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Nonparametric approach to multitrait selection for yield in groundnut (*Arachis hypogaea* L.)

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Summary. Eight characters related to nitrogen fixation and pod development measured 30 days after flowering were evaluated for their correct grading of the relative yield performance of 17 genetically diverse lines of groundnut (*Arachis hypogaea* L.). Each line was assigned a high or low yield status based on its pod yield, shelling percentage, and 100-kernel weight. Seventeen character combinations were examined for their relative merit in correct identification of the yield status of lines. The character sets, nitrogenase activity alone or in combination with nitrogen percent or shoot weight identified the status of 77% of lines correctly. The extent to which various characters accounted for the variation in pod yield was also checked by multiple regression analysis. While the character combination, nitrogen percent plus leaf area explained 75% of variation in pod yield, nodule mass, nitrogenase activity, and leaf area occurred in some other combinations that explained yield variation to a lesser extent. These analyses point to the profitability of involving crop physiological traits such as leaf area and nitrogen percent in selecting for relative yield performance in groundnut.

Key words: Selection index – Nitrogen fixation – Peanut – Nonparametric method – Stepwise regression

Introduction

Visual selection for yield and its components, which is possible in certain crops, is not feasible in groundnut due to the subterranean nature of pods. Selection is therefore limited most often to post-harvest observations. This will imply digging of pods of every plant, drying, and taking a number of post-harvest observations, which are laborious and time-consuming activities. During earlier studies, pod yield and its direct yield components, shelling percentage, and 100-kernel weight, were found to be favorably associated with some physiological characters, such as leaf area and biomass. Other leaf attributes were related to photosynthesis and characters indicative of biological nitrogen fixation, such as nodule mass and nitrogenase activity (Arunachalam et al. 1983; Pungle 1983). The optimum period for measuring those characters was found to be 30 days after flowering, corresponding to 10–12 weeks before harvest. Some of those characters could then be used for an indirect relative evaluation of yield and a first stage selection of desired lines. Intensive screening on a large spectrum of post-harvest observations including pod yield could then be confined only to those lines, which would result in an efficient allocation of time and resources. A feasibility study was hence conducted on populations representing available variability, with these major aims in view, the results of which are reported here.

Materials and methods

Three hundred and four breeding lines maintained as part of a national project on groundnuts were evaluated for their performance for a number of characters related to physiological growth, biological nitrogen fixation (BNF), and yield (Prabhu 1986). Seventeen lines were selected to represent their overall variability, and therefore can be treated as representative of the normal variation met with in breeding material of groundnut. They were grown in a randomized block design with four replications. Each line was assigned one row of 10 m. Rows were spaced 60 cm apart with plants at 10-cm intervals in the row.

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In earlier studies (Pungle 1983; Koteswara Rao 1984), as many as 50 characters spanning the seedling, flowering, post-flowering, and post-harvest stages were examined for their efficiency in a relative evaluation of the genetic potential of groundnut genotypes. It was found that characters indicative of nitrogen fixation and photosynthesis were important in addition to pod yield and its direct components. Hence, the following eight characters besides pod yield, shelling percentage, and 100-kernel weight were measured: leaf area per plant (cm^2, LA), specific leaf weight (cm^{-2}, SL), shoot weight per plant (g, SW), root weight per plant (g, RW), nodule mass per plant (mg, NM), nodule number per plant (NN), nitrogen percent (NP), and nitrogenase activity (μmol $hour^{-1}$ $plant^{-1}$, NG). Those characters were recorded 30 days after anthesis, as this stage was found to be the earliest adequate for a relative evaluation of lines (Pungle 1983). Two samples of three plants each were taken for every observation except NG, which was recorded on a sample of six plants from the same two samples on which other observations were recorded. Dried leaves, stem, pods, roots, and nodules of plants in each sample were separately ground to a fine powder. They were mixed thoroughly and two homogeneous samples were used to estimate nitrogen percent using the microkjeldahl method. Nitrogenase activity was measured by the acetylene reduction technique as described by Dart et al. (1972).

Following the procedure outlined by Arunachalam and Bandyopadhyay (1984), the differences in means among lines were tested for their significance (at the 5% level) by Duncan's multiple range test (DMRT). Arranging the means in descending order of magnitude, overlapping groups were obtained. The topmost group was assigned a score of 1, the next best a score of 2, and so on. When a culture was included in two or more groups, it was assigned a score equal to the mean of the scores of the respective groups. The scores were standardized by dividing them by the number of groups obtained for that character, so that the maximum score could at the most be equal to one. Then the scores were added across characters to provide a final score for each line. The mean of the final scores ($=M$) was used as the norm to allocate each line a high or low status. Lines whose individual final scores were equal to or less than M were classified to be of high and the rest to be of low status.

Arunachalam and Bandyopadhyay (1984) have emphasized several desirable features of this nonparametric procedure. (a) The variable magnitudes of error variance for various traits do get accounted for in the DMRT. (b) The assigning of logically defensible scores makes the assessment of the potential of various lines scale-independent across characters. (c) The method provides a unique advantage in aggregating the performance of a line across several characters of interest through a total score. (d) As long as the characters are chosen with care and caution, the nonparametric procedure is simple and efficient for grading the relative performance of a line. (e) The procedure also is flexible enough to assign a line to one of several classes (instead of high and low, which was found to be adequate for the objectives of this paper).

Obviously a status can be assigned to lines on the basis of one or more chosen characters. A base status of productivity was assigned to each line jointly on the characters pod yield (PY), shelling percentage (SP), and 100-kernel weight (TW), as they are the direct yield components (often showing negative correlation among themselves), hereafter to be denoted as yield status. On this basis, 11 of the 17 lines obtained high and the rest low yield status. High or low status was then assigned on chosen sets of characters and the percentage of agreement with yield status was worked out. This was used to compare the relative efficiency of character sets in identifying the yield status.

In addition, stepwise multiple regression analysis was used to determine the percentage of variation in pod yield accounted for by various characters. The coefficient of determination R^2 was used as an index to compare the relative efficiency of character sets in explaining variation in pod yield. Such an analysis was repeated using nitrogen percent and nitrogenase activity as dependent variables.

Results

The variation among lines was significant for all characters except specific leaf weight, root weight, and shelling percentage (Table 1). The high range of mean values for various characters allowed up to five overlapping groups (for TW) based on DMRT, confirming the broad spectrum of variation in the test material. In particular, the variation in pod yield, nitrogen percent, and leaf area could form four groups while, for most others, there were three groups except for shelling percentage (two groups) and specific leaf weight (one group). The differences in the magnitudes of variation for various characters were thus obvious. It would then be useful to identify opti-

Table 1. ANOVA (mean squares) and range of variation for various characters in groundnut

Source	*df*	LA	SL	SW	RW	NM	NN	NP	NG	PY	SP	TW
Lines	16	609,817.0*	1.07	87.56*	0.059	21,191.2*	13,365.7*	0.426*	3.95*	7.00*	56.52	40.34*
Replicates × lines	48	129,584.0	1.03	23.03	0.035	1,837.9	3,373.3	0.061	1.70	2.92	42.55	14.55
Sampling error	68	73,847.3	0.73	11.66	0.013	2,297.9	1,874.7	0.022	+	+	+	+
Range of variation		5.4 –17.6	6.4 –7.8	7.5 –24.7	0.51 –0.84	134[a] –257	99[a] –182	1.69 –2.69	1.26[a] –4.17	3.2 –9.1	57.0 –69.5	26.9 –38.6
No. of groups based on range test		4	1	3	3	3	3	4	3	4	2	5

* Significant at 5% level
+ = Based on plot means and hence replicates × lines m.s. was used as error variance
[a] Eliminating the non-nodulating line

Table 2. Percentage agreement (p) of the status assigned by a character set with base status given by pod yield, shelling percentage, and 100-kernel weight

Character set	p			Order of superiority
	High	Low	Total	
NG	100	33	77	1
NG, NP	91	50	77	1
NG, SW	100	33	77	1
NP	91	33	70	2
NG, LA	73	50	65	3
NP, NG, NM	55	67	59	4
NP, SW, LA	73	33	59	4
NM, LA	55	50	53	5
SW, LA	64	33	53	5
NM, NG, LA	55	50	53	5
NP, NM, SW, LA	64	33	53	5
NM, NG	36	67	47	6
NP, LA	46	50	47	6
NP, NM	55	33	47	6
NM	36	50	41	7
NP, SW	36	50	41	7
LA	27	50	35	8

Table 3. Significant (at 5% level) correlation coefficients (r) among physiological, BNF, and yield traits in groundnut

Character	r	Character	r
LA–SW	0.80	RW–NM	0.50
LA–RW	0.69	NM–NN	0.90
LA–NM	0.82	NM–NP	0.75
LA–NN	0.69	NM–NG	0.57
LA–NP	0.66	NN–NP	0.78
SL–NG	0.70	NN–NG	0.50
SL–SP	0.52	NP–NG	0.72
SW–NM	0.87	NP–PY	0.70
SW–NN	0.75	NP–SP	0.49
SW–NP	0.75	NG–PY	0.60
SW–NG	0.61	PY–SP	0.76

mum combinations of characters that can adequately identify the relative yield performance of lines.

Hence, 17 sets of characters relevant for practical breeding and which could be measured long before harvest were chosen, and their efficiency in correct identification of the yield status of lines was worked out. Three sets of characters – nitrogenase activity, nitrogenase activity plus nitrogen percent, and nitrogenase activity plus shoot weight – identified the status of 77% of lines (Table 2), followed closely by nitrogen percent alone. NG, LA, NM, and SW occurred in combinations that ranked third or fourth in the order of superiority. Leaf area alone was the last in order of merit, identifying the stauts of only 35% of lines. The results were corroborated, in general, by the correlations between characters that were directly or indirectly relevant to pod yield. For

Table 4. The extent of variation in pod yield, nitrogen percent, and nitrogenase activity accounted by various characters based on regression equations in groundnut

Dependent variable	Independent variables		Cumulative R	R given by variables[a]
	No.	Order of priority		
PY	10	SP[a,b], NP[a,b], LA[a,b], RW[b], TW[b], SW[b], NM, NN, NG, SL	92.5	82.7
PY	8	NP[a,b], LA[a,b], RW[a,b], SW, NM, NG, SL, NN	83.4	75.7
PY	4	NP[a,b], NM[a,b], NG, NN	63.0	48.4
PY	4	SL[b], SW[b], RW[b], LA[b]	47.0	
NP	7	NN[a,b], NG[a,b], LA[b], RW[b], SL, SW, NM	86.9	76.0
NP	4	SW[a,b], SL[b], RW[b], LA[b]	78.6	53.6
NP	3	NN[a,b], NG[a,b], NM[b]	76.0	76.0
NG	7	NP[a,b], SL[a,b], SW[b], RW[b], NM, LA, NN	90.1	69.3
NG	6	SL[a,b], SW[a,b], RW[a,b], NM, LA, NN	89.9	85.3
NG	4	SL[a,b], SW[a,b], RW[a,b], LA	85.7	85.3
NG	3	NP[a,b], NN[b], NM	56.2	51.9

[a] Variables selected by stepwise regression
[b] Variables accounting for 95% of cumulative R

instance, leaf area, which could not be used alone as a yield marker, had significant and high positive correlation with many other characters like SW, RW, NM, NN, and NP (Table 3). The correlation coefficient of NM with NN was as high as 0.9, followed by its correlation coefficient with NP and NG. NP, in turn, was correlated with PY ($r=0.7$), NG ($r=0.72$), and SP ($r=0.49$).

As a confirmation of the efficiency of specific traits in selection, stepwise regression analyses were done with pod yield, nitrogen percent, and nitrogenase activity as dependent variables (Table 4). Further, to remove the suspicion that, in the presence of direct yield components in the regression equation, the contribution of physiological and BNF variables can be low, SP and TW were included along with the eight traits in the regression analysis. They could explain 93% of variation in PY, of which the contribution of NP+LA alone was 75%, a reasonably high value. Likewise, characters relevant to BNF, NP+NM+NG+NN explained 63% of the variation in PY, of which NP and NM accounted for 48%. On the other hand, 76% of variation in NP was accounted for by NN+NG, while 56% of variation in NG was explained by NP (Table 4). Thus, SP and TW can be used in the laboratory to evaluate lines selected at the first stage based on the nonparametric approach using characters such as NP and LA.

Discussion

When several variables are involved in selection where the relative weight of each variable cannot be determined a priori, nonparametric procedures that are independent of the scale of measurements are useful and simple for evaluating a line simultaneously for a set of variables. They will permit identification of character combinations and delineation of a range of classes to reflect the variation in the expected relative yield performance, in contrast to the arbitrary truncation into high or low classes for each character in isolation.

Stepwise regression has now been accepted as an efficient procedure for identifying variables that have a high power of discrimination of genetic potential. In this procedure, variables having highest correlation are introduced into the regression step-by-step. At every step, the individual contribution of every variable in the regression is reassessed. If it is below a norm set by the experimenter, it is removed and another variable is entered. Through repeat cycles, variables are arranged according to their contribution to total variation. Variables thus selected are almost the "best" in the statistical sense. It has also been shown that the proportion of misclassification on stepwise regression is minimized and hence it scores over comparable procedures of selection (Weiner and Dunn 1966).

A scrutiny of the contribution of several combinations of variables to pod yield substantiates the importance of the two processes, biological nitrogen fixation and photosynthesis. While nitrogen fixation is an important process influencing yield in legumes, it has an energy cost which, in turn, is dependent on photosynthetic mechanisms. The results have correctly underlined the role of a few key variables related to those processes, such as total nitrogen percent and leaf area in relation to yield predictability. Those two variables could explain a substantial part (75%) of variation in pod yield (Table 4).

Several studies have reported that nitrogenase activity and nodule mass are vital parameters indicative of nitrogen fixation (e.g., Sinclair et al. 1980 in soybean; Ratner et al. 1979; Arunachalam et al. 1984 in peanuts). But opinion is gaining ground that nitrogenase activity measured by the acetylene reduction technique, which involves destructive sampling of plants, is too uncritical to be used with confidence (Schubert and Wolk 1982; Leif-Skot 1983; Nambiar and Dart 1983). Alternatively, nitrogen percent (on whole-plant basis) has been shown to be positively associated with nitrogenase activity, in general (Ratner et al. 1979; Lodha et al. 1983), and also with total dry weight in tropical legumes (Haydock and Norris 1967). In general, several traits related to BNF such as NG, NM, and NP have a strong association with crop physiological traits like leaf area index and leaf area (Viands et al. 1981; Pungle 1983; and also this study). This study has thus confirmed the role of total nitrogen percent (BNF trait) and leaf area (physiological character) in grading relative yield performance. While leaf area is highly relevant to efficient photosynthesis, which is a requisite of BNF, total nitrogen percent reflects the availability of nitrogen during critical stages of reproductive growth which, when combined with efficient translocation to growing fruit, would result in better pod and seed filling in groundnut.

As mentioned, this study is based on representative variation. The identification of key characters such as NP and LA is thus important in an indirect and relative evaluation of pod yield, where elimination of suboptimal and selection of a few desirable genotypes are critical, so that the selected genotypes can further be subjected to detailed post-harvest analysis in the laboratory.

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