



PREDICTION AND VALIDATION OF THE FREQUENCY OF RECOMBINANT INBRED LINES SURPASSING PRE-DETERMINED STANDARDS FOR QUANTITATIVE TRAITS IN DOLICHOS BEAN (*LABLAB PURPUREUS* L. SWEET) VAR. LIGNOSUS

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Abstract- Development and use of an objective criterion for selecting a few potential crosses to maximize the frequency of superior pure-lines would help increase the pace and efficiency of any self-pollinating species with Dolichos bean being no exception to this. Probability of RILs that transgress the parental and/or any pre-determined range can be predicted based on additive [d], additive \times additive epistatic gene effects [i] and additive genetic variance (σ^2_A) estimated in initial selfing and/or backcross generations derived from a cross. An investigation was conducted to predict and validate the frequency of F_5 RILs derived from HA 10-8 \times RIL 180 that surpass the limits of their parental phenotypes and of check variety, HA 4 for nine quantitative traits. A fairly good agreement was observed between observed and predicted frequency of RILs that transgressed higher scoring parent only for primary branches plant⁻¹. On the other hand, good agreement was noticed between observed and predicted frequency of RILs that transgressed the lower scoring parent for dry pod yield plant⁻¹ and 100-dry seed weight. The agreement between predicted and observed frequency of RILs that transgressed either of the parent was rather poor for plant height, primary branches plant⁻¹, racemes plant⁻¹, raceme length, dry pods plant⁻¹ and dry seed plant⁻¹. The poor agreement between predicted and observed frequency of RILs for these traits was attributed to linkage disequilibrium between the genes controlling these traits.

Keywords- Dolichos bean, Prediction, Recombinant Inbred Lines, Validation.

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Introduction

Dolichos bean (*Lablab purpureus* L. Sweet) var. Lignosus is one of the grain legume crops known for its food [1-3] and fodder [4] values. Its origin traces to Indian sub-continent [5]. Fresh pods containing immature grains are the economic products, which are consumed as a vegetable [1-3]. The pods of *Lablab purpureus* var. Lignosus exude oily substances that emit characteristic fragrance [6], a highly preferred trait by farmers and consumers [1-3].

In India, dolichos bean is primarily grown both as an inter-crop and pure crop [7]. In pure crop stands, the productivity of dry seed yield is 1.2 t ha⁻¹ (raithamitra.co.in) while it is 0.4 to 0.5 t ha⁻¹ in inter-cropping system [3]. When it is grown for forage, it produces green fodder of 2.20 to 2.75 t ha⁻¹ under rain fed conditions [4]. In India, nearly 90 per cent of both area and production of dolichos bean is from Karnataka state (raithamitra.co.in).

Pedigree selection of desirable genotypes derived from planned crosses is the most widely used breeding method to develop improved cultivars in dolichos bean [8]. However, breeders are often constrained to select a few F_1 's from among a large number of crosses to develop high yielding varieties through pedigree selection. Therefore, development and use of an objective criterion for selecting a few potential crosses to maximize the frequency of superior pure-lines would help increase the pace and efficiency of dolichos bean breeding. Earliest possible prediction of seed-yield potential of early segregating populations in autogamous crops helps enhance breeding efficiency besides saving time and resources. Theoretical basis and analytical procedure to predict the frequency of recombinant inbred lines (RILs) which transgress pre-determined standards have been reported [9,10]. Additive genetic effect [d] and/or additive \times additive epistatic gene effects [i] (if significant) and additive genetic variance (σ^2_A) are used as the

predictors for *a priori* prediction of the frequency of RILs which transgress predetermined standards [10]. In this article, we predict and validate the frequency of RILs (derived from a cross between parents contrasting for pod yield and its component traits) which transgress the limits of parental phenotypes and of check variety, HA 4 for nine quantitative traits in dolichos bean.

Material and methods

Basic genetic material and development of experimental material

HA 10-8 (P_1) and RIL 180 (P_2) which are contrasting for pod yield and its component traits constituted the basic genetic material for the study. While HA 10-8 is high yielding, RIL 180 is a low yielding advanced breeding line [8]. HA 10-8 and RIL 180 were crossed during 2013 rainy season to obtain F_1 's (HA-10-8 \times RIL 180). The plants of the F_1 's were grown and selfed during 2014 summer. F_2 population derived from the F_1 's along with their parents and F_1 's were grown in 2014 rainy season at the experimental plot of Department of Genetics and Plant Breeding (GPB), University of Agricultural Sciences (UAS), Bengaluru, India which is located at an altitude of 930 m above mean sea level 12° 58' North and 77° 35' East latitude and longitude, respectively. The selfed seeds from the parents and F_1 and F_2 plants were collected, dried, treated and stored in -20°C. The five basic generations (P_1 , P_2 , F_1 , F_2 and F_3) of the cross, HA 10-8 \times RIL 180 constituted the experimental material.

Evaluation of experimental material

The non-segregating generations, P_1 , P_2 and their F_1 (HA 10-8 \times RIL 180) were evaluated in a randomized complete block design with 2 replications at the experimental plot of the Department of GPB, Bengaluru during 2015 rainy season.

The seeds of P₁, P₂ and F₁ were sown in a single row of 3m length. Ten days after sowing, the seedlings were thinned by maintaining intra-row spacing of 0.20 m within a row in each replication. The F₂ and F₃ generations were evaluated in two separate contiguous blocks. The seeds of the F₂ plants were sown in 20 rows. Ten days after sowing, seedlings were thinned maintaining 10 plants in each row with intra-row spacing of 0.20 m and 0.45m between rows. Seeds collected from 30 randomly selected F₂ plants were sown in plant-to-row progenies. Each F₃ progenies were grown in a single row of 2 m length and 10 plants were maintained in each row with an intra-row spacing of 0.20 m and 0.45 m between rows. Data were recorded on five randomly selected plants in P₁, P₂ and F₁, all F₂ plants and 5 randomly selected plants from each F₃ progenies on nine quantitative traits, namely, plant height (cm), primary branches plant⁻¹, racemes plant⁻¹, raceme length (cm), dry pods raceme⁻¹, dry pods plant⁻¹, dry pod yield plant⁻¹ (g), dry seed plant⁻¹(g) and 100 dry seed weight (g) based on descriptors[11].

Statistical analysis of data

Mean of the data recorded on five plants in P₁, P₂, F₁ and data recorded on individual F₂ and F₃ plants (within each of 30 progenies) were used for all the following statistical analysis. For prediction of the frequency F₅ RILs (derived from HA 10-8 × RIL 180), mid parental value [m], additive gene effects [d], additive × additive epistatic gene effects [i] and additive genetic variance (σ²_A) were computed using the following procedure.

Estimation of [m], [d] and [i].

The predictor parameters namely, [m], [d] and [i] were estimated following five parameter model [12].

$$\begin{aligned} [\hat{m}] &= \bar{F}_2 \\ [\hat{d}] &= 1/2(\bar{P}_1 - \bar{P}_2) \\ [\hat{i}] &= \bar{P}_1 - \bar{F}_2 + 1/2\bar{P}_1 - 1/2\bar{P}_2 + 1/2[d] - 1/4[i] \end{aligned}$$

Statistical significance of [m], [d] and [i] were tested using 't' test [13].

Estimation of σ²_A

The predictor, σ²_A was estimated by equating observed and expected mean squares attributable to 'between F₃ families' and solving for σ²_A [14].

Prediction of frequency of RILs which transgress parental and check variety limits

Assuming that the data follow normal distribution, the probability (frequency) of

obtaining F₅ RILs that surpass HA 10-8 (P₁), the higher scoring parent and fall short of RIL 180 (P₂), the lower scoring parent and surpass the check variety (HA 4) was estimated as

$\frac{[d]}{\sigma_A}$, $\frac{-[d]}{\sigma_A}$ and $\frac{Meanofcheckvariety-\hat{m}}{\sigma_A}$, respectively, for traits for which additive-dominance (A-D) model was adequate; where, σ_A is the standard deviation of additive genetic effects.

For traits for which (A-D) model was not adequate, the probability of RILs that surpass the parental limits was estimated as

$\frac{[d]+[i]}{\sigma_A}$, $\frac{-[d]+[i]}{\sigma_A}$ and $\frac{Meanofcheckvariety-\hat{m}}{\sigma_A}$, respectively [9,10].

Validation of RILs

The material for validation consisted of 102 RILs derived from HA 10-8 × RIL 180. The seeds of each of 102 RILs were sown in a single row of 2 m length. Ten days after sowing, seedlings were thinned maintaining 10 plants in each row with intra-row spacing of 0.20 m and 0.45 m between rows. The data were recorded on five randomly selected plants from each of the 102 RILs for nine quantitative traits. The number of F₅ RILs surpassing the limits of their high scoring parent, HA 10-8 and low scoring parent, RIL 180 and the check variety, HA 4 were counted and expressed in *per cent*. The observed frequency of F₅ RILs that surpassed the phenotypic limits of parents and the check, HA 4 was compared with that of predicted to examine the reliability of prediction.

Results and Discussion

A-D model was inadequate to explain the inheritance of any of the nine quantitative traits. Therefore, additive × additive gene effects [i] were estimated in addition to main additive gene effects [d]. The additive effect of genes reflects those effects, which are expected to be manifested in a genotype to which the genes are being substituted for their alternate forms/alleles [13]. High magnitude of negative significant estimates of [d] and [i] [Table-1] coupled with σ²_A suggested possible role of high frequency of large effect decreasing genes controlling plant height, dry pods plant⁻¹, dry pod weight plant⁻¹ and dry seed weight plant⁻¹. On the other hand, traits such as primary branches plant⁻¹, raceme plant⁻¹, raceme length and dry pods raceme⁻¹ are controlled by small and decreasing effect genes as indicated by either non-significant or significant but small magnitude of [d] and σ²_A.

Table-1 Estimates of mid parental value [m], additive gene effects [d], additive × additive gene effects [i] and additive genetic variance σ²_A for nine quantitative traits in dolichos bean

Trait	[m]	[d]	[i]	σ ² _A
Plant height (cm)	52.54**	12.96**	5.51	9.55**
Primary branches plant ⁻¹	-02.07**	-0.2 ±	4.67**	0.01**
Racemes plant ⁻¹	07.11**	-2.24**	1.89	2.30**
Raceme length (cm)	10.19**	-0.62**	-4.41**	0.02
Dry pods raceme ⁻¹	4.09**	-0.76**	0.14	0.03
Dry pods plant ⁻¹	24.92**	-10.00**	-2.42	44.34**
Dry pod weight plant ⁻¹ (g)	24.16**	-10.55**	-3.81	43.58**
Dry seed weight plant ⁻¹ (g)	20.89**	-9.24**	-4.03	22.32**
100 dry seed weight (g)	19.34**	-1.55**	-2.79**	0.10**

* Significant @ P = 0.05;

** Significant @ P = 0.01

Prediction and validation of frequency of RILs, which transgress, pre-determined standards

Transgressive segregants are the plants in F₂ or advanced generation, which surpass the limits of phenotypes of the parents from which they are derived [15]. In the present study, for most of the traits, majority of the F₅RILs surpassed higher scoring parent indicating that their parents differed for 'increasing effect' alleles. The frequency of RILs that transgress the better parent or any pre-determined target value can be predicted even before the initiation of selection. Prediction of

frequency of superior RILs enable discarding un-/less-productive crosses and concentrating efforts on the most promising crosses [16]. In the present study, the RILs transgressed higher scoring parent for raceme length, dry pods plant⁻¹, dry pod weight plant⁻¹, dry seed weight plant⁻¹ and 100 dry seed weight plant⁻¹ [Table-2]. This could be attributed to the involvement of negative additive × additive digenic interaction [i] controlling these traits [17]. On the other hand, higher frequency of RILs that transgressed lower scoring parent for primary branches plant⁻¹ could be attributed to complementary positive additive × additive gene

effects [i] [18].

Theoretically, it is expected that the frequency of RILs that transgress lower scoring parent is higher than those that transgress higher parent, if [i] is positive. However, in the present study, the frequency of RILs that transgressed higher

scoring parent was higher than those that transgressed lower scoring parent for plant height, raceme plant⁻¹ and dry pods despite that they are controlled by [i] type of epistasis. Factors such as linkage disequilibrium are more likely to cause observed results contrary to those expected [9].

Table-2 Estimates of frequency (%) of transgressive segregants for nine quantitative traits in *F₅* progenies of *dolichos* bean.

Traits	≥ Higher scoring parent	≤ lower scoring parent	≥ HA 4(Check)
Plant height (cm)	00.10	00.10	42.00
Primary branches plant ⁻¹	10.60	10.60	00.10
Racemes plant ⁻¹	00.40	00.30	42.00
Raceme length (cm)	00.10	00.10	00.10
Dry pods raceme ⁻¹	00.10	00.10	00.10
Dry pods plant ⁻¹	02.90	12.50	08.10
Dy pod weight plant ⁻¹ (g)	01.40	15.90	01.80
Dry seed weight plant ⁻¹ (g)	00.30	13.60	00.50
100-dry seed weight (g)	00.10	00.10	00.10

A fairly good agreement was observed between observed and predicted frequency of RILs that transgressed higher scoring parent only for primary branches plant⁻¹ [Table-3a]. On the other hand, good agreement was noticed between observed and predicted frequency of RILs that transgressed the lower scoring parent for dry pod weight plant⁻¹ and 100-dry seed weight [Table-3b]. Non-agreement between predicted and observed frequency of RILs that transgressed

either of the parents for rest of the traits could be due to linkage disequilibrium [9]. On the contrary, Snape *et al.* [19] in wheat; Pooni *et al.* [20] in *N.rustica*; Nanda *et al.* [21] in wheat; Engqvist and Becker [22] in oil seed rape and Mallikarjuna *et al.* [23] in rice observed a good agreement between observed and predicted frequency of transgressive segregants in RIL populations.

Table-3a Estimates of predicted and observed probabilities of transgressive segregants in *F₅* RILs derived from HA 10-8 × RIL 180 for nine quantitative traits in *dolichos* bean

Criteria	Plant height (cm)		Primary branches		Racemes plant ⁻¹		Raceme length (cm)	
	Predicted Probability (%)	Observed Probability (%)	Predicted Probability (%)	Observed Probability (%)	Predicted Probability (%)	Observed Probability (%)	Predicted Probability (%)	Observed Probability (%)
≥ Higher parent	0.1	60	10.6	09	0.4	27	0.10	07
≤ Lower parent	0.10	07	10.60	42	0.30	14	0.10	02
HA 4	42	72	0.10	13	42	31	0.1	02

Table-3b Estimates of predicted and observed probabilities of transgressive segregants in *F₅* RILs derived from HA 10-8 × RIL 180 for nine quantitative traits in *dolichos* bean

Criteria	Dry pods raceme ⁻¹		Dry pods plant ⁻¹		Dry pod weight (g)		Dry seed weight (g)		100 dry seed weight (g)	
	Predicted Probability (%)	Observed Probability (%)	Predicted Probability (%)	Observed Probability (%)	Predicted Probability (%)	Observed Probability (%)	Predicted Probability (%)	Observed Probability (%)	Predicted Probability (%)	Observed Probability (%)
≥ Higher parent	0.10	49	2.90	33	1.40 (8.10)	36	0.3	32	0.1	69
≤ Lower parent	0.10	39	12.50	23	15.90	15	13.60	20	0.10	01
HA 4	0.10	04	8.10	39	1.8	38	0.50	21	0.10	69

Higher frequency of RILs that transgressed the higher scoring parent for a few traits and those transgressed lower scoring parent for a few other traits could be attributed to 'between parents' dispersion of completely or incompletely dominant increasing and decreasing effect genes that display complementary epistasis [24],[15]. Dispersion of alleles was more reasonable rather than over-dominance for recovering maximum frequency of transgressive segregants in barley [25]. The presence of transgressive segregation constitutes evidence for quantitative control for a trait and contribution of different alleles from the parents towards the expression of a trait.

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