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Genotype-dependent growth and reproductive responses of Bambara groundnut to foliar-applied *Moringa oleifera*-mediated silver nanoparticle suspensions

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Abstract

Bambara groundnut remains largely unexplored in studies of foliar-applied biogenic silver nanoparticle formulations, particularly with respect to genotype-specific responses. We evaluated three Bambara groundnut genotypes (MOK 01, MOK 02 and PTG 01) exposed to 0, 20, 40 and 60 mg L^{-1} of *Moringa oleifera*-mediated silver nanoparticle (AgNP) suspension in a 3 × 4 factorial randomised complete block design with four replications. Vegetative, phenological and reproductive traits were analysed at the pot level using factorial models that included block, genotype, concentration and genotype × concentration interaction. Responses differed markedly among genotypes and traits. MOK 02 plant height increased from 18.75 ± 1.50 cm in the control to 28.50 ± 5.80 cm at 20 mg L^{-1}, whereas PTG 01 recorded its lowest height (17.75 ± 1.89 cm) and leaf number (27.50 ± 1.73) at 60 mg L^{-1}. Pod production increased at 40–60 mg L^{-1} in MOK 02 and PTG 01, while MOK 01 pod width declined significantly from 9.60 ± 0.87 mm in the control to 7.07 ± 0.13 mm at 20 mg L^{-1}. Days to 50% flowering and seeds per pod were not significantly affected by concentration. Principal component analysis of nine directly measured traits explained 74.6% of total variation in the first two components and separated trait responses primarily along plant-size, leaflet, pod-dimension and pod-number gradients. Overall, the treatment produced non-linear, genotype-dependent responses rather than a uniform stimulatory effect. These findings support further mechanistic and multi-environment evaluation, but do not justify a general concentration recommendation or attribution of the response to the nanoparticle fraction alone.

Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an indigenous African grain legume valued for its nutritional quality, adaptation to low-input production systems and contribution to food security. Despite these attributes, the crop remains

under-researched and is still cultivated predominantly as genetically heterogeneous landraces whose agronomic performance varies across environments (Esan et al., 2023; Linus et al., 2023; Dada et al., 2024). Multi-environment studies have documented substantial variation in plant architecture, pod production, seed weight and yield stability, underscoring the importance of genotype when new crop-management technologies are evaluated (Chipeta & Gimode, 2023; Esan et al., 2023; Osundare et al., 2023). Evidence on the response of Bambara groundnut to foliar-applied silver nanomaterials remains particularly limited.

Silver nanoparticles (AgNPs) have attracted attention in crop research because their small size, high surface reactivity and capacity to release Ag^+ can influence plant development and stress responses. Depending on particle characteristics, concentration, exposure route and developmental stage, AgNP exposure has been associated with changes in germination, root growth, photosynthesis, antioxidant metabolism, pathogen response and biomass accumulation (Santhoshkumar et al., 2024; Narware et al., 2024; dos Santos et al., 2024; Pan et al., 2024). These effects are not uniformly beneficial, and the underlying mechanisms remain strongly dependent on the physicochemical properties of the formulation and the biological system in which it is applied.

Concentration is an important determinant of plant response to AgNP-containing formulations. Stimulatory responses have been reported at relatively low or intermediate exposure levels, whereas higher or prolonged exposure may suppress growth, impair photosynthetic performance or disturb cellular redox balance (Mays et al., 2024; Noori et al., 2024; Pan et al., 2024). Differences among studies also reflect variation in particle size, coating, aggregation, dissolution, plant species, genotype and developmental stage. Consequently, evidence from one crop or formulation cannot be assumed to translate directly to another, and genotype-specific evaluation is essential before agronomic relevance can be inferred.

Green synthesis provides a biological route for reducing and stabilising metal nanoparticles. *Moringa oleifera* leaves contain phenolics, flavonoids, proteins and other metabolites capable of reducing Ag^+ and contributing to particle capping and stabilisation (Haris & Ahmad, 2024). The biological matrix is, however, an important interpretive consideration because *M. oleifera* leaf extract itself has documented biostimulant activity (Mashamaite et al., 2022). In the absence of an extract-only comparator, plant responses cannot be attributed exclusively to the nanoparticle fraction. Likewise, potential contributions from dissolved Ag^+ remain unresolved without an ionic-silver control. These limitations are particularly relevant when a green-synthesised formulation is evaluated directly in plants.

Against this background, the present study examined vegetative, phenological and reproductive responses of three Bambara groundnut genotypes to four foliar concentrations of a *M. oleifera*-mediated AgNP suspension. We tested whether the direction and magnitude of response depended on genotype and concentration. Because the experimental design did not include extract-only or $AgNO_3$ controls, the treatment is interpreted as a *M. oleifera*-mediated AgNP suspension rather than as an isolated nanoparticle effect.

Materials and Methods

Study Site and Plant Materials

The experiment was conducted at Lafiagi, Edu Local Government Area, Kwara State, Nigeria (approximately 8.85° N, 5.42° E; 110–120 m above sea level), within the Southern Guinea Savanna ecological zone (Aderibigbe et al., 2017). Planting was carried out on 15 June 2024. Three Bambara groundnut landraces were evaluated: MOK 01 and MOK 02, obtained from Niger State, and PTG 01, obtained from Kwara State.The experiment was conducted under natural rainfall conditions without supplemental irrigation or fertiliser application. Weeding was done manually as required, while plants were routinely monitored for pests and diseases. Crop establishment and morphological characterisation followed the

Bambara groundnut descriptors of BAMNET (2000).

Synthesis and Characterisation of AgNPs

Silver nanoparticles were synthesised using aqueous *Moringa oleifera* leaf extract. Five grams of air-dried leaf powder were heated in 100 mL double-distilled water at 90 °C for 1 h and filtered. A 2.5-mL aliquot of the extract was mixed with 100 mL of 1 mM $AgNO_3$ under continuous stirring at room temperature. The resulting particles were recovered, washed and dried at 55 °C. The AgNP preparation was characterised by ultraviolet-visible spectroscopy (UV–Vis), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and dynamic light scattering (DLS). The preparation showed a surface-plasmon absorption maximum at 408.5 nm, a mean hydrodynamic diameter of 44.71 nm and a polydispersity index (PDI) of 0.202. Zeta potential and elemental silver concentration by AAS or ICP-MS were not determined and are therefore not reported. A fuller physicochemical characterisation of the preparation is the subject of a separate manuscript currently under review. The dried AgNPs were resuspended in distilled water to prepare 20, 40 and 60 mg L^{-1} working suspensions for foliar application. The stated concentrations refer to the mass of dried AgNP powder per litre of suspension. The suspensions were mixed thoroughly before spraying to minimise particle settling and ensure uniform application.

Experimental Design and Crop Establishment

The experiment was arranged as a 3 × 4 factorial in a randomised complete block design with four replications. The factors were genotype (MOK 01, MOK 02 and PTG 01) and suspension concentration (0, 20, 40 and 60 mg L^{-1}), giving 48 pots as experimental units. Five seeds were sown in each 20-L container and thinned to three seedlings at 7 days after emergence. Foliar applications were made at 30, 34 and 37 days after sowing. AgNP suspensions were applied foliarly on the stated days using a hand-held sprayer until the foliage was uniformly wetted. Control plants received the same foliar application procedure using distilled water without AgNPs.

No surfactant was added. Applications were made in the morning, and care was taken to minimise spray runoff to the soil and cross-drift between treatments. Measurements obtained from individual plants within a pot were averaged before analysis, so the pot, rather than the individual plant, was the unit of inference.

Data Collection

Plant height, number of leaves, terminal leaflet length and terminal leaflet width were measured at 60 days after sowing. Days to 50% flowering, pods per plant, pod length, pod width, seeds per pod, seed yield per pot and 100-seed-equivalent weight were recorded at reproductive maturity. Seed yield per pot was the total mass (g) of all harvested seeds recovered from all pods in an experimental pot and was measured using a weighing balance. Because many pots produced fewer than 100 seeds, seed mass was standardised to a 100-seed equivalent by weighing the available counted seeds and calculating: 100-seed-equivalent weight (g) = [sample mass (g) / number of seeds weighed] × 100. Thus, the reported value represents a scaled equivalent where fewer than 100 seeds were available. Pod shape and pod colour were invariant and were excluded from inferential analysis.

Statistical Analysis

All analyses were conducted at the pot level (n = 48). Factorial analyses of variance were fitted by ordinary least squares with block, genotype, suspension concentration and genotype × concentration interaction as fixed terms, using Type II sums of squares. Residual normality was assessed with the Shapiro–Wilk test and homogeneity of variance with the median-centred Levene test. Assumptions were satisfactory for plant height, leaf number, leaflet length, leaflet width, days to 50% flowering, pods per plant and pod length; pod width showed heterogeneity of variance (Levene $P = 0.0016$). Residual normality was not supported for seed yield per pot or 100-seed-equivalent weight. These two traits were therefore reanalysed using factorial linear models with HC3 heteroskedasticity-robust inference and

Type III tests, retaining genotype, suspension concentration, genotype × concentration and block effects. Within-genotype comparisons for seed yield per pot and 100-seed-equivalent weight were based on HC3-robust contrasts with Holm adjustment for multiple comparisons. Because leaf number, pods per plant and seeds per pod are count traits, Poisson generalised linear models were fitted as sensitivity analyses. The leaf-number result was robust, whereas the genotype × concentration effect for pods per plant was not retained in the Poisson model; seeds per pod remained non-significant. Within-genotype concentration means for the remaining traits were compared using Tukey-adjusted pairwise contrasts based on the pooled residual error at $P \leq 0.05$. Principal component analysis (PCA) was performed on standardised genotype × concentration means for nine directly measured traits, excluding seed yield per pot and 100-seed-equivalent weight from the primary ordination because of their distributional characteristics. Pearson correlations were calculated across the 48 pots for the same nine traits and interpreted as pooled, not genotype-specific, associations. Analyses were performed in Python 3.11 using statsmodels 0.14.6, scikit-learn 1.8.0 and SciPy 1.17.0.

Results

Factorial Effects

Factorial effects differed substantially among traits (Table 1). Strong genotype × concentration interactions were detected for pod width ($P < 0.001$) and plant height ($P = 0.0059$), whereas evidence was weaker for leaf number ($P = 0.0430$) and pod length ($P = 0.0314$). The Gaussian model also indicated an interaction for pods per plant ($P < 0.001$). Days to 50% flowering and seeds per pod showed no concentration or genotype × concentration effects. Block effects were generally small, with a significant block term detected only for terminal leaflet length ($P = 0.0016$). HC3-robust analysis of seed yield per pot detected significant effects of genotype ($P = 0.0043$), concentration ($P < 0.001$) and genotype × concentration interaction ($P = 0.0103$). For 100-seed-equivalent weight, concentration was significant ($P < 0.001$), whereas genotype ($P = 0.1189$) and genotype × concentration interaction ($P = 0.8147$) were not significant.

Vegetative and phenological responses were strongly genotype dependent (Table 2A). MOK 02 showed the largest height response at 20 mg L^{-1}, increasing from 18.75 ± 1.50 cm in the control to 28.50 ± 5.80 cm. In MOK 01, leaf number increased to 49.75 ± 11.76 at 20 mg L^{-1} but declined to 25.50 ± 4.20 at 40 mg L^{-1}, below the control mean of 33.50 ± 7.94. PTG 01 showed no comparable height stimulation and recorded its lowest mean height and leaf number at 60 mg L^{-1}. Days to 50% flowering did not differ significantly among concentrations.

Reproductive responses were likewise non-uniform (Table 2B). MOK 02 produced 5.25 ± 1.26 and 5.25 ± 0.50 pods $plant^{-1}$ at 40 and 60 mg L^{-1}, respectively, compared with 1.50 ± 0.58 in the control. PTG 01 reached 5.75 ± 1.26 pods $plant^{-1}$ at 40 mg L^{-1}. In contrast, MOK 01 pod width declined significantly from 9.60 ± 0.87 mm in the control to 7.07 ± 0.13 mm at 20 mg L^{-1}. Seeds per pod changed little across concentrations. Seed yield per pot showed a significant genotype × concentration interaction in the HC3-robust analysis ($P = 0.0103$). In MOK 01, yield increased from 1.00 ± 0.00 g in the control to 4.25 ± 0.50 and 4.00 ± 0.00 g at 40 and 60 mg L^{-1}, respectively. MOK 02 increased from 1.00 ± 0.00 g in the control to 3.50 ± 1.73 and 5.00 ± 0.00 g at 40 and 60 mg L^{-1}, while all treated PTG 01 groups had greater yields than the control, reaching 5.50 ± 0.58 g at 60 mg L^{-1}. The 100-seed-equivalent weight showed a significant concentration effect ($P < 0.001$) without a significant genotype × concentration interaction ($P = 0.8147$); the highest numerical mean was recorded in PTG 01 at 60 mg L^{-1} (56.50 ± 7.90 g).

PCA and Trait Correlations

PCA of the nine directly measured vegetative, phenological and reproductive traits was conducted on the 12 genotype × concentration means after standardisation. The first two components explained 74.6% of the total variance (PC1 = 57.1%; PC2 = 17.5%; Figure 1; Table 3). PC1 was characterised by high positive loadings for

seeds per pod (0.961), terminal leaflet length (0.950), terminal leaflet width (0.935), plant height (0.859), pod width (0.824) and pod length (0.806). PC2 was associated most strongly with pods per plant (0.838) and days to 50% flowering (0.775). The ordination therefore represented coordinated variation in plant size, leaflet dimensions, pod dimensions and pod number rather than a single yield gradient. Loading vectors were scaled uniformly for visualisation; numerical loadings are given in Table 3.

Pearson correlations among the same nine traits were calculated across all 48 pots (Figure 2). Because observations were pooled across genotypes and concentrations, the matrix describes overall covariance in the experiment and is not used to infer genotype-specific relationships. Significant coefficients are indicated in the matrix, which uses a diverging scale centred on zero.

Discussion

The central finding of this study is that Bambara groundnut responded to the *M. oleifera*-mediated AgNP suspension in a genotype- and trait-specific manner, with no evidence of a uniformly beneficial concentration. The strongest interaction evidence occurred for pod width and plant height, while effects on leaf number and pod length were weaker. Although the Gaussian analysis indicated a genotype × concentration interaction for pods per plant, that effect was not reproduced in the Poisson sensitivity analysis and should therefore be regarded cautiously. This pattern of heterogeneous responses is more informative than a simple stimulation narrative because it identifies both responsive traits and conditions under which apparent benefits were absent or reversed.

Table 1: Factorial model summary from the 48 raw experimental units, including block and residual terms.

Trait	Effect	df	Mean square	F	P	R^2
Plant height	Genotype	2	61.750	8.05	0.0014	0.630
	Dose	3	27.167	3.54	0.0250	
	Genotype × dose	6	28.750	3.75	0.0059	
	Block	3	17.833	2.33	0.0927	
	Residual	33	7.667			
Leaves	Genotype	2	245.271	3.39	0.0457	0.566
	Dose	3	382.472	5.29	0.0043	
	Genotype × dose	6	179.493	2.48	0.0430	
	Block	3	131.917	1.83	0.1618	
	Residual	33	72.280			
Leaflet length	Genotype	2	0.617	4.15	0.0247	0.515
	Dose	3	0.106	0.71	0.5528	
	Genotype × dose	6	0.135	0.90	0.5034	
	Block	3	0.951	6.39	0.0016	
	Residual	33	0.149			
Leaflet width	Genotype	2	1.193	15.11	<0.001	0.609
	Dose	3	0.331	4.19	0.0129	
	Genotype × dose	6	0.061	0.77	0.5991	
	Block	3	0.103	1.31	0.2879	

	Residual	33	0.079			
Days to 50% flowering	Genotype	2	15.771	13.34	<0.001	0.587
	Dose	3	0.521	0.44	0.7256	
	Genotype × dose	6	2.271	1.92	0.1067	
	Block	3	2.910	2.46	0.0799	
	Residual	33	1.182			
Pods per plant	Genotype	2	10.187	10.51	<0.001	0.778
	Dose	3	16.722	17.24	<0.001	
	Genotype × dose	6	6.493	6.70	<0.001	
	Block	3	0.833	0.86	0.4718	
	Residual	33	0.970			
Pod length	Genotype	2	39.154	6.30	0.0048	0.612
	Dose	3	48.124	7.74	<0.001	
	Genotype × dose	6	16.661	2.68	0.0314	
	Block	3	0.154	0.02	0.9946	
	Residual	33	6.220			
Pod width	Genotype	2	82.690	79.58	<0.001	0.887
	Dose	3	11.426	11.00	<0.001	
	Genotype × dose	6	11.166	10.75	<0.001	
	Block	3	0.456	0.44	0.7269	
	Residual	33	1.039			
Seeds per pod	Genotype	2	0.333	2.85	0.0719	0.355
	Dose	3	0.132	1.13	0.3513	
	Genotype × dose	6	0.111	0.95	0.4726	
	Block	3	0.132	1.13	0.3513	
	Residual	33	0.117			
Seed yield per pot	Genotype	2	5.674	6.46	0.0043	0.851
	Dose	3	100.798	114.70	<0.001	
	Genotype × dose	6	2.974	3.38	0.0103	
	Block	3	0.615	0.70	0.5587	
	Residual	33	0.879			
100-seed-equivalent weight	Genotype	2	170.278	2.27	0.1189	0.766
	Dose	3	3342.929	44.63	<0.001	
	Genotype × dose	6	36.323	0.48	0.8147	
	Block	3	108.969	1.45	0.2448	
	Residual	33	74.907			

Note: Type II sums of squares are reported for traits meeting the Gaussian-model assumptions. For seed yield per pot and 100-seed-equivalent weight, mean squares, F and P values are from HC3 heteroskedasticity-robust Type III tests; R^2 is reported once per trait, and residual rows provide the error mean square.

Table 2A: Vegetative and phenological responses of Bambara groundnut genotypes to foliar-applied *Moringa oleifera*-mediated AgNP suspension concentrations.

Genotype	Dose (mg L^{-1})	Height (cm)	Leaves (no.)	Leaflet L (cm)	Leaflet W (cm)	Days to 50% flowering
MOK 01	0	18.00 ± 1.83^{a}	33.50 ± 7.94^{ab}	5.45 ± 0.53^{a}	1.77 ± 0.19^{a}	37.75 ± 0.50^{a}
MOK 01	20	18.75 ± 2.22^{a}	49.75 ± 11.76^{a}	5.72 ± 0.46^{a}	2.02 ± 0.26^{a}	38.00 ± 1.41^{a}
MOK 01	40	17.75 ± 2.50^{a}	25.50 ± 4.20^{b}	5.62 ± 0.95^{a}	1.65 ± 0.37^{a}	36.50 ± 0.58^{a}
MOK 01	60	20.00 ± 2.16^{a}	47.25 ± 13.38^{a}	6.03 ± 0.71^{a}	2.02 ± 0.05^{a}	37.00 ± 1.15^{a}
MOK 02	0	18.75 ± 1.50^{b}	31.50 ± 1.73^{a}	5.85 ± 0.10^{a}	1.95 ± 0.30^{a}	35.25 ± 1.89^{a}
MOK 02	20	28.50 ± 5.80^{a}	43.25 ± 3.59^{a}	6.18 ± 0.30^{a}	2.40 ± 0.28^{a}	36.00 ± 1.41^{a}
MOK 02	40	21.50 ± 4.04^{b}	38.50 ± 15.78^{a}	6.12 ± 0.63^{a}	2.15 ± 0.50^{a}	36.00 ± 1.63^{a}
MOK 02	60	20.25 ± 1.50^{b}	42.50 ± 12.04^{a}	5.92 ± 0.15^{a}	2.48 ± 0.46^{a}	35.25 ± 0.50^{a}
PTG 01	0	19.00 ± 3.46^{a}	28.50 ± 7.94^{a}	5.67 ± 0.28^{a}	1.65 ± 0.17^{a}	36.50 ± 1.00^{a}
PTG 01	20	19.00 ± 0.82^{a}	37.50 ± 5.74^{a}	5.73 ± 0.15^{a}	1.93 ± 0.15^{a}	37.00 ± 0.82^{a}
PTG 01	40	20.75 ± 3.50^{a}	35.25 ± 4.50^{a}	5.72 ± 0.29^{a}	1.62 ± 0.13^{a}	37.75 ± 0.50^{a}
PTG 01	60	17.75 ± 1.89^{a}	27.50 ± 1.73^{a}	5.50 ± 0.08^{a}	1.65 ± 0.17^{a}	38.25 ± 1.26^{a}

Values are mean ± SD (n = 4 pots). Superscript letters compare concentrations within genotype and trait.

Table 2B: Reproductive traits, seed yield per pot and 100-seed-equivalent weight of Bambara groundnut genotypes across suspension concentrations.

Genotype	Dose (mg L^{-1})	Pods $plant^{-1}$	Pod length (mm)	Pod width (mm)	Seeds pod^{-1}	Seed yield pot^{-1} (g)	100-seed-equivalent weight (g)
MOK 01	0	2.25 ± 0.96^{ab}	11.88 ± 2.27^{a}	9.60 ± 0.87^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{b}	20.00 ± 0.00^{b}
MOK 01	20	3.75 ± 1.50^{a}	12.45 ± 0.38^{a}	7.07 ± 0.13^{b}	1.00 ± 0.00^{a}	1.50 ± 0.58^{b}	36.25 ± 12.33^{ab}
MOK 01	40	1.75 ± 0.96^{b}	12.20 ± 0.20^{a}	9.80 ± 0.89^{a}	1.00 ± 0.00^{a}	4.25 ± 0.50^{a}	47.50 ± 5.00^{a}
MOK 01	60	2.75 ± 1.26^{ab}	14.85 ± 2.44^{a}	8.95 ± 1.44^{ab}	1.25 ± 0.50^{a}	4.00 ± 0.00^{a}	41.66 ± 16.67^{ab}
MOK 02	0	1.50 ± 0.58^{b}	11.53 ± 2.39^{b}	9.22 ± 0.79^{b}	1.00 ± 0.00^{a}	1.00 ± 0.00^{b}	20.00 ± 0.00^{b}
MOK 02	20	2.75 ± 0.50^{b}	19.50 ± 2.85^{a}	12.75 ± 1.84^{c}	1.50 ± 0.58^{a}	1.00 ± 0.00^{b}	48.96 ± 11.97^{a}
MOK 02	40	5.25 ± 1.26^{a}	16.98 ± 3.80^{a}	13.88 ± 1.97^{ac}	1.50 ± 0.58^{a}	3.50 ± 1.73^{a}	50.00 ± 0.00^{a}
MOK 02	60	5.25 ± 0.50^{a}	15.82 ± 0.30^{ab}	15.38 ± 0.49^{a}	1.25 ± 0.50^{a}	5.00 ± 0.00^{a}	50.00 ± 0.00^{a}
PTG 01	0	1.75 ± 0.96^{b}	10.60 ± 1.00^{b}	8.75 ± 0.30^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{b}	22.50 ± 5.00^{b}
PTG 01	20	4.00 ± 0.82^{a}	13.35 ± 2.50^{ab}	8.70 ± 0.08^{a}	1.25 ± 0.50^{a}	5.00 ± 2.71^{a}	49.00 ± 12.27^{a}
PTG 01	40	5.75 ± 1.26^{a}	16.15 ± 2.39^{a}	8.93 ± 0.13^{a}	1.00 ± 0.00^{a}	5.25 ± 0.50^{a}	51.50 ± 10.75^{a}
PTG 01	60	5.25 ± 0.50^{a}	16.40 ± 4.00^{a}	9.15 ± 0.19^{a}	1.00 ± 0.00^{a}	5.50 ± 0.58^{a}	56.50 ± 7.90^{a}

Values are mean ± SD (n = 4 pots). Superscript letters compare concentrations within genotype and trait. For seed yield per pot and 100-seed-equivalent weight, letters are based on HC3 heteroskedasticity-robust contrasts with Holm adjustment for multiple comparisons. Seed yield per pot represents the total harvested seed mass per pot (g), while 100-seed-equivalent weight was calculated from the mass and number of available seeds when fewer than 100 seeds were produced. For the remaining traits, means followed by the same superscript letter are not significantly different according to Tukey-adjusted comparisons at $P \leq 0.05$; letters compare concentrations within genotype only.

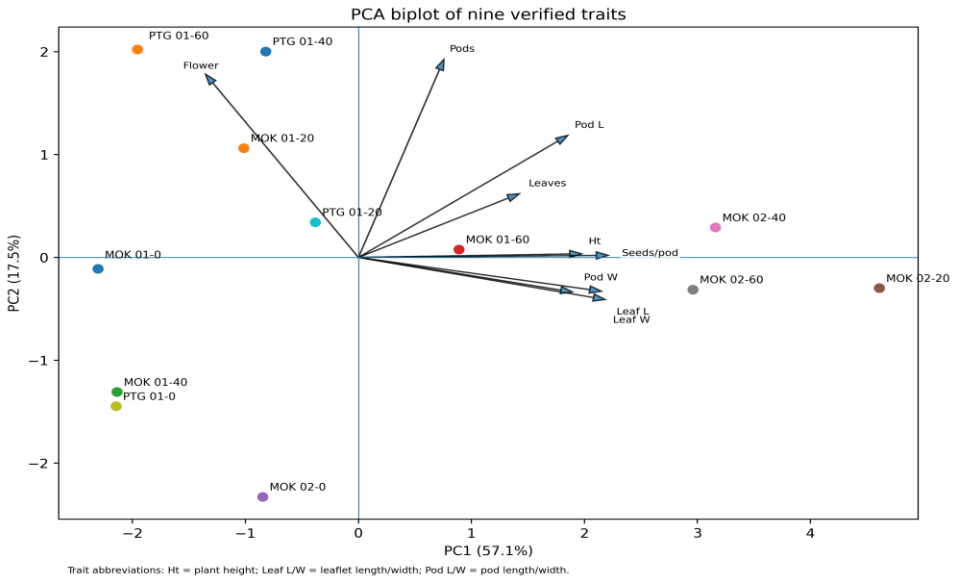

Figure 1: PCA biplot of standardised genotype × concentration means for nine directly measured traits.

Table 3. PCA loadings for the first two principal components.

Trait	PC1	PC2
Plant height	0.859	0.015
Leaves	0.620	0.269
Leaflet length	0.950	-0.178
Leaflet width	0.935	-0.143
Days to 50% flowering	-0.589	0.775
Pods per plant	0.329	0.838
Pod length	0.806	0.516
Pod width	0.824	-0.146
Seeds per pod	0.961	0.009

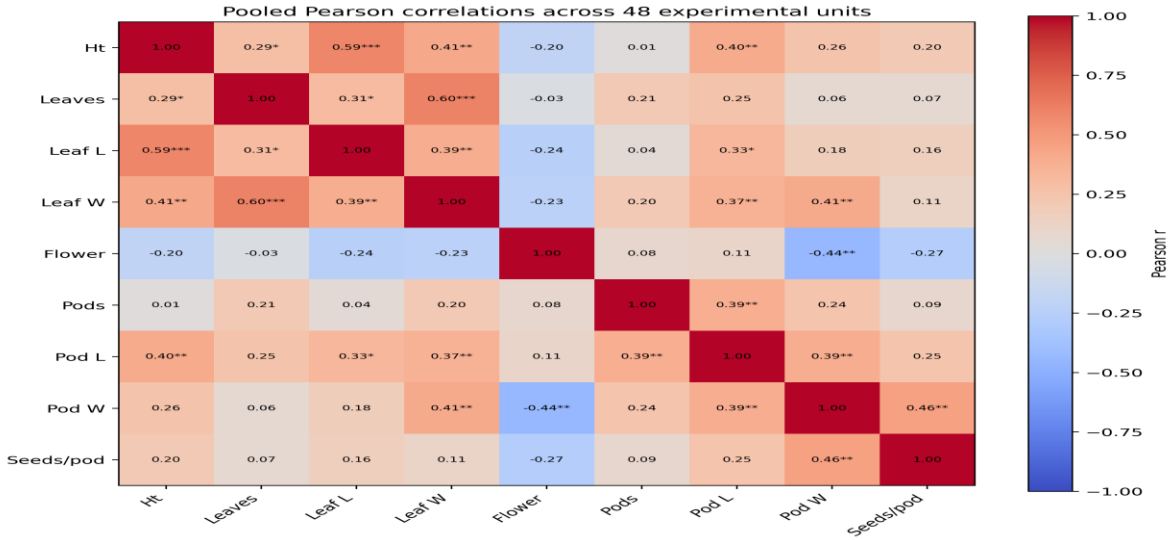

Figure 2: Pooled Pearson correlation matrix for nine traits across 48 experimental pots. * P < 0.05, ** P < 0.01, *** P < 0.001. Correlations represent overall experimental covariance and should not be interpreted as genotype-specific associations.

The response of MOK 02 illustrates this non-linearity. Plant height increased markedly at 20 mg L^{-1}, whereas the larger pod-number means occurred at 40 and 60 mg L^{-1}. Seed yield also showed a genotype-dependent response in the HC3-robust analysis, with the genotype × concentration interaction remaining significant, while 100-seed-equivalent weight responded primarily to concentration without a significant genotype × concentration interaction.

Such separation among vegetative, reproductive and yield-related responses is consistent with evidence that plant responses to AgNP exposure depend on concentration, developmental stage and biological context (Noori et al., 2024; Pan et al., 2024).

The inhibitory responses are equally consequential. MOK 01 pod width was reduced at 20 mg L^{-1}, its leaf number at 40 mg L^{-1} was below the untreated mean, and PTG 01 recorded its lowest plant height and leaf number at 60 mg L^{-1}. Together, these observations rule out a monotonic dose-response relationship and caution against identifying a single concentration as optimal across genotypes or traits. They also reinforce the importance of reporting negative responses alongside positive ones when nanomaterial formulations are evaluated for crop use.

The mixed pattern observed here is consistent with reports in other crops in which AgNP-containing treatments produced stimulation at some exposure levels and inhibition at others (Narware et al., 2024; dos Santos et al., 2024; Mays et al., 2024; Noori et al., 2024). Interpretation is further complicated by the synthesis matrix. *M. oleifera* leaf extract has recognised biostimulant activity (Mashamaite et al., 2022), while AgNP suspensions may also release biologically active Ag^{+}. Because the present design lacked extract-only and $AgNO_3$ controls, the observed responses cannot be partitioned among nanoparticles, dissolved silver and residual Moringa-derived compounds. The data therefore support treatment-associated effects, not nanoparticle-specific causation.

The multivariate results reinforce this interpretation. PC1 captured coordinated variation in plant height, leaflet dimensions, pod dimensions and seeds per pod, whereas PC2 was driven more strongly by pods per plant and flowering time. The treatment combinations therefore separated according to suites of correlated traits rather than along a single productivity axis. Because the PCA was based on 12 treatment means, it should be regarded as descriptive support for the univariate results rather than as an independent inferential test.

Several limitations define the scope of inference. The study was conducted at one location in one season with four blocks, and the design did not include extract-only, ionic-silver or matched dispersant controls. Tissue silver concentration, nanoparticle dissolution, physiological status and biochemical responses were not measured. In addition, 100-seed-equivalent weight was necessarily extrapolated from smaller counted seed samples in pots that produced fewer than 100 seeds. These limitations do not invalidate the observed genotype-dependent phenotypic responses, but they restrict mechanistic interpretation and preclude recommendation of a concentration for agronomic use. Future experiments should include appropriate formulation controls, direct exposure measurements, physiological endpoints and validation across environments.

Conclusion

Foliar application of the *M. oleifera*-mediated AgNP suspension elicited contrasting responses among Bambara groundnut genotypes and traits. MOK 02 showed a marked height response at 20 mg L^{-1} and higher pod-number means at 40–60 mg L^{-1}, whereas MOK 01 and PTG 01 also exhibited inhibitory responses at specific concentrations. The combined evidence therefore supports a genotype-dependent, non-linear treatment response rather than a universal growth-promoting effect. Because the formulation contained biologically active components that were not separated experimentally, the observed effects cannot be attributed specifically to the nanoparticle fraction. Further work should incorporate extract-only and ionic-silver controls, improved exposure

characterisation, physiological measurements and multi-environment validation before agronomic recommendations are considered.

Declarations

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Author Contributions

DMC: Conceptualization, methodology, investigation, data collection and writing—original draft. DOAY: Methodology, investigation, supervision and writing—review & editing. GAA: Investigation, data collection and writing—review & editing. AH: Investigation, data curation and writing—review & editing. AA: Methodology, nanoparticle synthesis and characterisation, and writing—review & editing. JY: Conceptualization, methodology, formal analysis, data interpretation, visualization, writing—original draft, writing—review & editing.

Conflict of interest

The authors declare no competing interests.

Data availability

The replicate-level dataset and analysis outputs supporting the findings of this study are available from the corresponding author upon reasonable request.

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