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N_2 fixation, grain mineral accumulation, and water-use efficiency in 30 field-grown groundnut (*Arachis hypogaea* L.) genotypes in Mpumalanga, South Africa, measured using ^{15}N and 13^C natural abundance techniques

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African soils are inherently low in mineral nutrients. Incorporating N_2-fixing legumes into cropping systems can improve soil fertility and increase crop yields. This study assessed N_2 fixation, carbon assimilation, grain mineral accumulation and water-use efficiency of 30 groundnut genotypes grown in the field at the Mpumalanga Province, South Africa, using the ^{15}N and ^{13}C natural abundance techniques. The results revealed marked differences in symbiotic performance between and among the groundnut genotypes, with IS-07273 and ICGV13910 exhibiting greater symbiotic dependency on N_2 fixation for their N nutrition and higher amounts of N-fixed. The two high N_2-fixing symbioses (IS-07273 and ICGV13910) also accumulated significantly high levels of K, Na, Zn, Cu, Mn, and B in their grain. As a result, there were strong correlations between amounts of N-fixed and K, Na, B, Cu, Zn and Mn for genotype ICGV13910. Genotype IS-07273 also showed significant correlations between N-fixed and S, N concentration (%N) and P, %N and K, as well as nodule number and Ca. As to be expected, genotypes with the highest shoot %N accumulated the most protein in their grain. Out of the 30 groundnut genotypes tested in the field, YENYAWOSO, ICGV13848, ICGV13851, ICGV15033 and ICGV131065 showed greater shoot δ13C values, and hence higher water-use efficiency. The high N_2 fixation in genotypes ICGV13910 and IS-07273 correlated positively with macro- and micro-nutrient concentrations in their grain, indicating their potential for use in breeding programmes to enhance nutritional security in groundnut.

KEYWORDS

N_2-fixation, grain yield, soil N uptake, seed protein content, grain mineral content

Introduction

Groundnut (*Arachis hypogaea* L.) is an important source of protein (25%–28%), oil (48%–50%), and vitamins for human consumption and cash income for farmers (Nyambok, 2011; Janila et al., 2013). Groundnut is also a source of symbiotic nitrogen for plant growth and for boosting crop production in farmers' fields because of its ability to form effective symbioses with soil rhizobial bacteria (Vieira et al., 2010). Groundnut is nodulated by *Bradyrhizobium* bacteria (Zhang et al., 1999; Chen et al., 2003; Yang et al., 2005), *Rhizobium giardini*, and *Rhizobium tropici* (Taurian et al., 2006; Ibañez et al., 2008; Ibanez et al., 2009). The groundnut plant has been shown to meet much of its N requirement from symbiotic N_2 fixation and to contribute to the N economy of cropping systems (Mokgehle et al., 2014).

Groundnut can contribute a considerable amount of nitrogen to cropping systems in Africa. In fact, many studies (Dakora and Keya, 1997; Bado et al., 2006; Nyemba and Dakora, 2010) have found that significant levels of nitrogen have been contributed by groundnut in African cropping systems. In South Africa, groundnut is reported to fix between 58 and 188 kg N.ha^{-1} (Mokgehle et al., 2014), thus improving soil N fertility in resource-poor farmers' fields and increasing the yields of intercrops and succeeding cereal crops (Kumawat et al., 2022).

N_2-fixing rhizobia in root nodules can convert nitrogen (N_2) in the air to NH_3, which is then incorporated into amino acids and protein, and stored in leaves and seeds. This explains why the edible leaves and seeds of legumes (or pulses) are rich in protein. Soybean and cowpea grain can contain up to 40% seed protein (Oso and Ashafa, 2021; Zarkadas et al., 2007; Dakora and Belane, 2019), common bean 20%–25% (Broughton et al., 2003; de Paiva Gouvêa et al., 2024), Bambara groundnut 20.6% (Hlanga et al., 2021; Mazahib et al., 2013), Kersting's bean 21.3% (Ikujenlola et al., 2022; Ayenan and Ezin, 2016), pigeon pea 27%–29% (Obala et al., 2020; Saxena and Singh, 1987) mungbean 21%–31% (Yi-Shen et al., 2018), chickpea 21.8%–25.8% (Xu et al., 2016), and groundnut 20%–30% (Toomer, 2018; Syed et al., 2021). Furthermore, cowpea can contain 34.9% protein in edible leaves and up to 40% in some genotypes (Dakora and Belane, 2019).

In addition to protein, the edible leaves and grain of legumes contain significant levels of nutritionally important minerals that are required for human nutrition and health, especially to combat trace element deficiency and promote brain development. The leaves and seeds of cowpea respectively contain 142–626 mg.kg^{-1} Fe, 49–104 mg.kg^{-1} Zn, 196–394 mg.kg^{-1} Mn, 8.6–19.7 mg.kg^{-1} Cu, and 42–55 mg.kg^{-1} B (Belane and Dakora, 2011). Groundnut grain also contained 22.6 mg.kg^{-1} Fe, 33.1 mg.kg^{-1} Zn, 6.7 mg.kg^{-1} Mn, and 7.5 mg.kg^{-1} Cu (Toomer, 2018), while chickpea seed is reported to have 500.0 mg.kg^{-1} Fe, 405.0 mg.kg^{-1} Zn, 480.0 mg.kg^{-1} Mn, and 85.0 mg.kg^{-1} Cu (Xu et al., 2016).

African soils are inherently low in nutrients and require fertilization for high crop yields. However, the high cost of chemical fertilizers and their damaging effect on the environment have prohibited their use. There is therefore a need to develop sustainable, green, and affordable technologies to increase crop yields and nutritional quality of grains for smallholder farmers in Africa. Furthermore, with climate change, there is a need to develop drought-tolerant groundnut genotypes that can produce economic yields even under low rainfall conditions. Carbon isotope discrimination during photosynthesis in C3 plants is known to correlate with water-use efficiency (WUE) and can therefore be used as a measure of plant WUE (Farquhar et al., 1989; Oteng-Frimpong and Dakora, 2018; Muhaba and Dakora, 2020; Mohammed et al., 2022; Pampa et al., 2023). Plants experiencing water limitation tend to show high WUE, measured as greater shoot $\delta^{13}C$ values. However, with adequate soil moisture, plants exhibit lower shoot $\delta^{13}C$ values due to increased discrimination against $^{13}CO_2$ (Cernusak et al., 2013). Carbon isotope discrimination has therefore been successfully employed to assess plant water relations in legumes and other C3 species (Belane et al., 2014; Mokgehle et al., 2014; Mapope and Dakora, 2016). The aim of this study was to evaluate groundnut genotypes for increased plant growth, high symbiotic performance, greater WUE, and increased grain mineral accumulation under field conditions using the ^{15}N and ^{13}C natural abundance techniques.

Materials and methods

Site description, experimental design, and treatments

The experiment was conducted during the rainy season of **2019** at Blesbokfontein farm, Bronkhorspruit, Mpumalanga, South Africa. The area is characterized by warmer temperatures and low rainfall with a unimodal rainy season that starts in October each year and ends in April the following year. The map reference coordinates were 25°19'0" S, 31°6'0" E, and the elevation was 199 m above sea level. The optimal rainfall ranged from 500 mm to 900 mm, with a temperature range of 17.5°C to 26°C per annum. Before planting, the soil at Blesbokfontein farm was classified as sandy loam. The experiment was laid out in a randomized complete block design with three replicates in a total of 90 plots. Seeds were sown on the flat with 80 cm between the rows and 10 cm within each row. Each plot size was 6 m^2 (3 ^{m} × 2 m). Each plot contained three rows with 30 plants per row. Soil samples were collected at a depth of 15–20cm and processed for chemical analysis prior to planting. The characteristics of the 30 groundnut genotypes use in the study are shown in Table 1.

Plant sampling and processing

Plant sampling for dry matter yield and the ^{15}N isotopic analysis were done at the flowering to early pod-filling stage. Three plants were randomly sampled from the central rows of each plot. Plant samples were carefully separated into shoots, roots, and nodules, and the nodule number was recorded. The shoots and nodules were oven-dried at 60°C to a constant weight (48 h). The dried shoots were ground to a fine powder (0.85 mm sieve) and stored in vials

TABLE 1 Agronomic characteristics of the groundnut genotypes used in the study.

Genotype name	Botanical type	Plant growth habit	Estimated maturity	Disease resistance	100 seed wt (g)	Seed coat color
12CS098	Spanish	Erect-bunch	100	Late leaf spot tolerance	35	Tan
12CS116	Spanish	Erect-bunch	100	Late leaf spot tolerance	31	Tan
CHINESE	Spanish	Erect	90	None	38	Tan
ICGV131051	Spanish	Erect	105	Early and late leaf spot tolerance	32.18	Brown
ICGV131065	Spanish	Erect	105	Early and late leaf spot tolerance	33.13	Tan
ICGV131090	Spanish	Erect	105	Early and late leaf spot tolerance	34.23	Tan
ICGV131091	Spanish	Erect	105	Early and late leaf spot tolerance	31.25	Tan
ICGV131096	Spanish	Erect	105	Early and late leaf spot tolerance	34.48	Tan
ICGV13842	Spanish	Erect	105	Early and late leaf spot tolerance	32.85	Tan
ICGV13848	Spanish	Erect	105	Early and late leaf spot tolerance	35.75	Tan
ICGV13851	Spanish	Erect	105	Early and late leaf spot tolerance	32.92	Brown
ICGV13864	Spanish	Erect	110	Early and late leaf spot tolerance	35.93	Tan
ICGV13876	Spanish	Erect	110	Early and late leaf spot tolerance	34.60	Tan
ICGV13910	Spanish	Erect	110	Early and late leaf spot tolerance	34.90	Tan
ICGV13937	Spanish	Erect	110	Early and late leaf spot tolerance	33.80	Deep tan
ICGV13950	Spanish	Erect	110	Early and late leaf spot tolerance	35.65	Tan
ICGV13984	Spanish	Erect	110	Early and late leaf spot tolerance	34.47	Tan
ICGV141088	Spanish	Erect	110	Early and late leaf spot tolerance	42.52	Tan
ICGV14849	Spanish	Erect	110	Early and late leaf spot tolerance	31.47	Tan
ICGV14857	Spanish	Erect	110	Early and late leaf spot tolerance	36.10	Brown
ICGV14876	Spanish	Erect	110	Early and late leaf spot tolerance	35.55	Tan
ICGV14877	Spanish	Erect	110	Early and late leaf spot tolerance	35.37	Deep tan
ICGV14880	Spanish	Erect	110	Early and late leaf spot tolerance	33.17	Tan
ICGV15028	Spanish	Semi-spreading	105	Early and late leaf spot tolerance	41.00	Deep tan
ICGV15033	Spanish	Semi-spreading	110	Early and late leaf spot tolerance	46	Tan

(Continued)

TABLE 1 Continued

Genotype name	Botanical type	Plant growth habit	Estimated maturity	Disease resistance	100 seed wt (g)	Seed coat color
ICGV86124	Spanish	Erect	105	Early and late leaf spot tolerance	37.00	Tan
IS-07273	Spanish	Bunch	120	Early and late leaf spot tolerance	46.65	Deep tan
IS-07947	Spanish	Semi-spreading	110	Early and late leaf spot tolerance	33.00	Deep tan
IS-13834	Spanish	Erect	90	Early and late leaf spot tolerance	34.6	Tan
YENYAWOSO	Spanish	Semi-erect	95	Rosette	46	Dark red

prior to 15N and 13C analysis. Nine non-leguminous weed species (namely, *Verbena hybrid* L., *Digitaria bicomis* L., *Commelina benghalensis* L., *Ruella tuberosa* L., *Brassica juncea* L., *Anisomelos indica* L., *Desmodium panicultum* L., *Asplenium nidu* L., and *Eleusine indica* L.) growing within the experimental plots were sampled as reference plants to determine the soil N uptake by the groundnut plants (Table 2).

Measurement of shoot N_2 fixation and C accumulation

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

The analyses of $^{15}N/^{14}N$ isotopic ratios were performed at the Stable Light Isotope Laboratory, University of Cape Town, Rondebosch, South Africa. For this, 2.0 mg of finely ground groundnut shoots and 2.5 mg of the reference plant samples were weighed into tin aluminum capsules and loaded onto a Thermo 2000 Elemental Analyzer coupled with a Thermo Conflo IV Plus stable light isotope mass spectrometer (Thermo Corporation, Bremen Germany). Samples were combusted in an evacuated quartz tube and analyzed to determine the ratio of $^{15}N/^{14}N$ and the N concentration (%N) in the plant material. An internal standard of *Nasturtium* spp. was included after every five runs of the plant samples to correct for machine errors during the isotopic fractionation. The results were normalized against in-house reference material and reported relative to an international standard (N in air). The $^{15}N/^{14}N$ ratio was used to calculate the isotopic composition (δN), as in Mariotti et al. (1981):

$$\delta^{15}N = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{atm}}{(^{15}N/^{14}N)_{atm}} \times 1000$$

Where $^{15}N/^{14}N_{sample}$ is the abundance ratio of ^{15}N and ^{14}N in the sample and $^{15}N/^{14}N_{atm}$ is the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

Shoot N content

The N content of the shoots was calculated as the product of shoot %N and shoot dry matter, using the method of Pausch et al. (1996), where %N was obtained directly from the mass spectrometer: $N_{content}$ = $\%N_{shoot}$ x dry mass $_{shoot}$.

The B-value

The ^{15}N natural abundance technique was used for N fixation analyses. It is based on the principle that an effectively nodulated legume growing on a medium free from combined N (mineral N or organic N) is expected to be completely reliant upon symbiotic N_2 fixation for its growth, hence, the isotopic composition of the legume would be expected to be similar to that of atmospheric N_2. A legume's $\delta^{15}N$ value, when grown in soil containing mineral N, is expected to resemble that of the soil mineral N (Unkovich et al., 2008). Different non-legume plant species were collected from the experimental plots and used for the $\delta^{15}N$ analyses and a B-value of -2.70 ‰ (Nyemba & Dakora, 2010) was used for the calculations.

Percent N derived from atmospheric fixation

The proportion of N derived from the atmospheric N_2 fixation (%Ndfa) was estimated as in Shearer and Kohl (1986):

$$\%\,Ndfa = \frac{\delta^{15}N_{ref} - \delta^{15}N_{leg}}{\delta^{15}N_{ref} - Bvalue} \times 100$$

Where $\delta^{15}N_{ref}$ is the ^{15}N natural abundance of the reference plant, $\delta^{15}N_{leg}$ is the ^{15}N natural abundance of the legume, and the B-value is the ^{15}N natural abundance of groundnut plants deriving all their N nutrition from N_2 fixation.

Amount of N-fixed

The amount of fixed N (N-fixed) by the groundnut plant was calculated as in Unkovich et al. (2008): N-fixed = %Ndfa x legume biomass N

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

Soil N uptake

The soil N uptake by the groundnut plant was calculated as in Unkovich et al. (2008):

Soil N uptake = (100 - %Ndfa) x legume biomass N

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

Sub-samples of the ground shoots were analyzed at the Stable Light Isotope Laboratory, University of Cape Town, Rondebosch, South Africa as described above for N. The results were normalized and reported relative to Pee Dee Belemnite (PDB). The C concentration (%C) was also concurrently determined following combustion of the sample. The ratio of $^{13}C/^{12}C$ in each shoot sample was used to calculate $\delta^{13}C$ (Mohale et al., 2014):

$$\delta^{13}C = [\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1] \times 100$$

Where $(^{13}C/^{12}C)_{sample}$ is the isotopic ratio of the sample and $(^{13}C/^{12}C)_{standard}$ is the isotopic ratio of PDB (Craig, 1957).

C content

The C content of each genotype was calculated as the product of %C and the shoot dry mass (biomass) of each plant.

Measurement of nutrient accumulation in groundnut grain

To measure the mineral elements P, K, Ca, Mg, Cu, Zn, Mn, Fe, and B in groundnut grain, 1 g of ground sample was ashed in a porcelain crucible at 500°C overnight. This was followed by dissolving the ash in 5 ml 6 M HCl (analytical grade) and placing it in an oven at 50°C for 30 min, after which 35 ml de-ionized water was added. The mixture was filtered through Whatman No. 1 filter paper. The mineral element concentration in the plant extracts was determined from three replicate samples using inductively coupled plasma mass spectrometry (ICP-MS) (Ataro et al., 2008).

TABLE 2 Reference plants used for estimating $\delta^{15}N$ (‰).

Latin name	$\delta^{15}N$ (‰)
Verbena hybrid L	+1.887119
Digitaria bicornis L	+320247
Commelina benghalensis L	+5.982533
Ruella tuberosa L	+4.745336
Brassica juncea L	+3.83671
Anisomelos indica L	+3.892596
Desmodium panicultum L	+4.311906
Asplenium nidu L	+438764
Eleusine indica L	+4.380219
Average	+3.98

Data analysis

The data were subjected to analysis of variance (ANOVA) to compare the means of the treatments using the STATISTICA program (version 10) and GenStat 11th edition. Where there were differences, Duncan's multiple range test was used to separate the means at $p \leq 0.05$. Correlation analysis was performed to ascertain if any relationship existed between symbiotic parameters (e.g. nodule number, N-fixed, and %N) and mineral nutrient concentrations in grain/shoots.

Results

Soil analysis

The physico-chemical properties of soil samples collected before planting were measured. The soil contained 0.070% N, 39 mg $P.kg^{-1}$, 196 mg $K.kg^{-1}$, 117 mg $Ca.kg^{-1}$, 60 mg $Mg.kg^{-1}$, 91.35 mg $Fe.kg^{-1}$, 1.20 mg $Cu.kg^{-1}$, 34.18 mg $Mn.kg^{-1}$, 5 mg $Na.kg^{-1}$, and 3.18 mg $Zn.kg^{-1}$.

Shoot $\delta^{15}N$ of the reference plants

Nine non-legume reference plant species were sampled and analyzed to calculate the %Ndfa of the groundnut genotypes. The $\delta^{15}N$ values of the reference plants ranged from +1.89‰ to +5.98‰ with a combined mean of +3.98‰ (Table 3).

Plant growth, nodulation, and grain yield

A one-way ANOVA revealed significant differences in plant growth and root nodulation between and among the 30 groundnut genotypes (Table 2). Genotype IS-07273 produced higher nodule numbers and high dry matter. The shoot DM ranged from 24 $g.plant^{-1}$ in genotypes 12CS116 and CHINESE to 53 $g.plant^{-1}$ in genotype IS-07273. Nineteen of the 30 groundnut genotypes produced significantly more dry matter compared to 12CS116 and CHINESE, which recorded the lowest biomass together with genotype IS-07273. The other genotypes with greater nodulation included IS-07947, ICGV13864, ICGV13910, ICGV13851, ICGV13842, and 12CS116. Furthermore, 28 of the 30 groundnut genotypes produced high nodule numbers (Table 2). Due to poor rainfall, grain yield was generally low among the 30 groundnut genotypes, with ICGV131096, ICGV13950, ICGV14877, ICGV14857, ICGV14876, ICGV13848, and ICGV13876

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TABLE 3 Plant growth, symbiotic performance, and grain yield of groundnut genotypes planted in the field at Blesbokfontein, South Africa, in 2019. Values with dissimilar letters in a column are significantly different at $P \leq 0.05$.

Genotype	Shoot DM	Nodule no	Shoot N	N content	$\delta^{15}N$	Ndfa	N-fixed	SN uptake	Grain yield
	$g.plant^{-1}$	Per plant	%	$mg.plant^{-1}$	‰	%	$kg.ha^{-1}$	$kg.ha^{-1}$	$kg.ha^{-1}$
12CS116	24.44 ± 2.7d	63 ± 8.8bc	1.98 ± 0.08abc	490 ± 0.06b	+2.43 ± 0.08ab	40.86 ± 0.96ab	25.51 ± 54b	36.17 ± 4.60b	236.8 ± 5a-d
12CS098	28.44 ± 3.9cd	53 ± 5.1bc	2.00 ± 0.04abc	570 ± 0.08ab	+2.53 ± 0.07ab	39.73 ± 0.88ab	28.47 ± 4.13b	43.13 ± 6.43ab	284.1 ± 5a-d
CHINESE	24.00 ± 3d	54 ± 7.0bc	1.93 ± 0.08bc	450 ± 0.05b	+2.66 ± 0.06ab	38.24 ± 0.78ab	21.26 ± 2.30b	34.64 ± 4.26b	330.3 ± 6.2a-d
YENYAWO	31.55 ± 3.9b-d	57 ± 8.5bc	2.16 ± 0.11ab	660 ± 0.08ab	+2.24 ± 0.06ab	43.07 ± 0.54ab	35.63 ± 4.30ab	47.11 ± 5.54ab	219.2 ± 1.6cd
IS-07273	52.55 ± 8.9a	102 ± 4.5a	2.03 ± 0.09ab	1100 ± 0.24a	+2.32 ± 0.08ab	42.21 ± 0.91ab	58.07 ± 2.26a	80.64 ± 7.34a	194.3 ± 3.8d
IS-07947	25.00 ± 4.8d	77 ± 10.9b	1.83 ± 0.09bc	460 ± 0.07b	+2.38 ± 0.14ab	41.44 ± 1.56ab	22.68 ± 4.02b	31.77 ± 5.33b	305.3 ± 5.4a-d
IS-13834	36.44 ± 4.3b-d	61 ± 7.5bc	1.96 ± 0.07bc	690 ± 0.06ab	+2.45 ± 0.17ab	40.67 ± 1.92ab	35.91 ± 3.74ab	51.16 ± 4.63ab	348.2 ± 6.8abc
ICGV13842	45.33 ± 5.9abc	64 ± 8.3bc	1.84 ± 0.05bc	850 ± 0.13ab	+2.40 ± 0.12ab	41.23 ± 1.36ab	44.01 ± 6.46ab	62.75 ± 5.58ab	274.3 ± 2a-d
ICGV13848	30.44 ± 5.7b-d	55 ± 7.8bc	1.77 ± 0.04bc	540 ± 0.09b	+2.48 ± 0.19ab	40.38 ± 2.19ab	27.57 ± 5.46b	39.52 ± 6.91ab	358.4 ± 3.8abc
ICGV13851	36.00 ± 6.3b-d	65 ± 8.0bc	2.03 ± 0.06ab	740 ± 0.14ab	+2.60 ± 0.11ab	38.93 ± 1.22ab	35.93 ± 6.79ab	56.58 ± 4.58ab	266.3 ± 2.7a-d
ICGV13864	45.11 ± 5.7abc	68 ± 7.2bc	2.03 ± 0.08ab	920 ± 0.12ab	+2.68 ± 0.10ab	38.08 ± 1.19ab	42.8 ± 5.43ab	71.76 ± 5.09ab	299.8 ± 2.9a-d
ICGV13876	333 ± 4.9b-d	51 ± 4.6bc	1.87 ± 0.05bc	610 ± 0.07ab	+2.45 ± 0.09ab	40.73 ± 1.06ab	30.72 ± 54ab	45.24 ± 6.08ab	348.5 ± 5abc
ICGV13910	45.55 ± 6.0ab	65 ± 6.9bc	1.89 ± 0.14bc	820 ± 0.08ab	+2.64 ± 0.17ab	38.54 ± 1.96ab	38.82 ± 26ab	64.17 ± 7.93ab	285.3 ± 4.7ab
ICGV13937	29.77 ± 3.1b-d	52 ± 4.5bc	1.86 ± 0.05bc	550 ± 0.05b	+2.28 ± 0.17ab	42.64 ± 1.91ab	28.82 ± 2.29b	39.74 ± 4.67ab	222.6 ± 5.4cd
ICGV13950	27.44 ± 5.4d	55 ± 8.6bc	1.91 ± 0.06bc	520 ± 0.11b	+2.33 ± 0.14ab	42.01 ± 1.56ab	26.63 ± 4.53b	38.73 ± 8.86ab	385.8 ± 4.7ab
ICGV13984	27.56 ± 3.9d	51 ± 8.4bc	1.77 ± 0.06bc	480 ± 0.06b	+2.09 ± 0.15b	44.82 ± 1.71a	26.79 ± 38b	32.3 ± 4.47b	377.7 ± 6.4ab
ICGV14849	34.88 ± 4.3b-d	56 ± 8.1bc	2.38 ± 0.11a	820 ± 0.10ab	+2.42 ± 0.19ab	40.99 ± 2.16ab	40.65 ± 4.33ab	61.50 ± 9.09ab	281.1 ± 3.8a-d
ICGV14857	37.11 ± 4.9a-d	51 ± 7.6bc	2.11 ± 0.06ab	770 ± 0.09ab	+2.68 ± 0.12ab	38.02 ± 1.34ab	37.06 ± 4.78ab	59.91 ± 6.81ab	379.8 ± 51.7ab
ICGV14876	34.67 ± 5.2b-d	48 ± 8.5c	1.88 ± 0.06bc	640 ± 0.09ab	+2.32 ± 0.12ab	42.15 ± 1.43ab	33.83 ± 4.97ab	46.68 ± 6.56ab	377.5 ± 4.8ab
ICGV14877	36.78 ± 5.5a-d	57 ± 9.6bc	2.01 ± 0.07abc	740 ± 0.12ab	+2.49 ± 0.07ab	40.25 ± 0.79ab	37.64 ± 6.35ab	55.72 ± 9.15ab	383.6 ± 7.8ab
ICGV14880	44.67 ± 8.3abc	61 ± 9.9bc	1.90 ± 0.08bc	860 ± 0.18ab	+2.39 ± 0.12ab	41.33 ± 1.39ab	44.64 ± 9.55ab	63.82 ± 4.34ab	357.4 ± 5.6bc
ICGV15028	30.88 ± 4.8b-d	58 ± 6.4bc	1.57 ± 0.07c	490 ± 0.08b	+2.19 ± 0.06ab	43.62 ± 0.78ab	26.94 ± 4.55b	34.32 ± 5.53b	318.1 ± 6.6a-d
ICGV15033	31.33 ± 4.9b-d	58 ± 6.7bc	1.83 ± 0.09bc	580 ± 0.09ab	+2.37 ± 0.11ab	41.58 ± 1.23ab	29.52 ± 4.74b	42.83 ± 7.55ab	193.6 ± 6.6d
ICGV86124	28.88 ± 3b-d	54 ± 7.2bc	1.88 ± 0.07bc	540 ± 0.06b	+2.51 ± 0.11ab	40.05 ± 1.29ab	27.33 ± 53b	40.38 ± 4.75ab	308.9 ± 4.4a-d
ICGV131051	27.56 ± 3.6b-d	54 ± 9.1bc	1.87 ± 0.06bc	520 ± 0.06b	+2.76 ± 0.09a	37.14 ± 1.03b	24.15 ± 53b	40.65 ± 5.32ab	255.7 ± 2.8a-d

(Continued)

TABLE 3 Continued

Genotype	Shoot DM	Nodule no	Shoot N	N content	$\delta^{15}N$	Ndfa	N-fixed	SN uptake	Grain yield
	$g.plant^{-1}$	Per plant	%	$mg.plant^{-1}$	‰	%	$kg.ha^{-1}$	$kg.ha^{-1}$	$kg.ha^{-1}$
ICGV131065	26.67 ± 2.4d	61 ± 8.5bc	1.92 ± 0.11bc	500 ± 0.05b	+2.46 ± 0.15ab	40.58 ± 1.67ab	25.43 ± 2.28b	37.46 ± 4.05b	310.6 ± 5.5a-d
ICGV131090	30.22 ± 3.8b-d	62 ± 8.5bc	1.75 ± 0.07bc	530 ± 0.07b	+2.18 ± 0.13ab	43.77 ± 1.47ab	29.57 ± 4.57b	36.66 ± 4.42b	351.9 ± 5.8abc
ICGV131091	30.22 ± 2.9b-d	45 ± 5.8c	1.89 ± 0.07bc	570 ± 0.06ab	+2.38 ± 0.13ab	41.45 ± 0.74ab	29.44 ± 2.96b	41.79 ± 4.41ab	346.6 ± 31.9abc
ICGV131096	26.00 ± 5d	56 ± 7.7bc	1.97 ± 0.08abc	510 ± 0.06b	+2.40 ± 0.10ab	41.24 ± 1.18ab	26.30 ± 43b	36.89 ± 4.12b	392 ± 5.2a
ICGV141088	38.88 ± 3.6a-d	67 ± 4.7bc	1.84 ± 0.06bc	720 ± 0.07ab	+2.33 ± 0.08ab	42.09 ± 1.00ab	37.87 ± 3.92ab	51.71 ± 4.80ab	338.2 ± 4.7a-d
F-staitstics	*2.20****	*1.67**	*36****	*2.71****	*1.74**	*1.74**	*2.56****	*2.73****	*1.87***
CV	*44.1*	*41.1*	*12.3*	*46.5*	*14.8*	*10.1*	*46.5*	*48.2*	*41.5*

Means (±S.E.M) followed dissimilar letters in a column are significantly different at *$p \leq 0.05$, ** $p \leq 0.01$, and ***$p \leq 0.001$. Where CV=coefficient of variation.

producing relatively higher yields (Table 2). Genotypes ICGV15033, IS-07273, and YENYAWOSO recorded the lowest grain yields (Table 2).

N concentration and N content

The N concentration and N content differed between and among the 30 groundnut genotypes, with ICGV14849 showing the highest N concentration (2.3%), followed by genotypes YENYAWOSO, ICGV14857, ICGV13851, ICGV13864, IS-07273,12CS098, and ICGV14877. In contrast, genotypes ICGV15028 and ICGV131090 exhibited the lowest N concentrations (1.**57%** and 1.75**%, r**espectively; Table 2). Shoot N content was highest in genotype IS-07273 (which recorded the highest dry matter of 1,100 $mg.plant^{-1}$), followed by genotypes ICGV13864, ICGV14880, and ICGV13842, and lowest in genotypes CHINESE and IS-07947 (Table 2). The shoot N concentration ranged from 1.75% to 2.3%, and shoot N content from 450 to 1,100 $mg.plant^{-1}$ (Table 2).

$\delta^{15}N$ and %Ndfa values of groundnut plants

The $\delta^{15}N$ values of groundnut genotypes ranged from +2.09‰ in genotype ICGV13984 to +2.76‰ in genotype ICGV131051 (Table 2). The shoot $\delta^{15}N$ of all the groundnut genotypes were statistically similar except for genotype ICGV131051. The genotypes with much lower $\delta^{15}N$ values (i.e. ICGV13984, ICGV131090, ICGV15028, and YENYAWOSO) derived the most N from atmospheric N_2 fixation, while those with the highest $\delta^{15}N$ values (i.e. genotypes CHINESE, ICGV14857, and 12CS098) recorded the lowest %Ndfa (Table 2). Of the 30 groundnut genotypes tested, 23 derived 40% or more of their N nutrition from symbiotic fixation, but none obtained up to 50% of its N nutrition from symbiosis.

Amount of fixed N

The amount of N-fixed by groundnut ranged from 21 $kg.ha^{-1}$ for genotype CHINESE to 58 $kg.ha^{-1}$ for IS-07273 (the highest fixer), which was followed by genotypes ICGV14880, ICGV13842, ICGV13864, and ICGV14849 with 44.64, 44.01, 42.8, and 40.65 kg N-fixed.ha^{-1}, respectively (Table 2). The genotypes that produced the most symbiotic N recorded the largest biomass, while genotypes CHINESE, 12CS116, ICGV131096, ICGV131065, and ICGV131051, which contributed the least N, produced the lowest biomass (Table 2). Moreover, 14 of the 30 groundnut genotypes (namely, YENYAWOSO, IS-07273, IS-13834, ICGV13842, ICGV13851, ICGV13864, ICGV13876, ICGV13910, ICGV14849, ICGV14857, ICGV14876, ICGV14877, ICGV14880, and ICGV141088) contributed over 30 kg N.ha^{-1} from symbiotic N_2 fixation (Table 2).

Soil N uptake

The 30 groundnut genotypes differed markedly in their levels of soil N uptake. A comparison of the source of N utilized by the groundnut genotypes showed that 11 of the 30 genotypes (namely, IS-07273, IS-13834, ICGV13842, ICGV13851, ICGV13864, ICG13910, ICGV14849, ICG14857, ICGV14877, ICGV14880, and ICGV141088) obtained more N from the soil than from symbiotic fixation for their N nutrition (Table 2).

Carbon concentration and C content

Genotypes ICGV14849, ICGV131051, ICGV86124, ICGV13937, ICGV13864, and 12CS098 showed the highest shoot C concentration, with genotypes ICGV15033, ICGV131090, and ICGV131065 having the lowest. Genotypes IS-07273, ICGV13864, ICGV14880, and ICGV13910 recorded the highest C content per plant due to their greater shoot biomass (Table 4).

Shoot C:N ratio and $\delta^{13}C$

The C/N ratio of groundnut shoots was highest in genotypes ICGV14876, ICGV131051, ICGV86124, ICGV15033, ICGV15028, ICGV13950, ICGV13937, ICGV13848, ICGV13842, and IS-07947, and lowest in YENYAWOSO, IS-13834, ICGV14849, and ICGV13851 (Table 4). Shoot $\delta^{13}C$ values were much greater in genotypes ICGV13851, YENYAWOSO, and ICGV13950, followed by ICGV131090 and ICGV131091 (-25.58 to -25.63‰). Furthermore, 24 of the 30 groundnut genotypes recorded similar shoot $\delta^{13}C$ values, which ranged from -25.99‰ to -25.46‰.

Protein content and macronutrient and micronutrient concentrations in groundnut grain

The levels of P, K, Ca, N, Mg, and S in the grain differed significantly among and between the groundnut genotypes (Table 5), with 10 of the 30 groundnut genotypes (namely, IS-07273, IS-07947, 12CS116, ICGV13842, ICGV14876, ICGV14877, ICGV86124, ICGV131051, ICGV131090, and ICGV131096) recording more than 0.50% P. Genotypes ICGV1315, ICGV13910, IS-07273, and ICGV15028 accumulated more than 1% K in groundnut grain. Genotypes IS-07273, ICGV13910, and ICGV 86124 also showed a high concentration of Mg in their grain (Table 5). The protein content in groundnut grain ranged from 20.64% in genotype ICGV131090 to 29.82% in cultivar ICGV13937 (Table 5). The N concentration in groundnut grain was greater in cultivars ICGV13937, 12CS098, ICGV14857, ICGV131091, ICGV13842, and CHINESE, and lowest in ICGV131090 (Table 4). The Ca and S concentrations were higher in cultivars ICGV1315 and ICGV131091 (Table 5). The highest concentration of Na in groundnut grain was in genotype 12CS098, followed by ICGV13910, ICGV13987, and ICGV14877, and the lowest in genotypes ICGV14880, ICGV15028, ICGV1315, and IS-07273 (Table 5). The trace elements in groundnut grain differed significantly between and among the 30 groundnut genotypes (Table 5). The Fe concentration in groundnut grain was highest in genotype 12CS116, followed by IS-13834, 12CS098, CHINESE, ICGV1315, and IS-07273 and lowest in ICGV131051, ICGV131065, ICGV131096, and ICGV141088 (Table 5). The concentration of Cu was also highest in genotypes IS-07273, ICGV13910, ICGV86124, ICGV15028, and IS-07947 and lowest in ICGV13851, ICGV1315, and ICGV13987 (Table 5). Seed Zn concentration of the groundnut grain was significantly higher in cultivar YENYAWOSO, followed by ICGV13842 and 12CS116, and lowest in ICGV1315, ICGV13987, and ICGV14849 (Table 5). The Mn concentration in groundnut grain was highest in genotype ICGV14876, followed by ICGV13848, CHINESE, and ICGV13876, and lowest in ICGV1315, ICGV15033, and ICGV15028 (Table 5). The concentration of B was relatively higher in cultivars IS-07273, IS-07947, and ICGV15028 (Table 5).

Discussion

Low soil N fertility is a major factor affecting agricultural yields in sub-Saharan Africa. The selection and incorporation of symbiotically superior grain legumes into cropping systems can improve soil N fertility and increase crop yields. In this study, the ^{15}N and ^{13}C natural abundance technique was used to evaluate N contribution and plant water relations of 30 groundnut genotypes grown at Bleskbokfontein farm, near Bronkhorspruit in Mpumalanga, South Africa. The results revealed differences in plant growth, symbiotic performance, grain mineral accumulation, and shoot $\delta^{13}C$ (Mohammed et al., 2021, 2022; Ngwenya et al., 2024; Samago and Dakora, 2024). There were also differences in root nodulation among the groundnut genotypes. For example, genotype IS-07947 produced greater shoot biomass from increased nodulation, which resulted in higher N concentration and N content and a greater amount of N-fixed (Table 2). In contrast, genotypes 12CS116 and CHINESE performed poorly in terms of plant growth, N content, and amount of N-fixed, indicating genotypic differences in plant growth and symbiotic performance among the 30 groundnut genotypes. However, these differences could also be attributed to erratic rainfall and intermittent drought (Table 2). Furthermore, 22 of the 30 groundnut cultivars exhibited greater biomass accumulation. As a result, the N level was higher in 26 of the 30 genotypes (Table 2). Possibly due to the poor rainfall, all 30 genotypes (except for ICGV13984) showed markedly higher shoot $\delta^{15}N$ values, and hence lower %Ndfa values. Genotype IS-07273 was an exception as it obtained the most N from symbiosis and still took up higher amounts of soil N (Table 2). Moreover, 17 of the 30 groundnut genotypes produced a grain yield of around 300 kg ha^{-1} (Table 2), which was quite low due to poor rainfall. The low grain yield and impaired symbiotic performance found in this study contrast with the findings by Mokgehle et al. (2014) where rainfall was optimal.

The shoot concentration of C in plants is directly linked to the photosynthetic activity in leaves. In this study, the C concentration

TABLE 4 Comparison of dry matter yield, %C, C content, C/N ratio, $\delta^{13}C$, and grain yield of groundnut genotypes planted in the field at Blesbokfontein, South Africa, in 2019.

Genotype	Shoot DM	C	C content	$\delta^{13}C$	C:N ratio
	$g.plant^{-1}$	%	$g.plant^{-1}$	‰	$g.g^{-1}$
12CS116	24.44 ± 2,7d	38.20 ± 1,27ab	940.53 ± 111,64b	-25.87 ± 0,14abc	19.38 ± 0,44a-d
12CS098	28.44 ± 3.9cd	39.08 ± 0,51ab	1118.16 ± 162,68ab	-25.74 ± 0,17abc	19.57 ± 0,37a-d
CHINESE	24,00 ± 3d	37.27 ± 0,73ab	891.73 ± 117,11b	-25.97 ± 0,12abc	19.60 ± 0,87a-d
YENYAWO	31.55 ± 3.9b-d	37.01 ± 1,99ab	1131.18 ± 127,66ab	-25.51 ± 0,18a	17.17 ± 0,28d
IS-07273	52.55 ± 8,9a	38.43 ± 0,39ab	2019.09 ± 346,04a	-26.23 ± 0,16abc	19.28 ± 0,88a-d
IS-07947	25,00 ± 4,8d	37.24 ± 1,29ab	912.81 ± 166,96b	-26.36 ± 0,23abc	20.58 ± 0,62a-d
IS-13834	36.44 ± 4,3b-d	36.95 ± 1,34ab	1309.31 ± 122,33ab	-25.58 ± 0,10abc	18.55 ± 0,41a-d
ICGV13842	45.33 ± 5,9abc	37.46 ± 0,87ab	1701.92 ± 239,27ab	-25.81 ± 0,19abc	20.45 ± 0,64a-d
ICGV13848	30.44 ± 5,7b-d	37.00 ± 0,91ab	1146.85 ± 234,66ab	-25.55 ± 0,19ab	20.53 ± 0,87a-d
ICGV13851	36,00 ± 6,3b-d	38.51 ± 1,06ab	1404.37 ± 259,83ab	-25.46 ± 0,09a	18.78 ± 0,45a-d
ICGV13864	45.11 ± 5,7abc	39.19 ± 0,39ab	1758.95 ± 216,79ab	-25.81 ± 0,19abc	19.52 ± 0,75a-d
ICGV13876	333 ± 4,9b-d	36.80 ± 1,19ab	1187.32 ± 137,58ab	-25.67 ± 0,09abc	19.55 ± 0,47a-d
ICGV13910	45.55 ± 6,0ab	36.66 ± 1,23ab	1627.84 ± 170,74ab	-25.77 ± 0,24abc	19.98 ± 0,87a-d
ICGV13937	29.77 ± 3,1b-d	39.35 ± 0,31ab	1176.60 ± 128,70ab	-25.99 ± 0,11abc	21.29 ± 0,53ab
ICGV13950	27.44 ± 5,4d	38.22 ± 0,79ab	1050.40 ± 209,74ab	-25.65 ± 0,21abc	20.13 ± 0,40a-d
ICGV13984	27.56 ± 3,9d	38.49 ± 0,49ab	1056.51 ± 143,56ab	-25.99 ± 0,17abc	21.91 ± 0,55a-d
ICGV14849	34.88 ± 4,3b-d	40.58 ± 0,74a	1402.58 ± 166,60ab	-25.96 ± 0,24abc	17.33 ± 0,71cd
ICGV14857	37.11 ± 4,9a-d	38.38 ± 0,99ab	1403.74 ± 158,59ab	-25.75 ± 0,13abc	18.20 ± 0,36bcd
ICGV14876	34.67 ± 5,2b-d	37.76 ± 0,51ab	1314.56 ± 205,70ab	-26.26 ± 0,12abc	22.01 ± 0,99a
ICGV14877	36.78 ± 5,5a-d	38.36 ± 0,64ab	13957 ± 197,74ab	-25.62 ± 0,20abc	19.30 ± 0,66a-d
ICGV14880	44.67 ± 8,3abc	37.86 ± 1,28ab	1699.98 ± 332,76ab	-25.96 ± 0,16abc	20.08 ± 0,69a-d
ICGV15028	30.88 ± 4,8b-d	36.53 ± 1,18ab	1142.02 ± 190,02ab	-26.51 ± 0,09bc	21.50 ± 0,63ab
ICGV15033	31.33 ± 4,9b-d	34.63 ± 2,17b	10925 ± 187,32ab	-25.58 ± 0,33abc	20.99 ± 1,19a-d
ICGV86124	28.88 ± 3,3b-d	40.24 ± 0,47ab	1154.66 ± 126,48ab	-26.05 ± 0,13abc	21.64 ± 0,74ab
ICGV131051	27.56 ± 3,6b-d	40.29 ± 0,41ab	1110.98 ± 142,36ab	-26.56 ± 0,29bc	21.66 ± 0,64ab
ICGV131065	26.67 ± 2,4d	36.41 ± 1,59ab	966.44 ± 95,36b	-25.58 ± 0,13abc	20.25 ± 0,76a-d
ICGV131090	30.22 ± 3,8b-d	35.66 ± 1,77ab	1049.85 ± 114,94ab	-25.63 ± 0,17abc	20.47 ± 1,05a-d
ICGV131091	30.22 ± 2,9b-d	36.99 ± 0,84ab	11133 ± 106,48ab	-25.77 ± 0,20abc	19.78 ± 0,68a-d
ICGV131096	26,00 ± 3,5d	38.73 ± 0,44ab	999.88 ± 128,32b	-26.19 ± 0,14abc	19.72 ± 0,52a-d
ICGV141088	38.88 ± 3,6a-d	38.31 ± 0,91ab	1489.20 ± 138,11ab	-25.92 ± 0,10abc	20.86 ± 0,43a-d
F-statistic	*2,20****	*1,59**	*2,34****	*2,70****	*3,24****
CV	*44.1*	*8.5*	*43.7*	*2.1*	*10.3*

Values with dissimilar letters in a column are significantly different at P ≤ 0.05.
Means (±S.E.M) followed dissimilar letters in a column are significantly different at *p≤ 0.05, and ***p≤0.001. Where CV=coefficient of variation.

of groundnut shoots ranged from 34.6% in genotype ICGV15033 to 40.6% in ICGV14849 (Table 4), levels similar to those reported by Sprent et al. (1996) for nodulated legumes. Although genotypes ICGV86124, ICGV14849, ICGV131065, ICGV13937, and 12CS098 exhibited the highest C concentrations, they did not produce the highest C content due to very low dry matter (Table 4). The groundnut genotypes that produced the most C content also recorded the highest biomass (Table 4), which was consistent with previous reports (Oteng-Frimpong and Dakora, 2019; Taiz and Zeiger, 2002).

TABLE 5 Mineral accumulation in the grain of field-grown groundnut genotypes planted at Bronkhorspruit, South Africa, in 2019.

Genotype	N	Protein	P	K	Ca	Mg	S	Na	Fe	Cu	Zn	Mn	B	Al
	%	%	%	%	%	%	%	mg.kg^{-1}	mg.kg^{-1}	mg.kg^{-1}	mg.kg^{-1}	mg.kg^{-1}	mg.kg^{-1}	mg.kg^{-1}
12CS116	4.668abc	25.49abc	0.523ab	0.783n	0.05b	0.24efg	0.16e-h	41.67ab	49.67a	6.977ab	48.96abc	22.49d-j	18.72c-j	47.67b
12CS098	5.031ab	27.47ab	0.486abc	0.81mn	0.05b	0.21i	0.18abc	85a	47.96a	5.963ab	42.29hij	23.08c-g	17.52k-n	76ab
CHINESE	4.995ab	27.27ab	0.47abc	0.87i-m	0.05b	0.23gh	0.16e-h	39.67b	452a	8.39b	45.41c-h	24.76abc	18.11g-l	19.33d
YENYAWOSO	4.723abc	25.79abc	0.493abc	0.96def	0.05b	0.25c-f	0.15gh	37.33b	39.2a	6.707ab	52.38a	22.39e-j	19.5bcd	35b
IS-07273	4.403bcd	24.04bcd	0.54a	1.01cd	0.05b	0.26a	0.19a	32.67b	38.17a	10.683a	48.53bc	21.06h-l	24.18a	24.67cd
IS-07947	4.776abc	26.08abc	0.503abc	0.961def	0.04b	0.25a-e	0.17bcd	49ab	323a	9.5b	43.78f-j	19mno	23.89a	24.67cd
IS-13834	4.89ab	26.7ab	0.476abc	0.973de	0.05b	0.26abc	0.16e-h	333b	48.51a	5.797ab	46.64c-g	19.4lmn	17.67j-n	9.47e
ICGV13842	4.949ab	27.02ab	0.506abc	0.773n	0.05b	0.24efg	0.17b-f	44.67ab	36.86a	8.37b	50.9ab	23.1c-f	18.06h-m	95a
ICGV13848	4.913ab	26.83ab	0.486abc	0.87i-m	0.05b	0.25b-f	0.18abc	42.67ab	35.93a	6.093ab	431g-j	25.27ab	18.39e-l	12.33d
ICGV13851	4.859abc	26.53abc	0.466abc	0.95d-h	0.05b	0.26a	0.17b-f	35.33b	33.72a	3.67bc	45.48c-h	21.11h-l	16.65n	8.7d
ICGV13864	4.603a-d	25.13a-d	0.493abc	0.93e-i	0.05b	0.26a	0.16d-h	48ab	33.65a	8.03ab	48.38bcd	20.75i-m	17.02mn	12d
ICGV13876	4.841abc	26.43abc	0.446abc	0.926e-i	0.04b	0.24c-f	0.17b-f	39b	32a	6.34ab	46.52c-g	24.29a-d	18.25g-l	13.67d
ICGV13910	4.496bcd	24.55bcd	0.491abc	1.0867b	0.04b	0.26a	0.18abc	633ab	33.17a	10.887a	45.23c-h	22.71f-k	19.11c-h	17.33d
ICGV13937	5.462a	29.82a	0.466abc	0.82lmn	0.05b	0.24def	0.17b-f	533ab	30.88a	4.543bc	41.74hij	21.92e-k	18.82c-j	25.67cd
ICGV13950	4.552bcd	24.86bcd	0.443abc	0.81mn	0.05b	0.22gh	0.16c-g	32.33b	31.48a	4.127ab	42.16hij	21.36f-k	18.68d-j	14d
ICGV13984	4.766abc	26.02abc	0.446abc	0.96d-g	0.05b	0.25b-f	0.16e-h	49ab	31.35a	7.067ab	42.75hij	21.28g-k	18.16g-l	18.33d
ICGV14849	4.554bcd	24.86bcd	0.416bc	0.80mn	0.05b	0.25a-e	0.18abc	32.33b	30.67a	7.597ab	41.43ij	22.3e-j	17.95i-m	12.67d
ICGV14857	5.014ab	27.38ab	0.496abc	0.91e-j	0.05b	0.26a	0.18abc	40.33ab	30.55a	10.28ab	44.67d-j	20.24k-n	17.68j-n	56.67ab
ICGV14876	4.537bcd	24.77bcd	0.521ab	0.91e-j	0.05b	0.26a	0.18ab	44ab	30.1a	6.053ab	48.83abc	26.02a	19.78bc	18.67d
ICGV14877	4.814abc	26.29abc	0.523ab	0.82k-n	0.05b	0.25b-f	0.17b-e	533ab	29.91a	7.463ab	47.15b-f	22.86d-h	18.32f-l	9.7e
ICGV14880	4.262bcd	227bcd	0.49abc	0.81mn	0.04b	0.25b-f	0.16e-h	30.67b	29.75a	7.043ab	47.92b-e	243cde	18.5d-k	19d
ICGV15028	4.924ab	26.89ab	0.486abc	1.076bc	0.04b	0.21hi	0.15h	30.67b	29.71a	8.653ab	44.52e-i	17.28op	20.43b	10.9d
ICGV15033	4.41bcd	24.08bcd	0.48abc	0.89f-k	0.04b	0.2i	0.15gh	39b	29.42a	6.773ab	42.42hij	15.9p	19.43b-e	30cd
ICGV86124	4.919ab	26.86ab	0.51abc	0.89g-l	0.04b	0.26a	0.18abc	35.67b	29.2a	8.9b	44.63d-i	249b-e	18.46d-k	7.83e
ICGV131051	4.692abc	25.62abc	0.54a	0.87i-m	0.04b	0.25b-f	0.17b-f	433ab	28.69a	7.073ab	47.89b-e	22.27e-j	19.18c-g	21.67cd
ICGV131065	4.795abc	26.18abc	0.47abc	0.84j-n	0.04b	0.23fg	0.15fgh	433ab	28.21a	7.367ab	40.71j	18.64no	18.98c-i	9.67e

(Continued)

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TABLE 5 Continued

Genotype	N	Protein	P	K	Ca	Mg	S	Na	Fe	Cu	Zn	Mn	B	Al
	%	%	%	%	%	%	%	mg.kg-1	mg.kg-1	mg.kg-1	mg.kg-1	mg.kg-1	mg.kg-1	mg.kg-1
ICGV131090	3.781d	20.64d	0.50abc	0.86i-m	0.04b	0.24c-f	0.16c-g	40.67ab	28.19a	6.463ab	43.82f-j	22.56d-j	18.34f-l	10.47d
ICGV131091	4.966ab	27.12ab	0.49abc	0.88h-l	0.05b	0.25b-f	0.15h	44.33ab	36.92a	7.083ab	42.74hij	20.63j-m	16.72n	8.03e
ICGV131096	4.016cd	21.93cd	0.55a	0.86i-m	0.04b	0.24def	0.17b-f	41.67ab	26.95a	6.59ab	44.47e-i	21.21h-k	19.4b-f	9.53e
ICGV141088	4.748abc	25.92abc	0.48abc	0.92e-i	0.04b	0.26abc	0.17b-f	36.67b	25.39a	6.05ab	434g-j	21.47f-k	17.35lmn	14.67d
ICGV1315	4.767abc	26.03abc	0.3967c	1.206a	0.09a	0.24def	0.11h	33b	42.5a	4.96ab	333k	12.58pq	9.11o	50bcd
CV %	*5.4*	*5.4*	*7.2*	*2.4*	*6.6*	*1.9*	*2.6*	*31.4*	*30.4*	*28.7*	*2.5*	*2.5*	*1.7*	*52.4*
F-statistic	*4.71***	*4.71***	*2.83***	*57.25***	*22.72***	*35.20***	*32.71***	*2.01**	*162.24***	*2.21***	*31.63***	*78.20***	*168.79***	*25.52***

Values with dissimilar letters in a column are significantly different at P ≤ 0.05.
Means (±S.E.M) followed dissimilar letters in a column are significantly different at *p≤ 0.05, and ** p≤0.01. Where CV = coefficient of variation.

The C/N ratio of N_2-fixing species tends to be less than 24 g.g^{-1} while non-legumes have C/N ratios greater than 24 g.g^{-1} (Hobbie Macko & Shugart, 1998). In this study, the C/N ratios ranged from 17.2 g.g^{-1} in genotype YENYAWOSO to 22.0 g.g^{-1} in genotype ICGV14876, which are values much lower than 24 g.g^{-1} (Table 4). Two groundnut genotypes (namely, ICGV131090 and ICG13984) showed an increase in C/N ratios as the δ^{15}N levels decreased, indicating a relationship between C and N in symbiotic species (Table 4).

Shoot $\delta\ ^{13}$C is often considered an integrated measure of water-use efficiency in C3 species, which include legumes such as groundnut (Yoneyama et al., 1998). The δ^{13}C of C3 plant species has therefore been used as an indicator of WUE (Farquhar et al., 1989). Here, the higher the δ^{13}C (i.e., less negative or lower discrimination), the greater the WUE, whilst the lower the ^{13}C (i.e., more positive or high discrimination), the lower the WUE (Muhaba and Dakora, 2020). In this regard, genotypes YENYAWOSO, ICGV13848, ICGV13851, ICGV15033, and ICGV131065 showed less ^{13}C discrimination (i.e., less negative δ^{13}C value) and therefore had much greater WUE (Table 4).

The 30 field-grown groundnut genotypes in this study were evaluated for grain protein content. The results revealed marked differences in the levels of protein in groundnut grain. Seed protein levels ranged from 20.6% for genotype ICGV131090 to 29.8% in genotype ICGV13937, with five other genotypes recording approximately 27% grain protein, levels consistent with previous reports for protein in groundnut (Namrata et al., 2016; Dhakar, 2015; Janila et al., 2016; Toomer, 2018). As expected, the genotypes with the highest shoot %N accumulated the most protein in their grain, which is an indication of a direct link between shoot %N and protein level in groundnut grain (Dakora and Belane, 2019). The seeds of N_2-fixing legumes, such as groundnut, are rich in N due to the species' ability to reduce N_2 into NH_3 and subsequently into nitrogenous solutes for plant use (Belane et al., 2014).

In this investigation, there were significant differences in macro- and micronutrient (P, K, Ca, Mg, S, Fe, Cu, Zn, Mn, and B) uptake and accumulation in the grain. For example, Fe concentration was highest in genotypes 12CS116 (49.6 mg.kg^{-1}) and IS-07273 (48.5 mg.kg^{-1}), followed by 12CS098 (47.9 mg.kg^{-1}), CHINESE (45 mg.kg-1), ICGV1315 (42 mg.kg^{-1}), YENYAWOSO (39.2 mg.kg^{-1}), IS-07273 (38.2 mg.kg^{-1}), and ICGV141088 (25.4 mg.kg^{-1}). However, the grain concentrations of the macronutrients P, K, Ca, Mg, and S were low when compared to the micronutrients (Table 5). Grain P concentration was highest in nine of the 30 groundnut genotypes. However, all 30 groundnut genotypes showed high levels of Zn in edible groundnut seed. However, Mn concentration was highest in genotype ICGV14876 (26.0 mg.kg^{-1}) and lowest in ICGV15033 (15.9 mg.kg^{-1}). While genotype 12CS098 recorded relatively higher Na concentration in the grain (85 mg.kg^{-1}), genotypes ICGV14880 (30.7 mg.kg^{-1}) and ICGV15028 (30.7 mg.kg^{-1}) showed the lowest Na. Genotype IS-07273 was consistent in recording higher accumulation of P (0.54%), Mg (0.26%), S (0.19%), Cu (10.68%), and B (24.18 mg.kg^{-1}) in its grain (Gunununu and Dakora, 2024; Table 6). The greater uptake of P, Mg, S, Cu, and B and their accumulation in the grain would no doubt enhance the nutritional quality of groundnut seeds relative to the other genotypes, with implications for human nutrition/health. Furthermore, genotypes IS-07273, ICGV13842, ICGV13864, ICGV13910, ICGV14880, and ICGV13876, which recorded higher mineral concentrations in the grain, also fixed the most N in shoots. This is evidenced by the strong correlation found between the amount of N-fixed and K, Na, B, Cu, Zn, and Mn in genotype ICGV13910 and the positive correlation obtained between N-fixed and S, nodule number and Ca, %N and P, and %N vs. K in genotype IS-07273 (Table 6). An earlier report by Belane et al. (2014) found a strong relationship between N_2-fixing efficiency and mineral accumulation in nodulated cowpea by showing that rhizobia with higher symbiotic efficiency induced greater mineral accumulation in shoots, while strains with low N_2-fixing ability elicited reduced mineral accumulation. In this study, the high-fixing groundnut genotype ICGV13910 also accumulated significantly increased levels of K, Na, Zn, Cu, Mn, and B in the grain, in the same manner that the high-fixing genotype IS-07273 also showed greater concentrations of Ca, P, K, and S in grain (Table 5). As a result, K, Na, Cu, Zn, Mn, and B were strongly correlated with the amount of N-fixed in genotype ICGV13910, while Ca, P, K, and S were also positively correlated with symbiotic parameters (nodule number, N-fixed and %N) in genotype IS-07273 (Table 6). These results are therefore consistent with the report by Belane et al. (2014), which indicates that this relationship between N_2 fixing and mineral accumulation is not unique to cowpea. Taken together, genotype ICGV13910 and IS-07273 were high in N_2-fixation, which also correlated well with macro- and micronutrients in their grain, and therefore have huge potential for inclusion in breeding programs. While the mechanisms underlying the increase in mineral accumulation with high N_2 fixation remain unknown, there are many ways that symbiotic rhizobia can increase mineral supply to their host plants. For example, rhizobia are capable of solubilizing bound-P for use by their host plants and producing siderophores for increased Fe availability to plants (Dakora et al., 2024). Rhizobial bacteria can also synthesize and release plant hormones, including indole acetic acid (IAA), abscisic acid (ABA), lumichrome, riboflavin, cytokinins, and gibberellins that promote root proliferation and root hair formation for greater nutrient uptake (Dakora, 2015).

TABLE 6 Correlation analysis of symbiotic parameters (N-fixed,nodule number, and %N) and grain mineral accumulation in two groundnut genotypes.

Genotype	Parameters	R-value	P-value
ICGV13910	N-fixed vs. boron	1.000	0.0001
	N-fixed vs. potassium	1.000	0.0001
	N-fixed vs. sodium	1.000	0.0001
	N-fixed vs. copper	1.000	0.0001
	N-fixed vs. zinc	1.000	0.0001
	N-fixed vs. manganese	1.000	0.0001
IS-07273	Nodule Number vs. calcium	0.9979	0.041
	N-fixed vs. sulfur	0.9991	0.027
	%N vs. phosphorus	0.9994	0.022
	%N vs. potassium	0.9994	0.022

Conclusion

In conclusion, we found marked differences in symbiotic performance between and among 30 groundnut genotypes, with IS-07273 and ICGV13910 exhibiting greater symbiotic dependency on N2 fixation for their N nutrition, and higher amounts of N-fixed. These two high N2-fixing symbioses (IS-07273 and ICGV13910) also accumulated significantly higher levels of K, Na, Zn, Cu, Mn, and B in their grain. As a result, there were strong positive correlations between the amount of N-fixed and K, Na, B, Cu, Zn, and Mn for genotype ICGV13910. Genotype IS-07273 also showed significant correlations between N-fixed and S, %N and P, %N and K, and nodule number and Ca. The genotypes with the highest shoot %N accumulated the most protein in their grain. Of the 30 groundnut cultivars tested in the field, YENYAWOSO, ICGV13848, ICGV13851, ICGV15033, and ICGV131065 showed greater shoot $\delta^{13}C$ values, and hence higher water-use efficiency. The high N2 fixation genotypes ICGV13910 and IS-07273 correlated positively with macronutrient and micronutrient concentrations in the grain, indicating their potential for use in breeding programs to enhance nutritional security in groundnut.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

TN: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. RF: Resources, Supervision, Visualization, Writing – review & editing. FD: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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