RESEARCH ARTICLE

Symbiotic performance, shoot biomass and water-use efficiency of three groundnut (*Arachis hypogaea* L.) genotypes in response to phosphorus supply under field conditions in Ethiopia

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Abstract   Phosphorus is a key nutrient element involved in energy transfer for cellular metabolism, respiration and photosynthesis and its supply at low levels can affect legume nodulation, N_2 fixation, and C assimilation. A two-year field study was conducted in Ethiopia in 2012 and 2013 to evaluate the effects of P supply on growth, symbiotic N_2 nutrition, grain yield and water-use efficiency of three groundnut genotypes. Supplying P to the genotypes significantly increased their shoot biomass, symbiotic performance, grain yield, and C accumulation. There was, however, no effect on shoot $\delta^{13}C$ values in either year. Compared to the zero-P control, supplying 40 kg·ha^{-1} P markedly increased shoot biomass by 77% and 66% in 2012 and 2013, respectively. In both years, groundnut grain yields were much higher at 20 and 30 kg·ha^{-1} P. Phosphorus supply markedly reduced shoot $\delta^{15}N$ values and increased the %Ndfa and amount of N-fixed, indicating the direct involvement of P in promoting N_2 fixation in nodulated groundnut. The three genotypes differed significantly in $\delta^{15}N$, %Ndfa, N-fixed, grain yield, C concentration, and $\delta^{13}C$. The phosphorus × genotype interaction was also significant for shoot DM, N content, N-fixed and soil N uptake.

Keywords   shoot yield, N-fixed, %Ndfa, $\delta^{15}N$, $\delta^{13}C$, water-use efficiency

1   Introduction

Grain legumes are an important component of food crops and are vital in achieving food and nutritional security worldwide and in Africa[1]. Legumes also form an integral part of Ethiopian smallholder farming systems, especially in legume/cereal rotations[2]. Grain legumes are also a source of protein, food, forage and cash income for smallholder producers in Ethiopia, in addition to their contribution of symbiotic N to soil fertility[3]. However, grain legume production in Ethiopia is constrained by both biotic and abiotic factors as a result of cultivation on marginal infertile soils usually under rainfed conditions.

Phosphorus is the third most important essential soil nutrient element after soil C and N but P is the second most limiting mineral after N in promoting plant growth and crop yields[4,5]. Phosphorus is involved in cellular energy transfer, respiration and photosynthesis, thus its supply at low levels can affect legume nodulation and N_2 fixation[6,7]. Furthermore, due to the high P sink strength of nodules, the requirement for P in nodulated legumes is higher than in non-legumes[6,8–10]. Phosphorus can also increase stomatal conductance, CO_2 assimilation, water uptake and water-use efficiency in plants[11,12]. Under conditions of limited soil moisture, plants increase their water-use efficiency via stomatal closure[13] as well as developing a deeper root system, especially in perennials. However, stomatal closure to reduce water loss can also limit CO_2 intake and reduction via photosynthesis[14]. Thus, an increase in water capture and water-use efficiency via P application can be attributed to its positive effect in promoting root growth, which then enables more water uptake from deeper depths[15]. Songsri et al.[16] identified

groundnut (*Arachis hypogaea*) genotypes that had greater water-use efficiency with deeper root systems. Phosphorus supply has also been reported to increase water-use efficiency in a number of crops such as pearl millet[17], black gram[18], spring wheat[19] and lettuce[15]. Therefore, P supply seems to have profound effects on the growth and physiology of land plants[20].

Groundnut is a high P-demanding legume. As a result, P deficiency can be detrimental to groundnut production[21]. Most smallholder farmers in Africa cultivate groundnut with little or no P input. In Ethiopia, low groundnut yields are attributed to largely unreliable rainfall, low soil P, and the use of low-yielding cultivars. So far, there is little information on P requirements for groundnut in Ethiopia which is mainly focusing its effect on grain and biomass yield. Therefore, studies are currently needed to ascertain optimum P levels for increasing groundnut production in Ethiopia which can further help groundnut farmers to decide the amount based on the purpose of production. The aim of this study was to assess the effect of P supply on the growth, symbiotic N_2 fixation, water-use efficiency and grain yield of three groundnut genotypes grown in north-east Ethiopia under rainfed field conditions.

2   Materials and methods

2.1   Experimental site

The experiment was conducted at Bedeno in Dewachefa district, north-east Ethiopia, during the 2012 and 2013 cropping seasons. The study site is located at latitude 10° 35′ N and longitude 39° 41′ E at an altitude of 1000–2500 m above sea level and with a mean annual temperature range of 12–33°C. The area has bimodal rainfall, with the short rainy season beginning in January and ending in March and the long rainy season starting in June and ending in mid-October. The mean annual rainfall was 924 and 1078 mm in the 2012 and 2013 cropping seasons, respectively.

2.2   Experimental design and treatments

The experiments were laid out as a factorial randomized complete block design with four replicate plots per treatment. Each plot had an area of 3 m × 2 m (6 m²) with five rows and with intra and inter row spacing of 60 and 10 cm, respectively. The three groundnut genotypes used were obtained from Werer Agricultural Research Centre in Ethiopia and selected based on farmers' preference and their agroecological adaptation. The experimental treatments consisted of five P levels (0, 10, 20, 30 and 40 kg·ha^{-1} P) and three groundnut genotypes (Roba, Werer-961 and Werer-962). Triple superphosphate (TSP 46% P_2O_5) was used as the P source.

2.3   Collection and analysis of bulk soil samples

Before sowing and fertilizer P application, 20 soil samples were randomly collected to 20 cm depth from the entire experimental plot using a soil auger. The soil samples were pooled and thoroughly mixed and the composite sample was air-dried in the laboratory and sieved (2.0 mm) for analysis of particle size distribution, pH, cation exchange capacity (CEC), total N, available P and exchangeable cations. Particle size distribution was determined using the method of Bouyoucos[22], soil total N was determined by the Kjeldahl digestion method, available P as described by Bray and Kurtz[23], organic C as reported by Walkley and Black[24], and CEC and exchangeable cations (Na, K, Ca and Mg) by the ammonium acetate method. Soil pH was determined in a soil:water (1:10, w/v) suspension using a pH meter at the Agricultural Division of the Agricultural Research Council Institute for Soil, Climate and Water, Pretoria, South Africa.

2.4   Plant sampling and processing

Sampling of plants for dry matter (DM) yield and for ^{15}N and ^{13}C isotopic analysis was done at flowering to early pod-filling stage. Five random plants were carefully dug up from the inner three rows of each plot and separated into shoots, roots and nodules. The shoots were oven-dried (60° C), weighed and ground to a fine powder (0.85 mm) for storage before ^{15}N and ^{13}C analysis. Four replicate plants of different non-legume species growing within the experimental plots were sampled as reference plants for determining the amount of N derived from the atmosphere and then calculate the soil N uptake by groundnut. The shoots of the reference plants were also oven-dried (60°C), weighed, ground to a fine powder (0.85 mm) and similarly stored for ^{15}N isotopic analysis. Pod and grain yields were assessed at physiologic maturity by harvesting all plants from the three inner rows of each plot.

2.5   Measurement of N_2 fixation

2.5.1 $^{15}N/^{14}N$ isotopic analysis

About 1.0–2.0 mg of finely-ground groundnut shoots and 2.5 mg of reference plant samples were weighed into tin aluminum capsules and loaded onto a Thermo 2000 Elemental Analyzer coupled via a Thermo Conflo IV to a Thermo Delta V Plus stable light isotope mass spectrometer (Thermo Corporation, Bremen, Germany). The samples were combusted in an evacuated quartz tube and analyzed for $^{15}N/^{14}N$. An internal standard of *Nasturtium* spp. was included after every five runs to correct for machine error. The $^{15}N/^{14}N$ was used to calculate the isotopic composition ($\delta^{15}N$) as[25]:

$$\delta^{15}N(\text{‰}) = \frac{\left(^{15}N/^{14}N\right)_{\text{sample}} - \left(^{15}N/^{14}N\right)_{\text{atm}}}{\left(^{15}N/^{14}N\right)_{\text{atm}}} \times 1000 \quad (1)$$

where $\left(^{15}N/^{14}N\right)_{\text{sample}}$ is the ratio of ^{15}N and ^{14}N abundance in the sample and $\left(^{15}N/^{14}N\right)_{\text{atm}}$ is the ratio of ^{15}N and ^{14}N abundance in the atmosphere.

2.5.2   Shoot N content

The N content of shoots was calculated as the product of shoot %N (obtained from mass spectrometry) and shoot DM as[26]:

$$N_{\text{content}} = \%N_{\text{shoot}} \times \text{drymass}_{\text{shoot}} \quad (2)$$

2.5.3   Percent N derived from the atmospheric fixation (%Ndfa)

The proportion of N derived from atmospheric N_2 fixation was estimated as[27]:

$$\delta^{15}N(\text{‰}) = \frac{\delta^{15}N_{\text{ref}} - \delta^{15}N_{\text{leg}}}{\delta^{15}N_{\text{ref}} - B_{\text{value}}} \times 100 \quad (3)$$

where $\delta^{15}N_{\text{ref}}$ is the ^{15}N natural abundance of a non-nitrogen-fixing reference plant, $\delta^{15}N_{\text{leg}}$ is the ^{15}N natural abundance of the legume, and B_{value} is the ^{15}N natural abundance of groundnut plants deriving all of their N nutrition from symbiotic N_2 fixation. Here, the B_{value} used for estimating %Ndfa of groundnut shoot was −2.70‰[28].

2.5.4   Amount of N-fixed

The amount of N-fixed was calculated as[29]:

$$N-\text{fixed} = \%Ndfa \times \text{legume biomass N} \quad (4)$$

where legume biomass N is the N content of groundnut shoots.

2.5.5   Soil N uptake

Soil N uptake was calculated as[29]:

$$\text{Soil N uptake} = \text{Total legume biomass N}$$
$$- \text{amount of N-fixed} \quad (5)$$

2.6 $^{13}C/^{12}C$ isotopic analysis

To analyze for $^{13}C/^{12}C$, 2.0 mg of finely-ground groundnut shoot samples were weighed into aluminum capsules and run on a mass spectrometer as described for $^{15}N/^{14}N$ isotopic ratio. The ^{13}C natural abundance, or $\delta^{13}C$ (‰), was calculated as[30]:

$$\delta^{13}C(\text{‰}) = \left[\frac{\left(^{13}C/^{12}C\right)_{\text{sample}}}{\left(^{13}C/^{12}C\right)_{\text{standard}}} - 1 \right] \times 1000 \quad (6)$$

where $\left(^{13}C/^{12}C\right)_{\text{sample}}$ is the isotopic ratio of the sample and $\left(^{13}C/^{12}C\right)_{\text{standard}}$ is the isotopic ratio of PDB, a universally accepted standard from the Belemnite Pee Dee limestone formation[31]. Shoot C content was calculated as the product of %C (obtained from mass spectrometry) and shoot DM.

2.7   Statistical analysis

All data obtained were subjected to a test of normal distribution before being subjected to analysis of variance. Shoot biomass, grain yield, water-use efficiency and symbiotic parameters were analyzed by two-way analysis of variance using the SAS (System Analysis Software) version 9.0 package. Where significant differences were found, Duncan's multiple range test was used to separate treatment means at $P \leqslant 0.05$. Pearson correlation and linear regression analysis were done to determine relationships between measured parameters.

3   Results

3.1   Soil analysis

Analysis of bulk soils collected from plots before planting (Table 1) shows 26% sand, 24% silt and 50% clay, with pH 6.96–7.00, plant-available P 14.9–16.0 $mg \cdot kg^{-1}$, high cation exchange capacity and low sodium[32]. Total N was 1100 $mg \cdot kg^{-1}$ (medium fertility) in 2012 and 670 $mg \cdot kg^{-1}$ (low fertility) in 2013[33].

3.2 $\delta^{15}N$ of reference plants

The mean $\delta^{15}N$ of non-legume reference plant species

Table 1 Physicochemical properties of soils at Bedeno in north-east Ethiopia

Year	Texture	pH	Total N	Available P	OC	CEC	Exchangeable cations/($mg \cdot kg^{-1}$)			
		H_2O	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$	%	$Cmol(+) \cdot kg^{-1}$	Na	K	Ca	Mg
2012	Clay	7.00	1100	16.00	1.63	53.90	20.70	327.60	8080.0	1074.00
2013	Clay	6.96	700	14.88	1.44	56.66	35.65	598.65	5929.4	1086.72

sampled in 2012 ranged from +4.70 ‰ in *Zea mays* to +8.76 ‰ in *Brassica oleracea,* with a combined mean $\delta^{15}N$ value of +6.74 ‰. The $\delta^{15}N$ of reference plants in 2013 ranged from +6.65 ‰ in *Eragrostis tef* to +11.74 ‰ in *Cyperus esculentus,* with a combined mean of +9.06 ‰ (Table 2). It is these combined mean $\delta^{15}N$ values of the reference plant species that were used to estimate soil N uptake by groundnut.

3.3 Effect of P on shoot, pod and grain yields

There were significant effects of P supply on shoot DM, pod number plant^{-1} and grain yield during the 2012 and 2013 cropping seasons (Table 3; Table 4). Supplying 30 or 40 kg·ha^{-1} P to groundnut in 2012 markedly increased shoot DM by 62% and 77%, respectively, over the zero P control. Shoot biomass accumulation in 2013 also differed between the P levels (Table 4), with the highest shoot DM occurring at 40 kg·ha^{-1} P, representing a 66% increase over the zero-P control. Pod numbers plant^{-1} were maximum at 30 kg·ha^{-1} P, a 44% and 41% increase over the control in 2012 and 2013, respectively, during both 2012 and 2013 cropping seasons (Table 3; Table 4).

Grain yield in groundnut was also significantly increased by P supply during both 2012 and 2013 cropping seasons (Table 3; Table 4). Grain yield was significantly increased by P application in 2012 relative to the control but was similar at 10 to 40 kg·ha^{-1} P (Table 3). The maximum grain yield in 2013 was recorded in groundnut supplied with 20 kg·ha^{-1} P, an increase of about 34% over the zero-P control, followed by 30 kg·ha^{-1} P (Table 4).

3.4 Effect of P on symbiotic N nutrition in groundnut

There were significant differences in symbiotic response by groundnut to applied P in the 2012 and 2013 cropping seasons (Table 3; Table 4). Shoot N concentration ranged from 3.5% at zero P to 3.7% at 40 kg·ha^{-1} P in 2012 (Table 3). However, P supply had no significant effect on shoot N concentration in 2013 (Table 4). Groundnut shoot N content increased linearly in 2012 with increasing P supply from 1.4 g·plant^{-1} N at zero-P treatment to 2.6 g·plant^{-1} N at 40 kg·ha^{-1} P. Similarly, shoot N content increased from 1.4 g·plant^{-1} N in the zero-P control to 2.4 g·plant^{-1} N at 40 kg·ha^{-1} P in 2013 (Table 4).

Phosphorus application markedly decreased the shoot $\delta^{15}N$ of groundnut in both 2012 and 2013 cropping seasons (Table 3; Table 4). The minimum shoot $\delta^{15}N$ occurred in plants supplied with 40 kg·ha^{-1} P (+2.33 ‰) in 2012, followed by 30 kg·ha^{-1} P (+2.65 ‰) and 20 kg·ha^{-1} P (+2.83 ‰) (Table 3). Shoot $\delta^{15}N$ similarly decreased with increasing P application in 2013. The minimum $\delta^{15}N$ (−0.39 ‰) was recorded in plants supplied with 40 kg·ha^{-1} P and the maximum (+1.29 ‰) in zero-P control plants (Table 4). In general, shoot $\delta^{15}N$ values were lower in 2013 than in 2012, possibly due to low endogenous soil N in the experimental plots used in 2013 (Table 4).

Estimates of N derived from atmospheric N_2 fixation differed markedly with P application in 2012 and 2013 (Table 3; Table 4). Shoot %Ndfa increased significantly with increasing P supply from 40% at 10 kg·ha^{-1} P to 47% at 40 kg·ha^{-1} P in 2012. However, the groundnut genotypes derived 50% of their N nutrition from the symbiosis in 2012 (Table 3). In 2013, however, P supply markedly increased the percentage of N derived from N_2 fixation with the maximum (80%) obtained at 40 kg·ha^{-1} P and the minimum (66%) in the zero-P control. In general, percent N derived from fixation was greater in 2013 than in 2012 due to the lower shoot $\delta^{15}N$ in the former (Table 4).

The amount of symbiotically-fixed N in the shoots in

Table 2 $\delta^{15}N$ values of reference plants sampled in 2012 and 2013 to determine N_2 fixation by groundnut at Bedeno, Ethiopia

2012		2013	
Species	$\delta^{15}N/\%$	Species	$\delta^{15}N/\%$
Amaranthus hybridus	6.63	*Commelino benghalensis*	10.00
Argemone mexicana	7.24	*Cyperus esculentus*	11.74
Brassica oleracea	8.76	*Datura stramonium*	6.83
Eragrostis tef	5.99	*Eragrostis tef*	6.65
Galinsoga parviflora	6.91	*Malva verticilata*	9.63
Guizotia abyssinica	6.72	*Parthenium hyterophorus*	9.44
Parthenium hysterophorus	6.86	*Setaria pumila*	7.01
Portulaca oleracea	7.47	*Solanum nigrum*	7.12
Solanum nigrum	6.97	*Sorghum bicolor*	11.61
Sorghum bicolor	5.01	*Xanthium strumarium*	8.66
Xanthium strumarium	7.60	*Zea mays*	8.63
Zea mays	4.70	Mean	9.06±0.582
Mean	6.74±0.319		

Table 3 Shoot biomass, grain yield and symbiotic performance of three groundnut genotypes supplied with P and planted at Bedeno in north-east Ethiopia, in 2012

Treatment	Shoot DM	Pod number	Grain yield	N	N content	$\delta^{15}N$	Ndfa	N-fixed	Soil N uptake
	$g \cdot plant^{-1}$	$plant^{-1}$	$kg \cdot ha^{-1}$	%	$g \cdot plant^{-1}$	%	%	$kg \cdot ha^{-1}$	$kg \cdot ha^{-1}$
Added P/($kg \cdot ha^{-1}$)									
0	39.8e	18.5d	1173b	3.5b	1.4e	3.20a	38d	88e	145d
10	51.9d	22.0c	1365a	3.6ab	1.9d	3.01ab	40cd	123d	188c
20	59.6c	24.3b	1478a	3.6ab	2.1c	2.83bc	41bc	146c	207bc
30	64.3b	26.6a	1438a	3.6ab	2.3b	2.65c	43b	167b	219ab
40	70.6a	25.6b	1397a	3.7a	2.6a	2.33d	47a	202a	230a
Genotype									
Roba	59.3a	24.2a	1431a	3.6a	2.1a	2.66b	43a	156a	200a
Werer-961	56.1a	22.9a	1379ab	3.6a	2.0a	2.78b	42a	143b	195a
Werer-962	56.3a	23.0a	1301b	3.6a	2.0a	2.96a	40b	137b	198a
Two-way ANOVA									
F-Statistics									
Phosphorus (P)	52.73***	16.77***	5.43**	1.66*	47.80***	18.67***	18.66***	76.45***	20.78***
Genotype (G)	1.92ns	1.44ns	2.77*	0.87ns	1.89ns	6.29**	6.31**	6.70**	0.21ns
P×G	3.24**	1.83ns	1.17ns	1.09ns	3.15**	0.83ns	0.83ns	3.18**	2.32*
CV/%	9.92	11.66	12.85	4.25	11.00	9.59	6.84	11.72	12.74

Note: Values (means±SE) within columns followed by the same letter are not significantly different at * $P \leqslant 0.05$; ** $P \leqslant 0.01$; *** $P \leqslant 0.001$; and ns, not significant.

Table 4 Shoot biomass, grain yield and symbiotic performance of three groundnut genotypes supplied with P and planted at Bedeno, in north-east Ethiopia, in 2013

Treatment	Shoot DM	Pod number	Grain yield	N	N content	$\delta^{15}N$	Ndfa	N-fixed	Soil N uptake
	$g \cdot plant^{-1}$	$plant^{-1}$	$kg \cdot ha^{-1}$	%	$g \cdot plant^{-1}$	%	%	$kg \cdot ha^{-1}$	$kg \cdot ha^{-1}$
Added P/($kg \cdot ha^{-1}$)									
0	40.9e	25.9d	1120c	3.3a	1.4d	1.29a	66e	152d	77b
10	52.9d	29.2c	1274b	3.4a	1.8c	0.74b	71d	210c	88a
20	57.1c	33.2b	1499a	3.4a	2.0bc	0.46c	73c	238c	87a
30	62.4b	36.5a	1443a	3.4a	2.1b	0.18d	76b	268b	87a
40	67.8a	34.9ab	1337b	3.5a	2.4a	−0.39e	80a	320a	77b
Genotype									
Roba	55.2a	33.4a	1341a	3.6a	2.0a	0.78a	71c	239ab	96a
Werer-961	55.4a	31.4a	1346a	3.2c	1.8b	0.47b	73b	220b	77b
Werer-962	58.1a	31.1a	1318a	3.4b	2.0a	0.12c	76a	2546a	77b
Two-way ANOVA									
F-Statistics									
Phosphorus (P)	49.64***	16.48***	41.68***	0.45ns	29.40***	165.66***	165.45***	43.56***	4.34*
Genotype (G)	2.09ns	2.27ns	0.71ns	8.49**	5.46**	76.50***	75.97***	5.51**	24.92***
P×G	2.63ns	1.28ns	1.09ns	0.06ns	1.32ns	4.10**	4.11**	0.81ns	4.22***
CV/%	8.95	11.62	5.98	9.39	12.53	37.13	1.96	13.89	11.48

Note: Values (means±SE) within columns followed by the same letter are not significantly different at * $P \leqslant 0.05$; ** $P \leqslant 0.01$; *** $P \leqslant 0.001$; and ns, not significant.

2012 was determined and the data show increasing amounts of N-fixed (Table 3). In fact, N-fixed ranged from 88 kg·ha^{-1} N in the zero-P control in 2012 to 202 kg·ha^{-1} N in plants supplied with 40 kg·ha^{-1} P. The maximum P treatment (40 kg·ha^{-1} P) increased the N contribution by 130% over the zero-P control. Phosphorus

supply in 2013 similarly stimulated N_2 fixation in groundnut with the N contribution ranging from 152 kg·ha⁻¹ N in the zero-P control to 320 kg·ha⁻¹ N at 40 kg·ha⁻¹ P. These amounts were much higher than those in 2012 (Table 4).

3.5 Effect of P on soil N uptake by groundnut

Soil N uptake by groundnut was increased linearly by P application during the 2012 cropping season (Table 3). The increase in soil N uptake ranged from 145 kg·ha⁻¹ N in the zero-P control to 230 kg·ha⁻¹ N at 40 kg·ha⁻¹ P. In all instances, soil N uptake by groundnut in 2012 was much higher than the amount contributed by N_2 fixation (Table 3). The addition of 10, 20 and 30 kg·ha⁻¹ P in 2013 significantly increased soil N uptake over the zero-P control and 40 kg·ha⁻¹ P (Table 4). However, soil N uptake in 2013 was much lower than the amount contributed by symbiosis in 2012 (Table 4).

3.6 Effect of P on shoot C concentration, C content, C/N ratio and $\delta^{13}C$ of groundnut genotypes

There was a significant effect of P application on shoot C concentration, C content, C/N ratio and $\delta^{13}C$ of groundnut genotypes planted during the 2012 and 2013 cropping seasons (Table 5). C concentrations in shoots were increased markedly by P supply in 2012 with the maximum %C being obtained at 20 kg·ha⁻¹ P. As a result, shoot C content also rose with P supply and attained a 79%

increase over the zero-P control at 40 kg·ha⁻¹ P. Shoot C/N ratio was increased significantly by 20 and 30 kg P ha⁻¹ but was similar at 0, 10 and 40 kg·ha⁻¹ P (Table 5).

Shoot %C and C/N ratio were similar in 2013. Shoot C content ranged from 16.2 g·plant⁻¹ at zero-P to 28.0 g·plant⁻¹ at 40 kg·ha⁻¹ P with an increase of 73% (Table 5).

3.7 Effect of groundnut genotype on shoot biomass, N nutrition, grain yield, C accumulation and $\delta^{13}C$ values

The three groundnut genotypes used here were similar in shoot biomass, pod number plant⁻¹, shoot N concentration, content and soil N uptake in 2012 (Table 3). Genotype Roba, however, produced more grain yield than Werer-962 (Table 3). Shoot $\delta^{15}N$ was much higher in Werer-962 in 2012. As a result, Ndfa and N-fixed were lower in Werer-962 compared to Roba and Werer-961 (Table 3). Soil N uptake was, however, similar in all three genotypes in 2012.

The 2013 results were similar to the 2012 data in that shoot DW, pod number and grain yield were not different among the three genotypes (Table 4). However, Roba showed higher shoot N concentrations than Werer-961 and Werer-962 but was similar to Werer-962 in N content. Shoot $\delta^{15}N$ in 2013 was much lower in Werer-962, followed by Werer-961 and was greater in Roba. As a result, %Ndfa and N-fixed were much greater in Werer-962 than in the other two genotypes (Table 4). Soil N uptake was, however, much higher in Roba in 2013. Although %C

Table 5 Shoot biomass, %C, C content, C/N-fixed and $\delta^{13}C$ of 16 groundnut genotypes grown at Bedeno in north-east Ethiopia, in 2012 and 2013

Treatment	2012				2013			
	C	C content	C/N-fixed	$\delta^{13}C$	C	C content	C/N-fixed	$\delta^{13}C$
	%	g·plant⁻¹	g·g⁻¹	%	%	g·plant⁻¹	g·g⁻¹	%
Added P/(kg·ha⁻¹)								
0	41.5b	16.5d	11.4b	−26.7a	39.7a	16.2e	12.0a	−28.2a
10	41.7b	21.6c	11.6b	−26.8a	40.3a	21.3d	11.8a	−28.14a
20	42.7a	25.5b	12.4a	−26.7a	40.8a	23.2c	12.0a	−28.0a
30	42.3ab	27.2b	12.0a	−26.7a	41.6a	25.9b	12.3a	−28.1a
40	41.7b	29.5a	11.4b	−26.8a	41.5a	28.0a	11.9a	−28.2a
Genotype								
Roba	42.1a	24.9a	11.8a	−26.8a	42.1a	23.2a	11.7b	−28.1ab
Werer-961	41.9a	23.5a	11.8a	−26.8a	40.7ab	22.6a	12.8a	−27.8a
Werer-962	42.0a	23.7a	11.7a	−26.7a	39.7b	23.0a	11.5b	−28.2b
Two-way ANOVA								
F-Statistics								
Phosphorus (P)	3.60*	52.08***	10.05***	0.71ns	1.15ns	67.14***	0.24ns	0.64ns
Genotype (G)	0.24ns	1.93ns	0.26ns	1.42ns	4.54*	0.62ns	6.02**	3.58*
P×G	1.27ns	2.98**	3.21**	0.47ns	0.24ns	2.68*	0.28ns	0.74ns
CV (%)	2.25	10.19	3.90	−0.85	6.33	8.35	10.61	−1.10

Note: Values (means±SE) within columns followed by the same letter are not significantly different at * $P \leqslant 0.05$; ** $P \leqslant 0.01$; *** $P \leqslant 0.001$; and ns, not significant.

was higher in Roba in 2013, shoot C content was similar among the three genotypes (Table 5). Shoot C/N ratio and $\delta^{13}C$ were higher in Werer-961 than in the other genotypes (Table 5).

3.8 Interactive effects of P and genotype

The P × genotype interaction was significant for shoot DM, N content, N-fixed and soil N uptake, but not for pod number, grain yield, $\delta^{15}N$ and %Ndfa in 2012 (Table 3). Analysis of this interaction shows that Roba and Werer-961 produced more shoot DM at zero-P than did Werer-962 (Fig. 1(a)). Except for 10 kg·ha^{-1} P, Roba produced the same shoot biomass as Werer-962 at 30 kg·ha^{-1} P and more DM than the other two genotypes at 40 kg·ha^{-1} P (Fig. 1(b)). Roba had higher shoot N content followed by Werer-961, and Werer-962 had the least (Fig. 1(b)). At 10 and 40 kg·ha^{-1} P, Roba and Werer-962 showed higher N contents than Werer-961 but all three genotypes were similar in N content at 20 and 30 kg·ha^{-1} P (Fig. 1(b)).

Whether at 0, 30 or 40 kg·ha^{-1} P, Roba consistently fixed more N than Werer-961 or Werer-962 but all three varieties had similar fixation rates at 10 and 20 kg·ha^{-1} P (Fig. 1(c)). With soil N uptake, Roba took up more N at zero-P than did Werer-961, followed by Werer-962, and again had greater N uptake at 40 kg·ha^{-1} P than Werer-961 but not Werer-962 (Fig. 1(d)).

The P × genotype interaction was significant for shoot $\delta^{15}N$, %Ndfa and soil N uptake but not for shoot DM, pod number, grain yield or N-fixed (Table 4). As shown in Fig. 2(a), at 0, 10, 20 and 30 kg·ha^{-1} P, genotype Werer-962 consistently had much lower shoot $\delta^{15}N$, followed by Werer-961 and Roba. Even at 40 kg·ha^{-1} P, Werer-962 still showed a much lower $\delta^{15}N$ value, followed by Roba. As a result, shoot %Ndfa was generally higher in Werer-962 than Roba or Werer-961, but the difference was significant at only 0, 10 and 20 kg·ha^{-1} P (Fig. 1(b)). Soil N uptake was greater in Roba at all P levels except 40 kg·ha^{-1} P. The lower %Ndfa of Roba was therefore due to greater soil N uptake at 0, 10, 20 and 30 kg·ha^{-1} P (Fig. 1(c)).

3.9 Correlation and regression analysis

Both correlation and regression analyses were conducted on various measured parameters (e.g., shoot biomass, symbiotic performance and water-use efficiency). As shown in Fig. 3(a), shoot DM was positively correlated with N-fixed ($r = 0.95***$). However, shoot $\delta^{15}N$ was inversely correlated with %Ndfa ($r = -1.00***$), as well as with N-fixed ($r = -0.84***$).

4 Discussion

4.1 Effect of P on shoot biomass and symbiotic performance

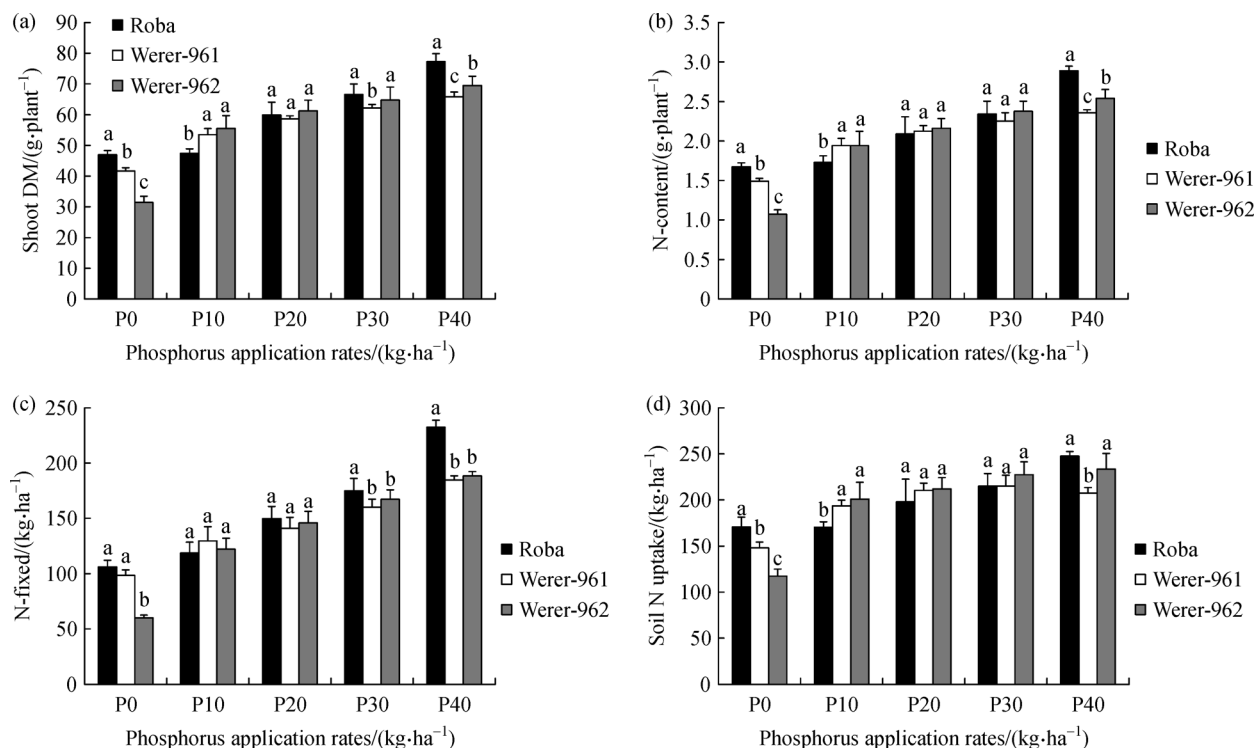Plant biomass, photosynthesis, N_2 fixation and grain yield

Fig. 1 Interactive effect of P × genotype on (a) shoot DM, (b) N content, (c) N-fixed, and (d) soil N uptake in field-grown groundnut at Bedeno in north-east Ethiopia, in 2012. Vertical lines on bars represent SE.
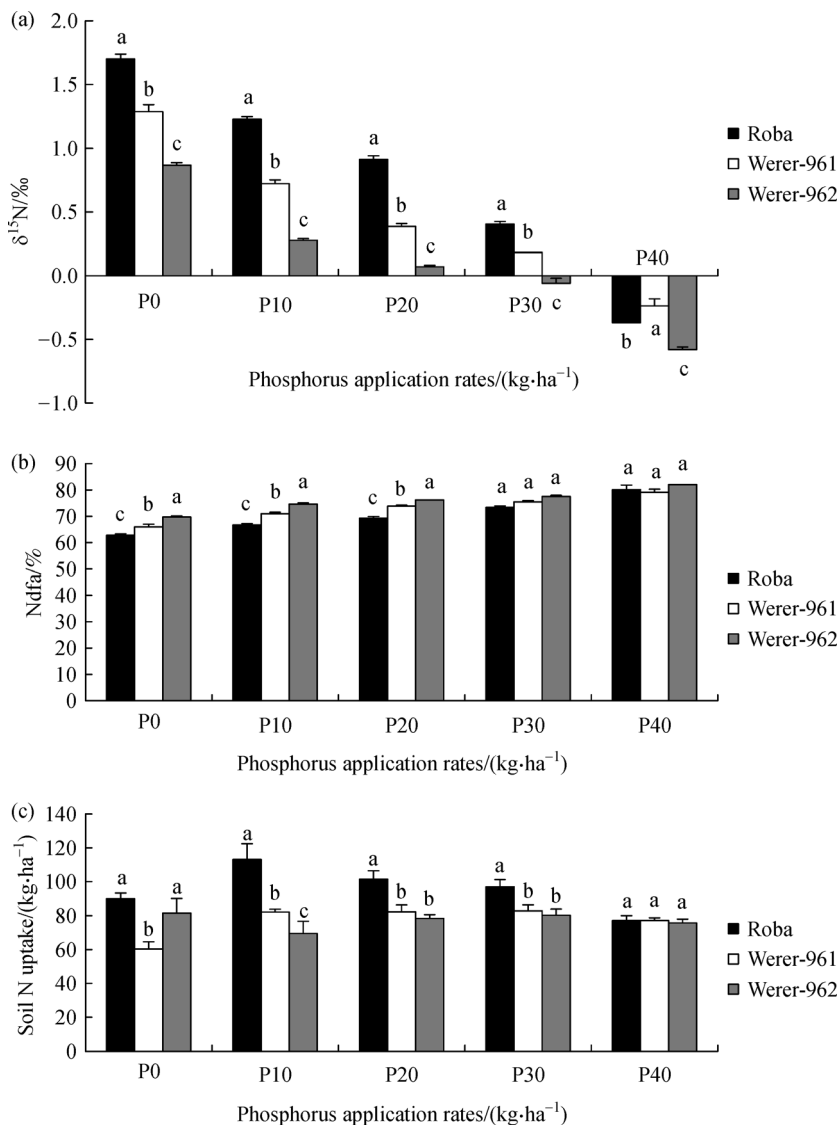
Fig. 2 Interactive effect of P × genotype on (a) δ^{15}N, (b) %Ndfa, and (c) soil N uptake in field-grown groundnut at Bedeno in north-east Ethiopia, in 2013. Vertical lines on bars represent SE.

of groundnut are highly dependent on optimal P nutrition[21]. Phosphorus application has been widely reported to have significant positive effects on nodulation and N$_2$ fixation in legumes[6]. A low yield due to P deficiency is therefore not unexpected in Africa. Although P requirements of groundnut have been documented under field and glasshouse conditions[21,34–36], little is known about P effects on groundnut performance in Ethiopian cropping systems. Here, we assessed three recommended groundnut genotypes cultivated by farmers for their growth and symbiotic responses to moderate P levels in Ethiopia using the ^{15}N natural abundance technique to measure N$_2$ fixation.

The precision of the technique was indicated by the difference between the combined mean δ^{15}N of reference plants and the highest δ^{15}N of groundnut genotypes. The values obtained here (+3.54 ‰ and +5.45 ‰ in 2012 and 2013, respectively) were much greater than +2.00 ‰, the recommended figure for accurate measurement of N-fixed using the ^{15}N natural abundance method[37]. Thus, the N contribution estimated in nodulated groundnut in this study can be considered to be highly reliable. An earlier study[28] also used the ^{15}N natural abundance method to estimate symbiotic N$_2$ fixation by groundnut in farmers' fields in Zambia.

Applying moderate levels of P to groundnut markedly reduced δ^{15}N and increased the proportion of N derived from symbiotic fixation (Table 3; Table 4). This suggests a high P demand by nodulated groundnut for its N$_2$ fixation. This is consistent with reports of increased symbiotic
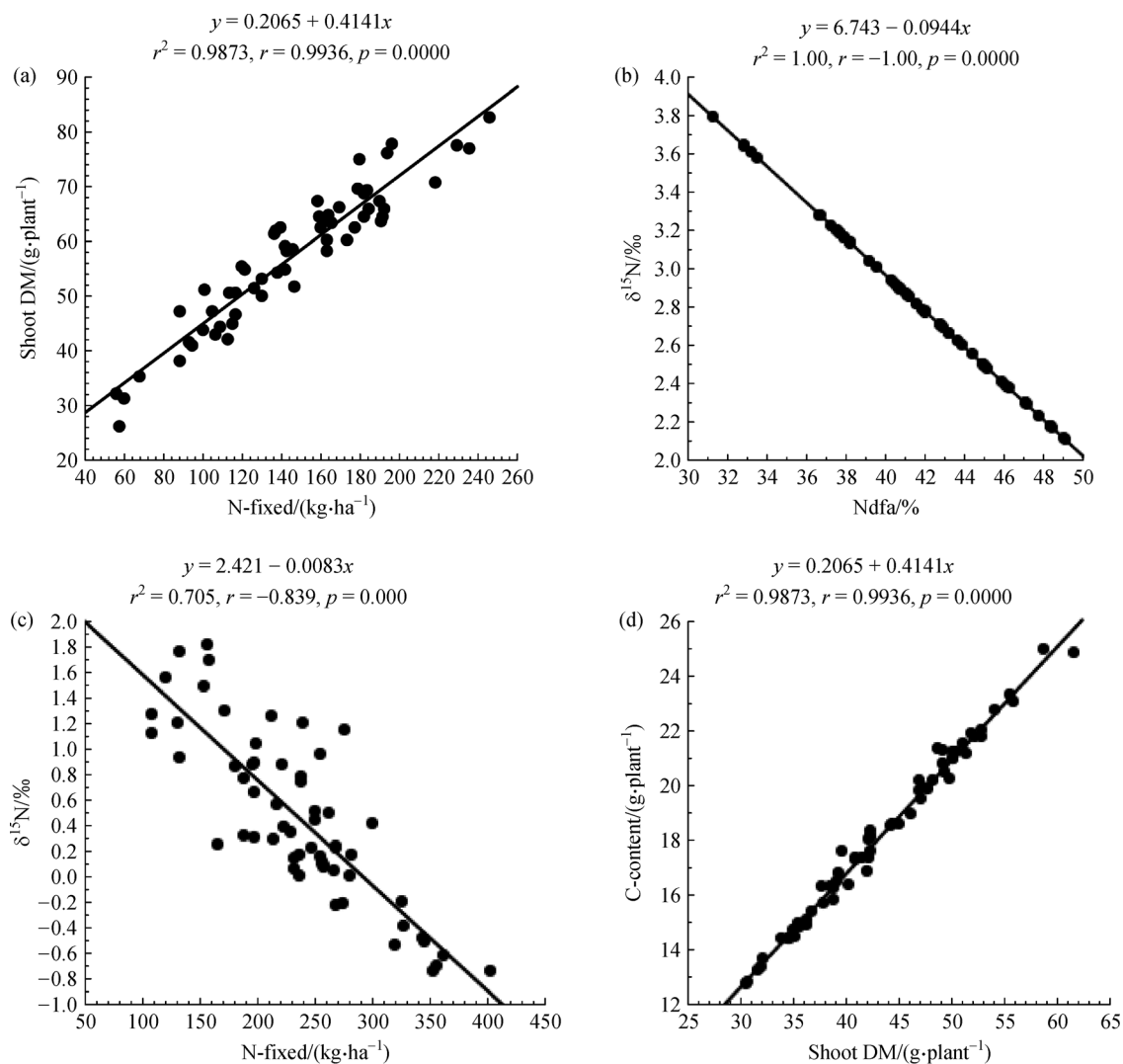
Fig. 3 Correlation and regression between (a) shoot DM and N-fixed, (b) δ^{15}N and %Ndfa, (c) δ^{15}N and N-fixed, (d) C-content and shoot DM for groundnut genotypes planted at Bedeno in north-east Ethiopia.

performance with P supply[38,39] and the direct involvement of P in promoting N_2 fixation in nodulated legumes[10,40].

The high %Ndfa caused by added P when combined with the increased DM yield of P-fed plants automatically raised the amounts of N-fixed by groundnut during the 2012 and 2013 cropping seasons (Table 3; Table 4). Independent of P supply, the N contribution by groundnut was greater in 2013 than 2012, and this may be attributed to the higher endogenous soil N concentration in 2012 which inhibited nodulation and N_2 fixation. The 0.07% soil N concentration in 2013 was lower when compared to 0.11% N in 2012 (Table 1) but high soil NO_3 has been reported to impair nodule development and decrease nitrogenase activity and N_2 fixation in legumes[41,42]. It is, however, also possible that the low fixation in 2012 might be due to a low population of native soil effective rhizobia since in legumes a direct relationship exists

between soil rhizobial numbers, their effectiveness, and the level of N_2 fixation[43]. The data obtained here clearly show that symbiotic legumes tend to increase N supply from symbiosis to meet their N requirements, especially where the soil N concentration is low. However, they increase soil N uptake if fixation is inhibited by endogenous soil N.

The higher pod numbers plant^{-1} obtained at 30 kg·ha^{-1} P and the greater grain yields at 20 kg·ha^{-1} P in this study would be exciting to farmers. In Ethiopia, as elsewhere in Africa, resource-poor smallholder farmers cannot afford chemical inputs such as P fertilizers. Thus, the increases in grain yield of 26% in 2012 and 34% in 2013 from application of a low rate (20 kg·ha^{-1} P) may potentially boost groundnut production in Ethiopia. The yield increase of applied P over zero-P was 16% to 14% at 10 kg·ha^{-1} P in 2012 and 2013. These data suggest that applying as little as 10 to 20 kg·ha^{-1} P can result in yield increases as high as

15% to 30%. Another study[35] also found a similar grain yield increase in groundnut with the application of 20 kg·ha⁻¹ P.

The three groundnut genotypes used here were found to differ significantly in δ^{15}N, %Ndfa, N-fixed and grain yield. The observed differences in symbiotic performance between the three genotypes are consistent with data obtained by for 25 groundnut genotypes in South Africa[44]. The amounts of N-fixed here ranged from 137 to 156 kg·ha⁻¹ N in 2012 and 220 to 254 kg·ha⁻¹ N in 2013. Although there were no significant differences in shoot DM between the genotypes, N contribution was found to increase with greater %Ndfa and lower δ^{15}N values, indicating a functional relationship between symbiotic N₂ fixation and biomass production[44,45].

The groundnut genotypes used here differed in soil N uptake in 2013, with Roba taking up more mineral N than the other two genotypes (Table 4). It is interesting to note that at zero-P, Roba took up more soil N than Werer-961 or Werer-962 in 2012 and again accumulated more soil N in 2013 than Werer-961 or Werer-962 at all P levels studied except 40 kg·ha⁻¹ P and, to some extent, at zero-P (Fig. 1(d); Fig. 2(c)). These findings suggest a relationship between N and P nutrition in some genotypes, with low soil P promoting greater soil N uptake and high P promoting symbiotic N nutrition.

Here, P supply significantly increased the growth, grain yield and symbiotic performance of three groundnut genotypes. Overall, higher values were obtained in plants supplied with 40 kg·ha⁻¹ P, the highest P level. However, grain yield was similar from 10 to 40 kg·ha⁻¹ P in 2012, but higher at 20 and 30 kg·ha⁻¹ P. Grain yield was again greater at 20 and 30 kg·ha⁻¹ P in 2013, indicating that 20 kg·ha⁻¹ P is capable of providing the same grain yield as 30 kg·ha⁻¹ P. This finding indicates good news for the economics of groundnut production in Ethiopia.

4.2 Effect of P nutrition on C accumulation and water-use efficiency

The current study also evaluated the effect of P supply on shoot biomass, C accumulation and water-use efficiency of the three groundnut genotypes. The results show that P supply significantly increased shoot DM, C concentration, C content, and C/N ratio, without effect on shoot δ^{13}C (Table 5). However, the magnitude of the shoot DM yield response to added P was more marked than the other parameters. For example, at 40 kg·ha⁻¹ P shoot biomass accumulation during the 2012 and 2013 cropping seasons increased by 77% and 66%, respectively, over the zero-P control. The effect of P on shoot biomass accumulation and C concentration resulted in an increased shoot C content with increasing P supply. However, the effect of P nutrition on plants has been attributed to increased stomatal conductance, photosynthesis, CO₂ assimilation and water-use efficiency[46,47]. Therefore, the increase in

shoot biomass and C accumulation obtained in this study is also likely due to increased plant growth, photosynthesis and C assimilation[11,48].

Although there was no significant effect of P on δ^{13}C values during the 2012 and 2013 cropping seasons, values were lower in 2013 than 2012, indicating greater amounts of rainfall received in 2013 compared to 2012. Plants growing under high rainfall conditions usually exhibit lower δ^{13}C values than those growing under water-stressed conditions[49,50]. The amount of rainfall received was high in 2013 (1078 mm) relative to 2012 (924 mm), therefore the higher amounts of rainfall during the 2013 cropping season may have increased ^{13}C discrimination and decreased the δ^{13}C values, leading to low water-use efficiency. This argument is supported by negative correlations found between mean annual precipitation and δ^{13}C of C₃ plants[50–52].

The groundnut genotypes used also differed significantly in C concentration, C/N ratio and δ^{13}C in 2013, with genotype Werer-961 exhibiting greater %C, high C/N ratio and greater δ^{13}C values than the other two genotypes (Table 5). The greater C/N ratio in genotype Werer-961 may be due to the lower N content, while the lower C/N ratio obtained in genotype Roba might be the result of the greater N contribution and tissue N concentration (see Table 4). This was confirmed by the negative correlation between C/N ratio and N concentration. It is also interesting to note that genotype Werer-961 was greater in water-use efficiency (higher δ^{13}C value) but lower in symbiotic N contribution but Werer-962 was lower in water-use efficiency and greater in N₂ contribution. Thus, the findings of this study have shown that genotypes greater in N contribution were lower in water-use efficiency (δ^{13}C value) and vice versa. The genotypes also showed significant differences in shoot C and C/N ratio at the different P levels (Fig. 3), indicating that there is genotypic variability in response to soil P availability in groundnut.

Phosphorus supply markedly increased shoot biomass, shoot C accumulation and C/N ratio of groundnut. Shoot biomass and C content were greater in plants supplied with 40 kg·ha⁻¹ P. However, P application had no effect on the δ^{13}C of the groundnut genotypes.

5 Conclusions

Supplying exogenous P to three groundnut genotypes markedly increased plant growth, symbiotic performance and grain yield. Phosphorus supply markedly reduced shoot δ15N values and increased the %Ndfa and amount of N-fixed, indicating a direct involvement of P in promoting N2 fixation in nodulated groundnut. Of the five P levels (0, 10, 20, 30 and 40 kg·ha⁻¹ P) applied to field plants, the highest grain yield was produced with supplying only 20 kg·ha⁻¹ P in both 2012 and 2013. This means that even

resource-poor farmers in Ethiopia can significantly increase groundnut yields with the application of only 20 kg·ha^{-1} P.

Compliance with ethics guidelines Sofiya K. Muhaba and Felix D. Dakora declare that they have no conflicts of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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