

Growth and Yield Response of Groundnut (*Arachis hypogaea* L.) to Rhizobial and Arbuscular Mycorrhiza Fungal Inoculations in the Western Highlands of Cameroon

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Abstract: This study sort to investigate the effect of rhizobial and arbuscular mycorrhizal fungi (AMF) on the growth, yield and nutritional quality of groundnut. A field experiment conducted in a split-plot design was used to determine the effect of rhizobia, AMF, co-inoculation of rhizobia and AMF on the growth and yield of two groundnut genotypes of the subsp. *hypogaea* (village/Virginia) and subsp. *fastigiata* (Garoua/Fastigiata). Inoculations and a control treatment were repeated three times and groundnut seeds were inoculated before sowing. Results showed improved growth of inoculated plants compared to non-inoculated. 60 days after planting (DAP), the highest plant height (13.67cm) was recorded for the village type inoculated with AMF and 12.18cm for the Garoua type inoculated with combined rhizobium and AMF compared to the control (12.40 and 10.63cm respectively). The number of leaves plant⁻¹ was significantly ($p < 0.05$) higher in inoculated plants than non-inoculated plants for both varieties at 60 DAP. A similar trend was observed for dry aboveground biomass with the village type (213.88g) significantly ($p < 0.05$) higher than the Garoua type (90.40g). The village type was significantly more productive (51 pods plant⁻¹) than the Garoua type (7 pods plant⁻¹) for the most productive treatment (AMF inoculation). The Garoua type produced more nodules (264) especially in mycorrhizal and co-inoculation than the village type (213). Yield obtained from the village type (5.3 t ha⁻¹ for AMF inoculation) was significantly higher than yield obtained from the Garoua type. Total sugar and lipid content of grains were higher in inoculated plants and was significantly higher for the Garoua type 6.14% and 43.14%, respectively) than the village type. This study showed that inoculation of groundnut with rhizobia and AMF had a positive impact on the growth, nodulation, yield and nutritional quality of peanuts.

Keywords: Groundnut, *Rhizobium*, AMF, Inoculation, Growth, Yield, Quality

1. Introduction

Groundnut also known as peanut (*Arachis hypogaea* L.) is a food legume that occupies the fourth position as a source of

edible oils and the thirteenth important source of vegetable protein globally [2, 34]. It is a grain legume and an essential source of food in the tropics and subtropics. According to FAO, world production of unshelled groundnut amounted to 45.55 million tons on 25.44 hectares giving an average yield

of 1.77 T ha⁻¹ [15]. Groundnut is composed of about 48.32% oil, 22-25% protein, 20% carbohydrate, 5% fibre and ash, vitamin E, K and B [34]. It is used for human and animal feeding, in oil mills and in the manufacture of compost or green manure. From a monetary point of view, groundnut is ranked second after cotton in Cameroon and is cultivated in all the regions with the northern part representing 43% of the cultivated area [10].

Cameroon's contribution to groundnut production is relatively low due to poor agronomic performance. Average yield of groundnuts in Africa is appreciably 1-ton ha⁻¹ with that of Cameroon being 1.36 T ha⁻¹ against a world average of 1.77 T ha⁻¹, with greater quantities being cultivated in China and the United States [15, 27]. Non-availability of seeds, insect pests and diseases, soil fertility issues and inappropriate farm management practices are amongst the key factors affecting the growth, yield and the quality of groundnuts [3, 25]. The use of chemical fertilizers to resolve soil nutrient deficits in tropical and subtropical soils has been practiced with environmental drawbacks. Also, leguminous crops such as groundnut have been integrated into cropping systems to maintain soil fertility and enhance crop yield especially in the developing countries [17].

In crop production, biodiversity contributes directly or indirectly in different forms to the well-being of the biosphere, thereby playing an important role in food security, regulates the soil moisture quality and quantity. The soil microflora including rhizobia and fungi in symbiotic associations with the roots of cultivated crops such as nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (AMF) respectively, play a key role in natural processes that ensures coexistence and ecosystem dynamics including soil fertility issues [18, 31, 25, 30]. Some of the soil microorganisms enhance plant nutrition by either solubilizing and mobilizing or increasing nutrient availability in soils. Previous studies have shown that phosphorus and nitrogen are amongst the most limiting nutrients for plant growth [29]. Thus, the application of commercial rhizobia and AMF has been used as a strategy to improve sustainable agricultural practices through soil nutrient uptake enhancement that facilitates plant growth and improves yield. In addition, tripartite symbiotic associations improve plant nutrition and crop production [32, 19, 24].

In order to assess the ability of plants to acquire nutrients, mycorrhizal fungi and rhizobia are two important symbiotic partners. They play a key role in natural agroecosystems influencing plant productivity, nutrition and inhibition of pathogens [12]. These symbiotic relationships benefit the host plant by mobilizing phosphorus in the soil and providing nitrogen through fixation of atmospheric N [28]. In addition, previous findings showed that rhizobium-mycorrhizal inoculations had stimulatory and inhibitory effects respectively, on plant growth [13, 33, 16]. Other findings

showed that double inoculation of AMF and rhizobia induces synergistic benefits for the host legume since they act as biofertilizers and such interactions in legumes has resulted to increased phosphorus and nitrogen availability [14, 19, 1].

Contrarily, other studies reported that the mechanisms controlling the interactions rhizobia, AMF and plant roots, and their activities in the soil is very difficult to generalize due to the variation of such interactions with microbial species and plant varieties [6]. Some authors suggested there is a high degree of specificity between bacteria associated with AMF [5, 29]. Thus, the objective of this study was to evaluate the effect of commercial rhizobial and AMF inoculations on the growth, yield and quality of two groundnut varieties in the western highlands of Cameroon.

2. Materials and Methods

2.1. Experimental Site

The field experiment was conducted at the experimental site of Institute of Agricultural Research for Development (IRAD-Dschang), located in Dschang district of Menoua division in the West Region of Cameroon. This area is located at latitude 5°27'00" North and longitude 10° 04'00" East and is at 1600 m above sea level. The climate is cool and humid, linked to the intersection of moist oceanic air and dry continental air masses. It is characterized by a rainy season from March to October and a dry season from November to February. Annual rainfall is about 1800 mm and the average annual temperature is about 20.3°C with a maximum of 27.5°C and a minimum of 13.4°C.

The soil in the study zone is ferralitic and moderately acidic (pH-H₂O = 6.71). Soil organic carbon content is greater than 2.5% and the organic matter content varies between 4.2 and 6%. The C/N ratio is greater than 20 indicating low total nitrogen content [21, 7].

2.2. Planting Materials and Biofertilizers

Two local varieties of groundnuts (*A. hypogaea* L.) obtained from Dschang local market were used in this experiment. These varieties were chosen based on their desired taste by the population and their characteristics as presented on Table 1.

The biofertilizers tested in this experiment are inocula based on rhizobia at a concentration of 10⁷ CFU ml⁻¹ and mycorrhiza consisting of mixture of spores, root pieces and hypae of fungal genera *Glomus*, *Gigaspora* and *Acaulospora* at 20³ propagules g⁻¹ of soil obtained from the soil microbiology laboratory of Biotechnology Centre, University of Yaoundé I and GIC AGRIBIOCAM (*Agriculture Biologique du Cameroun*), respectively.

Table 1. Characteristics of the groundnut varieties used in the study (IRAD-2012).

Variety	Type	Growth cycle (days)	No. of grains pod ⁻¹	Growth habit
Village var.	Virginia	120	2	Climbing
Garoua var.	Fastigiata	90	2	Erect

2.3. Land Preparation and Experimental Design

The experimental plot was cleared manually with a machete to get rid of weeds and plant residues of the previous crop (maize). A tractor was then used to plough the soil to the depth of 30 cm. The field was tilled manually with a hoe to breakdown soil aggregates to facilitate the demarcation of experimental units and other land preparatory activities before planting.

The experimental design was a randomized complete block arranged in split-plots with three repetitions. The main factor was the groundnut varieties: Village type and the Garoua type, and the secondary factor was the fertilization treatments consisting of non-inoculated or control, rhizobial inoculation, AMF inoculation and combined rhizobial + AMF inoculation. These factors together with the repetitions gave a total of twenty-four experimental units in the form of flat beds, each measuring 1 m × 1.5 m.

2.4. Planting and Field Management

Prior to sowing, groundnut seeds were coated with rhizobial inoculum using a powder milk slurry as a sticker while mycorrhiza was directly applied into the planting holes at the rate of 20g hole⁻¹. The powder milk slurry was prepared by thoroughly mixing 66 g of powder milk with 33 ml of the rhizobial inoculum. The mixture was homogenized until a medium dense paste was obtained. Seeds weighing 100g were added to the paste and mixed until the seeds were completely coated.

Sowing was done manually at the depth of 2-3 cm after air-drying the coated seeds for about an hour. Planting distance was 30 cm between the lines and 30 cm between plants, giving a planting density of 20 plants per experimental unit or 111,111 plants ha⁻¹. Weeding was conducted manually three times throughout the experiment; one month after sowing and the others at two weeks interval.

2.5. Data Collection

2.5.1. Growth and Yield Variables

Data on growth variables (plant height and number of leaves), root colonization (mycorrhizal colonization, number of nodules, nodule efficiency), yield and yield attributes (number of pods per plant, weight of pods) for both varieties were collected throughout the experiment. Data on plant height and number of leaves were collected at 60 days after planting (DAP).

Destructive plant sampling was conducted at 50% flowering stage for the evaluation of plant biomass, AMF root colonization and root nodulation. Similarly, plant samples were collected for the evaluation of yield and yield attributes at harvest. Plant height was measured using a graduated ruler from the base of the stem to the highest point while the number of leaves and root nodules were counted manually. Plant aboveground biomass, the weight of nodules, yield attributes and yield were determined by

weighing sampled parts after oven-drying to constant weight at 65°C.

2.5.2. Root Colonization and Nodule Efficiency

Plant roots obtained at 50% flowering stage were washed properly and treated with 10% KOH before staining [26, 20]. Root colonization by AMF was determined by observing 20 stained roots on mounted slides [22, 11]. Nodule efficiency was determined by dissecting and observing a red internal colour indicating active nodules [8]. Nodule efficiency was determined as a percentage of all active nodules relative to the total number of nodules dissected.

2.5.3. Leaf Total Chlorophyll, N and P

Leaf total chlorophyll, leaf N and P were determined after sampling at 60 DAP. Samples were oven-dried, ground and extracted using standard methods [4, 23].

2.6. Data Analysis

Data collected were entered and organized in the form a spreadsheet in Microsoft Excel 2013. Spreadsheets were imported into the statistical package for social sciences (SPSS) version 26 for analysis of variance (ANOVA). Means of variables were separated using Tukey test at 5% probability threshold.

3. Results and Discussions

3.1. Growth Response of Peanut to Rhizobial and AMF Inoculations

Generally, in terms of growth, the village var. was superior to the Garoua var. Plant height and aboveground biomass (fresh and dry) showed not significant difference amongst treatments for both varieties but was significantly higher for the village var. than the Garoua var. (Table 2). Amongst the treatments for both varieties, the combined inoculation showed a relatively higher plant height (13.33cm and 12.18cm) and higher dry aboveground biomass (142.70g and 97.78g) (Table 2). Interestingly, plant number of leaves at 60 DAP showed significant differences ($p < 0.05$) with the highest number of leaves observed on the combined inoculation treatment and the least on the control treatments for both varieties (Table 2). Growth trends observed is probably due to the natural presence of rhizobia and AMF in the soils of the study site. However, the combined inoculation showed a better growth performance in terms of plant height, number of leaves and aboveground biomass (Table 2) compared to the other treatments indicating a positive synergistic interaction between the groundnut variety, inoculated rhizobia and AMF. This corroborates previous findings of a tripartite symbiotic association (Rhizobium-AMF-Legume) [32]. There was no significant interaction amongst the studied varieties under various treatments but the village var. showed a better growth performance than the Garoua var. due to the fact that it best adapted to the study zone.

Table 2. Growth response of groundnut (*Arachis hypogaea* L.) to rhizobia and AMF inoculations 60 DAP.

Variety	Treatments	Plant height (cm)	No. of leaves plant ⁻¹	Plant aboveground biomass (g plant ⁻¹)	
				Fresh	Dry
Village var. (Virginia)	Control	12.40±0.42a	103b	184.05±28.78a	124.74±9.64a
	Rhizobium	11.87±1.11a	100ab	202.51±74.83a	124.41±31.66a
	Mycorrhiza	13.67±0.48a	111ab	199.66±13.53a	126.66±14.32a
	Rhizobium+ Mycorrhiza	13.33±0.44a	112a	208.72±27.48a	142.70±4.91a
Garoua var. (Fastigiata)	Control	10.63±1.12a	61b	108.74±7.06a	50.01±15.09a
	Rhizobium	10.40±0.25a	73ab	114.70±12.07a	56.50±6.73a
	Mycorrhiza	11.31±0.84a	83ab	183.56±23.82a	85.33±6.99a
	Rhizobium+ Mycorrhiza	12.18±0.80a	85a	190.29±23.93a	97.78±10.16a
Village var. (Virginia)		12.82±0.72a	106a	198.74±37.01a	129.60±16.19a
Garoua var. (Fastigiata)		11.13±0.81b	76b	149.32±27.58b	72.40±14.82b
Variety*Treatment	ns		ns	ns	ns

DAP: Days after planting. Values (mean±standard error) with the same letter within a column are not significantly different at 5% probability threshold according to Tukey test.

3.2. AMF Root Colonization, Nodulation and Nodule Efficiency

Results showed that there was a significant difference ($p < 0.05$) in AMF colonization between the inoculated and non-inoculated plants for both groundnut varieties (Table 3).

Root colonization by AMF at 50% flowering growth stage ranged from 25.33% to 63.11%. The highest AMF root colonization occurred in the co-inoculated and the sole AMF treatments for both varieties. However, the village var. significantly showed a higher AMF root colonization rate than the Garoua var. (Table 3). The number of nodules and nodule efficiency showed no significant difference amongst treatments for both groundnut varieties and there was no significant interaction between variety and treatments. This is probably due to the fact that groundnut is nodulated by a myriad of rhizobia and was considered a highly “promiscuous” species [9]. Similarly, previous results in which AMF inoculation had no significant effect on nodulation had been reported [19]. Nodule efficiency ranged from 73.33 to 86.67% in both varieties (Table 3).

3.3. Leaf Total Chlorophyll, N and P Content at 60 DAP

At 60 DAP, leaf chlorophyll content showed no significant difference between the inoculated and non-inoculated plants for both varieties. But the chlorophyll content of the leaves for the control treatments were lower than that of the inoculated

plants. Total chlorophyll content of the leaves ranged between 18.26mg g⁻¹ and 19.48mg g⁻¹ for the two groundnut varieties under investigation (Table 4).

Contrarily, leaf N and P content showed a significant difference between inoculated and non-inoculated plants. That is, rhizobial and AMF inoculations influenced leaf N and P content. However, there was a significant interaction between the varieties and the treatments for total leaf N probably due to the fact that the village var. was more adapted to the study zone than the Garoua var.

3.4. Groundnut Dry Matter and Yield Attributes

Generally, in terms of dry matter and yield attributes (number of pods plant⁻¹, weight of pods plant⁻¹, weight of grains plant⁻¹), the village var. showed a better agronomic performance than the Garoua var. The dry aboveground biomass at harvest was significantly higher ($p < 0.05$) for the village var. (213.8g plant⁻¹) than the Garoua var. (90.20g plant⁻¹). Inoculated plants performed better than non-inoculated plants with AMF inoculated plants producing the highest aboveground dry matter (250.74 and 103.97), number of pods plant⁻¹ (51 and 7) and weight of pods plant⁻¹ (48.17 and 8.21) for the village var and Garoua var., respectively (Table 5). This reflects the leaf N and P content shown on Table 4 indicating enhanced N and P uptake in inoculated plants compared to non-inoculated plants.

Table 3. Nodulation and root colonization of peanuts under rhizobial and AMF inoculation.

Variety	Treatments	No. of nodules plant ⁻¹	Nodule DW (mg plant ⁻¹)	%	
				AMF colonization	Nodule efficiency
Village var. (Virginia)	Control	169±78a	13.33±8.82	25.33±3.01d	76.67±8.82a
	Rhizobium	278±77a	10.00±5.77	34.00±7.37bcd	73.33±3.33a
	Mycorrhiza	233±9a	70.00±65.06	53.83±3.56ab	76.67±3.33a
	Rhizobium+ Mycorrhiza	173±46a	100.00±70.95	63.11±5.66a	70.00±10.00a
Garoua var. (Fastigiata)	Control	195±31a	66.67±29.63	28.56±1.64cd	66.67±6.67a
	Rhizobium	234±41a	103.33±31.80	32.67±1.26bcd	86.67±6.67a
	Mycorrhiza	252±20a	66.67±27.28	49.67±2.33abc	73.33±8.82a
	Rhizobium+ Mycorrhiza	378±87a	16.67±6.67	59.11±4.47a	83.33±3.33a
Village var. (Virginia)		213±58b	48.33±47.28a	44.08±10.12a	75.16±6.26a
Garoua var. (Fastigiata)		265±60a	63.33±28.85a	42.50±7.85a	77.50±7.44a
Variety*Treatment	ns		ns	ns	ns

DW: Dry weight. Values (mean±standard error) with the same letter within a column are not significantly different at 5% probability threshold according to Tukey test.

Table 4. Leaf total chlorophyll, nitrogen and phosphorus content at 60 DAP.

Variety	Treatments	mg g ⁻¹		
		Total chlorophyll	Nitrogen	Phosphorus
Village var. (Virginia)	Control	19.12±0.43a	9.27±2.28c	0.62±0.03c
	Rhizobium	19.28±0.57a	18.96±1.08a	0.80±0.03abc
	Mycorrhiza	19.64±0.68a	17.00±0.71a	0.67±0.03bc
	Rhizobium+ Mycorrhiza	19.48±0.62a	21.05±1.17a	1.02±0.14a
Garoua var. (Fastigiata)	Control	18.26±0.05a	13.13±2.32bc	0.53±0.01c
	Rhizobium	19.18±0.49a	19.09±1.19a	0.82±0.03abc
	Mycorrhiza	19.61±0.89a	17.98±1.23a	0.74±0.02abc
	Rhizobium+ Mycorrhiza	18.95±0.26a	14.64±0.48abc	0.95±0.09ab
Village var. (Virginia)		19.38±0.51a	16.57±2.95a	0.78±0.11a
Garoua var. (Fastigiata)		19.00±0.54a	16.21±1.92a	0.76±0.10a
Variety*Treatment		ns	s	ns

DAP: Days after planting. Values (mean±standard error) with the same letter within a column are not significantly different at 5% probability threshold according Tukey test.

3.5. Yield and Grain Quality

In a similar manner as the yield attributes, the village var. produced more grains than the Garoua var. with the AMF inoculated plants producing better yield than non-inoculated plants for both varieties under investigation. Grain weight (g plant⁻¹) ranged from 19.78 to 26.89 for the village var. and 0.30-0.81 for the Garoua var. while yield (ton ha⁻¹) ranged from 2.20-2.99 and 0.03-0.09 for the village var. and Garoua var., respectively (Table 6).

In terms of the grain quality, the total sugar content of the Garoua var. was higher than the village var. Contrarily, the Garoua var. had a lower lipid content compared to the village var. (Table 6). The total sugar and lipid content were highest in plants inoculated with both rhizobia and AMF.

4. Conclusion

In crop production systems, microorganisms play a vital role in the mobilization of nutrients in the soil or the enhancement of nutrient uptake. The results of this study show a positive tripartite symbiotic association that plays a vital role

in groundnut production in the western highlands of Cameroon. The most adapted variety (village var.) demonstrated a superior growth and yield performance over the less-adapted variety (Garoua var.).

Inoculated plants for both varieties showed an improve growth and yield performance relative to the non-inoculated plants. However, plants inoculated with both rhizobia and AMF showed better agronomic performance compared to the sole inoculations. Similarly, at harvest, dry aboveground biomass, yield and yield attributes (number of pods plant⁻¹, pod weight plant⁻¹) were higher in inoculated plants compared to the control treatment indicating the important role of these microorganisms in enhancing nutrient uptake or mobilizing nutrients in the soil and possibility of integrating these biofertilizers in crop nutrient management programs.

In terms of quality, grains of the village var. had a higher lipid content but lower sugar content compared to the Garoua var. Consequently, rhizobial and AMF inoculations influenced the grain sugar content with inoculated plant having a relatively higher sugar content compared to non-inoculated plants.

Table 5. Aboveground biomass and yield attributes of groundnuts at harvest.

Variety	Treatments	No. of pods plant ⁻¹	DW of pods (g plant ⁻¹)	Aboveground biomass at harvest (g plant ⁻¹)	
				Fresh	Dry
Village var. (Virginia)	Control	42±6a	34.51±3.27b	386.60±10.76bc	156.06±5.55b
	Rhizobium	46±12a	38.58±4.50ab	550.51±48.20ab	208.03±19.59a
	Mycorrhiza	51±10a	48.17±4.10a	609.61±102.03ab	250.74±51.11a
	Rhizobium+ Mycorrhiza	41±6a	37.62±6.11ab	631.69±45.41a	239.08±19.58a
Garoua var. (Fastigiata)	Control	6±1b	7.99±0.39c	203.70±31.37c	68.61±9.12d
	Rhizobium	5±1b	6.65±0.45c	269.53±14.39c	92.84±3.11c
	Mycorrhiza	7±3b	8.21±1.05c	310.66±20.76c	103.97±6.07bc
	Rhizobium+ Mycorrhiza	6±3b	7.78±0.75c	266.34±28.19c	95.35±5.03c
Village var. (Virginia)		45±9a	39.72±5.00a	544.60±177.83a	213.48±33.29a
Garoua var. (Fastigiata)		6±2b	7.66±0.71b	262.56±31.15b	90.20±9.53b
Variety*Treatment	ns		ns	ns	ns

DW: Dry weight. Values (mean±standard error) with the same letter within a column are not significantly different at 5% probability threshold according Tukey test.

Table 6. Yield and grain quality of groundnuts.

Variety	Treatments	Grain weight (g plant ⁻¹)	Yield (Ton ha ⁻¹)	%	
				Total sugar	Lipids
Village var. (Virginia)	Control	20.14±1.06a	2.24±0.12a	0.91	42.50
	Rhizobium	23.08±3.38a	2.56±0.38a	1.61	42.85
	Mycorrhiza	26.89±2.68a	2.99±0.30a	4.10	43.35
	Rhizobium+ Mycorrhiza	19.78±2.65a	2.20±0.29a	5.66	43.88
Garoua var. (Fastigiata)	Control	0.73±0.11b	0.08±0.01b	3.43	40.33
	Rhizobium	0.30±0.06b	0.03±0.01b	5.95	40.35
	Mycorrhiza	0.81±0.48b	0.09±0.05b	6.53	40.40
	Rhizobium+ Mycorrhiza	0.49±0.18b	0.05±0.02b	8.66	43.10
Village var. (Virginia)		22.47±2.80a	2.49±0.31a	3.07	43.14
Garoua var. (Fastigiata)		0.58±0.25b	0.06±0.03b	6.14	41.04
Variety*Treatment	ns		ns		

Values (mean±standard error) with the same letter within a column are not significantly different at 5% probability threshold according Tukey test.

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