

Predicting the Crosses for their Potential to Uncover Transgressive RIL's in Cowpea (*Vigna unguiculata* (L.) Walp)

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ABSTRACT

Crop breeding efficiency can be increased by removing ineffective crosses early on and managing sizable segregating populations that are produced from a small number of promising crosses. Early generation identification and removal of the inefficient population helps breeders with better allocation of resources. Biparental-derived breeding population with higher estimates of mean, standardized range, Genotypic coefficient of variability (GCV), Phenotypic coefficient of variability (PCV), heritability, usefulness index (UI) and transgressive segregation index (TSI) were considered to identify potentiality of F₂ populations. We predicted aforesaid parameters in six F₂ breeding populations of cowpea to identify potential population with the higher number of transgressants. Population derived from cross EC244046 x IC263015 recorded the highest train mean, absolute range and usefulness index. However, the standardized range was found to be higher in the population derived from EC738260 x IC263015. Estimates of heritability were high for grain yield across F₂ populations. The breeding population derived from cross EC738260 x IC263015 was found to be the potential to recover transgressants considering the standardized range, TSI and UI.

Keywords : Transgressive segregation index, Usefulness index, Breeding potential, Phenotypic coefficient of variability, Genotypic coefficient of variability

COWPEA [*Vigna unguiculata* (L.) Walp], is a versatile legume crop that grows in semi-arid to arid regions. It is an adaptable crop due to its high tolerance to heat and drought stress as well as its relationship with bacteria that fix nitrogen (Boukar *et al.*, 2018). Thus, a valuable species to cultivate as the effects of climate change intensify. Cultivar improvement rates in cowpea are frequently influenced by the degree of success attained in introducing desired gene(s) into varieties or pooling desirable genes from different genetic backgrounds

to a single genotype by hybridizing contrasting parents with desirable traits. Lack of variation is one of the major problems observed in self-pollinated crops which can be overcome by hybridization. Cowpea has very little productivity when compared with other major pulses (Ravelombola *et al.*, 2017). Combining desirable genes from the gene pool into a single variety is expected to enhance grain yield plant⁻¹ and 100 grain weight and thus productivity in addition to targeting pods plant⁻¹, pod length and seeds pod⁻¹ are other yield attributing traits. Plant breeders

are constantly involved in designing and optimizing a pipeline to deliver a product profile after breeding targets have been established and the product profile has been created with input from stakeholders.

Breeders create a lot of breeding populations (BPs) on a regular basis in order to use pedigree selection to identify and isolate superior recombinant inbred lines (RILs). In addition to being excessively resource-intensive, managing a large number of BPs decreases breeding process efficiency by producing less-than-satisfactory genetic gain. Over 99 per cent of the resources are used in crosses between parental combinations that do not result in viable cultivars (Witcombe *et al.*, 2013). Plant breeding efficiency is expected to be improved by the early removal of subpar crosses and resource allocation solely to large-sized BPs derived from a few promising crosses chosen according to an objective criterion. This process is expected to increase the likelihood of identifying desirable RILs for use as pure-line cultivars (Chahota *et al.*, 2007 and Witcombe *et al.*, 2013). Crop breeding efficiency can be increased by removing ineffective crosses early on and managing sizable segregating populations that are produced from a small number of promising crosses. Moreover, there has been less success with direct selection for economically significant traits like pod yield and its component traits. It is probably more successful to use indirect selection based on readily observable, assayable and scorable traits for such hard-to-breed traits. Nevertheless, this approach necessitates the pre-identification of characteristics that are highly linked to traits that are challenging to breed. There is a need for the use of the most appropriate criteria to evaluate and identify crosses with high breeding potential in terms of recovery of high frequency of pure lines with high quantitative trait means across traits, absolute and standardized range, variances and frequency of transgressive segregants estimated in F_2 and F_3 generations. An increasing trend in quantitative trait means and phenotypic coefficient of variation (PCV) from F_2 to F_3 generations suggested better breeding potential in advanced generations (Suresh *et al.*, 2016).

In this background, the objectives of our study are to assess the breeding potential of crosses based on first and second-degree statistics and their combination.

MATERIAL AND METHODS

Parental genotypes differing for 100-grain weight and seed coat colour were used to generate six biparental crosses during the 2022 rainy season. The F_1 s of six crosses were raised and hybridity was confirmed using male parent-specific traits and seed coat colour in each cross. The size of the F_2 population varied from 99 to 151 (Table 1). The true hybrids of these crosses were forwarded to generate six F_2 populations, which were sown during Summer 2023 at the Experimental plots of 'K' block, Department of Genetics and Plant Breeding, University of Agricultural Sciences (UAS), GKVK, Bengaluru (N12 °58, E77 °35'). Each line was sown in a 2m row with a spacing of 0.6m between rows and 0.2m between plants within a row. All the recommended agronomic practices were adopted to raise a good crop of cowpeas. The data were recorded on each F_2 individual plant at their maturity for grain yield expressed as grain yield plant⁻¹ (g).

Data Analysis

Quantitative trait means and variances in F_2 generations were used as criteria to assess the breeding potential of crosses. Data recorded on individual F_2 plants were used to estimate descriptive

TABLE 1

The details of the size of the F_2 populations derived from six different crosses

Crosses	F_2 population size
EC724826 x EC725102	140
EC724826 x EC738216	99
EC244046 x IC263015	107
IC263015 x EC738260	146
EC738260 x IC268262	136
EC738260 x IC263015	151

first-degree statistics such as mean, absolute range (AR), standardized range (SR), transgressive segregation index (TSI) and second-degree statistics such as absolute phenotypic variance (σ_p^2), phenotypic standard deviation (σ_p) and standardized statistics, *i.e.*, phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) to assess and quantify the genetic variability in F_2 breeding populations. The formulae for these statistics are described under.

Quantitative Traits Mean (\bar{X})

$$\bar{X} = \frac{\text{All plants in each of the } F_2 \text{ population}}{\text{Number of plants}}$$

Quantitative Traits Range

Absolute range (AR) = Highest trait mean - Lowest trait mean

Standardized range (SR) = $\frac{\text{Highest trait mean} - \text{Lowest trait mean}}{\text{Trait mean}}$

Transgressive Segregation Index (TSI)

TSI was estimated as the proportion of the difference between the parents and the corresponding range for a phenotype in the segregating (F_2) populations. Based on descriptive statistics, TSI was estimated (Koide *et al.*, 2019) as :

$$\text{(TSI)} = \frac{\text{Absolute range in segregating population}}{\text{Trait mean difference between parents}}$$

Phenotypic (PCV) and Genotypic Coefficient of Variability (GCV)

Phenotypic variance (σ_p^2) was standardized as the phenotypic coefficient of variation (PCV) (Burton and Devane, 1953) to facilitate comparison across QTs.

$$\text{PCV (\%)} = \frac{\sigma_p}{\bar{X}} \times 100$$

$$\text{GCV (\%)} = \frac{\sigma_g}{\bar{X}} \times 100$$

Where, σ_p = Phenotypic standard deviation,

σ_g = Genotypic standard deviation,

\bar{X} = Quantitative traits mean.

The ' σ_g ' was estimated as the square root of σ_g^2 ; The σ_g^2 was estimated as $\sigma_p^2 - \sigma_e^2$, where, σ_e^2 for F_2 populations was estimated as the average of phenotypic variance in non-segregating populations (F_1, P_1, P_2)

$$\sigma_e^2 = \frac{2V_{F1} + V_{P1} + V_{P2}}{4}$$

Heritability

Heritability was estimated to measure the degree of variation in a phenotypic trait in a population that is due to genetic variation between individuals in that population. In F_2 populations, broad sense heritability (h_{bs}^2) estimates for five quantitative traits were estimated according to the formula suggested by Lush (1945).

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

σ_g^2 = Genotypic variance; σ_p^2 = Phenotypic variance

Usefulness Index (UI)/ Expected Genetic Gain

UI accounts for trait mean, phenotypic variability and narrow-sense heritability and provides a more comprehensive and informative statistic in terms of genetic gain expected when different intensities of selection are imposed in segregating populations (Lehermeier *et al.*, 2017; Allier *et al.*, 2019 and Ongom *et al.*, 2021).

In F_2 populations, the UI for each trait was estimated as:

$$U_i = \text{Trait mean} + (k \times \frac{\sigma_g^2}{\sigma_p})$$

where, k = standardized selection differential at different selection intensities; k = 2.67 and 2.06 at 1 per cent and 5 per cent selection intensities, respectively (Bernardo, 2020).

Criteria to Assess the Breeding Potential of Crosses

Potential breeding populations vary with the statistical parameter used and trait under study. Hence, a comprehensive strategy has to be used which takes all the parameters into consideration to identify the potential population. One such approach is the rank-sum method.

The rank sum (RS) method was used as a criterion to assess the breeding potential of individual crosses. Rank sum calculated from sums of rank standardized range, rank TSI and rank usefulness index across quantitative traits of each cross was compared with each other (Pathy *et al.*, 2018). In computing rank sums, equal weightage was given to all genetic parameters. Ranks for genetic parameters were given in descending order, *i.e.* highest value was given lowest rank. Further, ranks of genetic parameters for all quantitative parameters were pooled to give overall rank to crosses. Later, the overall rank was assigned to crosses by pooling ranks across the F_2 generations.

RESULTS AND DISCUSSION

The arithmetic mean is often used as a measure of central tendency in summarizing the data points. It picturizes the tendency of the individuals in the population to congregate in the distribution. Trait mean indicates the presence of a gene that enhances

the phenotype and a higher mean increases the likelihood that selected progeny will have a high mean, even when the selection is not precise (Bernardo, 2020). The trait mean of grain yield was found to be highest in the breeding population derived from EC244046 x IC263015 which was followed by the population derived from EC724826 x EC738216 amongst six crosses (Fig. 1).

Absolute range being one of the measures of dispersion, denotes variability present in the population. However, a standardized range is helpful when traits with different units of measurement are to be compared within and among segregating populations. Thus, apart from absolute range, standardized range was also considered for assessing breeding potential of the populations. The absolute range was found to be highest in a population derived from the cross involving EC244046 X IC263015 (Table 2) which also recorded the highest trait mean (Fig.1). However, the standardized range was found to be higher in a population derived from EC738260 X IC263015 (Table 2).

Transgressive segregation (TS) refers to the phenomenon wherein a few genotypes in F_2 or later segregating generations exhibit phenotypes outside the range of both parents. The transgressive segregation index indicates the greater chance of

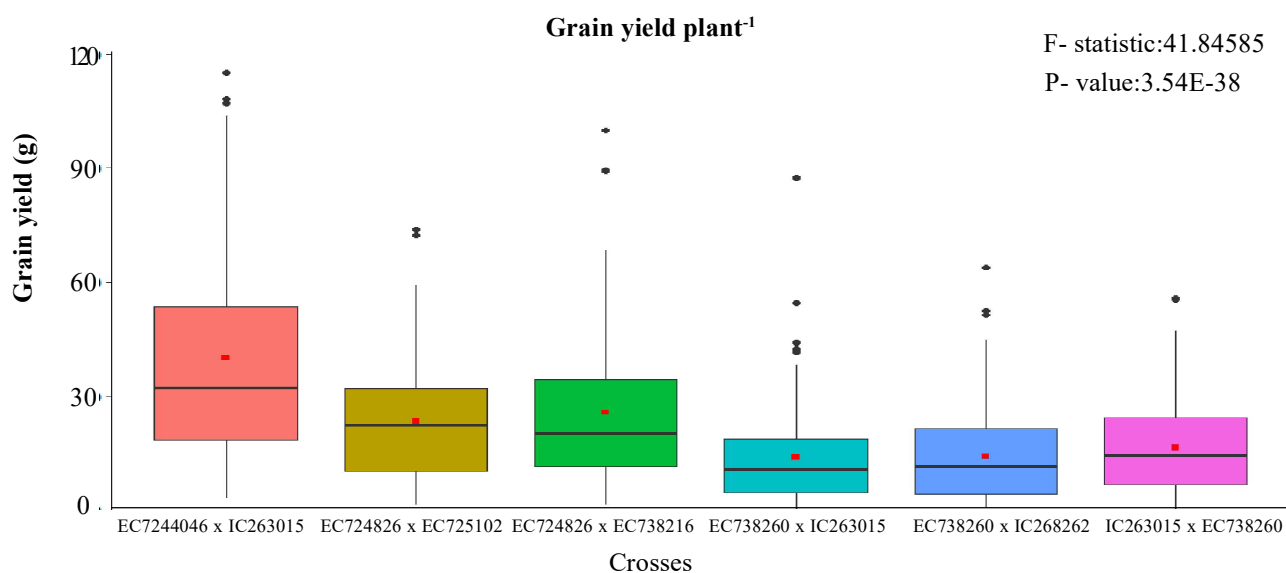


Fig. 1 : Box-plot showing significant differences in means of grain plant⁻¹ in F_2 population derived from cowpea crosses

TABLE 2
Estimates of first and second-degree statistics in F₂ generations derived from six crosses
for grain yield plant⁻¹ in cowpea

Crosses	Absolute range (g)	Standardized range (g)	PCV (%)	GCV (%)	Heritability
EC724826 x EC725102	72.614	3.11	64.42	63.07	95.86
EC724826 x EC738216	98.497	3.83	75.27	72.56	92.95
EC244046 x IC263015	111.955	2.80	71.45	70.29	96.80
IC263015 x EC738260	55.325	3.37	73.96	72.52	96.15
EC738260 x IC268262	63.081	4.46	85.74	83.44	94.71
EC738260 x IC263015	87.055	6.27	92.23	90.23	95.72

Levene's test : F-value = 19.604 , p- value < 2.2e-16 ***

recovering transgressive segregants which were found to be highest in the breeding population derived from EC738260 x IC268262 indicating the possibility of a higher amount of transgressants in subsequent generations (Fig. 2).

The higher the variance, higher the scope for the selection of genotypes with the desired combination

of traits. If the genetic variance and the mean are relatively high, selected progeny can perform to their maximum potential (Bernardo, 2020 and Anilkumar *et al.*, 2021). Phenotypic variability as a measure of dispersion confounds the genetic and environmental variability. Thus, the narrow difference between PCV and GCV indicates little influence of the environment on the expression of a trait. Such differences can be

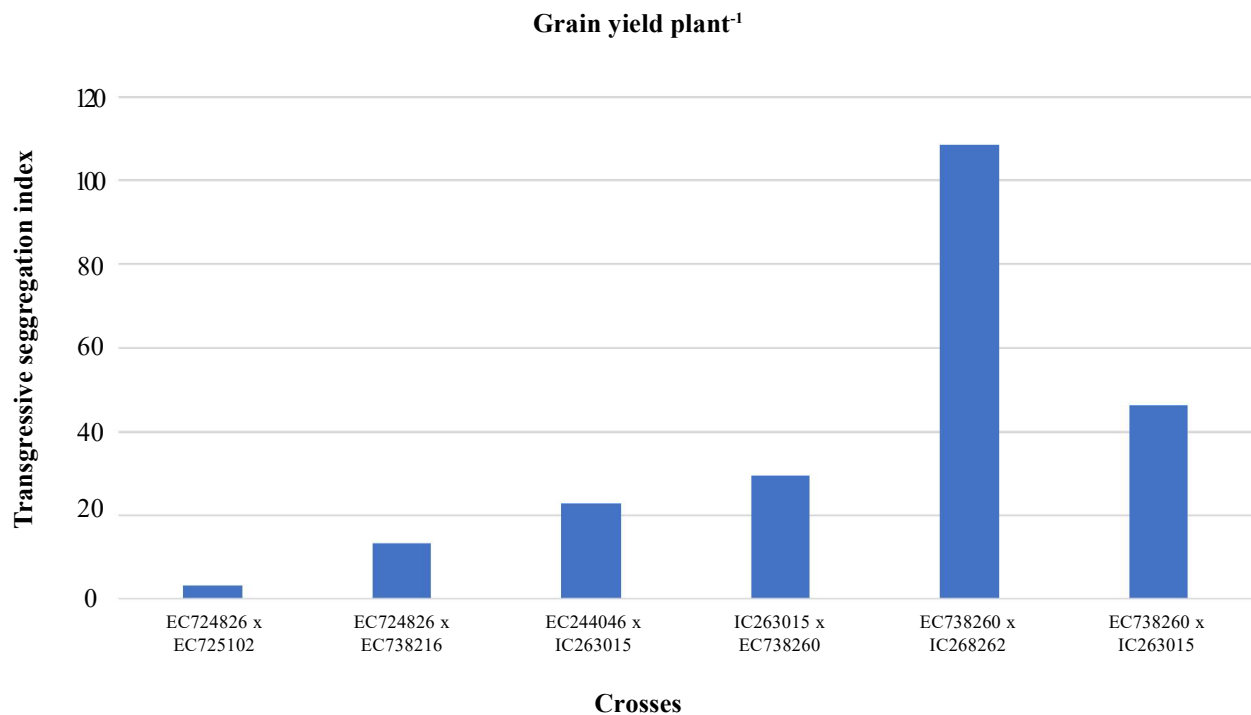


Fig. 2 : Bar graph showing differences in estimates of transgressive segregation index for grain yield plant⁻¹ in F₂ population derived from cowpea crosses

observed to be reflect another variability parameter, broad sense heritability. The variability measures PCV and GCV which are true reflections of variability were found to be higher in EC738260 x IC263015 for grain yield plant⁻¹. While broad sense heritability was found to be high in the F₂ population from a cross EC244046 x IC263015 (Table 2). The crosses whose parents differ for a larger number of dispersed genes are likely to exhibit higher traits means and variances than those whose parents differ for fewer genes (Kearsey & Pooni 1996 and Bernardo 2020).

Melchinger (1987) suggested utility of Schnell’s (1983) concept of ‘usefulness’ as a valuable tool for determining the potential of breeding populations (BPs). Millicent *et al.* (2020) and Ongom *et al.* (2021) in cowpea, suggested that UI helps in making informed decisions for implementing selection among a large number of BPs routinely developed in crop breeding programs. UI has been suggested as an unambiguous objective method of selecting early segregating generations to increase the frequency of recovering superior RILs for use as pure-line cultivars in self-pollinated crops like cowpea (Chandana and Ramesh, 2021). In the present study, UI for selection intensities at 1 per cent was found to be higher than

5 per cent and at both intensities breeding population from the cross EC244046 x IC263015 was found to be highest (Fig. 3).

The breeding potential of crosses is their genetic worth in terms of recovery of superior recombinant inbred lines (RILs) in the advanced generations. From the preceding results and discussion, it is evident that the breeding potential varies with the trait studied and the statistics used. This creates an ambiguity while selecting a cross with better breeding potential. Hence, it is necessary to use a more comprehensive quantitative measure which effectively combines different first and second-degree statistics to assess the breeding potential of crosses. The breeding potential of crosses at early segregating generations indicates if there’s any need to go beyond the F_{2,3} generation of the cross if the transgressive RILs are predicted to be low (Basangouda *et al.*, 2022). UI, which is a summary statistic that accounts for a trait’s mean, variance and heritability could be used as an objective tool for selecting the segregating populations with good breeding potential to derive superior RILs for use as pure line cultivars in self-pollinated crops and rank-sum method can be used to identify the population with higher breeding potential. The

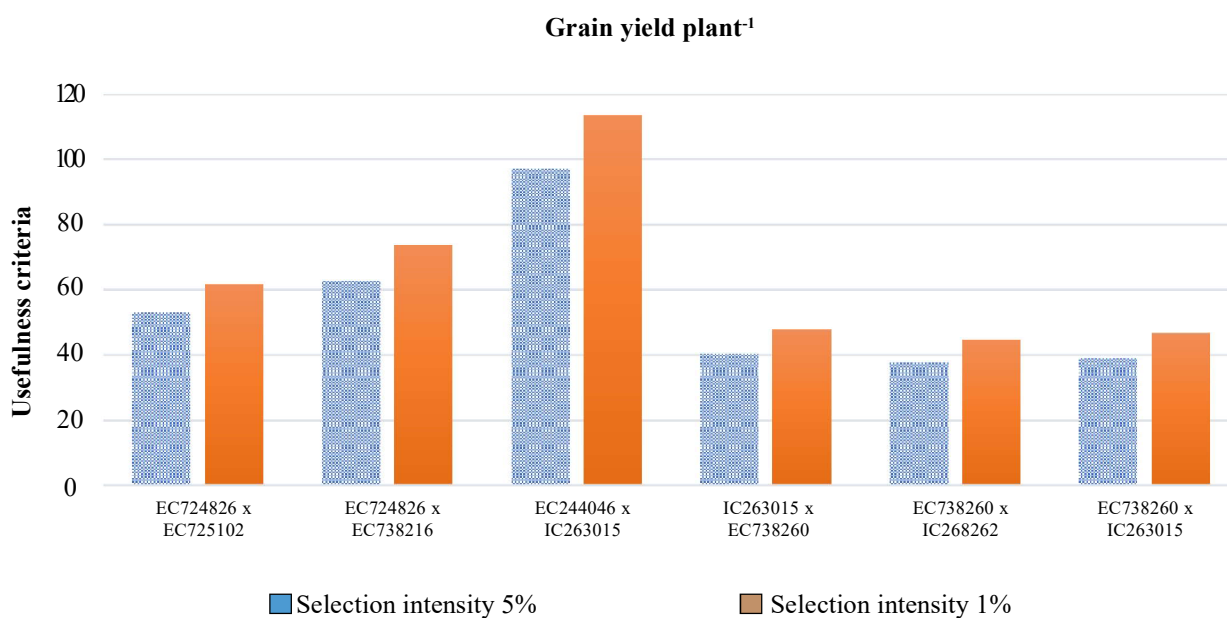


Fig. 3 : Bar graph showing differences in estimates of usefulness index for grain yield plant⁻¹ in F₂ population derived from cowpea cross

TABLE 3
Rank-sum for grain yield plant⁻¹ in F₂ breeding populations derived from six crosses in cowpea

Crosses	Standardized range	Rank a	TSI	Rank b	Usefulness index	Rank c	Rank sum (a+b+c)	Overall rank
EC724826 x EC725102	3.11	5	3.20	6	53.04	3	14	5
EC724826 x EC738216	3.83	3	13.19	5	62.86	2	10	3
EC244046 x IC263015	2.80	6	22.53	4	96.88	1	11	4
IC263015 x EC738260	3.37	4	29.43	3	40.47	4	11	4
EC738260 x IC268262	4.46	2	108.76	1	37.77	6	9	2
EC738260 x IC263015	6.27	1	46.31	2	39.16	5	8	1

breeding population derived from crosses EC738260 x IC263015 (Table 3) was found to be potential considering the standardized range, TSI and UI in the rank-sum method. However, the population derived from EC244046 x IC263015 recorded the highest mean and UI. Hence, potential productive populations can be further forwarded to recover the superior RILs.

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