PDW34 with 246 florets and 76 embryos (30.89% formation rate). PDW274 had 264 florets and 67 embryos (25.37% formation rate), while WHD943 had 188 florets and 32 embryos (17.02% formation rate). It was noted that PDW291 produced the most embryos, while WHD943 produced the fewest.

S.no	Durum wheat × Himalayan rye	Number of Spikes pollinated	Number of Florets pollinated	Number of Embryos formed.	Embryos formation percentage
1	PDW291	10	210	78	37.14%
2	PDW274	10	264	67	25.37%
3	PBW34	10	246	76	30.89%
4	WHD943	10	188	32	17.02%

Table 2. Crossability percentage of Durum Wheat × rye

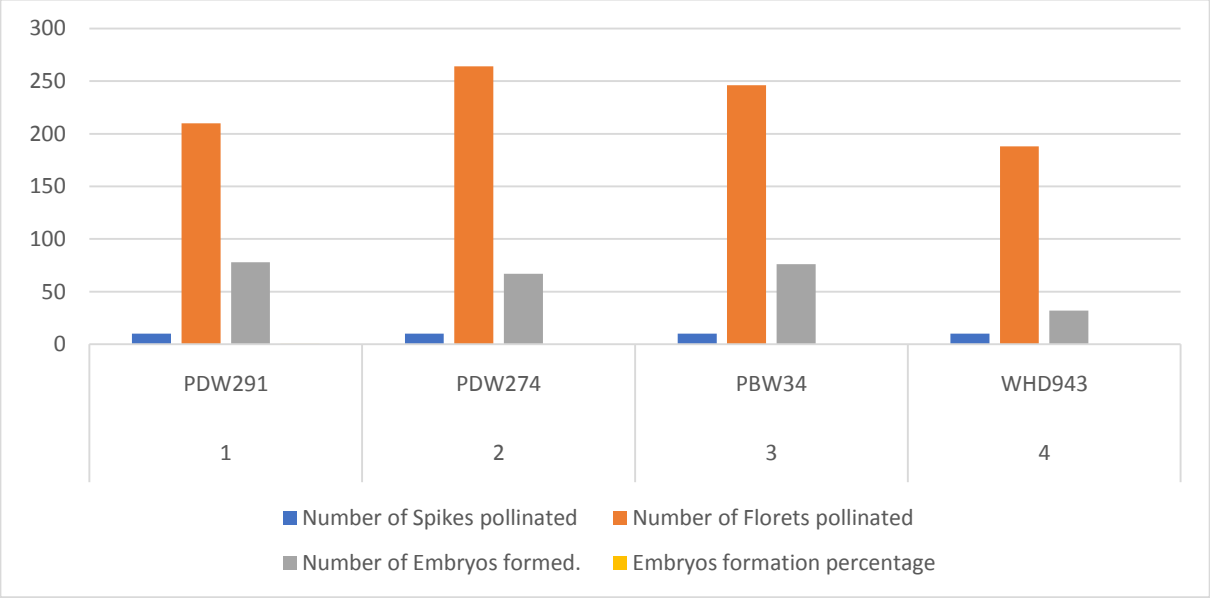

Figure 2. Bar graph representation of Durum wheat × rye

Regeneration frequency of Bread wheat × rye seeds

The regeneration frequency percentage of seeds resulting from crosses between bread wheat and rye was assessed by sowing the crossed seeds in pots under optimal conditions for germination. A total of 150 seeds were sown, out of which 15 seeds germinated. The regeneration frequency ranged from 11.42% to 8.69% among the four genotypes, with an average of 9.99% across all bread wheat genotypes. Genotype PBW502 exhibited the highest germination, with 6 seeds sprouting, followed by WH1105 with 4 seeds, HD2967 with 3 seeds, and Unnat343 with 2 seeds. Interestingly, there was a disparity between regeneration

frequency and seed germination rates. WH1105 had the highest regeneration frequency at 11.42%, followed by HD2967 at 10.34%, PBW502 at 9.52%, and Unnat343 at 8.69%.

S.no	Genotypes	No. seeds sown	No. seeds germinated	Regeneration frequency percentage
1	HD2967	29	3	10.34%
2	WH1105	35	4	11.42%
3	PBW502	63	6	9.52%
4	Unnat 343	23	2	8.69%

Table 3. Regeneration frequency of Bread wheat × rye seeds

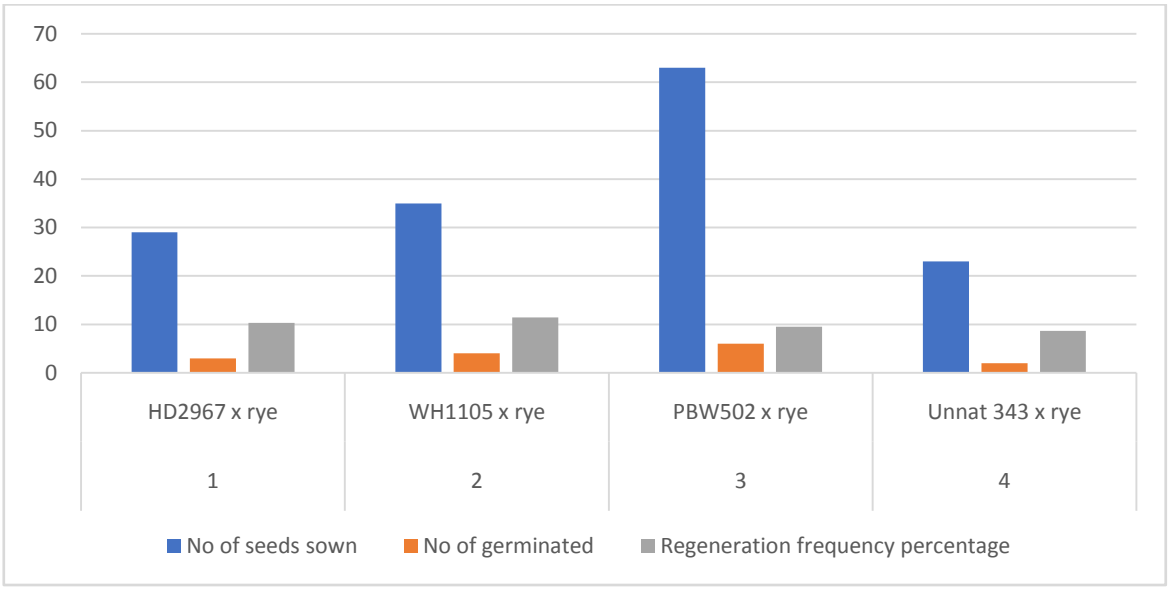

Figure 3. Bar graph representation of Germination frequency of Bread wheat x rye seeds

Regeneration frequency of Durum wheat × rye seeds

The regeneration frequency percentage of seeds resulting from crosses between durum wheat and rye was evaluated by sowing the crossed seeds. To determine the regeneration frequency percentage of embryos resulting from the cross, an embryo rescue procedure was conducted using MS media, as outlined in the materials and methods section. The embryos were provided with optimal conditions for germination, and the formation of plantlets was observed.

After embryo rescue, it was found that genotype PDW291 produced 2 plantlets, as did PDW274, while PBW34 produced 1 plantlet, and WHD943 also produced 1 plantlet. Across all durum wheat genotypes, the average regeneration frequency was calculated to be 2.32%. Notably, WHD943 exhibited the highest regeneration frequency percentage among the plantlets, at 3.12%, followed by PDW274 at 2.98%, PDW291 at 1.88%, and PBW34 at 1.31%.

S.no	Genotypes	No. of embryos formed	No. of plantlets formed	Regeneration frequency percentage
1	PDW291	78	2	2.56%
2	PDW274	67	2	2.98%
3	PBW34	76	1	1.31%
4	WHD943	32	1	3.12%

Table 4. Regeneration frequency of Durum wheat × rye seeds

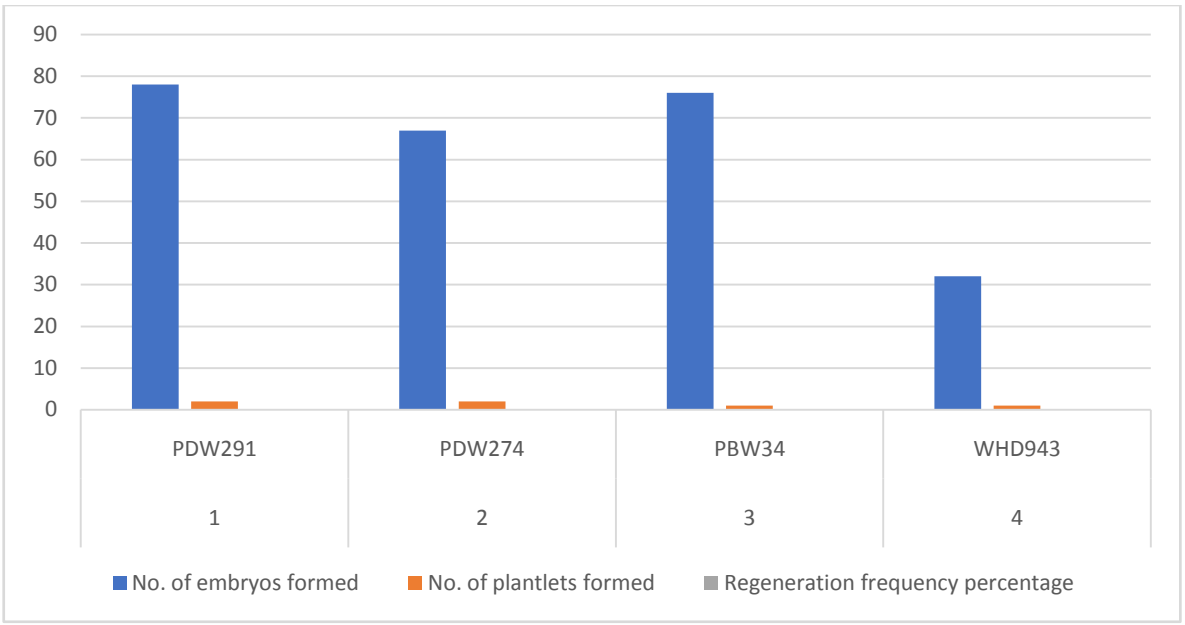

Figure 4. Bar graph representation of Regeneration frequency of Durum wheat x rye embryos.

SUMMARY

In bread wheat, the genotype PBW502 produced more seeds with 106 out of 197 florets pollinated highest number of seeds are formed in this genotype as compared to other genotypes and the crossability percentage is higher for this genotype at 31.97%. the regeneration frequency of this genotype is 9.52 %. In bread wheat, the genotype WH1105 produced 35 seeds out of 250 florets pollinated the crossability percentage of this genotype is 14% and the regeneration frequency percentage is 11.42% as compared to another genotype the regeneration frequency is higher for WH1105. In durum wheat, the genotype PDW291 embryo obtained was 106 out of 210 florets pollinated and with a crossability percentage of 50.47%. The regeneration frequency of this genotype is 1.88%. In durum wheat, the genotype WHD943 embryo obtained was 32 out of 188 florets pollinated and the embryo formation percentage is 17.02%. The regeneration frequency is 3.12%.

Conclusion.

The phenotypic characterization of a high-altitude Himalayan rye landrace in the northwest Indo-Gangetic plains revealed its adaptive traits, including compact growth habits, resilient leaf structure and strong stemshave a high crossability with *Triticumaestivum* and *Triticum durum* without the application of any Phyto-hormones-furthermore,the crossabilityof Rye is extremely self-incompatible, hence the only way to create inbred lines was using doubled haploid technology. The genotypes PBW502, WH1105, PDW291, and WHD943 have given better results after crossing with the Himalayan rye landrace.

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