Plant and Soil 121, 83–88 (1990).
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PLSO 8024

Light interception as a source of variation for nitrogen fixation in groundnut genotypes*

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Received 1 November 1988. Revised April 1989

Key words: acetylene reduction, *Arachis hypogaea*, genotypes, light, light interception, nitrogen fixation

Abstract

A range of groundnut (*Arachis hypogaea* L.) genotypes, representing the cultivated botanical groups, were grown at ICRISAT Center, India. In 3 experiments, 3–8 genotypes were grown at various plant-population densities. In a fourth experiment, 27 genotypes were grown at a constant spacing. Acetylene reduction (AR) and fractional light interception (f) by these cultivars were measured at several stages of crop growth. Plant population (density), sample date and genotype influenced both the AR rate m^{-2} and the fraction of light intercepted; variables that were well correlated. In 3 experiments, *ca.* 90% of the statistical variation in AR rate m^{-2} was attributed to variations in f. In the remaining experiment, genotypic variance was 46% of the explained variance; one genotype (Gangapuri) had consistently low AR across the range of populations, however in the other experiments Gangapuri did not differ from other cultivars in AR/f, when sampled at earlier stages of development.

Introduction

One research avenue extensively investigated is the genetic improvement of the host plant (Nutman, 1984). Substantial genotypic variation in groundnuts for biological N_2 fixation rate has been reported (Nambiar *et al.*, 1982; Wynne *et al.*, 1982), and this variation has been shown to be heritable (Isleib *et al.*, 1980). Similarly, differences in the total amount of N fixed by different cultivars have been demonstrated (Gillier *et al.*, 1987). Therefore, the prospects of increasing biological N_2 fixation through breeding and selection are considered good.

The estimation of N_2 fixation rate by the destructive acetylene reduction (AR) assay (Dart *et al.*, 1972) has been shown to be influenced by the method of assay (Minchin *et al.*, 1983b), environmental and cultural conditions (Nambiar and Dart, 1983), and crop growth stage (Dutta *et al.*, 1988; Tonn and Weaver, 1981). Despite these shortcomings, observations of acetylene reduction activity (ARA) on groundnuts (grown in conditions of limited soil N) can correlate well with final N accumulation (Arunachalam *et al.*, 1984; Dutta *et al.*, 1988), suggesting that this method can provide a quantitative index of N_2 fixing ability for use in breeding programs (Coale *et al.*, 1985; Duhigg *et al.*, 1978; Graham and Temple, 1984). Usually the described genotype variations in ARA are based on isolated plants, or on plants in widely spaced rows, *i.e.*, conditions where the fraction of incoming light intercepted by the crop is less than one.

At the plant level, variations in light interception (created by defoliation) have been shown to vary ARA (Osman *et al.*, 1983), while Wynne *et al.* (1982) reported that total leaf area and N_2 fixation were associated in a range of groundnut genotypes. This is expected because of the dependence of N_2 fixation on energy derived from photosynthesis. In groundnuts, there are wide genotypic differences in canopy structure which can interact with plant

* Submitted as Journal Article No. 838.

spacings to influence photosynthesis by changing the fraction of light intercepted (f) by the plants. In a growth study conducted at ICRISAT (ICRISAT, 1983), genotypes having large differences in N_2 fixation rate (Nambiar *et al.*, 1982) did not differ in their crop growth rates. In these N_2 fixation studies, the plants were grown in rows 0.6 or 0.9 m apart, while in the crop-growth studies, the populations were adjusted for canopy characteristics to ensure that light interception was complete.

While there is ample evidence that leaf expansion is strongly influenced by N nutrition (Seetharama *et al.*, 1982; Trewavas, 1985), it is incorrect to attribute all variations in leaf area to N nutrition or to the symbiosis. It is possible that the reported genotypic differences in the N_2 fixation rate of genotypes are confounded by morphologically mediated differences in f. If this were true, it would have considerable implication for the conduct of a breeding effort aimed at improving N_2 fixation rates.

This study was conducted to examine the influence of canopy-based variations in light interception on the ARA m^{-2} of groundnut genotypes.

Materials and methods

Four experiments were conducted in the post-rainy seasons (Nov–April) of 1983/84 and 1984/85 at ICRISAT Center, near Hyderabad in India. Since these plants were grown in Alfisols with a history of successful groundnut nodulation no inoculants were added. During land preparation, 60 kg ha^{-1} P_2O_5 was incorporated into the soil, and 400 kg ha^{-1} of gypsum was top-dressed at flowering. The intended plant populations were achieved by sowing more seeds than needed and thinning to the required spacing after seedling establishment. Irrigation (*ca.* 50 mm) was initially applied at 10-day intervals until the evaporative demand increased in February, after which irrigation was applied weekly. Plots were kept weed-free by using herbicides (Alachor and Glyphosate at recommended rates), and by hand weeding.

Plant sampling for the ARA was done 2 or 3 days after irrigation, between 0930 and 1130 h on clear sunny days. Sample areas were guarded from other treatments, and from previously sampled areas by at least 50 cm, or by another row of plants in the wider spacings. Immediately before samplings for ARA, light interception (as a fraction of the flux) was measured with a Licor Instruments line-quantum sensor placed above and below the canopy. Plants were then removed by digging with a garden fork and the root systems gently shaken free of soil. The root systems of plants in a sample were cut from the tops and placed in a 10-litre plastic bucket, immediately after the top was sealed acetylene was introduced to create a 10% (v/v) mixture of acetylene and air. The gas mixture was sampled at the start of the incubation period, and the jars were covered with wet sacking to keep them cool and incubated for 30 min before a second sample was made. Full details of the AR methods are described by Nambiar and Dart, (1983). Hydrogen evolution was not measured. All the plants in a sample were assayed as a bulk, however the numbers of plants within a sample varied depending on the population density, and AR rates were adjusted for the land area involved in the sample.

Experiment 1

Three genotypes (NC Ac 2821, Kadiri 3, and ICGS 15) were sown at 3 spacings (15 × 15, 30 × 30, and 60 × 60 cm) in a randomised-block design with 4 replications in 1983/84. Plots 1.5, 3.0, and 4.5 m^2 for the 15, 30, and 60 cm spacings, respectively, were sampled for estimation of ARA at 88 days after sowing (DAS).

Experiment 2

In 1983/84, 4 genotypes (NC Ac 2821, Kadiri 3, Gangapuri, and JL 24) were sown in 3 inter-row spacings (30, 45 and 60 cm) with both 10- and 20-cm intervals between plants within the rows. Treatments were replicated 4 times. Samples for AR assay were made at 80 and 140 DAS, and 30 days after flowering.

Experiment 3

Eight genotypes, previously characterised as high and low N_2 fixing (Nambiar *et al.*, 1982), were

Table 1. Genotypes classified by botanical group and N_2-fixing attributes

Botanical group	Genotype	N_2 fixing classification
Spanish	X-14-4-B-19B	High
	JL 24	Low
Valencia	NC Ac 490	High
	Gangapuri	Low
Virginia bunch	Egret	High
	Kadiri 3	Low
Virginia runner	NC Ac 2821	High
	M 13	Low

selected in 1984/85. The 4 morphologically distinct types, virginia runner, virginia bunch (ssp. *hypogaea* var. *hypogaea*), valencia (*fastigiata* var. *fastigiata*) and spanish (*fastigiata* var. *hirsuta*), were each represented by 2 cultivars (Table 1) in each of three replications. The inter-row spacings were 30, 60, 90, and 120 cm with a 10-cm in-row spacing. Two m lengths of row were sampled for AR assay 47, 61, 75, 90, and 116 DAS.

Experiment 4

In 1984/85, 27 genotypes (16 *fastigiata* ssp. and 11 *hypogaea* ssp.) were grown in 3 replications, with one spacing (30 × 10 cm). ARA m^{-2} and f of each genotype were measured 35 days after commencement of flowering. Sample size was the same as in Experiment 3.

Statistical analysis

In Experiments 1–3, statistical analysis started with analysis of variance (ANOVA) to determine if genotypes and spacings influenced ARA m^{-2}. The relationship between ARA m^{-2} and f was evaluated using linear regression techniques, and correlation coefficients for individual genotypes. Since ARA m^{-2} was usually related linearly to f, further ANOVA for genotype (G) effects was conducted using f and G in a linear model approach (SAS GLM procedure), where population effects were not considered. This provided an assessment of the relative contributions to variance of canopy differences (morphology and spacing dependent), and genotype after the effects of canopy were considered. This approach was the only one used in Experiment 4 where only one population was used.

Results

The mean maximum temperature was 31.2°C in 1983/84 and 33.1 in 1984/85. Mean minimum temperatures were 18.0 in both years; relative humidity was 35% in 1983/84 and 28% in 1984/85; and radiation averaged 17.6 and 18.7 MJ m^{-2} day^{-1} in these seasons respectively.

Experiment 1

Genotypes did not vary significantly after the effects of f had been taken into account (Table 2). In all 3 genotypes, the ARA m^{-2} increased linearly with f (r was between 0.69* and 0.84**); larger differences between genotypes existed at low f or wide spacings (different intercepts for the regression of ARA m^{-2}), but these decreased as f approached 1.0 in the close spacings because of the differences in regression coefficients (Table 3).

Experiment 2

No genotypic differences in ARA m^{-2} were found at 140 DAS, or 30 days after flowering (data not shown), but existed at 80 DAS, when both genotypes and f significantly influenced ARA m^{-2}. ARA m^{-2} was linearly related to f for 3 genotypes. Gangapuri differed from the other genotypes in that ARA m^{-2} did not increase with f, resulting in

Table 2. Analysis of variance for the effects of genotype and fractional light interception (f) on the acetylene reduction activity per unit area of groundnuts

Source	Experiment 1 (88 days after sowing)		Experiment 2 (80 days after sowing)	
	DF	F value	DF	F value
Genotypes	2	1.67^{NS}	3	14.34***
f	1	31.69***	1	18.57***
Error	32		64	
Total	36		69	

*** $P > 0.001$, ^{NS}Not significantly different.

Table 3. Regression analysis between acetylene reduction activity (per unit area) and fractional light interception (f) for genotypes of groundnut

Genotype	Intercept	(SE)±	b	(SE)±	r
Experiment 1. (88 days after sowing)					
Kadiri 3	−2058	689.9	37.3	8.61	0.81**
NC Ac 2821	14	217.5	10.6	3.46	0.69*
ICGS 15	−1368	435.6	27.1	5.56	0.84**
Experiment 2. (80 days after sowing)					
Kadiri 3	118	156.7	4.7	2.33	0.48*
NC Ac 2821	45	120.1	4.5	2.29	0.45*
Gangapuri	146	79.8	0.4	1.53	0.02
JL 24	−193	89.8	6.5	1.33	0.78**

$*p > 0.05$, $**p > 0.01$.

the significant genotype (G) effect (Table 2). The regression coefficients for both slope and intercept were similar for the other three genotypes (Table 3).

Experiment 3

The seasonal increase then decrease of ARA m^{-2} (Table 4) followed that previously reported by Nambiar *et al.* (1982). Only low ARA was observed after 80 DAS. Without considering f, genotypes displayed ARA m^{-2} consistent with their prior classification (Table 1) when averaged across the range of row spacings (Table 4). Although the rankings remained consistent, however, statistically significant differences between genotypes occurred at different dates. Wide row spacing resulted in low ARA m^{-2}, as demonstrated by the data

Table 4. Seasonal pattern of acetylene reduction ($\mu mol\ h^{-1}\ m^{-2}$), averaged across row spacings. Experiment 3

	Days after sowing				
	47	61	75	90	116
X-14-4-B-19B	454	820	1005	597	126
JL 24	646	925	976	587	95
NC Ac 490	543	819	1155	738	152
Gangapuri	392	664	887	505	128
Egret	503	824	1179	790	232
Kadiri 3	457	774	817	556	223
NC Ac 2821	525	954	1112	915	219
M 13	450	634	1045	677	192
LSD (5%)	153.5	236.4	240.4	237.6	66.6

Table 5. Acetylene reduction ($\mu mol\ h^{-1}\ m^{-2}$) by eight groundnut genotypes at four row spacings at 75 days after sowing. Experiment 3

Genotypes	Row spacing (cm)			
	30	60	90	120
X-14-4-B-19B	1791	1078	743	408
JL 24	1775	952	683	496
NC Ac 490	2101	1138	739	643
Gangapuri	1737	917	469	426
Egret	2459	1056	716	487
Kadiri 3	1217	955	567	529
NC Ac 2821	1983	1178	722	565
M 13	1662	1248	769	502
LSD (5%)		±735.8		

Table 6. Analysis of variance of acetylene reduction by eight genotypes between 47 and 75 DAS. Experiment 3

Source	DF	F Value
VAR	7	2.10*
DAS	2	9.39***
VAR*DAS	14	0.91^{NS}
RAD	1	611.79***
Error	263	
Total	287	

$***P > 0.001$.
$*\ P > 0.05$.
NS Not significantly different.

at 75 DAS (Table 5), when ARA m^{-2} was at its peak. However, ANOVA (GLM) of ARA m^{-2} as a function of f and G showed that although genotypes were a significant source of variation, their contribution to the overall sums of squares was only 2% (Table 6).

A multi-factor regression of ARA m^{-2} on DAS (between 47 and 75 DAS) and f was computed for individual genotypes, and the rate at which ARA m^{-2} changed as f and DAS increased was compared (Table 7). Diversity (for the regression term of ARA m^{-2} on f) was greatest among the virginia bunch types. Egret had the greatest AR response to f, and Kadiri 3 the lowest; these differences in response to f being statistically significant ($P > 0.05$). The slope terms of the regressions were not different for all the other genotypes; the *fastigiata* genotypes did not differ amongst them-

Table 7. Regression parameters for the effects of fractional light interception (f) and age (DAS) on acetylene reduction (μmol h^{-1} m^{-2}). Experiment 3

Genotype	Intercept	S.E. ±	f	S.E. ±	DAS	S.E. ±	r
X-14-4-B-19B	60	(329)	2307	(310)	−5	(6.2)	0.89
JL 24	376	(229)	2384	(206)	−11	(4.2)	0.83
NC Ac 490	−98	(279)	2308	(252)	−1	(5.1)	0.93
Gangapuri	−7	(296)	2058	(292)	−3	(5.5)	0.84
Egret	384	(300)	3128	(301)	−13	(5.8)	0.87
Kadiri 3	503	(234)	1644	(208)	−9	(4.5)	0.81
NC Ac 2821	645	(227)	2602	(191)	−15	(4.4)	0.90
M 13	116	(116)	1869	(252)	−3	(3.0)	0.82
Mean[a]	241	(100.9)	2253	(92)	−7	(1.9)	0.85

[a] based on model fitted across genotypes (35 degrees of freedom).

selves, or from the mean of the *hypogaea* ssp. and were not significantly less than the best genotype (Egret). The intercept terms were not significantly different from zero for all genotypes.

Although ARA m^{-2} increased with time until 75 DAS, the multi-factor regression showed that this was the effect of increased f with time, ARA $m^{-2}f^{-1}$ tending to decrease with age.

Experiment 4

As with Experiment 3, both the G and f were significant ($p > 0.01$) in accounting for the variations in ARA m^{-2}; however, f was associated with 90% and G with 6% of the explained variation in ARA m^{-2} (data not shown).

Discussion

In these experiments the emphasis is on relative 'fixation rates'. Thus, the effect of acetylene on nitrogenase (Minchin *et al.*, 1983b), becomes important only if the depression is not constant across genotypes. Since the ANOVA also shows that the genotypic effects are small, differences in sensitivity to acetylene are (if they exist at all in groundnuts) a small source of error. More importantly, fractional light interception (f) contributed substantially to the rate of fixation, this being significant for future work.

The differences in ARA m^{-2} between the four morphological classification groups were more dependent on variations in f, rather than inherent differences in symbiotic efficiency (Table 6). Thus, reports that *fastigiata* types have lower ARA than the *hypogaea* group (Nambiar *et al.*, 1982; Wynne *et al.*, 1982) may be due to canopy characteristics, with the experimental conditions favouring genotypes with greater branching and leaf production capabilities. Graham and Rosas (1978) observed an interaction in the fixation rate of bean genotypes with widely differing canopies over a range of populations. At low populations, were f of the 'bush' variety would have been less than that of the 'climber', the fixation per plant and per unit area of the 'climber' were several times greater than that of the 'bush' types. But at high population densities (that probably intercepted most radiation), both compact bush and climbing cultivar had the same ARA m^{-1}.

The possibility of comparing the fixation rate of genotypes, after adjusting for canopy effects on f can be evaluated using these experiments. Investigation of the AR rate of individual genotypes, after adjusing for f, showed that differences did exist, but these accounted for a small fraction of the variance (2–6%). It still needs to be determined whether the gains possible by breeding are economic.

Radiation interception is easily manipulated by changes of plant spacing and this alternative strategy to manipulate the fixation rate of groundnut seems more attractive than breeding. However, with improved methods of measuring ARA and the associated respiratory cost of fixation (Minchin *et al.*, 1983a) linked to measurements of the canopy

photosynthesis, it may be possible to demonstrate a genetic variation in fixation rate that is not confounded by the canopy and the acetylene effect.

Since the total N_2 fixed by a crop is a function of duration and mean rate of fixation and as these experiments show that the instantaneous rate of fixation by groundnuts is dominated by canopy considerations, there remains the possibility of improving the N_2 fixation by extending the duration of fixation. Nitrogen isotope technologies are able to evaluate the total amounts of N_2 fixed by legumes (Giller *et al.*, 1987), and these techniques combined with measurement of the fractional light interception, to overcome the confounding effect of morphologically derived differences in canopy, should allow selection of differences in duration.

In conclusion, these data indicate that direct selection to improve host-plant rates of N_2 fixation in groundnuts is unlikely to succeed if measurements of N_2 fixation are attempted without associated measurements of energy interception. Secondly, canopy-related effects probably are a confounding factor in previous reports of genotypic differences in symbiotic N_2 fixation.

Acknowledgements

Use of a trade name does not imply endorsement of this product over others of a similar nature. We wish to thank B Srinivas Rao and A Viswanath for their assistance in these experiments.

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