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Predicting the Frequency of Transgressive RILs and Minimum Population Size Required to Recover Them in Dolichos Bean [*Lablab purpureus* (L.) Sweet]

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ABSTRACT

Background: Allocation of resources only to a few promising segregating populations that are likely to result in high frequency of transgressive recombinant inbred lines (RILs) for use as pure-line cultivars would help enhance efficiency of breeding self-pollinated crops including dolichos bean. The use of an objective criterion to identify promising segregating populations is therefore assumes importance. The prediction of frequency of transgressive RILs that could be derived from advanced generations of segregating populations of crosses is one such criterion.

Methods: We predicted the frequency of RILs that transgressed the better parent (HA 5) from two reciprocal crosses derived from two elite but genetically diverse parents (HA 4 and HA 5) for four quantitative traits based on estimates of mid parental value [m], additive genetic effects [a] and additive genetic variance [σ^2_A].

Result: The frequency of transgressive RILs predicted from H 5 × HA 4 was higher than those predicted from H 4 × HA 5 for primary branches plant^{-1}, pods plant^{-1} and grain weight plant^{-1}, while it was comparable between the crosses for pod weight plant^{-1}. The required minimum population size was relatively smaller to recover the transgressive RILs from the cross which was predicted to result in higher frequency of RILs than that was predicted to result in lower frequency of RILs.

Key words: Additive genetic effects, Additive genetic variance, Minimum population size, Prediction, Transgressive segregation.

Abbreviation: RILs- Recombinant inbred lines; A-D- Additive dominance model; TS-Transgressive segregation; ANOVA- Analysis of variance.

INTRODUCTION

Dolichos bean is one of the ancient legume crops grown in dry and semi-arid regions of Asia and Africa. It is a versatile crop grown for vegetable, pulse, fodder and green manure purposes (Ramesh and Byregowda, 2016). As is true with other predominantly self-pollinated legume crops, pure-lines are the only cultivar types used for commercial dolichos bean production by farmers. Pedigree selection of desirable recombinant inbred lines (RILs) for use as pure-line cultivars from bi-parental crosses-derived segregating populations is the most widely used breeding method in dolichos bean (Ramesh and Byregowda, 2016). The breeders routinely develop a large number of bi-parental crosses-derived segregating populations to implement pedigree selection to identify superior RILs. Handling of a large number of segregating populations is not only prohibitively resource demanding, but also reduces the efficiency of breeding process in terms of less than satisfactory genetic gain. The crosses made between parental combinations that fail to produce useful cultivars consume over 99% of the resources (Witcombe *et al.,* 2013). Allocation of resources only to large-sized segregating populations derived from a few promising crosses selected based on an objective criterion is expected to enhance the chance of identifying desirable RILs for use as pure-line cultivarsand thus helps enhance breeding efficiency (Chahota *et al.,* 2007; Witcombe *et al.,* 2013; Bernardo, 2020). Given that resources are most often limited, determination of the minimum population size required to be raised to ensure(by say 95%) that at least one of the predicted RILs is recovered is equally important. Jinks and Perkins (1972) conceptualized the theory and out-lined the analytical procedure to predict the frequency of RILs that transgress pre-determined standards.The quantitative genetic parameters such as additive gene effect [a] and additive genetic variance [σ^2_A] reliably estimable from

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parental and early segregating generations (F_2 and F_3) are sufficient to predict the frequency of transgressive RILs (Jinks and Pooni, 1976). Thomas, (1987) demonstrated that even random sample of a few F_3 families provide a reliable estimate of [a] and [σ^2_A]. Kearsey and Pooni (1996) have described the method of predicting the minimum population size (n) required to recover at least one such predicted transgressive RIL. The objective of the present study is to predict the frequency of transgressive RILs and minimum population size required for their recovery from reciprocal crosses derived from two elite parents that complement the desirable traits in dolichos bean.

MATERIALS AND METHODS

The basic material consisted of two elite parents, namely HA 4 and HA 5 (Table 1). Both HA 4 and HA 5 are high yielding pure-lines cultivars released for commercial production of dolichos bean in eastern dry zone of Karnataka, India. While HA 4 produce relatively fewer branches, racemes and large-sizedcurved pods bearing large-sized grains, HA 5 produce relatively many branches, racemes and small-sized pods bearing small-sized grains (Table 1). The objective was to develop new pure-lines that producea large number of branches, racemes and large-sized pods and grains. Reciprocal crosses, namely HA 4 × HA 5 and HA 5 × HA 4 were synthesized during 2020 rainy season at the experimental plots of the Department of Genetics and Plant breeding (GPB), University of Agricultural Sciences, (UAS), Bangalore, India. A total of 20 and 15 well-filled F_1 seeds could be obtained from HA 4 × HA 5 and HA 5 × HA 4 crosses, respectively. The F_1 seeds were planted in 2020 post rainy season. All the F_1 seeds germinated and survived to maturity. The F_1 plants of the two reciprocal crosses were carefully inspected for the traits specific to male parents to confirm their true hybridity. Indeterminate growth habit of all the 20 candidate F_1 plants of HA 4 × HA 5 cross confirmed their true hybridity considering that indeterminacy is dominant over determinacy (Modha *et al.,* 2019; Basanagouda *et al.*, 2022). Production of curved pods (typical pod shape of HA 4) by all the 15 candidate F_1 plants of HA 5 × HA 4 confirmed their true hybridity considering that curved pod shape is dominant (Girish and Byregowda, 2009) over straight pods (typical pod shape of HA 5). The selfed pods from F_1's of the reciprocal crosses were harvested, hand-threshed and sun-dried to obtain F_2 seeds. F_2 plants from these two crosses were raised in 2021summer season. A spacing of 0.3m was maintained between F_2 plants of the reciprocal crosses. A total of 236 and 225 F_2 plants from HA 4 × HA 5 and HA 5 × HA 4 crosses, respectively survived to maturity. Selfed pods from each F_2 plants were manually harvested, hand-threshed and seeds were sun-dried for use in raising $F_{2:3}$ populations during 2021 rainy season. The seeds ofthe two parents and randomly selected 144 $F_{2:3}$ families derived from each of the HA 4 × HA 5 and HA 5 × HA 4 crosses were planted in asingle row of 3 m length in alpha-lattice design using two replications during 2021 post rainy season. Fifteen-days after planting, seedlings of two parents and 144 $F_{2:3}$ families were thinned to maintain a spacing of 0.3m between the plants and 0.6m between the rows. The recommended production package was practiced to raise two parents, F_1, F_2 and $F_{2:3}$ generations. A total 12 plants in each of the two parents and within each $F_{2:3}$ families in each replication survived to maturity.

Sampling of plants and data recording

Data were recorded on 10 randomly selected plants (avoiding border ones) fromtwo parents, their reciprocal F_1's and from each of the 144 $F_{2:3}$ progenies in each of the two replications and all the individual F_2 plants (236 and 225) derived from HA 4 × HA 5 and HA 5 × HA 4 crosses, respectively for four traits, namely, number of primary branches and pods and weights of sun-dried pods and grains. The average of these traits across ten sample plants in each replication was computed and expressed as primary branches plant^{-1}, pods plant^{-1}, pod weight plant^{-1} (g) and grain weight plant^{-1} (g).

Estimation of quantitative genetic parameters

Data recorded on 10 randomly selected individual plants in two parents and their two reciprocal F_1's and F_2 plants (236 and 225) and replication-wise mean data of 10 randomly selected plants from each of the 144 $F_{2:3}$ progenies were used for estimation of three quantitative genetic parameters, namely mid-parental value [m], additive gene effect [a] and additive genetic variance [σ^2_A] for use in prediction of the frequency of transgressive RILs that could be derived from HA 4 × HA 5 and HA 5 × HA 4 crosses. Assuming additive-dominance (A-D) model, the parameters, [m] and [a] were estimated using the multiple regression model (Kearsey and Pooni, 1996) implemented in SPSS software version, 16.0. Adequacy of A-D model was examined by joint scaling test

Table 1: Growth habit, response to photoperiod and pedigree/source of parents used to derive crosses in dolichos bean.

Parents	Growth habit	Response to photoperiod	Quantitative traits	Pedigree/source	Reference
HA 4	Determinate	Insensitive	Bear fewer number of branches, racemes andpods and produce large-sized pods and seeds	HA 3 × Magadi local	Mahadevu and Byregowda (2005)
HA 5	Indeterminate	Insensitive	Bear relatively a larger number of branches, racemes and pods and produce small-sized pods and seeds	HA 4 × GL153	Ramesh *et al.* (2018)

(Kearsey and Pooni, 1996) implemented in SPSS software version 16.0. The [σ^2_A] was estimated by equating observed and expected mean squares (MS) attributable to 'between $F_{2:3}$ families' from analysis of variance (ANOVA) of $F_{2:3}$ families and solving for σ^2_A using the formula; (van Ooijen, 1989).

$$\sigma^2_A = \frac{2 \times \text{MS due to 'between } F_{2:3} \text{ families' - MS due to error}}{\text{Number of replications}}$$

The analysis was implemented suing Microsoft Excel software.

Predictingthe frequency and minimum population size required for the recovery of transgressive RILs

Assuming that the data follow normal distribution, the probability (frequency) ofrecovering RILs that are likely to transgress the better parent (HA 5) was estimated as standard normal distribution integrals corresponding to quotient, (mean of HA 5-m)/σ_A for each trait considered in the present study; where, [m] is mid parental value and σ_A is square-root of σ^2_A (Jinks and Pooni, 1976). The minimum population size required to guarantee (say 95%) that RILs transgress HA 5 was predicted as the number (n) of RILs need to be raised such that probability of RILs that do not surpass HA 5 is less than 5% (Kearsey and Pooni, 1996). This probability was translated in to the equation,

$(1-P)^n \leq 0.05$,

where,

P= Probability of RILs that transgress HA 5.

(1-P) = Probability of RILs that do not transgress HA 5.

The equation was solved for 'n' by applying logarithm to both the sides and rearranging the terms as $n \geq \log 0.05/\log (1-P)$. If say 1% of RILs are predicted to surpass the HA 5, then 'n' was predicted as the ratio of log 0.05 to log 0.99, which is ≥298.

RESULTS AND DISCUSSION

ANOVA of $F_{2:3}$ families

ANOVA is a diagnostic step to detect and estimate variation attributable to target source (σ^2_A in the present study). ANOVA of $F_{2:3}$families suggested significant differences among means of F_3 families derived from reciprocal crosses for all the traits (Table 2). As most of the genetic variation between $F_{2:3}$ families are contributed by genes with additive effects, significant differences between $F_{2:3}$ families indicate significance of σ^2_A in controlling the inheritance of all the traits considered in the present study. Significance of σ^2_A is not surprising as, empirical studies across a range of crops have indicated that σ^2_A contributes over 50% and often close to 100% to observed variation across a range of traits (Bernardo, 2020). Bernardo (2020) theoretically demonstrated that the estimates of σ^2_A are always more than those of non-additive genetic variation even if there exists substantial interaction between alleles within each locus (dominance) and between loci (epistasis) controlling quantitative traits. In the present study, significance of σ^2_A is important considering that it is one of the predictors of the frequency of transgressive RILs.

Predicted frequency of transgressive RILs

Pure-line cultivars in most naturally self-pollinating crops including dolichos bean are developed by crossing parents, which are often cultivated varieties (as is true in the present study) and selecting RIL (s) with desired combination of traits. If no RIL was ever found which were betterthan their parents (or ancestors), referred to as transgressive segregants, plant breeding would notwork (Mackay, 2021). Though transgressive segregation (TS) occurs frequently enough that plant breeding works as amatter of routine, not all crosses display it and only a small proportion ofprogeny in any particular crossdisplay TS. It is therefore relevant to identify the potential crosses that uncover high frequency of TS. Predicting the frequency of transgressive RILs that could be derived from advanced generationsis an objective method of identifying potential cross (es). In the present study, the predicted frequency of RILs that are expected to transgress the better parent (HA 5) varied with the cross (Table 3). The predicted frequencies of RILs that transgressed HA 5 were higher from HA 5 × HA 4 than those from HA 4 × HA 5 for primary branches plant^{-1}, pods plant^{-1} and grain weight plant^{-1}. On the other hand, the predicted frequencies of RILs that transgressed HA 5 were comparable between HA 5 × HA 4 and HA 4 × HA 5 for pod weight plant^{-1}. Adequacy of A-D model (indicating lack of evidence for the role of epistasis), suggest that these predicted frequencies of transgressive RILs were reliable. Thus, our results clearly

Table 2: Analysis of variance of reciprocal crosses-derived $F_{2:3}$ progeny families evaluated in alpha-lattice design for four quantitative traits in dolichos bean.

Source of variation	Degrees of freedom		Primary branches		Pods plant^{-1} plant^{-1}		Pod weight plant^{-1} (g)		Grain weight plant^{-1} (g)	
	HA 5 × HA 4	HA 4 × HA 5	HA 5 × HA 4	HA 4 × HA 5	HA 5 × HA 4	HA 4 × HA 5	HA 5 × HA 4	HA 4 × HA 5	HA 5 × HA 4	HA 4 × HA 5
Between $F_{2:3}$ families	143	143	4.59**	4.05**	89**	66.43**	54.25**	76.2*	57.5**	67.6**
Replication	01	01	0.42	1.93	1.17	0.12	2.12	1.52	0.86	2.22
Blocks within replication	11	11	1.83	1.65	1.53	1.78	1.96	1.88	2.01	2.05
Residual	132	132	1.33	1.67	22.1	16.42	15.20	18.2	22.1	16.32

*Significant @ P=0.05 **Significant @ P=0.01.

Table 3: Predicted frequency of RILs which transgressed the limits of means of better parent 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			
	HA 4 × HA 5		HA 5 × HA 4	
	≥ Higher scoring parent (HA 5)	Minimum population size to recover predicted transgressive RILs	≥ Higher scoring parent (HA 5)	Minimum population size required to recover predicted transgressive RILs
Primary branches plant^{-1}	25.50	10.17	29.10	08.71
Pods plant^{-1}	17.90	15.18	22.70	11.63
Pod weight plant^{-1} (g)	12.50	22.40	12.10	23.43
Grain weight plant^{-1} (g)	26.44	09.77	28.08	09.11

suggest higher breeding potential of segregating population derived from the cross-involving HA 5 as female parent in terms of the frequency of RILs that transgress HA 5 for grain (marketable and consumable end-product) productivity.

Minimum population size required to recover predicted frequency of RILs

As expected, the minimum population size required to recover the transgressive RILs predicted from HA 5 × HA 4 was relatively smaller than those predicted from HA 4 × HA 5 (Table 3). These results also suggest the importance of the direction of the crosses to be developed for generating breeding populations to maximize the recovery of transgressive RILs for use as pure-line cultivars. Our results augur well with theoretical results of Bernardo, (2022), who demonstrated that breeding populations generated from good × good crosses as is the case in our study, would tend to uncover higher frequency of transgressive RILs in a predictable manner. Both quantitative genetic theory and empirical results indicate that TS mostly results from the combinations of complementary 'plus' and 'minus' alleles that are dispersed between parents (Rieseberg *et al*., 1999; Surma *et al*., 2000). In other words, individuals that receives 'plus' alleles from both parents or 'minus' alleles from both parents are likely to transgress the parents. Thus, TS most likely to occur when difference between the parents is small and σ^2_A is high. This is possible when the parents are genetically diverse, though phenotypically similar. Empirical results in other grain legumes such as lentil (Chahota *et al.,* 2007), dolichos bean (Shivakumar *et al.,* 2016) and horse gram (Chandana *et al*., 2022) provide adequate evidence for the robust reliability of the prediction approach used in the present study to assess breeding potential of crosses.

Breeding implications

It is possible to accelerate the process of identification of new crop varieties with a desired combination of both farmer and end-user preferred traits in breeding populations that are predicted to result in high frequency of transgressive RILs (Kochetov *et al.,* 2021). However, the genotypes that fall short of maximum trait expression are relatively common in segregating populations routinely developed by crop breeders (Bernardo, 2020). This is because, appearance of genotypes with all the desirable genes controlling a quantitative trait requires several precisely placed crossovers between all possible pairs of desirable and undesirable genes linked in repulsion phase in all the chromosomes (Witcombe *et al.,* 2013; Bernardo, 2020). However, the chance of occurrence of such precisely placed crossovers in high frequency is almost remote. Nevertheless, intermating such genotypes (falling short of maximum trait expression) selected from the same cross is expected to uncover relatively high frequency of genotypes with near-maximum trait expression even from small F$_2$/F$_{2:3}$ populations that breeders routinely handle (Bernardo, 2020). Our previous reports indicate that it is possible to increase the frequency of TS by one or more cycles of random mating between F$_2$ individuals resulting from biparental crosses in dolichos bean (Chandrakant *et al.,* 2015).

CONCLUSION

We believe that the method of identifying a few promising crosses at early segregating generations based on the frequency and minimum population size required for the recovery of transgressive RILs sounds practical as breeders develop F$_2$/F$_{2:3}$ populations from a large number of crosses on a routine basis. Based on our results and those reported, we opine that there is no reason why crop breeders ever need to go beyond F$_{2:3}$ generation of cross if the predicted frequency of transgressive RILs is low.

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Conflict of interest: None.

REFERENCES

Basanagouda, G., Ramesh, S., Chandana, B.R., Siddu, C.B. and Kirankumar, R. (2022). Inheritance of growth habit under photoperiod insensitivity BR background in dolichos bean [*Lablab purpureus* (L.) Sweet]. Genetic Resources and

Crop Evolution. 69 (7): 2535-2543. doi.org/10.1007/s10722-022-01390-x.

Bernardo, R. (2020). Breeding for Quantitative Traits in Plants. Third Edition. Stemma Press. Woodbury, Minnesota, USA.

Bernardo, R. (2022).Outliers and their distribution in breeding populations. Crop Science. 65: 1107-1114. https://doi.org/10.1002/csc2.20742.

Chahota, R.K., Kishore, N., Dhiman, K.C., Sharma, T.R. and Sharma, S.K. (2007). Predicting transgressive segregates in early generation using single seed descent method-derived micro-macrosperma gene pool of lentil (*Lens culinaris* Medikus). Euphytica. 156: 305-310.

Chandana, B.R., Ramesh, S., Kirankumar, R. and Basanagouda, G. (2022). Predicting the frequency of transgressive RILs and minimum population size required for their recovery in horse gram [*Macrotyloma uniflorum* (Lam.) Verdcourt]. Plant Genetic Resources. 20 (1): 73-75. doi.org/10.1017/s1479262122000077.

Chandrakant., Ramesh, S., Vaijayanthi, P.V., Byregowda, M., Mohan Rao, A., Keerthi, C.M. and Shivakumar, M.S. (2015). Impact of F_2 bi-parental on quantitative traits inter-relationships and frequency of transgressive segregants in dolichos bean (*Lablab purpureus* L.). Electronic Journal of Plant Breeding. 6(3): 723-728.

Girish, G. and Byregowda, M. (2009). Inheritance of qualitative characters in Dolichos bean *Lablab purpureus* (L.) Sweet. Environment and Ecology. 27: 571-580.

Jinks, J.L. and Perkins, J.M. (1972). Predicting the range of inbred lines. Heredity. 28: 399-403.

Jinks, J.L. and Pooni, H.S. (1976). Predicting the properties of recombinant inbred lines derived by single seed descent. Heredity. 36(2): 253-266.

Kearsey, M.J. and Pooni, H.S. (1996). The Genetical Analysis of Quantitative Traits. First Edition. Chapman and Hall, London.

Kochetov, A.A., Mirskaya, G.V., Sinyavina, N.G. and Egorova, K.V. (2021). Transgressive Breeding: A methodology for accelerated creation of new forms of plants with a predictable complex of economically valuable traits. Russian Agricultural Sciences. 47: 40-50.

Mackay, I.J., Cockram, J., Howell, P. and Powell, W. (2021). Understanding the classics: the unifying concepts of transgressive segregation, inbreeding depression and heterosis and their central relevance for crop breeding. Plant Biotechnology Journal. 19: 26-34. doi: 10.1111/pbi.13481.

Mahadevu, P. and Byregowda, M. (2005). Genetic improvement of dolichos bean (*Lablab purpureus* L.) through the use of exotic and indigenous germplasm. Indian Journal of Plant Genetic Resources.18: 1-5.

Modha, K., Kale, B., Borwal, D., Ramtekey, V. and Arpit, B. (2019). Inheritance patterns of photoperiod responsive flowering, growth habit and flower colour in Indian bean [*Lablab purpureus* (L.) Sweet]. Electronic Journal of Plant Breeding. 10(1): 297-302.

Ramesh, S. and Byregowda, M. (2016). Dolichos bean (*Lablab purpureus* L. Sweet Var. Lignosus) genetics and breeding -present status and future prospects. Mysore Journal of Agricultural Sciences. 50(3):481-500.

Ramesh, S., Byregowda, M., Keerthi, C.M., Reena, M., Ramappa, H.K. and Rajendra Prasad, B.S. (2018). HA 10-2 (HA 5): Promising high yielding advanced breeding line for use in commercial production of dolichos bean (*Lablab purpureus* L. Sweet). Mysore Journal of Agricultural Sciences. 52(1): 1-5.

Rieseberg, L.H., Archer, M.A. and Wayne, R.K. (1999). Transgressive segregation, adaptation and speciation. Heredity. 83: 363-372.

Shivakumar, M.S., Ramesh, S., Suresh, Showkath Babu, Keerthi, C.M., Byregowda, M. and Rao, A.M. (2016). Prediction and validation of frequency of recombinant inbred lines surpassing pre-determined standards for quantitative traits in dolichos bean (*Lablab purpureus* L. Sweet). International Journal of Agricultural Sciences. 8(18): 1325-1328.

Surma, M., Kaczmarek, Z. and Adamski, T. (2000). Predicted and observed frequencies of transgression effects in barley doubled haploids. (in Polish with English summary). Bulletin of Plant Breeding and Acclimatization Institute. 216: 195-199.

Thomas, W.T.B. (1987). The use of random F_3 families for cross prediction in spring barley. Journal of Agriculture Science. (Cambridge). 108: 431-436.

Van Ooijen, J.W. (1989). Estimation of additive genotypic variance with the F_3 of autogamous crops. Heredity. 63: 73-81.

Witcombe, J.R., Gyawali, S., Subedi, M., Virk, D.S. and Joshi, K.D. (2013). Plant breeding can be made more efficient by having fewer, better crosses. BMC Plant Biology. 13: 22 http://www.biomedcentral.com/1471-2229/13/22.