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COMPARATIVE PERFORMANCE OF F_2- AND BC_1F_1- DERIVED DOUBLED HAPLOIDS IN MAIZE (*ZEA MAYS* L.)

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ABSTRACT
Conventional method of developing homozygous lines for use as parents of hybrid cultivars in crops including maize is time and resource demanding process. Doubled haploid (DH) induction is the quickest method of developing homozygous lines. Breeders most frequently induce DHs from heterozygous generation such as F_1's of breeding crosses, referred to as breeding populations (BPs). Simulation experiments have indicated that induction of DHs from F_2 BPs not only help break undesirable linkages but also offer selection prior to induction. However, considering that F_2 and BC_1F_1 (backcrossing F_1 with superior parent) require same time for development, we hypothesized that *per se* performance of BC_1F_1-DH would be greater than those of F_2 DHs, especially when one parent of the breeding crosses is better than the other. This hypothesis is based on the rationale that frequency of favourable alleles would be increased in BC_1F_1. To test this hypothesis, we developed doubled haploids (DH) from F_2 and BC_1F_1 populations derived from four crosses using in vivo maternal induction at CIMMYT haploid induction facility located in Agricultural research station (ARS) Kunigal, Karnataka, India. These DHs were evaluated in two-replicated alpha lattice design for grain yield. The results indicated that the estimates of mean grain yield, frequency of transgressive segregation (TS) of DHs derived from BC_1F_1 BPs were greater than those derived from of F_2 BPs in all the four breeding crosses. Implications of these results are discussed in relation to adoption of appropriate strategies for rapid genetic gain in maize.

KEYWORDS: doubled haploid, grain yield, maize, back cross, breeding potential

INTRODUCTION

Maize (*Zea mays* L.) is a global staple crop that contributes to food security, animal feed, and industrial application (Tiah et al., 2026). It originated in the mountains of southern Mexico and Guatemala and is now the world's third most extensively cultivated crop, following rice and wheat (Hallauer & Carena, 2014). The vast majority of worldwide maize is farmed in temperate regions, with the United States, China, Brazil, and Mexico accounting for 70% of total production. Though India has 5% of the global maize area, it contributes only 2% of the world's produce (Erenstein et al., 2022). The significant disparity between global (5.88 t ha^{-1}) and Indian maize productivity (3.26 t ha^{-1}) may be bridged by exploiting heterosis through the development and deployment of superior hybrids (Sarangi et al., 2020). Development of superior hybrids requires development of homozygous lines, which can be obtained either by continuous selfing or by doubled haploid (DH) induction. DH induction is the most rapid method of developing homozygous lines. The other advantages of using DHs are as follows (i) selection among DH is more effective because within-line genetic variation is less, (ii) the issue of early generation testing becomes mute, (iii) with intervening selfing generations (F_3, F_4, F_5, etc.) not being present, the number of plants handled in the breeding program is substantially reduced, (iv) deleterious alleles are completely eliminated due to 100% homozygosity of DHs and (v) *per se* performance of DH increase due to selection pressure in haploid phase and/or during first generation of DHs. Most appropriate source / breeding population is more critical for induction of DH. Frequently DHs are induced from F_1 generation. Simulation and empirical experiments have indicated that induction of DHs from F_2 BPs not only help break undesirable linkages but also offer selection prior to induction. However, considering that both F_2 and BC_1F_1 require same time for their development and that favourable linkage between genes controlling target traits is preserved in BC_1F_1 due to low net recombination we hypothesized that *per se* performance of BC_1F_1-DH would be greater than those of F_2 DHs especially when one parent of the breeding crosses is better than the other. The present study is aimed at testing this hypothesis of comparative performance based on breeding potential of F_2- and BC_1F_1- derived doubled haploids in maize.

MATERIAL AND METHODS

Development of Experimental Material

The basic material for study consisted of F_2-derived and BC_1F_1- derived DH populations derived from four breeding crosses (Table 1). The seeds of the parents of these crosses were planted during 2021 post-rainy season (rabi). Considering NAI 317, SKV 50 and CML 451 as female parents, the male parents were chosen such that they are far superior than the female parents which means they contain favourable dominant alleles which are not present in the female parent. The plants of female parents were detasseled and manually crossed with their respective male parents in line × tester mating design (Kempthorne, 1957) to generate F_1 hybrids. F_1s were raised during 2022 summer. The seeds collected from each of the F_1s were selfed and seeds were collected from each of the F_2 plants. Parallelly, F_1s were back-crossed to the male parent (superior) and seeds were collected from each of the BC_1F_1 plants. The seeds so collected were sown to raise F_2 population and BC_1F_1 population along with maternal inducer line at Agriculture Research Station (ARS) Kunigal. The F_2 and BC_1F_1 plants derived from each of the four crosses were manually detasseled. The pollen grains were collected from maternal inducer lines and dusted onto detasseled parents. The true haploid seeds were identified using pigmentation as marker. The true haploids were treated with colchicine to develop DH plants at International Center for Maize and Wheat Improvement (CIMMYT) Maize Doubled Haploid (DH) facility in ARS, Kunigal, Karnataka.

Evaluation of Experimental Material

F_2- and BC_1F_1-derived DH populations from four breeding crosses were evaluated in the alpha lattice design with two replications at experimental plot of All India Coordinated Research Project (AICRP) on Soybean of Zonal Agricultural Research Station (ZARS), University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bengaluru during 2024 rainy season. The seeds of F_2- and BC_1F_1-derived DH families of all the crosses were planted in a single row of 3 m length spaced 0.6 m between rows. Fifteen days after sowing, seedlings were thinned-out to maintain a spacing of 0.2 m between plants within a row. A population of around ten individuals within each DH family were maintained. All the recommended package of practices were followed to raise a healthy crop.

Sampling of plants and data recording

.Five plants were randomly selected in DH families (avoiding border ones) in each of the two replications. Cobs were harvested from these five plants at physiological maturity from the two replications and were sun-dried. Sun-dried cobs were hand-threshed and the resulting grains were weighed using metric scale and expressed as average grain yield plant^{-1} (g).

Statistical Analysis

The replication-wise data on grain yield plant^{-1} of four F_2- and BC_1F_1- derived DH populations were used for statistical analysis. Analysis of variance was performed for both F_2 and BC_1F_1-DH separately according to alpha lattice design to partition the total variation into DH families, replication and blocks. This analysis was implemented in R software *ver* 4.5.3. After ascertaining significant mean squares attributable to DH families, mean grain yield plant^{-1} of F_2 and BC_1F_1-DH derived from all the four crosses were used to were used to compute the following descriptive statistics and genetic parameters. Mean and range for grain yield plant^{-1} was computed using the following formula. Mean ($\bar{X}$) = $\frac{\text{Sum of grain yield of DH families in each of the DH population}}{\text{Number of DH families}}$ Absolute range (AR) was estimated as, AR = Highest grain yield – lowest grain yield; Standardized range (SR) was estimated as, SR = $\frac{\text{Highest grain yield} - \text{lowest grain yield}}{\text{Mean grain yield}}$. Genetic parameters like phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) were estimated using the following formulae given by (Burton & De Vane, 1953) for mean grain yield plant^{-1}. PCV (%) = $\frac{\text{Phenotypic standard deviation } (\sigma_p)}{\text{Mean grain yield } (\bar{X})} \times 100$; GCV (%) = $\frac{\text{Genotypic standard deviation } (\sigma_g)}{\text{Mean grain yield } (\bar{X})} \times 100$ and TSF % as the percentage of number of TC hybrids with mean grain yield > better parent to the total number of TC hybrids evaluated and expressed in percent. Usefulness index (U_I)/ genetic gain was estimated using the following formula. U_I = Mean grain yield + k × $\frac{\sigma^2_g}{\sigma_p}$; where, k = standardized selection differential at different selection intensities; k = 2.67 and 2.06 at 1 and 5% selection intensities, respectively; σ^2_g = Genotypic variance, σ_p = Phenotypic standard deviation. Higher the estimates of mean grain yield, AR, SR, TSF, PCV, GCV, and U_I, better is the breeding potential of the cross (Bernardo, 2020) (Table 2).

RESULTS

The analysis of variance serves as a diagnostic step to detect variability for quantitative traits like grain yield plant^{-1}. The significant mean sum of squares indicated the presence of genetic variability for grain yield in both F_2 and BC_1F_1 populations among the DH families derived from all the four crosses. Significant variability could be attributed to greater differences between the parents for number of genes controlling grain yield (Table 3). The estimates of mean grain yield of BC_1F_1- derived DH were greater than F_2- derived DH in all the four crosses. From among the four BC_1F_1- DH populations, (NAI 137 × V 70) × V 70 showed greater estimates of mean grain yield compared to other crosses whereas (NAI 137 × V 70) showed greater estimates of mean grain yield among the F_2- DH populations (Figure 1).

Based on first-degree statistic criterion, the estimates of standardized range were higher in F_2 derived DH compared to BC_1F_1- derived DH in all the crosses. SKV 50 × CML 582 showed higher standardized range closely followed by SKV 50 × CML 581 compared to other crosses. Similar trend of greater estimates of absolute range was observed in all crosses except in SKV 50 × CML 581 and CML 451 × MAI 194. Higher estimates of absolute range was observed in NAI 137 × V 70 (Table 4). Based on second- degree statistic such as phenotypic and genotypic coefficient of variation F_2 derived DH showed higher estimates of PCV and GCV compared to BC_1F_1- derived DH in all the crosses. Highest estimate of PCV was found in SKV 50 × CML 581 closely followed by SKV 50 × CML 582 whereas SKV 50 × CML 582 showed higher GCV estimates closely followed by SKV 50 × CML 581 (Figure 2). In terms of variability, the estimates of both first- (absolute and standardized range) and second- degree statistic (PCV and GCV) parameters were greater in F_2 derived DH compared to BC_1F_1- derived DH with an exception of absolute range in two crosses.

(CML 451 × MAI 194) × MAI 194 showed greater TSF of 83.33% followed by F_2 -DH population of the same cross with TSF of 40%. Estimates of TSF was found higher in BC_1F_1- derived DH compared to F_2 derived DH in all the crosses. In the present study as is true with respect to mean grain yield, the estimates of TSF in DH derived from BC_1F_1 were greater than those derived from F_2 generation emanating from all the four crosses (Figure 3). Just like mean grain yield and TSF, U_I was found higher in case of BC_1F_1- derived DH compared to F_2 derived DH at both 1 and 5% selection intensities in all crosses except in SKV 50 × CML 582 where BC_1F_1- and F_2- derived DH showed similar estimates of U_I at 5% selection intensity (Figure 4). Among all the crosses, (CML 451 × MAI 194) × MAI 194 showed highest U_I of 97.04 at 1% selection intensity and 91.14 at 5% selection intensity. Overall, the results of the present investigation demonstrates that estimates of mean grain yield and TSF of BC_1F_1-DH were found greater compared to F_2-DH. However, the estimates of SR, PCV and GCV of BC_1F_1-DH were lower those of F_2-DH.

DISCUSSION

The DH populations with high mean and a high frequency of TSF for trait (s) of interest is considered as ideal ones for synthesizing and identifying heterotic hybrids (Bernardo, 2020). Higher mean grain yield in BC_1F_1- DH derived from all the four crosses is attributable to greater frequency of favourable alleles (Dudley, 1982; Melchinger, 1987). When favourable alleles are concentrated in one parent, backcross populations are expected to possess greater breeding potential because they increase the probability of recovering superior recombinants carrying a higher proportion of desirable alleles from the elite parent (Bernardo, 2020, 2023). The estimates of unit-dependent absolute range and unit-independent standardized range are the reflections of occurrence of extreme phenotypes. As expected, F_2- DH showed greater estimates of standardized range.

The PCV and GCV estimates represent an unbiased and true reflection of variability unlike absolute range and standardized range which are biased by extreme values. The differences in the estimates of quantitative trait means, absolute range, standardized range and PCV in F_2- and BC_1F_1- DH derived from the four crosses could be attributed to differences in the number of genes dispersed between their parents. The crosses whose parents differ for a larger number of dispersed genes would exhibit higher traits means and variances than those whose parents differ for fewer genes. The close correspondence between PCV and GCV in some populations such as (CML 451 × MAI 194) × MAI 194 and CML 451 × MAI 194 indicated that environmental influence on trait expression was minimal and that most of the observed variability could be attributed to genetic causes. (Bernardo, 2020; Kearsey & Pooni, 2020).

The TSF has been attributed as major cause for the success of plant breeding and without which, plant breeding does not work (Mackay et al., 2021). The greater estimates of TSF in the BC_1F_1- DH suggests that the BC_1F_1 generation itself provides sufficient opportunity for recovering superior recombinants. Thus, TSF reflecting the frequency of desirable transgressive segregants, it offers a more reliable assessment of breeding potential. Usefulness index (U_I) accounts for trait mean, genetic variance and phenotypic standard deviation. Thus, provides a more comprehensive and informative statistic in terms of genetic gain expected when different intensities of selection are imposed in segregating populations (Bernardo, 2020).

From practical breeding point of view, genetic variance of BC_1F_1-DH relative to F_2-DH is not of much significance, because the breeder selects those BC_1F_1-DH or F_2-DH which results in higher mean performance regardless of whether magnitude of variance is small or large. This further implies that breeders would develop breeding populations for the purpose of selection rather than variance estimation.

Estimates of U_I were found greater BC_1F_1-DH compared to F_2-DH except one cross. Neither the F_2-DH nor the BC_1F_1-DH is universally superior for AR in all crosses. Thus, the study establishes that the breeding potential of F_2-DH nor the BC_1F_1-DH is context-dependent and integrating mean performance, standardized range, absolute range, genetic variability, transgressive segregation, and UI provide a robust framework for identifying the most suitable population with efficient breeding potential. The present findings are in agreement with those of Bernardo (Bernardo, 2020, 2023), who proposed that the choice between F_2-DH and BC_1F_1-DH populations should be made on the basis of the distribution of favourable alleles rather than by assuming the inherent superiority of either population.

CONCLUSION

BC_1F_1-derived DH showed greater breeding potential in terms of mean grain yield, TSF and U_I compared to F_2-derived DH. BC_1F_1-derived DH are likely to uncover greater frequency of superior inbred lines for use in developing

heterotic hybrids. It is desirable to induce DH from BC_1F_1 rather than from F_2 populations derived from biparental crosses when one of their parents is better than the other one

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Authors' contributions
BSKS: carried out the data curation; investigation; preparing the draft manuscript. **TO and SR:** carried out the conceptualization; supervision and editing the draft manuscript. **KMS, KS and PKS:** contributed to conduct the field experiment and data curation. **ARG and HKBM:** contributed to data analysis. All authors read and approved the final manuscript.

Compliance with ethical standards
Conflict of interest: –Authors do not have any conflict of interests.

Ethical issues: "None".

Declaration of generative AI and AI-assisted technologies in the writing process
None of the AI tools were used during the preparation of this manuscript.

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Table 1. Pedigree of F_2-derived and BC_1F_1-derived doubled haploid populations of four breeding crosses

Pedigree of the cross	Breeding populations	No of DH families	Total no. of DH individuals
NAI 137 × V 70 (Better parent)	F_2	36	180
(NAI 137 × V 70) × V 70 (Better parent)	BC_1F_1	35	175
SKV 50 × CML 582 (Better parent)	F_2	45	225
(SKV 50 × CML 582) × CML 582 (Better parent)	BC_1F_1	70	350
SKV 50 × CML 581 (Better parent)	F_2	28	140
(SKV 50 × CML 581) × CML 581 (Better parent)	BC_1F_1	63	315
CML 451 × MAI 194 (Better parent)	F_2	30	150
(CML 451 × MAI 194) × MAI 194 (Better parent)	BC_1F_1	30	150

Table 2. Criteria for assessing breeding potential of F_2- and BC_1F_1- derived DH populations

Statistical parameters	Criteria	Inference
Mean		
Range (Absolute and standardized range)		
Transgressive segregation frequency (%)	High	High breeding potential
Coefficient of variance (PCV/GCV)		
Usefulness index (U_I)/ genetic gain		

Table 3. Analysis of variance of F_2 and BC_1F_1- derived DH families for grain yield per plant.

Source of variation	Degrees of freedom								Mean sum of squares							
	NAI 137 × V 70		SKV 50 × CML 582		SKV 50 × CML 581		CML 451 × MAI 194		NAI 137 × V 70		SKV 50 × CML 582		SKV 50 × CML 581		CML 451 × MAI 194	
	F_2	BC_1F_1	F_2	BC_1F_1	F_2	BC_1F_1	F_2	BC_1F_1	F_2	BC_1F_1	F_2	BC_1F_1	F_2	BC_1F_1	F_2	BC_1F_1
Replication	01	01	01	01	01	01	01	01	56.64	84.94	582.80**	0.49	172.59	55.96	98.82	2.02
DH families	35	34	44	69	27	62	29	29	418.15**	191.03***	328.80***	209.56***	301.93***	225.86***	276.81***	321.62***
Block	05	06	08	09	06	08	05	05	103.72	8.56	72.44	73.52	67.40	30.70	47.38	37.42
Residual	30	28	36	60	21	54	24	24	154.36	24.86	64.76	79.07	74.59	56.89	66.44	67.59

* Significant at P = 0.05, ** Significant at P = 0.01, *** Significant at P = 0.001

Table 4. Estimates of absolute and standardized range of F_2 and BC_1F_1- derived DH families for grain yield per plant.

Cross	Absolute range		Standardized range	
	F_2	BC_1F_1	F_2	BC_1F_1
NAI 137 × V 70	55.65	34.5	0.84	0.46
SKV 50 × CML 582	52.59	47.34	1.01	0.80
SKV 50 × CML 581	48.10	51.70	0.96	0.84
CML 451 × MAI 194	44.00	50.00	0.74	0.70

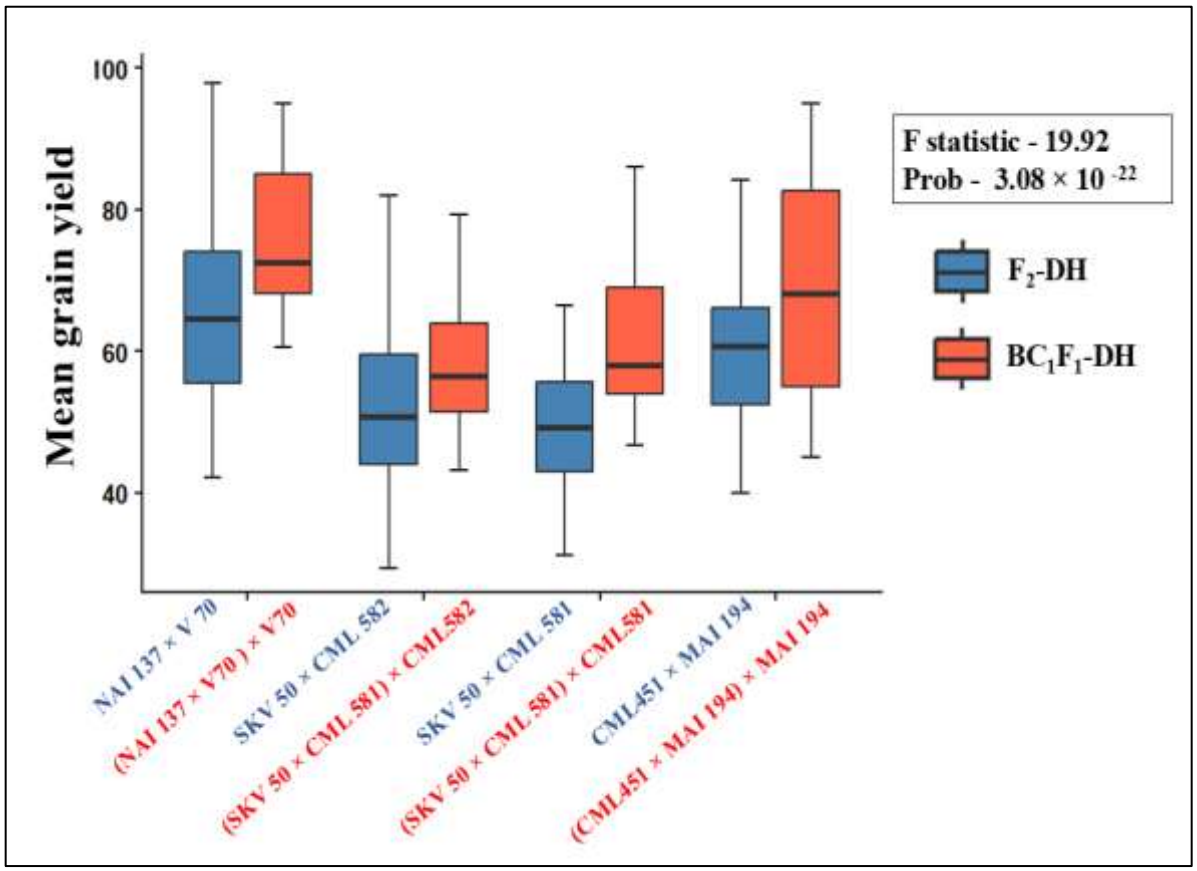


Figure 1. Box whisker plot depicting comparative mean grain yield of F_2- and BC_1F_1-derived DH families

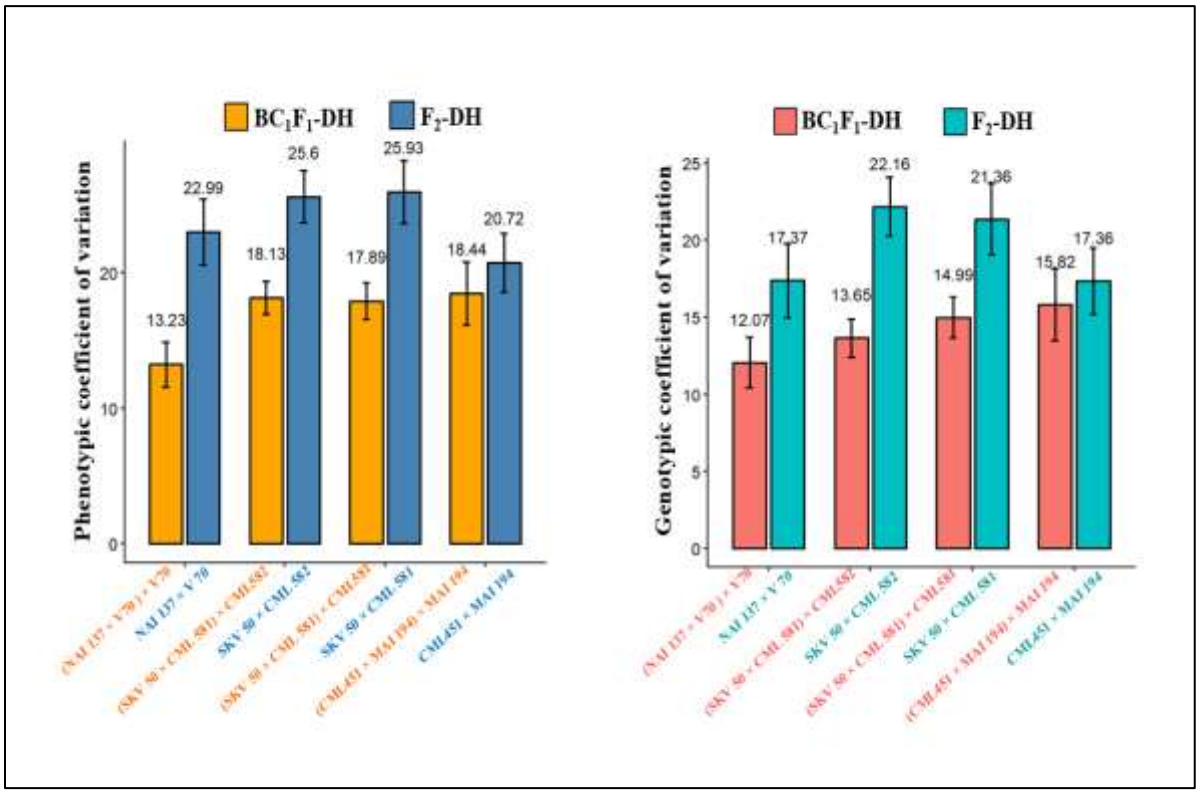


Figure 2. Bar graph depicting phenotypic and genotypic coefficient of variation of F_2- and BC_1F_1-derived DH families for grain yield

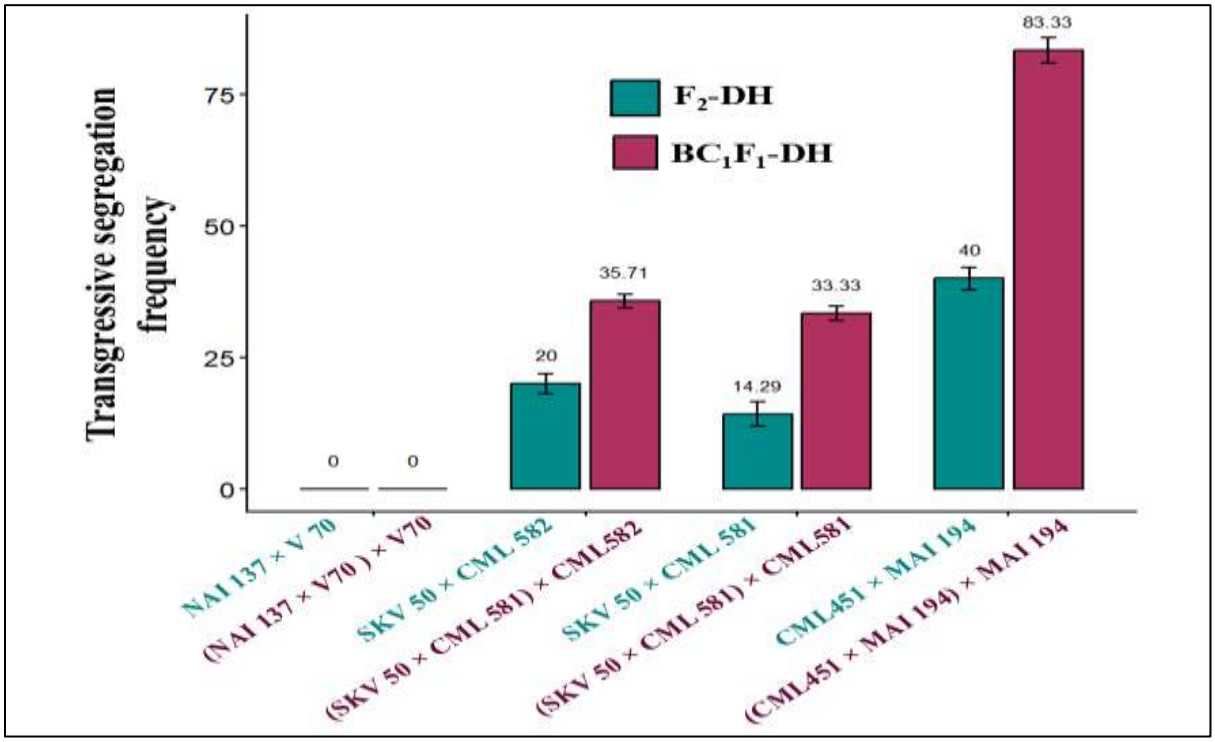


Figure 3. Bar graph depicting transgressive segregation frequency in F_2 and BC_1F_1- derived DH families for grain yield

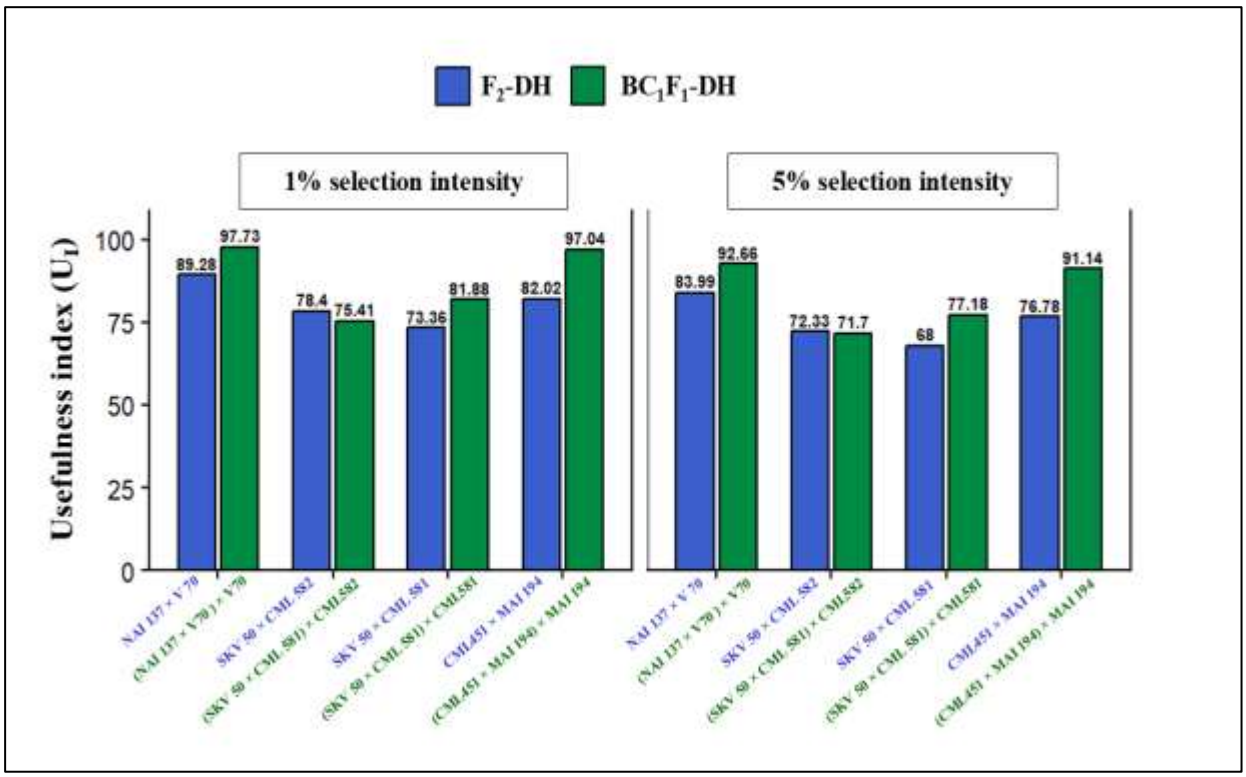


Figure 4. Bar graph depicting Usefulness index at 1% and 5% selection intensities in F_2 and BC_1F_1- derived DH families for grain yiel