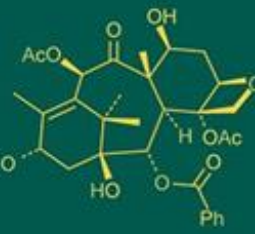
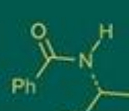
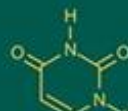
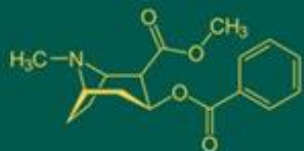


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Evaluation of backcross-derived maintainer lines for stigma Exsertion and associated agronomic traits in hybrid rice

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Abstract

Stigma exsertion is a key floral trait influencing outcrossing ability and hybrid seed production efficiency in rice. The present study evaluated 32 rice genotypes comprising parental lines, checks and BC₂ backcross-derived maintainer lines for stigma exsertion percentage (SE%) and associated agronomic traits. The experiment was conducted during Kharif 2025 in a randomized block design with two replications. Analysis of variance revealed significant genotypic differences for all traits, indicating exploitable variability. The SE% varied from 30.45% in BCN 41 to 86.87% in BCN 16.2, with a mean of 61.25%. Eight genotypes were grouped in the high stigma-exsertion class of 80-90%, while the largest frequency class was 40-50%, comprising 11 genotypes. The highest SE% was recorded in BCN 16.2, followed by BCN 22.2, BCN 31, BCN 17.2 and BCN 26. Correlation analysis showed significant positive association of SE% with panicle length and thousand grain weight, but negligible association with single plant yield. Supporting divergence analysis indicated that SE% contributed the highest proportion to total divergence (16.16%), followed by panicle length (15.72%). The study identified BCN 16.2, BCN 22.2, BCN 17.2, BCN 26, and BCN 31 as promising BC₂ lines with high seed exertion (SE). These lines can be further selected and advanced in the backcross breeding programme for the development of high-SE CMS lines.

Keywords: Hybrid rice, maintainer line, stigma exsertion, outcrossing, floral trait, genetic variability

1. Introduction

Hybrid rice is an important technology for increasing rice productivity, but its commercial value depends not only on heterosis but also on the ability to produce hybrid seed efficiently and economically. In the three-line hybrid rice system, cytoplasmic male sterile lines serve as female parents, while corresponding maintainer lines are required to maintain and improve these female-line backgrounds. Floral traits that improve pollen reception, stigma exposure and outcrossing therefore have direct practical relevance in parental-line improvement.

Among floral traits, stigma exsertion is one of the most important determinants of outcrossing ability. Stigma exsertion refers to the protrusion of one or both stigmas outside the spikelet after anthesis and spikelet closure. Exserted stigmas remain available for pollen capture for a longer period and can improve cross-pollination under hybrid seed production conditions. Classical studies on rice floral biology reported genetic variability for floral characters influencing outcrossing (Virmani & Athwal, 1973) [1], while later syntheses emphasized that pistil traits, including stigma exsertion, are among the most useful traits for improving hybrid seed production (Marathi & Jena, 2015) [2].

Recent literature continues to support this breeding priority. Ashraf *et al.*, (2024) [3] reviewed the role of floral traits and breeding technologies in hybrid rice production and emphasized that floral architecture, stigma traits and flowering behavior influence hybrid seed production success. Guo *et al.*, (2024) [4] described stigma exsertion rate as a key trait affecting the outcrossing ability of sterile lines and summarized molecular and quantitative genetic progress in stigma-exsertion improvement. Studies on grain shape and stigma exsertion have also shown that floral and spikelet structure can influence stigma exposure and outcrossing rate (Tan *et al.*, 2023; Zhu *et al.*, 2023) [5, 6].

Stigma exertion is not a simple single-gene trait. It is generally considered a quantitative floral trait controlled by multiple loci and influenced by genetic background, stigma size, style length, glume morphology, flowering behavior and panicle architecture. QTL studies have confirmed that multiple genomic regions contribute to stigma exertion rate in rice, and that high stigma exertion can be reconstructed through pyramiding of favourable loci (Tan *et al.*, 2022; Yang *et al.*, 2024)^[7,8]. This genetic complexity makes phenotypic screening of breeding material important, especially when the objective is to identify practical parental-line candidates rather than only map genomic regions.

Backcross-derived maintainer lines provide a useful context for such evaluation because they are expected to retain desirable maintainer-line backgrounds while carrying improved floral or agronomic attributes. However, high stigma exertion alone is not sufficient for breeding use. A line with high SE% but poor spikelet fertility or poor yield-related performance may be valuable as a donor but may not be suitable for direct advancement. Therefore, stigma exertion must be interpreted together with fertility, grain filling and agronomic traits.

The present study was conducted to evaluate BC₂ backcross-derived maintainer lines and related genotypes for SE% and associated agronomic traits. The objectives were to quantify variability for stigma exertion, identify high-SE maintainer-line derivatives, assess the relationship of SE% with fertility and yield-related traits, and develop a balanced selection interpretation for hybrid rice parentalline improvement.

2. Materials and methods

2.1 Experimental material and field evaluation

The experimental material consisted of 32 rice genotypes comprising four parental lines, five check varieties and 23 BC₂ backcross-derived maintainer lines. The parental lines included 14RR1, 14RR2, IR79156B and IR58025B. The check varieties included CO-51, US-314, GONTRA BIDHAN-3, VNR-2228 and 27P63. The backcross-derived maintainer lines consisted of BCN and BCP series lines developed in the hybrid rice breeding programme.

The field experiment was conducted during Kharif 2025 at ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, India. The experiment was laid out in a randomized block design with two replications. Standard crop management practices were followed to raise a healthy rice crop. Observations were recorded on representative competitive plants from each genotype and replication.

2.2 Trait recording

Observations were recorded for days to 50% flowering (DFF), plant height (PH), panicle length (PL), number of productive tillers (NPT), number of grains per panicle (NGP), number of filled grains per panicle (NFG), number of unfilled grains per panicle (UFG), spikelet fertility% (SF), thousand grain weight (TGW), single plant yield (SPY) and stigma exertion percentage (SE%). Rice trait observations were recorded following standard rice descriptor principles described in the Standard Evaluation System for Rice (IRRI SES, 2013).

Stigma exertion was recorded after anthesis from representative panicles. Spikelets were examined and classified as spikelets with no stigma exertion, single-stigma exertion or dual-stigma exertion. Total SE% was calculated as the percentage of spikelets showing either single or dual stigma exertion. For distributional interpretation, genotypes were

grouped into 10% SE classes from 0-10% to 90-100% (Ying and Zhang 1989)^[9]

2.3 Statistical analysis

Analysis of variance was performed to test genotypic differences among the 32 genotypes. Phenotypic correlation coefficients were estimated using Pearson's correlation approach (Pearson, 1895)^[10] to examine trait associations. Path coefficient analysis was performed following Dewey and Lu (1959)^[11] to partition correlations into direct and indirect effects. Statistical analyses were carried out using STAR version 2.0.1 (Statistical Tool for Agricultural Research, International Rice Research Institute), Microsoft Excel 365 and R version 4.4.2 using R Studio 2024.12.0+467 for figure generation.

3. Results

3.1 Significant genotypic variability for stigma exertion and associated traits

Analysis of variance revealed significant differences among genotypes for all traits studied, including SE% (Table 1). The genotype mean square was highly significant for DFF, PH, PL, NPT, NGP, NFG, UFG, SF, TGW, SPY and SE%, indicating the presence of substantial variability among parental lines, checks and backcross-derived maintainer lines.

The significance of genotypic variation for SE% was particularly relevant because this trait is directly associated with outcrossing ability. However, significant variation for fertility and yield-related traits indicated that high SE% should be evaluated together with reproductive and agronomic performance rather than used as an isolated selection criterion.

Table 1: Analysis of variance summary for stigma exertion percentage and associated agronomic traits.

Trait	Genotype mean square (df = 31)	Error mean square (df = 287)
DFF	106.33**	0.49
PH	816.00**	68.00
PL	20.88**	2.83
NPT	15.35**	4.43
NGP	5392.00**	944.00
NFG	7636.00**	812.00
UFG	6019.00**	420.00
SF%	3970.00**	188.00
TGW	66.72**	0.96
SPY	120.30**	20.50
SE%	3021.20**	0.60

Note: DFF = days to 50% flowering; PH = plant height; PL = panicle length; NPT = number of productive tillers per plant; NGP = number of grains per panicle; NFG=number of filled grains per panicle; UFG = number of unfilled grains per panicle; SF% = spikelet fertility%; TGW = thousand grain weight; SPY = single plant yield; SE%=stigma exertion percentage. ** indicates significance at $P \leq 0.01$

3.2 Distribution of stigma exertion and identification of high-SE lines

The high-SE parental lines 14RR1 and 14RR2 recorded SE values of 72.93% and 76.15%, respectively, confirming their usefulness as donor parents for stigma exertion improvement. Among the backcross-derived maintainer lines, BCN 16.2 recorded the highest SE% (86.87%), followed by BCN 22.2 (86.12%), BCN 31 (84.24%), BCN 17.2 (84.06%) and BCN 26

(81.66%). However, high SE% was not always associated with high spikelet fertility or single plant yield. Therefore, these lines should be considered high-SE sources requiring further validation for fertility, outcrossing ability and agronomic performance (Table 2).

Frequency distribution further clarified the structure of variation (Figure 1). No genotype occurred in the 0-10%, 10-

20% or 20-30% classes, and only one genotype occurred in the 30-40% class. The largest class was 40-50%, comprising 11 genotypes. Eight genotypes were grouped in the 80-90% class, indicating the presence of a distinct high-SE group. The 50-60% and 70-80% classes each contained five genotypes, while the 60-70% class contained two genotypes. No genotype was recorded in the 90-100% class.

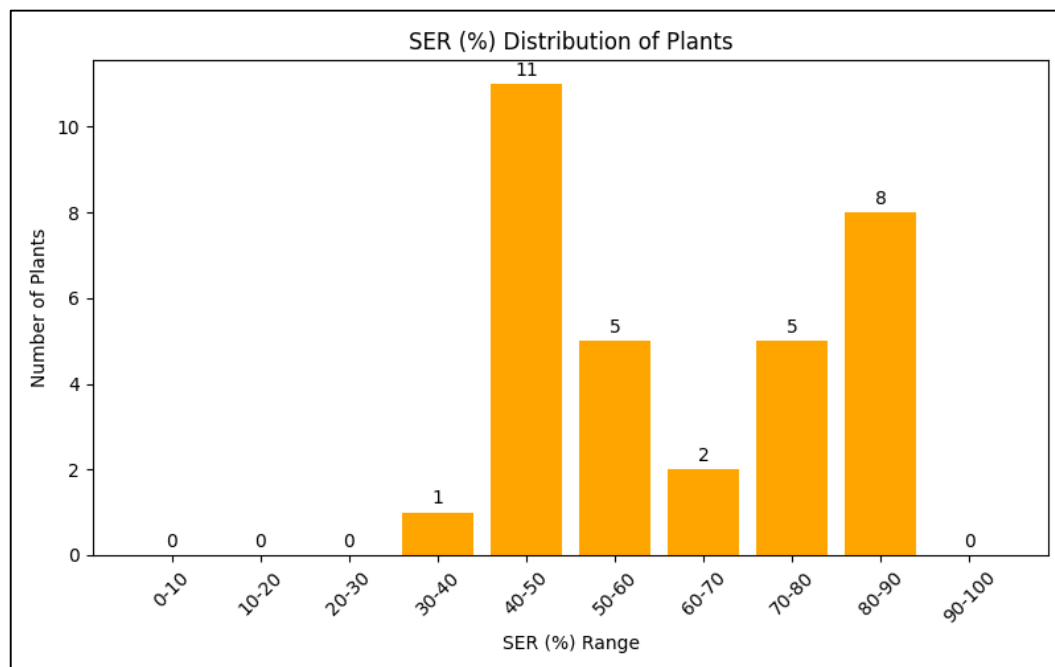


Fig 1: Frequency distribution of evaluated rice genotypes based on stigma exertion percentage classes.

Table 2: Selection summary of high-SE and fertility/yield-balanced genotypes.

Genotype	SE (%)	SF (%)	SPY (g plant ⁻¹)	Breeding interpretation
BCN 16.2	86.87	33.48	5.03	Highest SE%; donor-type candidate; needs fertility and yield improvement
BCN 22.2	86.12	51.21	11.60	High SE%; useful candidate for further selection
BCN 31	84.24	31.32	6.74	High SE but low fertility; use cautiously as donor
BCN 17.2	84.06	39.33	7.60	High SE; requires fertility and yield improvement
BCN 26	81.66	79.46	12.02	High SE with better fertility balance
BCN 44	45.30	73.58	18.62	Highest yield but low-to-moderate SE

3.3 High stigma exertion did not always coincide with fertility and yield superiority

The high-SE genotypes showed important differences in reproductive and yield-related performance (Table 2). BCN 16.2 recorded the highest SE% (86.87%) but showed low spikelet fertility (33.48%) and low single plant yield (5.03 g plant⁻¹). Similarly, BCN 31 recorded high SE% (84.24%) but had the lowest spikelet fertility among the selected high-SE lines (31.32%). These genotypes are therefore useful as high-SE sources but should not be considered directly superior breeding lines without further improvement or validation.

In contrast, BCP 16.2 and BCP 17.2, which are BC₂ backcross-derived maintainer lines, recorded high spikelet fertility values of 90.53% and 91.06%, respectively, while also expressing high SE% above 80%. BCN 26 also expressed high SE% (81.66%) with comparatively better spikelet fertility (79.46%) and single plant yield (12.02 g plant⁻¹). These lines appear more balanced for breeding use than genotypes that express high SE% but poor fertility.

BCN 44 recorded the highest single plant yield (18.62 g plant⁻¹) but only moderate SE% (45.30%), indicating that yield

superiority and stigma exertion were not necessarily expressed in the same genotype. This separation supports a two-level selection strategy: high-SE donor lines should be identified for floral improvement, while balanced lines should be identified for further advancement.

3.4 Trait associations relevant to stigma exertion-based selection

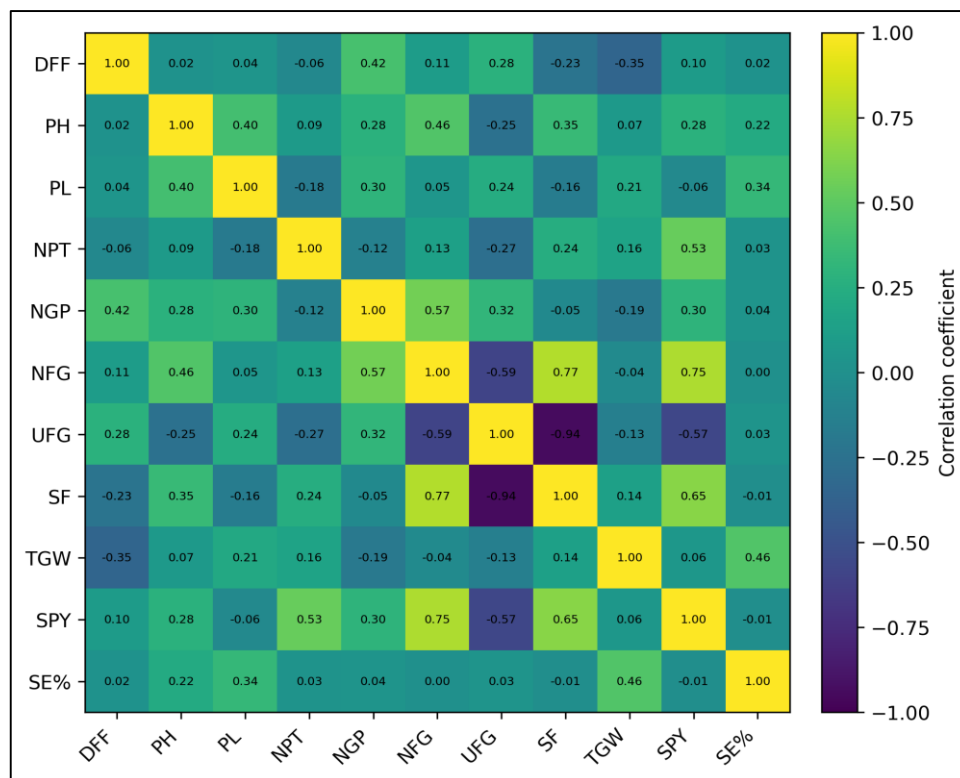
Correlation analysis revealed that SE% had a significant positive association with PL ($r=0.3355^{**}$) and TGW ($r=0.4582^{**}$), whereas its association with SPY was negligible and non-significant ($r=-0.0113$) (Table 3). Similarly, SE% showed weak and non-significant associations with NPT, NGP, NFG, UFG, SF% and DFF.

The correlation heat map further confirmed that SE% was more closely associated with panicle length and thousand grain weight than with direct yield expression (Figure 2). This result is important for breeding interpretation because it indicates that SE% should be treated primarily as an outcrossing-related floral trait rather than a direct yield trait.

Table 3: Phenotypic correlation of stigma exertion percentage with associated traits.

Trait	Correlation with SE%	Significance
DFF	0.0214	NS
PH	0.2218	NS
PL	0.3355	**
NPT	0.0259	NS
NGP	0.0353	NS
NFG	0.0028	NS
UFG	0.0346	NS
SF	-0.0144	NS
TGW	0.4582	**
SPY	-0.0113	NS

Note: indicates significance at $P < 0.01$; NS = non-significant.

**Fig 2:** Correlation heat map showing relationships among stigma exertion percentage and associated agronomic traits.

DFF = days to 50% flowering; PH = plant height; PL = panicle length; NPT = number of productive tillers per plant; NGP = number of grains per panicle; NFG = number of filled grains per panicle; UFG = number of unfilled grains per panicle; SF% = spikelet fertility%; TGW = thousand grain weight; SPY = single plant yield; SE% = stigma exertion percentage.

4. Discussion

The present study demonstrates that backcross-derived maintainer lines possess substantial exploitable variation for SE%. The observed range of 30.45-86.87% indicates that the evaluated material allowed clear differentiation between low, moderate and high stigma-exsertion types. The presence of eight genotypes in the 80-90% class is particularly useful for hybrid rice breeding because high stigma exertion improves stigma exposure and increases the opportunity for pollen capture in female-line seed production.

The importance of stigma exertion in hybrid rice is well established. Virmani and Athwal (1973)^[1] showed that rice floral traits influencing outcrossing are genetically variable. Marathi and Jena (2015)^[2] later emphasized that pistil traits are critical for improving hybrid seed production. More recent reviews and genetic studies have reinforced the same breeding

logic, identifying stigma exertion rate as a key determinant of outcrossing ability in sterile lines and an important target for molecular and phenotypic improvement (Ashraf *et al.*, 2024; Guo *et al.*, 2024; Yang *et al.*, 2024)^[3, 4, 8].

A major practical finding of the study is that high SE% did not always coincide with high fertility or yield. The high-SE parental lines 14RR1 and 14RR2 recorded SE values above 70%, confirming their usefulness as donor parents for stigma exertion improvement. Among the backcross-derived maintainer lines, BCN 16.2 recorded the highest SE% but had low spikelet fertility and low single plant yield, while BCN 31 also expressed high SE% but showed poor spikelet fertility. These genotypes may be useful as high-SE sources, but their direct advancement would require caution. Among the high-SE derived lines, BCN 26 showed comparatively better balance between SE%, spikelet fertility and yield-related performance. This distinction between high-SE donor value and advancement value is important for breeding decisions.

The weak association between SE% and SPY further clarifies the biological role of stigma exertion. SE% is not a direct yield trait under maintainer-line plot evaluation. Instead, it is an outcrossing-related floral trait that may improve hybrid seed production when expressed in the corresponding female-line

background. Grain yield per plant depends more directly on filled grains, spikelet fertility, productive tillers and grain number. Therefore, selection for SE% should be integrated with fertility and agronomic performance rather than used as a stand-alone criterion.

The significant positive association of SE% with PL and TGW suggests that stigma exposure may be linked with structural attributes of panicle and spikelet development. This interpretation is consistent with recent reports showing relationships between stigma exertion, grain shape and floral organ morphology (Tan *et al.*, 2023; Zhu *et al.*, 2023) ^[5, 6]. However, the moderate magnitude of these correlations indicates that PL and TGW cannot replace direct measurement of SE%. Direct phenotyping remains necessary for accurate identification of high-SE lines.

The supporting divergence analysis also indicated that SE% was the largest contributor to total divergence among the evaluated traits. This confirms that SE% was not a marginal descriptor but a major differentiating trait in the material. Nevertheless, full diversity analysis was not retained as a central section in the manuscript because the primary objective was to identify and interpret high-SE maintainer-line candidates. Treating divergence as supporting evidence keeps the article focused and avoids converting the study into a general diversity paper.

Overall, the most useful outcome is the identification of trait-specific breeding material. BCN 16.2, BCN 22.2, BCN 17.2, BCN 26 and BCN 31 are useful high-SE sources. BCN 31 should be used cautiously due to low fertility. Such lines should be evaluated further for flowering synchrony, actual outcrossing rate, seed set under hybrid seed production conditions and performance of corresponding male sterile-line backgrounds.

5. Conclusion

Overall, 14RR1 and 14RR2 were confirmed as high-SE parental donors, with SE values above 70%. Among the backcross-derived maintainer lines, BCN 16.2, BCN 22.2, BCN 31, BCN 17.2 and BCN 26 expressed high SE% and may serve as useful sources for improving stigma exertion. The trait SE expressed a positive correlation with the traits panicle length and thousand grain weight. Since, high SE% did coincide with high spikelet fertility or single plant yield, the selected lines require further validation for fertility, outcrossing rate and hybrid seed production performance before use in parentalline improvement.

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