Predicting, validation of frequency of transgressive Recombinant Inbred Lines and minimum population size required to recover them in Groundnut (*Arachis hypogaea* L.)

ABSTRACT

Background: Identification of transgressive recombinant inbred lines (RILs) that can be use as pure-line cultivars is the prime objective of a breeder in self-pollinated crops including groundnut.

Aim: Thus, identification of such promising segregating generations assumes importance. This can be achieved by predicting the transgressive RILs that could be derived from advanced generations of segregating population.

Material and methods: In our study we predicted and validated transgressive RILs derived from cross TMV 2 × GPBD 4 for five quantitative traits based on estimates of mid parental value [m], additive genetic effects [a] and additive genetic variance [σ^2_A].

Results: the frequency of transgressive RILs was higher for all the five traits. Primary branches per plant had highest frequency of predicted transgressive RILs with minimum population size of 6.95. Narrow difference between the predicted and realised frequency of RILs was seen.

Conclusion: Our results indicate that this approach could be the efficient in selecting the best breeding population out of many number of crosses made.

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

INTRODUCTION

Groundnut is one of the important oilseed crop, majorly grown in arid and semi-arid regions in the world. Like any other self-pollinated crop, pure-lines are the only cultivar option in groundnut. The Recombinant Inbred Line (RILs) that transgress the better parent or the present day cultivar can be used as a new pure-line cultivar. Out of the breeding programs followed in groundnut, pedigree selection of developing RILs. These are generally developed from the segregating generation that is derived from bi-parental crosses. In this process of identifying superior RILs, breeders often tend to develop many number of bi-parental crosses.But handling large number of bi-parental crosses is tedious and also few crosses among them may show less genetic gain. Other than this the crosses made with certain parental combinations will yield less useful cultivars and consume 99 % of the resources (Witcombe *et al*., 2012). Therefore, allocation of resources on segregating populations derived from few promising crosses that are selected based on an objective will increase the chance of identifying desirable RILs that can be used as pure-lines and thus increases breeding efficiency (Chahota*et al*., 2007; Witcombe *et al*., 2013; Bernardo, 2020).By all this predicting the RILs that can be recovered in the advanced generation becomes important. Given the resources are limited, determination of minimum population size required to be raised, say 95 % so that at least one of the predicted RILs is recovered is also important. Jinks and Perkins (1972) dealt in detail the theory and out-lines the analytical procedure to predict the frequency of RILs that could transgress the threshold set for a particular trait. The quantitative genetic parameters such as additive gene effect [a] and additive genetic variance [σ^2_A] reliably estimated from parental and early segregating generations *viz*., F_2 and F_3 will be used in predicting the frequency of transgressive RILs (Jinks and Pooni, 1976).Method of predicting the minimum population size (n) required to recover superior RILs was given by Kearsey and Pooni (1996). With this background, present study was conducted to predict the frequency of transgressive RILs and minimum population size required to recover them from cross derived from two elite parents that complement the desirable traits in groundnut.

MATERIAL AND METHODS

The basic material consisted of two elite parents, namely TMV 2 and GPBD 4 (Table 1). Both TMV 2 and GPBD 4 are pure-lines cultivars and have farmer and end product user friendly characteristics. TMV 2 is farmer preferred variety as it has minimum yield loss even under adverse climatic conditions and has excellent pod filling which increases shelling percentage. On the other hand, GPBD 4 is high yielding with high shelling percentage and is resistant late leaf spot disease which is considered as one the most devastating disease of groundnut (Daniel *et al.* 2022). The objective of present study to obtain pure-lines that have characteristics of both the parents. Crossing was carried out between the parents in 2019, *summer* at experimental plots of the Department of Genetics and Plant Breeding, University of Agricultural Sciences, Bangalore, India. True F_1's were identified by performing hybridity test using SSR markers that were polymorphic between the parents. The obtained true F_1 seeds were planted in 2019, *kharif*. All the F_1 seeds germinated and survived to maturity. The selfed pods from F_1's were harvested, sun dried and shelled to obtain F_2 seeds.F_2 plants were sown in 2020, *summer* with spacing of 0.15 m between F_2 plants and 0.35 m between lines.Selfed pods from each F_2 plant was manually harvested and $F_{2:3}$ populations were raised during 2020, *kharif*. Ninety-four $F_{2:3}$families were raised in augmented design with a spacing of 0.15 m between plants and 0.35 m between lines. The recommended production packages were practiced to raise two parents, F_1, F_2 and $F_{2:3}$ generations.

Sampling of plants and data sampling

Data was recorded on 10 randomly selected plants from the two parents, their F_1's and from each of the 94 $F_{2:3}$ progenies and all the individuals of F_2 plants derived from cross TMV 2 × GPBD 4. Observations were recorded for five traits, primary branches per plant, pod per plant, pod yield per plant (g), kernel yield per plant (g) and shelling *per cent*sound mature kernel percentage (%).

Estimation of quantitative genetic parameters

Data recorded on ten randomly selected individual plants in parents, their F_1's, F_2's and 94 $F_{2:3}$ progenies were used to estimate three quantitative genetic parameters, namely mid-parental value [m], additive gene effect [a] and additive genetic variance [σ^2_A]. These estimates were used to predict frequency of transgressive RILs that could be derived from TMV 2 × GPBD 4. The parameters, [m], [a] and [σ^2_A] were estimated using the multiple regression model (Kearsey and Pooni, 1996) implemented in SPSS software *ver.* 16.0. Adequacy of A-D model was examined by joint scaling test (Kearsey and Pooni, 1996) implemented in SPSS software version 16.0.

Predicting the 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 was estimated as standard normal distribution integrals corresponding to quotient, (mean of better parent-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 obtain (say 95%) that RILs transgress better parent was predicted as the number (n) of RILs need to be raised such that probability of RILs that do not surpass GPBD 4 would be less than 5% (Kearsey and Pooni, 1996). This probability was translated in to the equation, $(1\text{-}P)n \leq 0.05$, where, P= Probability of RILs that transgress GPBD 4. (1-P) = Probability of RILs that do not transgress GPBD 4. 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 2% of RILs are predicted to surpass the HA 5, then ‘n’ was predicted as the ratio of log 0.05 to log 0.98, which is ≥ 148.

Validation of predicted frequency of transgressive RILs

The material for validation consisted of 94 F_6-families derived from TMV 2 × GPBD 4. The seeds of each of 94 F_6-families were sown in a single row of 3 m length. The data were recorded on all plants from each of the F_6-families for the same five quantitative traits for which frequency of transgressive RILs were predicted. Transgressive segregates were identified for each trait, as those F_6 plants whose values exceeded those of best parent, GPBD 4. The number of such F_6 segregates were counted and expressed in *per cent*. The observed frequency of F_6 plants that surpassed the phenotypic limits of GPBD 4 was compared with that of predicted to examine if observed and predicted frequency of RILs in F_6 generation were comparable for all the five quantitative traits. Narrower the difference between predicted and observed frequency of transgressive RILs, higher is the reliability and robustness of the prediction.

RESULTS AND DISCUSSION

ANOVA of $F_{2:3}$ families

Analysis of variance is an important method to detect and estimate the amount of variation present in population. In the present study, ANOVA of $F_{2:3}$ families recorded significant differences between the mean of F_3 families (Table 2).In case of between $F_{2:3}$ families, the genetic variation is due to genes with additive effects, significant difference between $F_{2:3}$ families indicates thatσ^2_A is significant in governing the inheritance of the traits that are investigated in the present study (Table 3). Empirical studies in different crops have suggested that σ^2_A(additive genetic variance) contributes to more than 50% of the total observed variation which is why σ^2_A is significant in our study also. Theoretical demonstration of estimates of σ^2_ABernardo (2020) revealed that additive genetic variance could be more than the sum of non-additive genetic variation even in the present of interaction of alleles within the locus (dominance) and between the loci (epistasis) controlling different quantitative traits. Hence in the present study, significant $\sigma^2_A$is an important parameter that has to be considered in predicting the frequency of transgressive RILs.

Predicted frequency of transgressive RILs

In self-pollinatedcrop like groundnut pure-lines are the only cultivar option. Transgressive recombinant inbred lines that perform better than the better parent or the ruling variety can be directly used as a new pure-line cultivar.If the RILs obtained did not perform better than the better parent, referred as transgressive segregants, plant breeding would not work (Mackay 2021). In plant breeding transgressive segregation is displayed by only few number of potential crosses derived from good parents that share favourable alleles. Therefore, selection of parents for crossing program should be done in such a way that the breeding population produces maximum transgressive RILs. One can identify such breeding population by predicting the frequency of transgressive RIL that can be recovered in advanced generations. In the present study, the predicted frequency of RILs that performed better than the better parent (GPBD 4) is presented in table 4. Primary branches per plant recorded highest predicted probability of transgressed RILs (34.90) followed by sound mature kernel *per cent* (32.54). kernel yield per plant recorded low probability of transgressive RILs (24.70) that could be obtained in advanced generation. Similar studies were conducted in different crops such as Dolichos bean by Basangouda*et al*. (2022) and Shivkumar *et al*. (2016) and predicted the frequency of transgressive RILs in early generations.Chahota*et al*. (2007) in Lentil, Carneiro *et al*. (2007) in Common bean, Nanda *et al*. (1990) and Yadava *et al*. (1998) in Wheat, Thomas *et al*. (1987) in barley and Jinks and Pooni (1972) in Tobacco also used similar approach to predict the frequency of transgressive RILs. Lack of epistasis as indicated by the adequacy of Additive- Dominance model suggest that the predicted frequencies of transgressive RILs are reliable. Thus our study clearly indicates TMV 2 and GPBD 4 form a good ×good cross and has higher breeding potential in generating superior transgressive RILs.

Minimum population size required to recover predicted frequency of RILs

The minimum population size required to recover the transgressive RILs is presented in Table 4. The results suggest the importance of direction of selection of cross that has to be developed in generating breeding populations to recover the maximum transgressive RILs that can be used as pure-lines cultivars.Our results were accordance with Bernardo (2022) who suggested that the breeding population developed good × good crosses as in case of our study, will yield higher frequency of transgressive RILs in a predictable manner. The present study, we can observe that as the probability of transgressive RILs is higher the minimum population size required to recover them is low. Primary branches per plant which recorded 34.90 probability of predicting transgressive RILs requires minimum population size of 6.95.

Whereas, kernel yield per plant having 24.70 predicted transgressive RILs require 10.5 minimum population size to recover them. The results suggest that higher the predicted probability of predicted transgressive RILs lesser is the population size required to recover them.Quantitative genetic theory suggest that transgressive segregation is possible when both the parents have complementary ‘plus’ and ‘minus’ alleles that are dispersed between them (Riesebergeт *et al*., 1999; Surma *et al*., 2000).Therefore, selection of parents should be done in such a way that the difference between both the parents and σ^2_Ashould be high. The parents should be genetically diverse yet 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)also suggested the robustness of the prediction method used in our study in order to assess the breeding potential of the cross.

Validation of frequency of transgressive RILs

There was a fair good agreement between the predicted and realized frequency of RILs in F_3 and F_6 generation, respectively that transgressed over better parent GPBD 4 for all the traits under study (Table 5). The results suggest the credibility of this approach in predicting the frequency of transgressive RILs. Similar results were reported by several researchers such as Carneiro *et al.* (2002) in common bean, Chahota*et al.* (2007) in lentil, Shivakumar *et al.* (2016) in dolichos bean and Chandana *et al*. (2022) in horse gram. Our study suggests this approach of identifying promising crosses in early segregating generations by predicting the frequency of transgressive RILs that could be recovered in advanced generations and minimum population to be raised to recover them is practical as plant breeders often develop F_2 /$F_{2:3}$ populations from large number of crosses. By using this strategy of identifying breeding potential of crosses one can select the best cross out of many crosses made in the process of development a pure-line cultivar.

CONCLUSION

The present study validated the effectiveness of using estimates of additive effects and additive genetic variance in predicting the frequency of transgressive RILs that can be recovered in advanced generations and also minimum population required to be raised in order to achieve it. Our study suggests this approach of identifying promising crosses in early segregating generations by predicting the frequency of transgressive RILs that could be recovered in advanced generations and minimum population to be raised to recover them is practical as plant breeders often develop F_2 /$F_{2:3}$ populations from large number of crosses.

FUTURE SCOPE: The robustness of this approach helps in identifying breeding potential of crosses and one can select the best cross out of many number of crosses made in the process of developing a superior pure-line cultivar in groundnut.

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Table 1: Growth habit and pedigree/source of parents used to derive cross in groundnut

Variety	**TMV 2**	**GPBD 4**
Varietal group	Spanish Bunch	Spanish Bunch
Origin	Mass selection from "Gudiatham Bunch" AH. 32.	KRG 1 × CS 16 (ICGV 86855)
Year of release	1940	2004
Duration in days	105	105
Kernel type	Small to medium	Small to medium
Growth habit	Erect	Erect
Yield t/ha	1.9	2.3
Special attributes	Wide adaptability **Shelling − 65.8 %,** Oil content − 49.7% Short duration Small to medium kernel type	Wide adaptability **Shelling − 67.7 %,** Oil content − 48.77%, Short duration Small to medium kernel type
Response to disease	Susceptible	Resistant

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

Source variation	Df	PBP	NPP	PYP	KYP	SMK
Between $F_{2:3}$ families	93	0.2*	70.93**	72.31**	18.07*	35.42**
Replication	1	0.07	0.2	0.59	0.27	0.21
Blocks within replication	11	0.19	32.71	39.87	12.79	30.39
Error	82	0.03	1.65	1.15	0.22	0.34

Df = Degrees of freedom
PBP = Primary branches per plant
NPP = Number of pods per plant
PYP = Pod yield per plant (g)
KYP = Kernel yield per plant (g)
SMK = Sound mature kernel *per cent* (%)
*Significant @P = 0.05
**Significant @P = 0.01

Table 3: Estimates of additive effects and additive genetic variance for five quantitative traits in groundnut

Trait	[m]	σ^2_A	[a]
Primary branches per plant	7.05	5.9*	0.27
Pods per plant	29.75	47.8*	3.96
Pod yield per plant(g)	21.30	50.01**	5.60
Kernel yield per plant (g)	11.35	51.28*	3.16
Sound mature kernel *per cent* (%)	71.35	47.8**	2.29
Shelling *per cent* (%)	66.75	16.7**	5.91

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

Trait	**Predicted probability of RILs that transgress the better parent and minimum population size required for their recovery**	
	≥ Higher scoring parent (GPBD4 or TMV 2)	Minimum population size required to recover predicted transgressive RILs
Primary branches per plant	34.90	6.95
Pods per plant	26.70	9.64
Pod yield per plant(g)	32.50	7.60
Kernel yield per plant(g)	24.70	10.5
Sound mature kernel *per cent* (%)	32.54	7.60

Shelling *per cent* (%)	25.69	10.0

Table 5: Predicted and realized frequency of RILs which transgressed the limits of better parent (GPBD 4) for five quantitative traits in TMV2 × GPBD4 of groundnut

Trait	**Predicted frequency of RILs ≥ GPBD4 based on estimates of predictors**	**Realized frequency of RILs ≥ GPBD4**
Primary branches per plant	34.90	36.7
Pods per plant	26.70	29.7
Pod yield per plant (g)	32.50	28.4
Kernel yield per plant(g)	24.70	25.7
Sound mature kernel *per cent* (%)	32.54	30.4
Shelling *per cent* (%)	25.69	23.8