

Predicting the Frequency of Transgressive RILs and determining the Minimum Population Size required for their Recovery in Dolichos bean (*Lablab purpureus* L. Sweet)

M.P. Kalpana*, M. Kemparaju, C.B. Siddu and G. Basanagouda
Department of Genetics and Plant Breeding, College of Agriculture,
University of Agricultural Sciences, Bangalore (Karnataka), India.

(Corresponding author: M.P. Kalpana*)

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ABSTRACT: Dolichos bean var. *lignosus* is one of the important and ancient food legumes extensively grown in southern India. It is commonly known as ‘hyacinth bean’, ‘field bean’, ‘Indian bean’, etc. It is a self-pollinated crop with $2n=22$ chromosomes and genome size of 367 Mbp. Efficiency in breeding self-pollinated crops like dolichos bean can be significantly improved by directing resources towards a select few segregating populations with the potential to yield a high frequency of transgressive Recombinant Inbred Lines (RILs) for use as pure-line cultivars. The utilization of an objective criterion for identifying promising segregating populations becomes crucial in this context. One such criterion involves predicting the frequency of transgressive RILs that could emerge from advanced generations of crosses within segregating populations. In our study, we predicted the occurrence of RILs surpassing the performance of the better parent (HA 10-2). This prediction was based on the genetic diversity between two elite parent lines, HA 10-8 and HA 10-2, specifically for grain yield, using estimations of mid parental value [m], additive genetic effects [a], and additive genetic variance [σ^2_A]. Remarkably, the minimum population size required for the recovery of transgressive RILs was relatively smaller, indicating the feasibility of this approach.

Keywords: Additive effects, transgressive RILs, better parent, grain yield.

INTRODUCTION

The dolichos bean, an ancient legume crop cultivated in arid regions of Asia and Africa, serves various purposes such as vegetable, pulse, fodder, and green manure production (Ramesh and Byregowda 2016). Similar to other primarily self-pollinating legume crops, commercial dolichos bean farming relies on pure-line cultivars. The predominant breeding method in dolichos bean involves pedigree selection to identify desirable recombinant inbred lines (RILs) from bi-parental crosses and segregating populations (Ramesh and Byregowda 2016). However, managing numerous segregating populations is both resource-intensive and less efficient for genetic progress, with over 99% of resources often allocated to unproductive crosses (Witcombe *et al.*, 2013). To enhance breeding efficiency, resources should be directed towards larger segregating populations resulting from selected promising crosses based on objective criteria (Chahota *et al.*, 2007; Witcombe *et al.*, 2013; Bernardo, 2020), especially considering limited resources. It is also crucial to determine the minimum population size necessary to ensure a high probability (e.g., 95%) of recovering at least one desired RIL, as outlined by Jinks and Perkins (1972) and predicted using quantitative genetic parameters like additive gene effect [a] and

additive genetic variance [σ^2_A] obtainable from parental and early segregating generations (F₂ and F₃) (Jinks and Pooni 1976). Thomas (1987) demonstrated that even a random sample of a few F₃ families can reliably estimate [a] and [σ^2_A]. Kearsey and Pooni (1996) provided a method for predicting this minimum population size (n) required for recovering at least one predicted transgressive RIL. The aim of this study is to estimate the frequency of transgressive RILs and determine the minimum population size needed for their recovery through reciprocal crosses between two elite parents with complementary traits in dolichos bean.

MATERIAL AND METHODS

The fundamental materials used in this study included two elite parent lines: HA 10-8 and HA 10-2 (Table 1). HA 10-8 represents an advanced breeding line, while HA 10-2 is a high-yielding pure-line cultivar specifically developed for commercial dolichos bean cultivation in the eastern dry zone of Karnataka, India. HA 10-8 typically produces fewer branches, racemes, and larger grains, whereas HA 10-2 tends to produce more branches, racemes, and smaller pods with smaller grains. The primary goal was to create new pure-line varieties capable of yielding higher grain yields. This experimental material was synthesized during the 2021

rainy season at the experimental plots located in the Department of Genetics and Plant Breeding (GPB) at the University of Agricultural Sciences (UAS) in Bangalore, India. A total of 15 well-filled F₁ seeds were obtained from the cross between HA 10-8 and HA 10-2. These F₁ seeds were planted after the 2021 rainy season, and all of them successfully germinated and reached maturity. To confirm their true hybrid nature,

the F₁ plants were carefully examined for traits specific to the male parents. The fact that all 15 candidate F₁ plants from the HA 10-8 × HA 10-2 cross exhibited an indeterminate growth habit confirmed their hybrid status, as indeterminacy is known to be dominant over determinacy (Modha *et al.*, 2019; Basanagouda *et al.*, 2022).

Table 1: Pedigree/source of parents used to derive crosses in dolichos bean.

Parents	Growth habit	Photoperiod sensitivity	Pedigree/Source	References
HA 10-2	Indeterminate	Insensitive	HA 4 × GL 153	Ramesh <i>et al.</i> (2018)
HA 10-8	Determinate	Insensitive	HA 4 × GL 153	Shivakumar <i>et al.</i> (2016)

The self-pollinated pods of the F₁ plants were harvested, hand-threshed, and sun-dried to obtain F₂ seeds. These F₂ plants were cultivated during the 2022 summer season, with a spacing of 0.3 meters maintained between F₂ plants. Out of these, 251 plants survived to maturity. Selfed pods from each F₂ plant were manually harvested, hand-threshed, and their seeds were sun-dried for use in generating F_{2:3} populations during the 2022 rainy and post-rainy seasons. The seeds of the two parent lines, as well as a randomly selected 144 F_{2:3} families, were planted in a single row of 3 meters in length using an alpha-lattice design with two replications during the 2022 rainy and post-rainy seasons. Fifteen days after planting, the seedlings of both parents and the 144 F_{2:3} families were thinned to maintain a spacing of 0.3 meters between individual plants and 0.6 meters between rows. The recommended agricultural practices were followed to cultivate the two parent lines, F₁, F₂, and F_{2:3} generations, resulting in a total of 12 surviving plants for each of the two parents and within each F_{2:3} family in each replication.

Plant sampling and data recording: The data pertaining to grain yield plant⁻¹ (g) were collected from 10 randomly chosen plants, excluding those on the borders. These measurements were taken from both parental plants, their F₁ hybrids, all 144 F_{2:3} progenies in each of the two replications, and every individual F₂ plant (251).

Estimation of quantitative genetic parameters: To estimate three quantitative genetic parameters—mid-parental value [m], additive gene effect [a], and additive genetic variance [σ^2_A] for the prediction of transgressive Recombinant Inbred Lines (RILs) potentially derived from the HA 10-8 × HA 10-2 cross the data from ten randomly selected individual plants in the parental plants and their F₁ offspring, as well as the 251 F₂ plants, and the mean data from ten randomly selected plants in each of the 144 F_{2:3} progenies per replication, were utilized. Assuming an additive-dominance (A-D) model, [m] and [a] were estimated through a multiple regression model (as outlined by Kearsey and Pooni 1996) implemented in SPSS software version 16.0. The suitability of the A-D model was assessed via a joint scaling test (Kearsey and Pooni 1996) also performed in SPSS software version 16.0.

The estimation of [σ^2_A] was achieved by equating the observed and expected mean squares (MS) attributed to 'between F_{2:3} families' obtained from the analysis of variance (ANOVA) of the F_{2:3} families. The formula used was: $\sigma^2_A = 2 \times [(MS \text{ due to 'between F}_{2:3} \text{ families' - MS due to error}) / \text{number of replications}]$, following the method outlined by van Ooijen (1989). This analysis was conducted using Microsoft Excel software.

Predicting the frequency and minimum population size needed to recover transgressive RILs: Assuming that the data follows a normal distribution, the probability (frequency) of recovering RILs that are likely to exceed the performance of the superior parent (HA 10-2) was determined as standard normal distribution integrals corresponding to the quotient, (mean of HA 10-2 - [m]) / σ_A , for each trait considered in the study. Here, [m] represents the mid-parental value, and σ_A is the square root of σ^2_A (following the approach of Jinks and Pooni, 1976). The minimum population size required to ensure, for instance, a 95% probability that RILs surpass HA 10-2 was predicted as the number (n) of RILs needed to be generated such that the probability of RILs falling short of HA 10-2 is less than 5%, as per the method detailed by Kearsey and Pooni (1996). This probability was translated into the equation (1-P) n ≤ 0.05, where P represents the probability of RILs exceeding HA 10-2, and (1-P) stands for the probability of RILs not exceeding HA 10-2. To solve for 'n', logarithms were applied to both sides of the equation, and the terms were rearranged as follows: n ≥ log 0.05 / log (1-P). For example, if it is predicted that 1% of RILs will surpass HA 10-2, 'n' would be calculated as the ratio of log 0.05 to log 0.99, resulting in a value greater than or equal to 298.

RESULTS AND DISCUSSION

Analysis of Variance (ANOVA) for F_{2:3} families: ANOVA serves as a crucial diagnostic step aimed at identifying and quantifying the variation originating from a specific source, denoted as σ^2_A in this study. The ANOVA performed on the F_{2:3} families revealed notable disparities among the means of the F₃ families, as presented in Table 2. Given that a substantial portion of the genetic variability within F_{2:3} families is attributed to genes with additive effects, the observed significant differences between F_{2:3} families underscore

the significance of σ^2_A in regulating the inheritance of grain yield in this particular investigation. The significance of σ^2_A is not unexpected, as empirical research across various crop species has consistently demonstrated that σ^2_A contributes to more than 50%, and often nearly 100%, of the observed variation in a wide range of traits (Bernardo, 2020). Furthermore, Bernardo (2020) theoretically established that estimates of σ^2_A consistently surpass those of non-additive

genetic variation, even in cases where there is considerable interaction between alleles within each genetic locus (known as dominance) and between different loci (referred to as epistasis) governing quantitative traits. In the context of this study, the significance of σ^2_A holds particular importance, given its role as one of the predictors of the frequency of transgressive Recombinant Inbred Lines (RILs), as detailed in Table 3.

Table 2: Pooled Analysis of variance of F_{2:3} progeny families derived from HA 10-8 × HA 10-2 for grain yield plant⁻¹ in dolichos bean.

Source of variation	Df	Mean sum of Sq	F value	P Value
Seasons	1	1620.91	42.58	3.23×10 ⁻¹⁰
Replication (season)	2	70.26	1.84	1.60×10 ⁻¹
Replication×Season	10	25.49	0.66	7.52×10 ⁻¹
F _{2:3} families	143	326.70	8.58	4.20×10 ⁻⁷
F _{2:3} families × season	143	202.46	5.31	4.8 × 10 ⁻⁴
Residuals	276	38.06		

Table 3: Estimates of additive effects and additive genetic variance for grain yield plant⁻¹ in F_{2:3} families in dolichos bean.

Trait	[m]	[a]	σ^2_A
Grain weight plant ⁻¹ (g)	21.82	6.24**	54.26**

* Significant @ P=0.05; ** Significant @ P=0.01

Predicted frequency of transgressive RILs: In most naturally self-pollinating crops, including the dolichos bean, pure-line cultivars are typically created through the crossing of parents, often consisting of cultivated varieties (as is the case in this study). The selection process involves identifying RILs that exhibit the desired combination of traits. It's worth noting that if no RILs were ever found that surpassed their parents (or ancestors), a phenomenon known as transgressive segregants, the field of plant breeding would not be effective (Mackay *et al.*, 2021). While transgressive segregation (TS) does occur with sufficient frequency to make plant breeding a routine practice, it's important to recognize that not all crosses exhibit TS, and only a small fraction of progeny from any given cross display this phenomenon. Therefore, it becomes crucial to pinpoint the potential crosses that are more likely to yield a high frequency of TS. One objective approach to identifying potential crosses is by predicting the frequency of transgressive RILs that can arise from advanced generations. In the context of this study, the anticipated frequency of RILs expected to surpass the superior parent (HA 10-2) is detailed in Table 4. The adequacy of the Additive-Dominance (A-D) model, which indicates the absence of evidence for the involvement of epistasis, lends support to the reliability of these projected frequencies of transgressive RILs. Consequently, our findings strongly suggest a greater breeding potential within the segregating population derived from crosses involving HA 10-8 as the female parent, particularly in terms of enhancing grain productivity, which is the end-product important for both markets and consumption.

The Minimum Population Size Required for Recovering the Predicted Frequency of RILs: As

anticipated, the minimum population size needed to recapture the transgressive RILs foreseen from the HA 10-8 × HA 10-2 cross was relatively smaller, as outlined in Table 4. These findings underscore the significance of carefully selecting the direction of crosses when creating breeding populations to maximize the retrieval of transgressive RILs for their utilization as pure-line cultivars. Our findings align well with the theoretical insights provided by Bernardo (2022), who illustrated that breeding populations originating from "good × good" crosses, as exemplified in our study, tend to consistently reveal a higher frequency of transgressive RILs in a predictable manner. Both the quantitative genetic theory and empirical data indicate that transgressive segregation (TS) primarily arises from the combinations of complementary 'plus' and 'minus' alleles that are distributed among the parental lines (Rieseberg *et al.*, 1999; Surma *et al.*, 2000). In simpler terms, individuals that inherit 'plus' alleles from both parents or 'minus' alleles from both parents are more likely to exceed the performance of the parents. Consequently, TS is most likely to manifest when the genetic difference between the parents is minimal, yet the additive genetic variance (σ^2_A) is substantial. This scenario is achievable when the parents exhibit genetic diversity while remaining phenotypically similar. Empirical findings from other grain legumes such as lentils (Chahota *et al.*, 2007), dolichos beans (Shivakumar *et al.*, 2016), and horse gram (Chandana *et al.*, 2022) provide substantial evidence supporting the robust reliability of the prediction methodology employed in our study to evaluate the breeding potential of specific crosses.

Table 4: Predicted frequency of RILs which transgressed the limits of means of better parent and minimum population size required for their recovery in dolichos bean.

Trait	Predicted probability of RILs that transgress the better parent and minimum population size required for their recovery	
	≥ Higher scoring parent (HA 10-2)	Minimum population size required to recover predicted transgressive RILs
Grain weight plant ⁻¹ (g)	31.30	05.67

Breeding Implications: The potential for expediting the identification of new crop varieties possessing a desired blend of traits preferred by both farmers and end-users lies in breeding populations that are projected to yield a substantial frequency of transgressive Recombinant Inbred Lines (RILs) (Kochetov *et al.*, 2021). However, it's important to recognize that genotypes falling short of achieving the maximum trait expression are quite prevalent in the segregating populations routinely developed by crop breeders (Bernardo, 2020). This occurrence is rooted in the fact that the appearance of genotypes containing all the desirable genes governing a quantitative trait necessitates a precise alignment of multiple crossovers between all possible pairs of desirable and undesirable genes linked in repulsion phase across all chromosomes (Witcombe *et al.*, 2013; Bernardo, 2020). Nevertheless, the likelihood of such precisely positioned crossovers occurring at a high frequency is exceedingly rare. Nonetheless, interbreeding these genotypes (which do not attain maximum trait expression) selected from the same cross is expected to uncover a relatively high frequency of genotypes displaying near-maximum trait expression, even within the confines of the relatively small F₂/F_{2.3} populations that breeders commonly manage (Bernardo, 2020). Our previous reports provide evidence that increasing the frequency of transgressive segregation (TS) can be achieved through one or more cycles of random mating between F₂ individuals stemming from biparental crosses in the case of dolichos beans (Chandrakant *et al.*, 2015).

CONCLUSIONS

The study, predicted the occurrence of RILs surpassing the performance of the better parent (HA 10-2). This prediction was based on the genetic diversity between two elite parent lines, HA 10-8 and HA 10-2, specifically for grain yield, using estimations of mid parental value [m], additive genetic effects [a], and additive genetic variance [σ^2A]. Remarkably, the minimum population size required for the recovery of transgressive RILs was relatively smaller, indicating the feasibility of this approach.

Ethical approval: This manuscript does not contain studies performed by any of the authors involving human or animals

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Conflict of Interest. None.

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