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RESEARCH PAPER

Mode of inheritance of promiscuous nodulation and combining abilities in soybean genotypes

Eric E. Agoyi*[1], **Khalid E. Mohammed**[1], **Thomas L. Odong**[1], **John B. Tumuhairwe**[1], **Godfree Chigeza**[2], **Phinehas Tukamuhabwa**[1]

[1]*Department of Agricultural Production, Makerere University, Kampala, Uganda*

[2]*International Institute of Tropical Agriculture, Avondale, Lusaka, Zambia*

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Abstract

Knowledge of the mode of inheritance of a trait can be a powerful decision-making tool in a breeding program, as it helps predicting selection gain, defining breeding strategy and choosing parental lines. This study aimed at estimating genetic parameters to infer the mode of inheritance of promiscuous nodulation in soybean. Half diallel crosses were made among nine parental lines. F2 progenies were field evaluated together with parents for nodulation characteristics and grain yield in response to *Bradyrhizobium* sp. strain USDA 3456. Data on nodule number (NN), percent of effective nodules (NE), fresh and dry weight of nodules (NFW and NDW), and grain yield were subjected to analysis of variance, and progenies' means regression against parents' was performed following Griffing's Method2/Model 1. General and specific combining abilities, broad and narrow sense heritabilities, and Baker's ratio were estimated. The study showed predominant GCA effect for all measured traits except NE. Broad and narrow sense heritabilities were high for grain yield and NDW, moderate for NN and NFW, and low for NE. Baker's ratio was high for all measured traits except for NE. Overall, additive gene action was more important for all measured traits, except NE where non-additive gene action was more important. The high to moderate heritabilities for most traits showed that substantial gain can be achieved through selection.

* **Corresponding Author:** Eric E. Agoyi ✉ ericagoyi@gmail.com

Introduction

Soybean is a legume crop of great potential in Africa as it is an excellent source of protein and oil for both human food and animal feed (Tefera, 2011; Agarwal *et al.,* 2013). In addition, soybean helps sustain and enhance soil nitrogen fertility through its efficient nitrogen fixing capacity. It can fix up to 337 kg of nitrogen per hectare when inoculated with effective *Bradyrhizobium* strains (Salvagotti *et al.,* 2008). However, most soybean cultivars are highly specific in their rhizobium requirement (Sanginga *et al*., 1997) and the *Bradyrhizobium japonium* required for nodulation of these cultivars is not endemic in African tropical soils (Sanginga *et al*., 1997). This makes it necessary to import and apply inoculants onto soybean seed before planting. Some soybean cultivars named "promiscuous nodulating" or "freely nodulating" have the capability of nodulating with *Bradyrhizobium* strains that are indigenous in African soils. For example, *Bradyrhizobium* spp. that belong to the cowpea cross-inoculation group are widely spread in African tropical soils (Abaidoo *et al*., 2007) and have been reported to nodulate promiscuous soybean cultivars (IITA, 1996). Therefore, developing promiscuous soybean cultivars has been suggested as one of the most sustainable approach to promote African soybean production.

Scientist at the International Institute for Tropical Agriculture (IITA) have bred and released 21 promiscuous soybean varieties for different African countries (Terefa, 2011). However, knowledge on the genetic basis of promiscuous nodulation in soybean is scarce. Kueneman *et al.* (1984) interpreted the results of a preliminary study carried out on soybean by Chowdhury and Doto (1981), they reported that there may be transgressive segregation in the F2 generation for promiscuous nodulation, and that promiscuity is a heritable trait. Gwata *et al.* (2004) reported on two segregation patterns, nodule dry weight and leaf color score. Through a backcross analysis between two contrasting parents (promiscuous and non-promiscuous), the authors reported that non-promiscuity is partially dominant and controlled by four loci for nodule dry weight and non-promiscuity is completely dominant and controlled by two loci for leaf color score. Gwata *et al.* (2005) later found two alleles at each loci with a dominant gene action controlling non-promiscuous phenotype. The authors found out that genotypes with dark green leaves were double recessive and any individual possessing at least one dominant allele at either locus was non-promiscuous. More recently, Gwata *et al.* (2013) detected the RAPD marker OPB06 (5′-TGCTCTGCCC-3′) which they reported to co-segregate with promiscuous nodulation.

In plant breeding, knowledge of inheritance pattern of quantitative traits enables plant breeders to design suitable breeding programmes (Nadeem and Azhar, 2004, Acquaah, 2012, Bernardo, 2014). For instance, breeders need to consider the relative contribution of the components of genetic variance (additive, dominance, and epistasis) and environmental variance in choosing the best selection method. High additive gene action and heritability lead to high response to selection (Acquaah, 2012, Bernardo, 2014). Several approaches are used to acquire knowledge on the mode of inheritance of traits, and the diallel technique is one of the most powerful approaches. The diallel technique is used for estimating genetic parameters such as general combining ability (GCA), specific combining ability (SCA), Baker's ratio, and heritability, which are used to determine good combiners among parents to be chosen to initiate a plant breeding program, and to predict selection gain in a breeding program (Acquaah, 2012, Bernardo, 2014, Bi *et al.,* 2015). It also generates new genetic combinations that may have higher performance compared to the parents (heterosis).

The present study aimed at using the diallel approach to estimate genetic parameters which would help soybean breeders to more effectively improve promiscuous nodulation in their breeding programmes.

Materials and methods

Germplasm

The germplasm used in this study consists of nine soybean genotypes with different responses to inoculation with *Bradyrhizobium* sp. USDA 3456 (Agoyi *et al.,* 2016) (Table 1).

Table 1. Description of the genotypes used in the half-diallel crosses (9 parental lines).

Genotype	Pedigree	Release year	Current use status	Source
Namsoy 4M	Nam 2 x GC00138-29	2004	Commercial	NARO, Uganda
Maksoy 2N	Maksoy 1N x Duiker	2010	Commercial	MAK, Uganda
Maksoy 4N	Duiker x Gc00138-29	2013	Commercial	NARO, Uganda
Maksoy 5N	Nam2 x Gc00138-29	2013	Commercial	NARO, Uganda
Maksoy IN	TG x 1835-10E	2004	Commercial	IITA
Namsoy3	Kabanyolo I x Nam I	1995	Parental line	Naro, Uganda
Maksoy3N	Gc00138-29 x Duiker	2010	Commercial	NARO, Uganda
Wondersoya	-	-	Parental line	IITA, Nigeria
UG 5	-	-	-	MAK, Uganda

NARO: National Agricultural Research Organization; MAK: Makerere University; IITA: International Institute for Tropical Agriculture.

All genotypes were crossed with each other in a 9 × 9 diallel mating design with no reciprocal matings. Out of the thirty-six cross combinations possible, Thirty-one (31) were successfully generated. F1 seeds were selfed, grown together with their corresponding female parent in plastic pots in screen house at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) to generate F2 seeds for evaluation and at the same time confirm that the F1s resulted from successful hybridizations. Morphological traits such as hypocotyl colour, pubescence colour on stem, leaf and pod, days to flowering, flower colour, maturity dates, plant height, were used to confirm F1 individuals and to eliminate plants that did not result from hybridization.

Experimental sites, design and planting

The experiment was carried out in the field at MUARIK during the second rainy season of 2015. MUARIK is located at 0°27′03.0″N and 32°36′42.0″E and is 1300 meters above sea level. With its two rainy seasons, MUARIK receives an average of 1300 mm of rain per year and the soils are classified as Acric Ferralsol, which are typical slightly acidic (5.3 – 6.2) and low soil fertility (Aniku, 2001).

The 31 F2 progenies plus the nine parents were planted in the field on 13 October 2015. A plot of land not previously inoculated with *Bradyrhizobia* was selected and prepared for sowing following common land preparation methods (Tukamuhabwa and Obua, 2015).

Seeds of the forty genotypes (31 progenies and 9 parents) were inoculated with *Bradyrhizobium* sp. strain USDA 3456 (cowpea-type inoculant) *Bradyrhizobium* spp are reported to nodulate promiscuous cultivars (IITA, 1996).

Inoculant was obtained from Biofix (Kenya), purified and incubated in Soil Science Biological Nitrogen Fixation (BNF) laboratory at Makerere University. *Bradyrhizobium sp.* strain USDA 3456 was grown to 7.91×10^9 cells g^{-1} using the most probable number (MPN) through serial dilution technique described in Somasegaran & Hoben (1994), and then formulated into inoculum using steam-sterilized peat as a carrier. Ten grams of sugar was dissolved into 300 ml of clean luke-warm water in a plastic bottle for use as a sticking agent following Use-Instructions given by the inoculant supplier BIOFIX. The sugar solution was manually coated on the seed followed by *Bradyrhizobia* inoculant and immediately planted. This is the most recommended method of inoculation to enhance association of plant-rhizobium.

The experimental layout was an alpha lattice design with three replicates. Each replicate consisted of five blocks that each contained eight plots. The plots consisted of single 4 m long rows with a 0.6 m inter-rows spacing. The spacing between plants was approximatively 0.05 m, giving an average of 80 plants per complete row.

For some plots, the rows were not complete due to few seeds available. Standard agronomic practices for weeding and pest control were applied (Tukamuhabwa and Obua, 2015).

Data collection and analysis

Data were collected on December 1st 2015, six weeks after emergence. Ten plants were randomly dug up from each plot and the root systems from plants were carefully washed. All nodules were harvested from sampled plants and counted to determine the number of nodules (NN), and weighed to determine the fresh weight of nodules per plant (NFW) using the high precision digital scale ADAM Pow 453e (Max 450g, d = 0.001g). Thereafter, all the nodules were split to assess their effectiveness and the percentage of effective nodules (NE) per plant was computed based on the presence of brownish or pinkish pigmentation inside nodules. Nodules were then oven dried at 65°C for four days (Gwata *et al.,* 2004) and weighed to determine the total nodule dry weigh (NDW) per plant. At maturity, plots were harvested and seed yield was estimated.

Data were analyzed using linear mixed model (LMM) in GENSTAT 14th Edition (VSN International Ltd, Hemel Hempstead, UK) (Payne *et al*., 2011). Data and residuals were tested for normality and homoscedacity, and no data transformation was required.

The mixed model used was as follows:

$$\boldsymbol{Y_{ijk} = \mu + G_i + R_j + \frac{R}{B_{k(j)}} + \varepsilon_{ijk}}$$

Where Y_{ijk} = observed value from each experimental unit, μ = population mean, G_i = effect of ith genotype, Rj = effect of the jth replicate, $R/B_{k(j)}$ = effect of the kth block nested to the jth replicate, ε_{ijk} is the experimental error. Genotype was considered as fixed and blocks and replicates as random.

The regression of progeny means against parents was performed using Griffing's Method2/Model 1 (Griffing, 1956) in GENSTAT 14th Edition. General combining ability (GCA) of parental lines and specific combining ability (SCA) for the F2 progenies were estimated. Student's t-test was applied to examine the significance of the general and specific combining ability for each of the traits. GCA/SCA components of variance were estimated together with Baker's ratio and broad sense and narrow sense heritabilities were calculated. The formulas used for that purpose were as follows:

$$Baker's\,ratio = \frac{2vcG_i}{(2vcG_i + 2vcS_{ij})}$$

$$h^2 = \frac{2vcG_i}{(2vcG_i + 2vcS_{ij} + msLee)} \quad \text{(Narrow sense heritability)}$$

$$H^2 = \frac{(2vcG_i + vcS_{ij})}{(2vcG_i + 2vcS_{ij} + msLee)} \quad \text{(Broad sense heritability)}$$

Where vcG_i is the GCA effect of parent i, vcS_{ij} is the SCA effect of cross i × j, and *msLee* is the mean square of lattice effective error.

Spearman correlation was calculated between pairs of measured traits to determine which of the traits have the greatest correlation with grain yield and how the measured traits are related to each other. That would enable soybean breeders using one or few traits to predict nitrogen fixation and subsequent yield increase.

Results and discussion

The analysis of variance showed significant ($P < 0.05$) difference among genotypes for all measured traits, except for the percentage of effective nodules (NE) (Table 2). It was observed that neither GCA and SCA was significant for NE, all the parents and progenies had mean score ranges of 70 – 84 (Table 4) and 71 – 92 (Table 5), respectively, so were non-significant and so are the deviation of individual mean scores from the overall mean expressed as GCA and SCA.

General combining ability mean square was significant ($P < 0.05$) for all measured traits except for NE (Table 3). Specific combining ability mean square was not significant ($P > 0.05$) for any of the traits measured. The GCA/SCA ratio was greater than unity for most measured traits, suggesting that additive gene action is predominant in the expression of these traits (Acquaah, 2012). Consistent with this, the Baker's ratio which reveals the relative importance of GCA effect with respect to SCA effect (Baker, 1978), was high in all measured traits (0.73, 1, 0.99, 0.99 for NN, NFW, NDW, and grain yield, respectively) except in NE, where Baker's ratio was equal to zero.

Table 2. General analysis of variance for the measured traits.

Sources	NN		NE		NFW		NDW		Yield	
	d.f	m.s.	d.f	m.s.	d.f	m.s.	d.f	m.s.	d.f	m.s.
Reps	2	2625.6^{**}	2	221.8^{ns}	2	1.12^{***}	2	0.012^{*}	2	3249^{ns}
Block/Reps	12	275.2^{ns}	11	86.8^{ns}	11	0.04^{ns}	11	0.003^{ns}	11	70313^{ns}
Genotypes	39	402.3^{**}	39	106.3^{ns}	39	0.06^{*}	39	0.005^{*}	39	89446^{*}
Error	65	189.4	66	86.8	66	0.04	66	0.003	63	49516
L.e.e.	64	201.1	77	87.9	77	0.04	77	0.003	66	53242
Mean	33.5		80.3		0.50		0.11		709	
S.e.d.	11.6		7.7		0.16		0.04		188	
L.S.D.	23.1		15.2		0.33		0.09		376	

NN=nodule number, NE=percent of effective nodules, NFW=fresh weight of nodules, and NDW=dry weight of nodules. d.f=degree of freedom, m.s.=mean square, S.e.d.=standard error of difference, and L.S.D.=least significant difference.

These confirm the great importance of additive gene action in the inheritance of these traits. As mentioned earlier by Bi *et al.* (2015), a large ratio between GCA and SCA effects reveals the relevance of additive gene effects while a small value signifies that the trait is under dominant and or epistatic gene effects. The higher values of GCA compared to SCA observed in this study are a good indication that great genetic progress can be achieved in breeding for promiscuity in soybean by focusing on the number of nodules (NN), fresh weight of nodules (NFW), dry weight of nodules (NDW), and grain yield.

Table 3. Analysis of variance from regression of progenies' means against parents.

Sources	NN		NE		NFW		NDW		Yield	
	d.f	m.s.	d.f	m.s.	d.f	m.s.	d.f	m.s.	d.f	m.s.
Crosses	39	132.8^{**}	39	32.3^{ns}	39	0.02^{*}	39	0.002^{ns}	39	29674^{*}
Gca	8	312.1^{***}	8	27.4^{ns}	8	0.05^{**}	8	0.003^{**}	8	69147^{***}
Sca	31	86.6^{ns}	31	33.5^{ns}	31	0.01^{ns}	31	0.001^{ns}	31	19488^{ns}
Lee	64	67.1	77	29.3	77	0.01	77	0.001	66	17747
Baker's ratio	0.73		0		1		0.99		0.99	
vcGca/vcSca	1.33^{ns}		0.047^{ns}		8.20^{***}		111.8^{***}		377^{***}	
NSH	0.37		0		0.34		0.97		0.98	
BSH	0.52		0.13		0.36		0.98		0.98	

NN=nodule number, NE=percent of effective nodules, NFW=fresh weight of nodules, and NDW=dry weight of nodules. d.f=degree of freedom, m.s.=mean square, Lee=lattice effective error, Gca=general combining ability, Sca=specific combining ability, NSH=narrow sense heritability, BSH= broad sense heritability standard error of difference, and L.S.D.=least significant difference.

Heritability estimates showed that broad sense and narrow sense heritabilities were both high (0.98 and 0.97 respectively) for NDW and grain yield, suggesting that very high proportion of the phenotypic variation is due to genetic effect (Holland *et al.,* 2003), indicating that substantial gain can be achieved from selection. However, the fact that the present study was performed in a single environment may have led to underestimating the environmental component. Hence, we recommend that similar study be carried out in multiple environments for more accurate estimates. Both heritabilities were moderate (0.3 to 0.5) for NN and NFW, indicating that those two traits are under both genetic and environmental control with a relatively low genetic implication. For such traits, selection in single environment may lead to some gain, however, performing selection based on the performances in different environments would ensure substantial gain (Bernardo, 2014). Narrow sense heritability was 0 for NE and broad sense heritability for the same trait was low (0.13), indicating very low genetic control for this trait. Response to selection might be hard to achieve while focusing on percent of effective nodules (NE). However, carrying out selection based on plants' performances in several environments, may result in some selection gain (Holland *et al.,* 2003). Based on GCA estimates of the parental lines (Table 4), the parent P6 (NamSoy 4M) was the best combiner for NN, NFW and grain yield. For NE, the parent P7 (MakSoy 1N) was the best combiner, and P1 (MakSoy 2N) was the best combiner for NDW.

Table 4. Mean scores and GCA estimates of parents.

N°	Parents	NN		NE (%)		NFW (mg)		NDW (mg)		Yield (kg/ha)	
		Mean score	GCA	Mean score	GCA	Mean score	GCA	Mean score	GCA	Mean score	GCA
P1	MakSoy 2N	23	-9.0^{***}	84.3	0.1	380.7	-71.3^{***}	80.5	55.5^{***}	551.6	-25.2^{***}
P2	UG 5	37	5.0^{***}	72	1.9^{***}	544.2	91.7^{***}	113.5	-14.5^{***}	730	43.1^{***}
P3	MakSoy 3N	27	-5.4^{***}	84.1	0.4	407.9	-44.3^{***}	99	15.5^{***}	941.6	-18.3^{***}
P4	MakSoy 4N	30	-2.7^{***}	74.2	-0.8^{***}	391.6	-60.3^{***}	88.5	-24.5^{***}	613.1	56.7^{***}
P5	MakSoy 5N	32	-0.4	78.4	-0.6^{*}	422.9	-29.3^{***}	84.6	-14.5^{***}	635.6	48.9^{***}
P6	NamSoy 4M	53	20.5^{***}	70.3	-3.1^{***}	654.2	204.7^{***}	167.3	-14.5^{***}	875.4	142.1^{***}
P7	MakSoy IN	40	8.1^{***}	80.9	2.3^{***}	53	77.7^{***}	117.8	5.5^{***}	598.8	-15.6^{**}
P8	NamSoy 3	24	-8.1^{***}	76.3	1.5^{***}	389.8	-62.3^{***}	69.1	5.5^{***}	480.1	-62.8^{***}
P9	WonderSoya	17	-5.2^{***}	73.3	-1.8^{***}	195.1	-81.3^{***}	47.3	-24.5^{***}	384.1	-149.6^{***}
Average		31.4	0.32	77.09	-0.03	382.2	2.81	96.4	-1.17	645.6	2.14
s.e.		3.6	3.17	1.72	0.59	58.46	32.98	11.42	8.50	59.57	27.68

NN=nodule number, NE=percent of effective nodules, NFW=fresh weight of nodules, and NDW=dry weight of nodules. s.e.=standard error, Gca=general combining ability.

Based on the specific combining ability estimates (Table 5), the cross P7xP1 (Maksoy 1N x Maksoy 2N) had the greatest performance for NN and NFW, while the cross P4xP2 (Maksoy 4N x UG5) was the best performing cross for NE and NDW, and P4xP1 (Maksoy 4N x Maksoy 2N) for grain yield.

The correlation analysis showed that NFW and NDW were significantly ($p<0.01$) correlated to grain yield (Table 6). Similar trend was observed in previous studies, authors have reported positive correlation between NDW and nitrogen fixation and between nitrogen fixation and increased yield in soybean and other legume crops (Rosas and Bliss, 1986, Mirza *et al.,* 1990, Sinclair *et al.,* 1991, Hafner *et al.,* 1992, Ankomah, 1995). NN was significantly correlated with grain yield ($p<0.05$), but had lower coefficient ($r= 0.34$) than NFW and NDW. Correlation between grain yield and NE was not significant ($p>0.05$).

Table 5. Mean scores and SCA estimates of progenies.

Progenies	NN		NE (%)		NFW (mg)		NDW (mg)		Yield (kg/ha)	
	Mean score	SCA	Mean score	SCA	Mean score	SCA	Mean score	SCA	Mean score	SCA
P7XP1	64	3.23^{***}	83.8	0.22	795.1	2.33^{*}	192.2	2.68^{**}	562.1	-0.87
P7XP4	47	0.90	85.6	0.77	641.8	0.77	139.4	0.71	872.3	1.01
P7XP6	34	-0.80	79.7	0.04	600.4	-0.07	126.3	-0.54	877.6	0.35
P7XP2	36	-1.59	84.8	0.05	538.4	-1.70	117.3	-1.04	778	0.34
P7XP3	22	-0.95	86.4	0.70	475.4	-0.18	101.5	-0.08	534.6	-1.15
P7XP5	28	-1.44	81.4	-0.12	426.2	-1.58	80.7	-1.42	949.3	1.70
P3XP1	38	1.54	72.8	-1.60	461.3	0.41	98.7	0.53	628.3	-0.30
P8XP1	29	0.26	81.9	0.02	374.1	-0.52	71.5	-0.5	674.5	0.44
P3XP2	23	-1.56	79.6	-0.60	345	-2.28^{*}	72.4	-1.49	448.8	-2.33^{*}
P6XP1	32	-0.73	71.6	-1.14	495.3	-0.34	99.3	-0.77	727.2	-0.81
P6XP3	29	0.26	80.9	0.68	437.8	-0.35	89.7	-0.71	838.7	0.05
P6XP5	38	0.24	74.3	-0.46	569.5	-0.04	116.8	-0.38	950.8	0.42

Progenies	NN		NE (%)		NFW (mg)		NDW (mg)		Yield (kg/ha)	
	Mean score	SCA	Mean score	SCA	Mean score	SCA	Mean score	SCA	Mean score	SCA
P6XP2	50	0.55	87.6	1.71	772.5	0.70	173.8	0.72	971.1	0.63
P4XP1	19	-2.38*	73.1	-1.31	207.2	-2.63*	36.9	-2.16*	989	2.04*
P4XP3	29	0.34	70.5	-1.89	437.8	0.08	74	-0.47	702	-0.37
P4XP2	60	1.94*	91.8	2.10	884.3	2.20*	212.9	2.87**	940.3	1.08
P5XP1	39	0.43	86	1.26	542.7	0.25	117.5	0.58	835.1	0.84
P5XP3	23	-0.41	81.9	0.38	610.1	1.42	134.7	1.57	793.8	0.45
P5XP4	41	0.59	85.7	1.37	540.2	0.12	130.2	0.88	877.8	0.52
P5XP9	36	0.60	67.1	-2.17	446.9	-0.17	102.1	0.32	469.4	-1.14
P8XP3	24	0.93	83.2	0.22	370.1	-0.03	66.3	-0.28	567.1	-0.49
P8XP4	17	-1.38	83.4	0.49	419.6	-0.19	82.9	-0.24	552.5	-1.23
P8XP6	32	0.48	82.3	0.73	495.6	0.09	122.3	0.35	943.4	1.28
P8XP2	36	-0.06	85.4	0.35	563.7	-0.31	125.7	0.28	716.1	0.22
P2XP1	33	-1.65	84.3	0.42	658.9	0.17	138.4	0.41	812.5	0.71
P9XP7	40	0.66	80.4	-0.07	543.8	0.43	133.7	-1.09	531.5	-0.1
P9XP1	27	-0.70	81.5	0.59	456.7	0.33	93.3	0.93	495.6	-0.31
P9XP3	20	-0.15	83.3	0.89	463.4	0.92	47.8	0.23	506.2	-0.28
P9XP4	32	-0.01	79.1	0.29	397	-0.35	73.1	-0.96	660.1	0.37
P9XP8	21	-0.23	84.9	1.00	478.3	0.96	103.9	-0.61	675.4	1.47
P9XP2	58	2.37*	84.5	0.83	717.7	1.21	157.9	0.91	651.3	0.40
Average	34	0.04	81.1	0.19	521.5	0.05	110.8	0.08	726.85	0.16
s.e.	12	0.22	5.6	0.18	142.2	0.20	39.64	0.2	168.89	0.17

NN=nodule number, NE=percent of effective nodules, NFW=fresh weight of nodules, and NDW=dry weight of nodules. s.e.=standard error, Sca=specific combining ability.

Table 6. Correlations between the measured traits.

	Yield	NN	NE	NFW
Yield				
NN	0.34*			
NE	0.20ns	0.15ns		
NFW	0.41**	0.74***	0.38*	
NDW	0.41**	0.77***	0.42**	0.94***

NN=nodule number, NE=percent of effective nodules, NFW=fresh weight of nodules, and NDW=dry weight of nodules.

A positive correlation between NDW and N_2 fixation in soybean (Shiraiwa *et al.,* 1994) and in other leguminous species such as common bean (Rosas and Bliss 1986), cowpea (Ankomah, 1995), and peanut (Hafner *et al.,* 1992) was reported. In addition, shoot (plant) dry weight was found to be correlated positively with NDW (Sinclair *et al.,* 1991) and with both leaf chlorophyll content and shoot total nitrogen in soybean (Mirza *et al.,* 1990). Therefore, NDW is a reliable measure of N2 fixation in the present study. With such correlation, phenotyping can be limited to dry weight of nodules in promiscuous cultivars to infer on potential grain yield, enabling breeding programs with limited resources and time to still make progress.

Among the nodulation traits, NDW and NFW had the strongest correlation (p<0.001; r=0.94), followed by NN and NDW (p<0.001; r=0.77). Similar trend was observed in a previous study where Agoyi *et al.* (2016) reported NDW and NFW with the highest correlation (p<0.001; r=0.96), followed by NN and NDW (p<0.001; r=0.79). Sinclair *et al.* (1991) also reported a positive correlation between NN and NDW. Unlike the report in the previous study by Agoyi *et al.* (2016), where correlation between NE and NN was highly significant (p<0.001; r=062), the present study has shown a non-significant correlation between NE and NN (p>0.05; r=0.15). This could be because the previous study was carried out in controlled environment with sterilized soil media where only the introduced *Bradyrhizobium* strain caused nodulation. In the present study carried out in the field, the nodules are occupied by a diversity of different bacterial strains existing in the soil, so nodules effectiveness might have been affected by a competition between strains. Ge and Xu (1982), in a previous study reported on such a competition between strains. Hence, the relationship between NN and NE must be regarded as due to environmental effect rather than genetic response.

The low heritability and insignificant GCA and SCA effects observed in nodules effectiveness, coupled with the inconsistent relationship between percentage of effective nodules and the other nodule-related traits are indications that the trait shows complexity. This observation is of great concern, because nodule effectiveness is one of the most important traits in biological nitrogen fixation (BNF), therefore it is expected that NE has strong correlation with grain yield. In the present study, one reason why NE and grain yield had low correlation could be that all the lines experienced equally sufficient NE corresponding to potential yields and this is reflected as NE not correlated with yield. However, there is need to develop more accurate method to score nodule effectiveness, as the current method is based on visual scoring that could be biased in many ways. For instance, a good eyesight is required to appreciate the inner color of the nodules, tinny nodules are hard to hold, cut and assess their effectiveness. Besides, inside nodules' color must be assessed immediately after they are cut, as oxidation begins inside the nodules that turn into brownish causing bias in the assessment.

Previous studies reported NDW as good indicator of nitrogen fixation and grain yield (Sinclair *et al.,* 1991), this was confirmed in the present study. Moreover, nodules being the powerhouse of nitrogen fixation, nodules 'effectiveness is an important trait to focus on while breeding for promiscuous nodulation. The parental lines Maksoy 4N and UG5 being best combiners for those two traits can be recommended as good candidate to initiate a breeding program. In addition, Maksoy 2N, good combiner for grain yield, NN and NFW can help achieve selection progress in a breeding program.

Conclusion

The diallel analysis of promiscuous nodulation in soybean showed that both additive and non-additive gene action were involved in nodulation traits. Additive gene action was predominant compared to non-additive in most of the traits measured, indicating potential achievable gain from selection for promiscuity in soybean. Heritability were moderate to high in most traits showing that for promiscuous nodulation in soybean, plant breeders can predict progeny's performance based on parental lines. Thus the study provides useful information that breeding programs can build upon to define adequate breeding strategies for nodulation in soybeans. As for the effectiveness of the nodules, the trait is largely controlled by non-genetic sources. We suggest that more investigations be carried out on nodules' effectiveness to clarify the inheritance pattern of this trait, so as to enable predicting while conducting a soybean breeding program. We recommend that full diallel analysis be carried out to search for eventual maternal effects to obtain more precise estimates of the heritability values. This might also be insightful in understanding the low heritability values observed for NE.

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