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An analysis of hybrid sterility in rice (*Oryza sativa* L.) using genetically diverse germplasm under temperate ecosystem

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Sixty cross combinations consisting of 15 intervarietal *indica*, 15 intervarietal *japonica* and 15 crosses each of inter-subspecific and three-way crosses were generated through different mating patterns in *kharif* 2008 and evaluated for hybrid sterility under two different agroecologies of Kashmir during *kharif* 2009. The estimates of pollen and spikelet sterility were very high in *indica/japonica* crosses followed by *indicalindica* and *japonicaljaponica* crosses. The magnitude of pollen and spikelet sterilities were observed high in $L_1 \times T_1$ followed by $L_4 \times T_3$, $L_1 \times T_3$, $L_5 \times T_3$, $L_3 \times T_3$ and so on all belonging to inter-subspecific group. The mean estimates of pollen and spikelet sterilities of different crossing blocks were very high when compared to their corresponding parental mean. Only inter-subspecific cross $L_1 \times T_2$ yielded pollen and spikelet sterilities of less than 50% and another three crosses revealed a range between 50 and 70% for these traits. Five crosses were grouped as highly sterile by depicting sterility estimates greater than 70%. The mean estimates of pollen sterility and spikelet sterility for inter-subspecific crosses got reduced to the level of 30.80 and 25.75% respectively through three-way crosses by deploying wide compatibility cultivar (WCV) (Dular) as bridging parent. The estimated mean percentage overcome was 36.62% for pollen sterility and 63.92% for spikelet sterility. Such kind of modest attempt has widened the genetic variability by combining the genetic background across the two sub species and tailors the new genotypes. This in turn has paved way for the concept of ideotype breeding for unique cold temperate agroecologies.

Key words: Hybrid sterility, inter-subspecific crosses, intra-subspecific crosses, rice, wide compatibility cultivar (WCV).

INTRODUCTION

The strong hybrid vigor in the F_1 s between *indica* and *japonica* subspecies of Asian cultivated rice (*Oryza sativa* L.) has attracted a large amount of research interest, with the hope for developing hybrid rice by making use of such heterosis (Yang et al., 1962; Yuan, 1994). However, hybrid sterility frequently occurs in such inter-subspecific

crosses (Ikehashi, 1982), the fertility of *indica-japonica* hybrids varies widely from fully fertile to almost completely sterile, with the majority of such hybrids showing significantly reduced fertility (Oka, 1988; Liu et al., 1996; Zhang et al., 1997). Hybrid sterility is a major form of post zygotic reproductive isolation that restricts

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gene flow between populations and inter-sub specific hybrids are usually sterile (Jiang et al., 2012). Wide compatibility cultivars (WCVs) produce fertile hybrids when crossed to both *indica* and *japonica* cultivars. The discovery of WCVs brought hope by breaking the fertility barriers between *indica* and *japonica* subspecies and provided possibility for exploiting the very strong heterosis demonstrated in cross between the two subspecies. Consequently, there has been considerable interest in understanding the mechanism underlying wide compatibility and hybrid sterility. Under Kashmir valley- a unique temperate climate, *indica* sub-species are grown at an altitude range of 1350 to 1900 m amsl, whereas, *japonica* sub-species find their habitat under foot hills at an altitude of 1950 to 2300 m amsl. *Indica* types have higher yielding ability, wide adaptability and good grain quality, while as *japonica* types possess high degree of cold tolerance, early maturity and fertilizer responsiveness. The objective to combine the traits across the two subspecies is to develop the varieties with traits most suitable for the two agroecologies of Kashmir to raise productivity, quality and resilience to biotic and abiotic stresses. However, many attempts were made in this direction but major bottleneck in this effort was the factor of hybrid sterility. A modest effort in this direction was to assess and estimate the magnitude of hybrid sterility in intra- and inter-subspecific crosses *viz-a-viz* to determine the level of overcome by deploying wide compatibility cultivar (WCV) as bridging parent through three-way crosses and to get the most suitable recombinants for two different agroecologies of Kashmir.

MATERIALS AND METHODS

The materials for the present study comprised of twelve genetically diverse cultivars of rice (*O. sativa* L.) belonging to two sub-specific groups viz. *indica* and *japonica*. The six typical *japonica* types [Koshihikari (P₇), K-332 (P₈), GS-503 (P₉), GS-504 (P₁₀), Kohsar (P₁₁) and K-508 (P₁₂)] and six typical *indica* cultivars including one wide compatibility cultivar (WCV) [Jhelum (P₁), SK-382 (P₂), Shalimar Rice-1 (P₃), China-1039 (P₄), Chenab (P₅) and Dular (P₆) (WCV)] were selected out of the germplasm collection maintained at Rice Research and Regional Station, Khudwani of Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir.

Fifteen intra-sub specific hybrids were created from each half-diallel-mating (Griffing, 1956) using six parental lines each of *indica* and *japonica* rices in *Kharif* 2008. In the same season five *indica* genotypes as lines Jhelum (L₁), SK-382 (L₂), SR-1 (L₃), China-1039 (L₄) and Chenab (L₅) were crossed with three typical *japonica* cultivars as testers Koshihikari (T₁), K-332 (T₂) and Kohsar (T₃) in a line x tester fashion (Kempthorne, 1957) to generate 15 inter-subspecific (*indica*/*japonica*) hybrids. Five intervarietal single crosses using *indica* parents each with Dular (WCV) as one of the parent were mated to three typical *japonica* genotypes viz., Koshihikari, K-332 and Kohsar to generate 15 three-way crosses.

A total of sixty cross combinations generated (viz. 15 *indica* x *indica*, 15 *japonica* x *japonica*, 15 *indica* x *japonica*, and 15 three-way F₁s) were evaluated at two research stations namely, Rice Research and Regional Station, Khudwani (1650 m amsl) and the High Altitude Rice Research Sub-Station, Larnoo (2285 m amsl)

during *Kharif* 2009. Each progeny line was evaluated in two-row of 3 m length in a randomized complete block design (RCBD) with three replications. Twenty-eight days old seedlings with single plant/hill were planted at inter- and intra- row spacing of 20 and 15 cm, respectively. Recommended crop management practices were followed.

Pollen sterility and spikelet sterility are the best indices of hybrid sterility (Zhang and Lu, 1993). Hence hybrid sterility was studied in terms of these two aspects. Pollen sterility was observed under a light microscope using an iodine potassium iodide (I₂KI) [0.1%] staining method (Anonymous, 1996). Samples for pollen were collected from at least 10 florets from individual plants at the heading stage (6th growth stage). At least three random microscopic fields were used to count non-sterile pollen grains (dark stained round) and sterile pollen grains (unstained withered, unstained spherical, or partially stained round). Pollen sterility was calculated as ratio of sterile pollen to the total number of pollen grains. Similarly main/primary panicles of five randomly selected plants were bagged before heading stage. The harvested panicles were observed for filled and empty spikelets and mean spikelet sterility was calculated. The mean of the pooled data over the locations for pollen and spikelet sterility were subjected to statistical analysis for interpretation of results. The programme R-Software was used for estimating the variance analysis.

RESULTS AND DISCUSSION

Analysis of variance for pollen and spikelet sterilities revealed significant mean squares for all the genotypes on the whole and as different groups of crosses; however, higher estimates were observed for inter-subspecific crossing block. It was observed that variance due to inter-subspecific crosses was much more than the total variance contributed by all the groups of crosses together. Similarly variances due to interactions of crossing blocks with respect to locations were found significant to highly significant for these traits (Table 1). Estimate of interactions mean square again were observed high for *indica* intra-subspecific crosses followed by inter-subspecific group of crosses. The estimates of pollen and spikelet sterilities over locations of each genotype when crossed to its own or other subspecies and averaged over all possible combinations are listed in the Table 2. Similarly it also depicts pollen and spikelet sterilities of cross progenies involving single cross (one parent as WCV) with *japonica* parents averaged over two locations. Generally it was observed that F₁ progenies of *indica* genotypes exhibited relatively high sterility when crossed with *japonica* group of genotypes. Similar kind of trend was observed in F₁s of *japonica* genotypes when crossed with *indica* genotypes in line x tester mating design, however, reverse was the case for F₁s of intra-subspecific groups (*indica* x *indica* and *japonica* x *japonica*). The estimates of both kinds of sterilities were found non-significant in case of intra-subspecific (*indica* x *indica*) F₁s, however, low but significant estimates were observed for intra-subspecific (*japonica* x *japonica*) F₁s. The estimates of hybrid sterility (pollen and spikelet sterilities) of all single crosses with WCV (Dular) as one of the parent exhibited moderate

Table 1. Analysis of variance of various crossing blocks of rice for pollen and spikelet sterilities.

Source	df		Mean squares	F-test
	Main	Partitioned		
Location	1		11.04	4.92*
Block (loc)	2		5.5	2.45
Lines	59		5.6	2.5**
Among groups		3	42.17	4.82**
Within i x i		14	52.97	3.25**
Within j x j		14	10.78	2.45*
Within i x j		14	140.6	9.19**
Within three-way		14	8.28	2.24**
Lines x Location	59		2.24	
Loc x among groups		3	8.75	4.91**
Loc x within i x i		14	16.30	9.15**
Loc x within j x j		14	4.40	2.47*
Loc x within i x j		14	15.30	8.59**
Loc x within three-way		14	3.70	2.07*
Lines x Block (loc)	118		1.78	1.78

*, ** indicate significance at 5 and 1% level, respectively.

expression. All but one single cross exhibited moderate levels of expression for one or other kind of sterility, whereas, their corresponding three-way crosses followed similar trend but the values were non-significant. The higher and significant estimates of pollen and spikelet sterilities in inter-subspecific crosses and their low and non-significant expression in three-way crosses suggest the intervention of WCV.

The intensity of pollen and spikelet sterility varied greatly among and within crossing blocks [*indica x indica*, *japonica x japonica* and *indica x japonica*] from highly sterile to fully fertile. Barring a few exceptions, the magnitude of hybrid sterility of intra-subspecific (*indica x indica* and *japonica x japonica*) crosses, were much lower than those recorded for inter-subspecific (*indica x japonica*) crosses (Table 3). Further high mean pollen sterility of 58.26% was observed for *indica x japonica* crosses ranging between 8.3 and 96.3 % followed by relatively medium range (9.27 and 85.40%) and mean (30.25%) for intra-subspecific (*indica x indica*) crosses. The spikelet sterility did not perform differently and observed the similar trend with higher mean (76.51%) and wide range (31.80 and 96.60%) for inter-sub specific crosses. The higher estimates of hybrid sterility in inter-subspecific (*indica x japonica*) crosses were reported by number of workers (Oka, 1964; Liu et al., 1996, 1997). Among intra-sub specific crosses the high value of pollen sterility (85.4%) was observed on cross combination P₂ x P₆ (*indica x indica*). The lowest mean pollen sterility (10.97%) was recorded on intra-subspecific (*japonica x japonica*) crosses, besides, a constricted range (6.99 to 19.6%). Further spikelet sterility of intra-subspecific (*indica x indica*) cross progenies revealed a mean estimate of 27.32%. The intra-subspecific (*Japonica x*

Japonica) crosses recorded a lower mean value for the spikelet sterility (16.78%) and relatively a narrower range (6.50 to 31.74%) in comparison to range of intra-subspecific (*indica x indica*) cross progenies (13.11 to 74.00%).

The magnitude of pollen and spikelet sterilities were observed high on cross combination L₁ x T₁ (95.85 and 75.81%) followed by L₄ x T₃ (95.3 and 93.05%), L₁ x T₃ (92.03 and 86.08%), L₅ x T₂ (92.60 and 91.50%) and L₃ x T₁ (91.66 and 84.20%) all belonging to inter-subspecific crossing block. The only cross combination P₂ x P₆ belonging to intervarietal (*indica x indica*) group depicted the higher estimates for pollen and spikelet sterilities, that is, 85.4 and 74.0%, respectively. The mean pollen sterility estimates for intra-subspecific groups, that is, *indica x indica* (30.25%) and *japonica x japonica* (10.97%) crosses were about thrice and twice when compared to their corresponding parental mean values of 9.39 and 5.72%, respectively. The mean spikelet sterility of intra-sub specific (*indica x indica*) crosses (27.32%) was almost fourfold than the corresponding mean parental value (7.7%), whereas, for intra-sub specific group (*japonica x japonica*) the estimate for said sterility (16.78%) was just half of the corresponding parental mean (33.32%). High mean spikelet sterility of *japonica* parents than expected was owing to higher value of the same recorded on two *japonica* parents GS-503 and GS-504. The reason was due to their late heading which coincided with early commencement of cold weather as a result of untimely snowfall on adjoining mountains of High Altitude Rice Research Sub-Station, Larnoo (2200 m amsl)-one of the locations. This infact caused the cold stress that increased the rate of spikelet sterility for these parents.

Table 2. Pollen and spikelet sterilities (\pm SD) in the F₁ s of each parent of both subspecies averaged over two locations.

Variety	Sub species	Crossed to all varieties	<i>Indica</i>	<i>Japonica</i>	Selfing
Jehlum	I	45.78 \pm 8.12** (34.82 \pm 7.12)**	26.27 \pm 14.10(16.38 \pm 10.42)	78.31 \pm 15.13** (64.56 \pm 15.86)**	7.35(6.59)
SK-382	I	36.12 \pm 8.21** (42.23 \pm 8.18)**	34.49 \pm 21.22(33.53 \pm 19.64)	38.84 \pm 9.22** (74.74 \pm 12.28)**	9.55(7.26)
SR-1	I	33.56 \pm 6.22** (45.43 \pm 12.12)**	22.28 \pm 14.20(26.81 \pm 16.24)	52.36 \pm 18.02** (79.78 \pm 18.92)**	12.20(10.12)
China 1039	I	41.2 \pm 4.04** (42.34 \pm 11.22**)	23.27 \pm 14.78(20.85 \pm 15.18)	71.13 \pm 15.01** (78.14 \pm 28.95)**	8.30(6.91)
Chenab	I	36.34 \pm 5.28** (47.56 \pm 12.37)**	27.75 \pm 16.21(24.89 \pm 17.12)	50.66 \pm 14.15** (82.59 \pm 9.23)**	12.65(9.28)
Dular	I ^w	39.45 \pm 25.86** (40.86 \pm 26.20)**	39.45 \pm 25.86(40.86 \pm 26.20)	-	6.29(6.46)
Koshihikari	J	36.90 \pm 9.95** (45.91 \pm 14.39**)	13.87 \pm 3.62** (69.71 \pm 18.46)**	62.66 \pm 17.17** (22.04 \pm 4.18)**	6.80(51.40)
K-332	J	30.87 \pm 9.50** (74.70 \pm 20.80)**	47.87 \pm 10.07** (77.32 \pm 20.11)**	13.87 \pm 3.60** (22.86 \pm 6.08)**	4.88(11.88)
GS-503	J	8.88 \pm 1.81** (16.57 \pm 4.59)**	-	8.88 \pm 1.81** (16.57 \pm 4.59)**	7.49(55.29)
GS-504	J	9.15 \pm 1.91** (15.04 \pm 5.10)**	-	9.15 \pm 1.91** (15.04 \pm 5.10)**	6.66(60.18)
Kohsar	J	38.59 \pm 10.24** (41.52 \pm 14.24)**	64.25 \pm 14.28** (82.51 \pm 18.57)**	12.92 \pm 4.65** (16.53 \pm 5.16)**	3.86(8.62)
K-508	J	9.86 \pm 3.03** (15.19 \pm 4.52)**	-	9.86 \pm 3.03** (15.19 \pm 4.52)**	4.65(9.57)
Jhelum x Dular	(I x D)	24.7 \pm 3.02** (15.85 \pm 8.82)	-	16.51 \pm 2.32** (16.89 \pm 3.18)**	-
SK-382 x Dular	(I x D)	85.4 \pm 12.56** (74.0 \pm 20.45**)	-	31.58 \pm 8.90** (38.99 \pm 8.40)**	-
SR-1 x Dular	(I x D)	28.0 \pm 17.81(59.87 \pm 25.6*)	-	24.46 \pm 7.01** (25.16 \pm 4.06**)	-
China-1039 x Dular	(I x D)	26.8 \pm 15.38(15.17 \pm 8.90)	-	44.23 \pm 13.62** (25.64 \pm 3.70)**	-
Chenab x Dular	(I x D)	32.72 \pm 4.89** (39.42 \pm 20.7*)	-	37.25 \pm 12.32** (22.09 \pm 4.19)**	-

Figures in parentheses denote spikelet sterilities; I=*indica* sub species, J=*japonica* sub species, I^w=wcv with *indica* background and D=*Dular* (WCV). *, **, significant at 5 and 1% level respectively.

One inter-subspecific cross L₁ x T₂ yielded pollen and spikelet sterilities of less than 50% and another three crosses showed sterility estimates between 50 and 70%. Five crosses were highly sterile (pollen and spikelet sterility) spikelet sterilities were also reported by earlier workers on the same phenomenon. The mean pollen and spikelet sterilities of inter-sub specific (*indica* x *japonica*) crosses viz. 58.26 and 76.51% were manifold when compared to mean of 8 parents (5 lines and 3 testers) for the same traits, that is, 18.19 and 14% respectively. It was also observed that for intra-sub specific (*indica* x *indica*) crosses and in three-way crosses, pollen sterility showed positive and significant association with spikelet sterility, however, no such kind of association was observed for other two crossing blocks. These

results suggest that the two phenomena can occur independently or can happen together and are responsible for low seed setting (grain yield). When pollen and spikelet sterilities were correlated with number of grains harvested per panicle (Data not shown) it was highly significant and negative suggesting that both these aspects are accountable for yield, but in a negative fashion. Song et al. (2005) also reported that the pollen and embryo sac sterility contributed almost equally to fertility. However, Zhang and Lu (1993) and He et al. (1994) pointed out that the hybrid sterility within Asian cultivated rice mainly resulted from pollen abortion, whereas, reduced affinity between the uniting gametes is also an important cause for sterility (Liu et al., 2004). Kubo et al. (2011) reported that in inter-sub specific crosses

the hybrid male sterility gene *S24* caused the selective abortion of male gametes carrying the *japonica* allele (*S24-j*) via an allelic interaction in the heterozygous hybrids. Yan et al. (2003) demonstrated that inter-subspecific hybrid sterility mainly resulted from pollen abortion with the pollen deteriorating at various stages, and the correlation between pollen and spikelet sterility was highly significant in typical *indica-japonica* crosses.

The inter-subspecific test crosses L₁ x T₂, L₂ x T₂, L₂ x T₃, L₃ x T₂, L₅ x T₁ and L₅ x T₃ showed pollen sterility of just 25%, however, very high spikelet sterility suggesting that the semi sterility might be due to female gamete abortion. Since 25% sterility means 75% fertile pollen grains and the proportion was quite enough to set the

Table 3. Relative comparison of pollen and spikelet sterility averaged over two locations for *indica x indica*, *japonica x japonica* and *indica x japonica* crosses.

<i>Indica x Indica</i>	Pollen sterility (%)	Spikelet sterility (%)	<i>Japonica x Japonica</i>	Pollen sterility (%)	Spikelet sterility (%)	<i>Indica x Japonica</i>	Pollen sterility (%)	Spikelet sterility (%)
Jhelum x SK-382 (P ₁ x P ₂)	25.12	14.35	Koshihikari x K-332 (P ₇ x P ₈)	15.21	32.07	Jhelum x Koshihikari (L ₁ x T ₁)	95.85	75.81
Jhelum x SR-1 (P ₁ x P ₃)	30.49	13.11	Koshihikari x GS-503 (P ₇ x P ₉)	8.19	11.91	Jhelum x K-332 (L ₁ x T ₂)	47.05	31.80
Jhelum x Ch 1039 (P ₁ x P ₄)	14.36	24.11	Koshihikari x GS-504 (P ₇ x P ₁₀)	11.25	9.79	Jhelum x Kohsar (L ₁ x T ₃)	92.03	86.08
Jhelum x Chenab (P ₁ x P ₅)	37.13	17.5	Koshihikari x Kohsar (P ₇ x P ₁₁)	14.09	25.05	SK-382 x Koshihikari (L ₂ x T ₁)	62.06	60.58
Jhelum x Dular (P ₁ x P ₆)	24.27	15.85	Koshihikari x K-508 (P ₇ x P ₁₂)	6.99	31.74	SK-382 x K-332 (L ₂ x T ₂)	17.25	81.09
SK-382 x SR-1 (P ₂ x P ₃)	16.9	19.82	K-332 x GS-503 (P ₈ x P ₉)	11.83	9.79	SK-382 x Kohsar (L ₂ x T ₃)	37.21	82.56
SK-382 x Ch-1039 (P ₂ x P ₄)	16.9	27.16	K-332 x GS-504 (P ₈ x P ₁₀)	10.77	10.80	SR-1 x Koshihikari (L ₃ x T ₁)	91.66	84.20
SK-382 x Chenab (P ₂ x P ₅)	28.15	32.33	K-332 x Kohsar (P ₈ x P ₁₁)	19.6	10.84	SR-1 x K-332 (L ₃ x T ₂)	20.79	85.64
SK-382 x Dular (P ₂ x P ₆)	85.4	74.00	K-332 x K-508 (P ₈ x P ₁₂)	11.98	10.75	SR-1 x Kohsar (L ₃ x T ₃)	44.65	69.52
SR-1 x Ch-1039 (P ₃ x P ₄)	26.74	21.99	GS-503 x GS-504 (P ₉ x P ₁₀)	7.33	25.29	Ch-1039 x Koshihikari (L ₄ x T ₁)	55.43	44.77
SR-1 x Chenab (P ₃ x P ₅)	9.27	19.29	GS-503 x Kohsar (P ₉ x P ₁₁)	7.72	26.55	Ch-1039 x K-332 (L ₄ x T ₂)	61.67	96.60
SR-1 x Dular (P ₃ x P ₆)	28.00	59.87	GS-503 x K-508 (P ₉ x P ₁₂)	9.37	9.35	Ch-1039 x Kohsar (L ₄ x T ₃)	96.3	93.05
Ch-1039 x Chenab (P ₄ x P ₅)	31.51	15.91	GS-504 x Kohsar (P ₁₀ x P ₁₁)	9.35	13.75	Chenab x Koshihikari (L ₅ x T ₁)	8.3	83.22
Ch-1039 x Dular (P ₄ x P ₆)	26.87	15.17	GS-504 x K-508 (P ₁₀ x P ₁₂)	7.08	17.63	Chenab x K-332 (L ₅ x T ₂)	92.60	91.50
Chenab x Dular (P ₅ x P ₆)	32.72	39.42	Kohsar x K-508 (P ₁₁ x P ₁₂)	13.88	6.50	Chenab x Kohsar (L ₅ x T ₃)	51.10	81.35
Mean	30.25	27.32		10.97	16.78		58.26	76.51
Range	9.27-85.40	13.11-74.00		6.99-19.60	6.50-32.07		8.3- 96.3	31.80-96.60
CD at 5% level	4.30	1.216		4.044	8.632		7.97	7.22
Parental mean	9.39	7.7		5.72	33.32		-0.15	0.17
Correlation coefficient (r)					0.68 **			

grains, however, the empty grains obtained could be solely attributed to spikelet sterility. Similar sort of suggestions came from Iekashi and Araki (1986) who reported that hybrid sterility involved egg killer and induced abortion of megaspore. Female gamete abortion in *indica japonica* hybrids was also observed by Chen et al. (2008). Other possible reason is that the actual fertility (germinability) of the pollen produced by the *indica x japonica* hybrids would be much lower than what is observed with I₂KI staining. Because of the drastic fall in night temperature pollen fertility observed with I₂KI staining did not coincided with actual pollen germination. Liu et al.

(1992) reported that low germinability of morphologically normal pollen (<10%) could be a factor for sterility in *indica x japonica* hybrids.

Further cold stress experienced under high altitude testing ecology (2285 m amsl) at late heading stage (last week of August) is suggested a possible reason for varied expression of hybrid sterility within and more particularly among various crossing blocks particularly for inter-subspecific crosses generated for the study. This is supposed a major probability for recording differential expression of panicle fertility or seed setting in the present experiment. Qi et al. (1993) and Liu et al. (2004) proposed that the differential

expressions of hybrid sterility were in fact determined by temperatures in inter-subspecific hybrids which are highly prone to low temperature.

The other objective of the study was to estimate overcome in hybrid sterility by deploying WCVs as the bridging parent. It was observed that estimates of mean pollen sterility (58.26%) and spikelet sterility (76.51%) for inter-subspecific crosses got reduced to the level of 30.80 and 25.75% through three way-crosses with mean percentage overcome of 36.62 and 63.92%, respectively (Table 4). The highest estimate for pollen sterility was recorded on inter-subspecific

Table 4. Comparative estimates of pollen sterility and spikelet sterility (%) averaged over two locations for *indica x japonica* (*i x j*) crosses and their corresponding three-way crosses using WCV (*Dular*).

S/N	Cross combination (<i>i x j</i> crosses)	Pollen/spikelet sterility (%)	Cross combination (Three-way crosses)	Pollen/spikelet sterility (%)	Percentage overcome in sterility
1	Jhelum x Koshihikari L ₁ x T ₁	95.85 (75.81)	(Jhelum x Dular) x Koshihikari (L ₁ x D) x T ₁	19.18 (15.44)	79.98 (79.63)
2	Jhelum x K-332 L ₁ x T ₂	47.05 (31.81)	(Jhelum x Dular) x K-332 (L ₁ x D) x T ₂	14.90 (21.69)	68.33 (31.69)
3	Jhelum x Kohsar L ₁ x T ₃	92.03 (86.08)	(Jhelum x Dular) x Kohsar (L ₁ x D) x T ₃	15.47 (13.56)	83.19 (84.25)
4	SK-382 x Koshihikari L ₂ x T ₁	62.06 (60.58)	(SK-382 x Dular) x Koshihikari (L ₂ x D) x T ₁	47.49 (40.39)	23.47 (30.67)
5	SK-382 x K-332 L ₂ x T ₂	17.25 (81.09)	(SK-382 x Dular) x K-332 (L ₂ x D) x T ₂	25.55 (47.35)	-48.11 (33.28)
6	SK-382 x Kohsar L ₂ x T ₃	37.21 (82.56)	(SK-382 x Dular) x Kohsar (L ₂ x D) x T ₃	21.71 (20.89)	41.65 (74.70)
7	SR-1 x Koshihikari L ₃ x T ₁	91.66 (84.20)	(SR-1 x Dular) x Koshihikari (L ₃ x D) x T ₁	39.26 (21.45)	57.17 (74.52)
8	SR-1 x K-332 L ₃ x T ₂	20.79 (85.64)	(SR-1 x Dular) x K-332 (L ₃ x D) x T ₂	26.43 (30.69)	-27.13 (64.16)
9	SR-1 x Kohsar L ₃ x T ₃	44.65 (69.52)	(SR-1 x Dular) x Kohsar (L ₃ x D) x T ₃	7.69 (23.34)	82.78 (66.43)
10	China-1039 x Koshihikari L ₄ x T ₁	55.43 (44.77)	(China-1039 x Dular) x Koshihikari (L ₄ x D) x T ₁	6.41 (20.41)	88.43 (54.41)
11	China-1039 x K-332 L ₄ x T ₂	61.67 (96.60)	(China-1039 x Dular) x K-332 (L ₄ x D) x T ₂	93.88 (43.74)	-52.23 (54.72)
12	China-1039 x Kohsar L ₄ x T ₃	96.3 (93.05)	(China-1039 x Dular) x Kohsa (L ₄ x D) x T ₃	32.40 (12.79)	66.35 (86.25)
13	Chenab x Koshihikari L ₅ x T ₁	8.3 (83.22)	(Chenab x Dular) x Koshihikari (L ₅ x D) x T ₁	7.53 (17.27)	9.28 (79.25)
14	Chenab x K-332 L ₅ x T ₂	92.60 (91.05)	(Chenab x Dular) x K-332 (L ₅ x D) x T ₂	91.40 (37.29)	1.30 (59.24)
15	Chenab x Kohsar L ₅ x T ₃	51.10 (81.35)	(Chenab x Dular) x Kohsar (L ₅ x D) x T ₃	12.84 (11.73)	74.87 (85.58)
	Mean	58.26 (76.51)		30.80 (25.75)	36.62 (63.92)
	Range	8.3-96.3 (31.80-96.60)		6.41-93.88 (11.73-54.10)	
	CD at 5 % level	7.97		7.65	
	Correlation coefficient (<i>r</i>)		0.64**		

Figures in parenthesis denote spikelet sterility]; D=Dular; L and T denote lines and testers, respectively.

cross L₁ x T₁ (95.85%) followed by L₅ x T₃ (96.3%), L₁ x T₃ (92.03%) and L₄ x T₃ (91.66%). Among three-way crosses that recorded low estimates for pollen sterility were (L₃ x D) x T₃ (7.69%), (L₅ x D) x T₁ (7.53%) and (L₄ x D) x T₁ (6.41%), whereas, larger estimates among three-way crosses were noticed on (L₄ x D) x T₂ (93.88%) followed by (L₅ x D) x T₂ (91.40%). The three-way cross (L₄ x D) x T₁ recorded highest overcome in pollen sterility (88.43%) followed by (L₁ x D) x T₃ (83.19%) and (L₃ x D) x T₃ (82.78%) and so on. The reduction was noticed in negative direction on three three-way crosses namely (L₂ x

D) x T₂ (-48.11%), (L₃ x D) x T₁ (-27.13%) and (L₄ x D) x T₂ (-52.23%) meaning that WCV could not overcome this type of sterility even to the smallest degree. Regarding the spikelet sterility, the three-way crosses narrowed down the range from a low 11.73 % to a high 54.10 % with mean reduction of 63.92% against the corresponding inter-subspecific single crosses. The magnitude of spikelet sterility was recorded highest for single cross L₄ x T₂ (96.60%) followed by L₄ x T₃ (93.05%) and L₅ x T₃ (91.50%). The estimates of spikelet sterility were relatively lower for L₁ x T₂ and L₄ x T₁ with magnitude of 31.80 and 44.77%,

respectively. The sterility got reduced by a big margin of 86.25% in three-way cross (L₄ x D) x T₃ against corresponding single cross L₄ x T₃. The high reduction for the trait was also observed for three-way crosses (L₅ x D) x T₃ (85.58%) and (L₁ x D) x T₃ (84.25%) when compared with corresponding single crosses L₅ x T₃ and L₁ x T₃, respectively. Yang et al. (2012) showed that wide compatibility gene *S₅ⁿ* can overcome the embryo-sac sterility between *indica-japonica* hybrids caused by the *S₅* locus located on chromosome 6. Embryo-sac fertility was more than 93% in the *S₅ⁿ* gene-harboring hybrids, whereas embryo-sac

fertility was relatively low in control hybrids between typical *indica* and *japonica* cultivars without the S_5^n gene, suggesting that S_5^n can overcome the sterility between *indica-japonica* hybrids.

The *hybrid sterility* analysis showed a considerable variation in F_1 s with the same WCV (Dular) in different *indica* and *japonica* backgrounds. Differential expression under different backgrounds suggests the inadequate nature or inability of wide compatibility gene (WCG) from Dular source only, although both kinds of sterilities got reduced to an appreciable extent in some cross combinations. The reason of deploying only one WCV in the present experiment was due to non-availability of such established lines in the germplasm bank at the Regional Rice Research Station, Khudwani together with scanty information regarding the germplasm pertaining to this phenomenon. The other possible reason for this variable sterility is that additional genes might be involved that modify hybrid fertility in the presence of wide compatible gene WCG, since a large number of genes exist that affect hybrid fertility in one way or another. Epistasis or non-allelic interactions may also be involved for differential degrees of expression in different backgrounds. Kumar and Chakraborty (2000) observed different degree of expression of WCGs and suggested the set of modifier gene(s) and epistasis. Kinoshita (1995) emphasized the role of additional genes modifying hybrid fertility in the presence of WCG in one way or another, whereas, Ikehashi and Wan (1996) proposed other loci with minor effect in addition to S_5 in improving hybrid fertility. Kubo and Yoshimura (2005) and Kubo et al. (2008) suggested the role of epistatic interactions for differential expression of hybrid semi sterility.

The present study demonstrates differential expression of hybrid sterility among and within crossing blocks in terms of both pollen and spikelet sterility. The findings give us clue about the genetic relatedness regarding the germplasm available at the research station and explore high level of genetic distinctness between *indica* and *japonica* genotypes followed by within *indica* and close relatedness within *japonica* types. Since this was the modest and first successful attempt under cold temperate conditions of Kashmir, although few attempts earlier were made but they failed to make any conclusion. Now the genetically distinct parents identified will be crossed *inter se* followed by pedigree method of selection under different agroecologies to throw suitable promising segregants. Besides ideotype breeding/super rice breeding can be initiated by combining desirable traits from *indica* genotypes (desirable grain shape, texture and quality) with *japonica* genotypes (lodging resistance, early maturity and cold tolerance) and reciprocally through combination breeding. Under the present investigation only one WCV was attempted, hence several more need to be assessed so that many improved WC cultivars with *indica* and *japonica* backgrounds usable as cultivars and parents in hybrid breeding could be developed for unique temperate

conditions of Kashmir and equivalent ecologies worldwide. The new materials developed/derived will definitely overcome the yield barriers and will prove useful at an altitude range of 1500 to 2300 m amsl unique rice grown temperate agro-ecologies.

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