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RECEIVED 25 September 2025

REVISED 01 December 2025

ACCEPTED 10 December 2025

PUBLISHED 17 February 2026

CITATION

Gunununu RP, Ngmenzuma TY-a, Dakora FD and Mohammed M (2026) Symbiotic N₂-fixation, carbon accumulation and water-use efficiency of 63 common bean (*Phaseolus vulgaris* L.) genotypes grown under field conditions at Malkerns, Eswatini using the ¹⁵N and ¹³C natural abundance technique. *Front. Agron.* 7:1713315. doi: 10.3389/fagro.2025.1713315

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Symbiotic N₂-fixation, carbon accumulation and water-use efficiency of 63 common bean (*Phaseolus vulgaris* L.) genotypes grown under field conditions at Malkerns, Eswatini using the ¹⁵N and ¹³C natural abundance technique

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Common bean (*Phaseolus vulgaris* L.) is consumed worldwide and is the third most important food legume after soybean and groundnut. The aim of this study was to evaluate plant growth, N₂-fixation, C accumulation and water use efficiency of 63 common bean genotypes grown in Malkerns, Eswatini under field conditions using the ¹⁵N and ¹³C natural abundance techniques. The data revealed marked ($p \leq 0.05$) differences in shoot dry matter (SDM) yield, N concentration and content, $\delta^{15}\text{N}$, the proportion of N derived from symbiotic fixation (%Nd_{fa}) and the N-fixed among the 63 common bean genotypes. Common bean plants' %Nd_{fa} values ranged between 19.6–88.4%, contributed 2.7–66.8 kg N-fixed ha⁻¹ to the soil, while soil N-uptake ranged from 3.7–54.01 kg ha⁻¹. Out of the 63 test genotypes, 40 genotypes obtained more N from the soil than from symbiosis, implying that the N₂-fixing efficacy of the microsymbionts nodulating common bean in Malkerns were low. The results further revealed shoot $\delta^{13}\text{C}$ values ranging from -30.11‰ to -26.33‰, indicating differences in plant water use efficiency amongst different common bean genotypes. There was a significant positive correlation between $\delta^{13}\text{C}$ and N content ($r=0.25^{**}$), and also between N-fixed and shoot dry matter ($r = 0.88^{***}$), indicating a functional link between N₂-fixation and photosynthesis. Taken together, the findings indicate that, increased N₂-fixation is linked to improved photosynthetic activity, plant growth and water use efficiency in the bean plants.

KEYWORDS

%Nd_{fa}, common bean, grain yield, nitrogen fixation, water-use efficiency

Introduction

Common bean (*Phaseolus vulgaris* L.) is a very important field legume crop used for human consumption and household cash income (Reinprecht et al., 2020). It is a staple food in Africa and South America (Broughton et al., 2003). Nitrogen availability is however a limiting factor for the crop's good yield. To overcome soil nutrient deficiency, farmers often use synthetic N fertilizers to increase yields (Kamfwa et al., 2015). Since not all the applied N fertilizer is used by the plant (Van kessel and Hartley, 2000), a significant proportion is lost through runoff, eventually contaminating groundwater.

Nitrogen (N) management is therefore critical in cropping systems as it has a significant effect on overall productivity (Farid et al., 2016). Relative to N fertilizers, biological nitrogen fixation (BNF) is a more cost-effective method of delivering nitrogen to legume crops (Thilakarathna and Raizada, 2018) as it eliminates the requirement for nitrogenous fertilizers, thus reducing N leaching, runoff, and pollution, which are common with synthetic N fertilization (Stevens et al., 2004; Good and Beatty, 2011).

The efficiency of the legume/rhizobia symbiosis often vary with legume species and bacterial strain. Compared to other legumes, the common bean is a low N_2 -fixer (Mnasri et al., 2007; Peoples et al., 2009; Mnasri et al., 2014), even though the N_2 fixation potential can vary amongst bean genotypes (Farid and Navabi, 2015). For example, in comparison to most Andean determinate bush-type cultivars, the climbing and Mesoamerican indeterminate common bean cultivars exhibit better nodulation and enhanced symbiotic nitrogen fixation (SNF) (Wilker et al., 2019). According to Sanyal et al. (2018), navy and pinto beans fix more nitrogen than kidney and black beans. However, several bean genotypes have been identified across different agroecological systems that can fix large amounts of N, with about 39% of their N nutrition being derived from the atmosphere (Peoples et al., 2009). Wilker et al. (2019) also found about 21 to 76% N derived from fixation in a collection of Mesoamerican and Andean bean genotypes. The huge differences in N_2 fixation can be attributed to various factors, including genotype, rhizobial strain, edaphic climatic conditions, legume growth stage and plant-available soil N (Unkovich and Pate, 2000; Wilker et al., 2019).

Water scarcity and deficiencies in soil N and available P are the main factors affecting crop yields in Africa (Van der Velde et al., 2014). Low soil moisture is currently the primary constraint to increased crop yields and food security in Africa (Shiferaw et al., 2014). Adequate soil moisture is essential for plant growth, especially in tissue expansion and biological processes (Farooq et al., 2009). It has been reported that inadequate soil moisture is responsible for a about 40% yield reduction in soybean and other grain legumes (Manavalan et al., 2009). The presence of mineral N in the soil inhibits both nodule formation and nitrogenase activity, while the deficiency of P can also decrease nodule number and weight as well as affect the physiological and metabolic processes in root nodules (Farid and Navabi, 2015).

Therefore, rainfed crops must have the ability to withstand drought, while producing enough biomass per unit of water used; this is termed water-use efficiency (WUE), and is expressed as the ratio of photosynthate produced per unit of water loss via

transpiration during photosynthesis (Condon et al., 2004). Water-use efficiency is important for determining drought tolerance in crops and can be measured using gas exchange parameters and/or carbon (C) isotope discrimination (Farquhar et al., 1989) in C3 plant species (Mohale et al., 2014). Common bean is regarded as a drought tolerant plant, however, there is little information on the crop's water use efficiency when grown under field conditions.

The $\delta^{13}C$ of C3 plants has been reported to correlate with WUE (Farquhar et al., 1989). During photosynthetic CO_2 fixation, plants tend to discriminate against $^{13}CO_2$ (the heavier isotope of C) in favour of the lighter $^{12}CO_2$ isotope, leading to a lower $^{13}C/^{12}C$ ratio in plants relative to the atmosphere (Farquhar et al., 1989; Unkovich et al., 2008; Mohale et al., 2014). However, partial stomatal closure during soil water stress often results in an intercellular CO_2 concentration sufficient to saturate photosynthesis, leading to less ^{13}C discrimination (Farquhar et al., 1982; Farquhar and Richards, 1984). This forms the basis for the use of tissue $\delta^{13}C$ as a tool for measuring WUE in C3 plants such as common beans (Wang et al., 2010).

Farquhar et al. (1989) were the first to report a relationship between ^{13}C discrimination during photosynthesis and WUE. Plants experiencing water stress usually exhibit high (less negative) $\delta^{13}C$ values, which is an indication of high WUE (Ferrio et al., 2003). As a result, the ^{13}C isotope technique has been used to study crop species in breeding programs (Condon et al., 2004; Blum, 2009).

Nutritionally, the common bean is an important source of protein for millions of people around the world (Broughton et al., 2003; Mwenda et al., 2018). Its production is extensive in the tropics, subtropics, and temperate regions (Singh, 1999), an indication of its importance as a major food security crop. However, little is known about its nodulation, N_2 fixation, and water relations. These alone should justify renewed research interest in this species and the superior dietary value of its grain as food. Water scarcity and insufficient soil moisture for agricultural production are common in Sub-Saharan Africa. For example, although Eswatini receives 800 to 1000 mm of rain per year, there is a need to select common bean genotypes that can thrive in a changing climate. Studies aimed at identifying common beans with high water-use efficiency and nitrogen fixation could promote common bean production under rainfed conditions and provide a long-term solution to food in security for smallholder farmers in Eswatini. In this study, we hypothesize that plant growth or biomass accumulation and grain yield of the test common beans will be driven by N uptake, whether from symbiosis or directly from endogenous soil N. The specific objectives were 1) to assess N_2 fixation and plant growth parameters of the 63 common bean genotypes in response to natural rhizobia population in the soil using the ^{15}N natural abundance technique, and 2) to determine the water use efficiency of common bean genotypes grown in the field using the carbon isotope discrimination technique. The generation of a baseline information regarding the symbiotic performance of the bean genotypes will provide bases for selection and crop improvement to further improve the common bean/rhizobia symbiosis for increased productivity in low N soils.

Material and methods

Study site description

A field experiment was conducted during the 2017/2018 cropping season at the Malkerns Agricultural Research Station, Eswatini, with reference co-ordinate 26°33'18.33" S; 31°10'10.29" E, and elevation of 700 m above sea level. The area is classified as sub-tropical with an average annual rainfall ranging between 800 and 1000 mm (Edje and Ossom, 2009; Dlamini et al., 2021). The daily temperatures recorded during the cropping season (December to May) ranged from 15.7 °C to 18.3 °C for minimum and 25.3 °C to 28.9 °C for maximum in the 2017/2018 cropping season (Table 1). The total rainfall recorded during cropping season from December 2017 to May 2018 was 952.5 mm. The mean monthly rainfall, temperature, and relative humidity are illustrated in Table 1.

Experimental design, planting and pest management

The 63 self-pollinated common bean genotypes were planted in a randomized complete block design (RCBD) using four (4) replicate plots per genotype. The genotypes were obtained from the International Center for Tropical Agriculture (CIAT–Malawi) and were selected according to farmers' preferences (grain yield, nutritive value, and pest tolerance) and their agroecological adaptation. The genotypes were planted on plots size measuring 3 m x 2 m (i.e., 6 m²) with four rows of 4 m and 60 cm inter-row, and 10 cm intra-row spacing. The experiment was established in mid-December. Ploughing of the field was done mechanically, and harrowing was done using a disc plough. The experiment was conducted under natural rainfed conditions without irrigation or any chemical input. Two seeds were planted per hole and later thinned to one plant. About four to five weeks post-emergence, weeds were controlled manually using hand hoes and pests were controlled chemically using a mixture of Chlorpyrifos and Beef oil before flowering. The 63 genotypes and their origin, agronomic

characteristics ranging from flowering date, flower colour, physiological maturity period, and seed colour are presented in Table 2.

Soil sampling and analysis

Before sowing, the soil chemical properties of the study site were determined. Bulk soil samples were randomly collected (0–20 cm depth) from ten (10) spots across the diagonals of the field using a soil auger. The experimental field was previously planted to maize. The soil samples were pooled and thoroughly mixed to form a composite sample that represented the experimental field, air-dried and sieved (2.0 mm). Sub-samples were used for the analysis of particle size distribution, pH, total N, available P, and cation exchange capacity (CEC). Particles size distribution was determined according to Bouyoucos (1962). Soil pH was determined in a soil: water (1:10, w/v) suspension using a pH meter. To determine total N, the Kjeldahl digestion method was used, while available P was determined using the method described by Bray and Kurtz (1945). CEC and exchangeable cation (Na, K, Ca and Mg) were determined using the citric acid method (Du plessis and Burger, 1965). All soil analysis was done at the Agricultural Research Council (ARC)-Institute for Soil, Climate and Water, Pretoria, South Africa. The bulk soil collected from the plots before planting is classified as deep red loam of the Malkerns Series (Kunene et al., 2019) and contained; clay (19%), silt (47%) and sand (34%), with pH of 4.7, total N (0.0587%), available P (14 mg. kg⁻¹), CEC (5.059 Cmol⁽⁺⁾ kg⁻¹), Na (0.038 Cmol⁽⁺⁾ kg⁻¹), K (0.322 Cmol⁽⁺⁾ kg⁻¹), Ca (1.778 Cmol⁽⁺⁾ kg⁻¹) and Mg (1.078 Cmol⁽⁺⁾ kg⁻¹) as shown in Table 3.

Plant sample collection, processing, and analysis

At the early podding (R4) stage of plant growth which corresponded with 60 days after planting, four (4) plants were randomly sampled from the two middle rows of each plot and

TABLE 1 Climatic data collected at the Malkerns Research Station, Eswatini, during the experimental period (2017/2018) cropping season.

Months	Temperature		Rainfall	Relative humidity	
	(°C)		(mm)	(%)	
2017/2018	Min.	Max.	-	Min.	Max.
December	16.9	26.8	228.6	66.6	95.8
January	16.9	28.9	100	55.6	95.5
February	18.3	28.3	175	64	96.8
March	16.8	28.5	200	62.4	97.8
April	15.7	26.7	76.6	62.6	97.8
May	16.92	25.3	36.2	51.9	96.7
Mean	16.9	27.4	136.1	60.5	96.7

TABLE 2 Characteristics of the 63 common bean genotypes used in the study. Flowering date and maturity period indicate days after planting.

Genotype	Origin/source	Flowering date	Flower colour	Maturity period	Seed colour	Size
NUA 35	Malawi CIAT	40	pink	88	cream	medium
NUA 735	Malawi CIAT	38	pink	87	red	large
NUA 743	Malawi CIAT	45	white	100	red	medium
NUA 720	Malawi CIAT	38	white	88	red	large
DAB 363	Malawi CIAT	37	pink	84	cream	large
DAB 369	Malawi CIAT	37	pink	86	cream	medium
DAB 370	Malawi CIAT	37	pink	83	cream	large
DAB 378	Malawi CIAT	38	pink	85	cream	medium
DAB 386	Malawi CIAT	38	pink	85	cream	large
DAB 387	Malawi CIAT	38	pink	83	cream	large
DAB 190	Malawi CIAT	40	pink	86	cream	medium
DAB 174	Malawi CIAT	40	pink	86	cream	medium
DAB 410	Malawi CIAT	37	white	85	cream	medium
DAB 411	Malawi CIAT	38	pink	85	cream	medium
DAB 447	Malawi CIAT	38	pink	85	cream	large
DAB 429	Malawi CIAT	38	white	86	cream	medium
DAB 470	Malawi CIAT	38	pink	86	cream	medium
MCA 98	Malawi CIAT	41	white	99	red	large
NUC 461	Malawi CIAT	46	white	101	yellow	medium
NUC 451	Malawi CIAT	49	white	97	yellow	medium
NUC 219	Malawi CIAT	43	white	99	red	medium
MCA 78	Malawi CIAT	41	white	99	red	medium
CIM-RM-03-03-45	Malawi CIAT	40	pink	95	red	medium
DAB 381	Malawi CIAT	35	pink	90	cream	medium
NAIN DEKYONDO	Malawi CIAT	44	white	99	white	small
CZ 113-15 (SAB 630)	Malawi CIAT	36	pink	91	cream	medium
DAB 559	Malawi CIAT	39	pink	94	white	medium
CZ 104-13	Malawi CIAT	42	pink	89	red	large
CAL 96	Malawi CIAT	40	white	90	red	medium
SMC 17	Malawi CIAT	42	white	91	white	medium
CODMLB 03	Malawi CIAT	40	pink	96	red	large
G 5207	Malawi CIAT	49	purple	98	black	small
AND 277	Malawi CIAT	40	white	88	red	medium
DFA 73	Malawi CIAT	40	white	88	red	medium
KAB 77 F7.2-52	Malawi CIAT	40	pink	89	red	medium
KG 98-36	Malawi CIAT	38	white	87	cream	medium
KG 98-42	Malawi CIAT	40	white	87	cream	medium
CIM-RK06-ALS-S1-2	Malawi CIAT	40	pink	90	red	large

(Continued)

TABLE 2 Continued

Genotype	Origin/source	Flowering date	Flower colour	Maturity period	Seed colour	Size
CIM-SUG-03-09-04	Malawi CIAT	38	pink	88	cream	large
CIM-RM02-76-4	Malawi CIAT	40	pink	93	red	medium
CIM-RM02-46-1	Malawi CIAT	38	pink	92	red	medium
CIM-KHAK02-14-1	Malawi CIAT	42	pink	92	cream	small
CIM-KHAK02-20-1	Malawi CIAT	42	white	90	cream	small
CIM-RM07-ALS-71-2	Malawi CIAT	42	pink	88	red	large
CIM-RM00-7-1	Malawi CIAT	40	pink	90	red	medium
CIM-RM01-92-3	Malawi CIAT	40	pink	90	red	medium
CIM-RM02-20-3	Malawi CIAT	42	pink	88	red	medium
CIM-RM00-21	Malawi CIAT	40	pink	90	red	medium
KAB 06 F2.8-51	Malawi CIAT	40	pink	90	red	medium
CIM-RM02-76-1	Malawi CIAT	40	pink	88	red	large
CIM-RM02-79-1	Malawi CIAT	40	pink	89	red	medium
CIM-RM02-62-1	Malawi CIAT	39	pink	88	red	large
CIM-RM00-109	Malawi CIAT	40	pink	88	red	large
KAB 10 F2.8-84	Malawi CIAT	40	white	90	red	medium
CIM-RM02-73-1	Malawi CIAT	40	pink	88	red	medium
CIM-RM01-97-8	Malawi CIAT	40	pink	90	red	medium
CIM-RM00-141	Malawi CIAT	39	pink	88	red	medium
NCB 281	Malawi CIAT	40	purple	88	black	small
DAB 207	Malawi CIAT	38	white	86	cream	medium
DAB 155	Malawi CIAT	40	pink	90	red	medium
DAB 270	Malawi CIAT	38	pink	88	red	medium
CIM-RM05-ALS-103	Malawi CIAT	40	white	88	red	medium
CIM-RM00-27-4	Malawi CIAT	38	pink	88	red	medium

separated into shoots, roots, and nodules. The shoots were stored in labelled brown paper bags and the roots with intact nodules were kept in plastic Ziploc bags and transported to the laboratory. Roots were then slightly washed to remove debris, and the nodules were detached and counted manually. The shoots were oven-dried at 65 °C for (72 h) and weighed to determine shoot biomass. The dried shoots were ground into fine powder (0.85 mm sieve) and stored in vials for ^{15}N and ^{13}C isotopic analysis. Three

replicates of non-legume reference species namely, *Bidens pilosa*, *Galinsoga parviflora*, *Pomoea purpurea*, *Hyparrhenia hirta*, *Oxalis corniculata*, *Cyperus esculentus*, *Solanum nigrum* and *Tagetes minuta* growing within the experimental plots were sampled as reference plants, for estimating %Ndfa values (Table 4). The shoots of the reference plants collected were also oven-dried at 65 °C (72 h) weighed, grounded to a fine powder, and similarly processed for $^{15}\text{N}/^{14}\text{N}$ isotopic analysis.

TABLE 3 Physicochemical properties of topsoil sampled at the malkerns research station.

Texture	pH	Total N	Available P	CEC	Exchangeable cations (Cmol (+) kg ⁻¹)		Ca	Mg
	H ₂ O	%	mg kg ⁻¹	Cmol (-) kg ⁻¹	Na	K		
Loam	4.73	0.0587	14	5.059	0.038	0.322	1.778	1.078

CEC, Cation Exchange Capacity.

TABLE 4 Reference plants sampled from the field during 2017/2018 cropping season for measuring N₂ fixation by common bean at Malkerns Research Station.

Common Name	Botanical name	δ ¹⁵ N (‰)
Blackjack	<i>Bidens pilosa</i>	+6.13
Potato weed	<i>Galinsoga parviflora</i>	+5.42
Morning glory	<i>Pomoea purpurea</i>	+6.62
Thatching grass	<i>Hyparrhenia hirta</i>	+7.68
Creeping sorrel	<i>Oxalis corniculata</i>	+6.23
Mexican marigold	<i>Tagetes minuta</i>	+5.34
Tiger nuts	<i>Cyperus esculentus</i>	+6.34
Black nightshade	<i>Solanum nigrum</i>	+8.91
	Combined mean	+6.58

Measurement of nitrogen fixation

¹⁵N/¹⁴N isotopic analysis

The ¹⁵N/¹⁴N isotopic ratios were determined at the Stable Light Isotope Laboratory, Department of Archaeology at the University of Cape Town, South Africa. For this analysis, 2.0 mg of finely ground common bean 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 via a Thermo ConFlo IV to a Thermo Delta V Plus stable light isotope mass spectrometer (Thermo Corporation, Bremen, Germany). The plant samples were combusted in an evacuated quartz tube and analyzed to determine the ¹⁵N/¹⁴N ratio according to the formula below (Shearer and Kohl, 1986):

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

where, ¹⁵N/¹⁴N_{sample} is the abundance ratio of ¹⁵N and ¹⁴N in the sample and ¹⁵N/¹⁴N_{atm} is the abundance ratio of ¹⁵N and ¹⁴N in the atmosphere.

The B-value

The ¹⁵N natural abundance technique was used to measure N₂ fixation. This technique is based on the principle that a nodulated legume growing in a medium free of combined N is completely reliant on N₂ fixation for its growth, and its δ¹⁵N can be designated as the 'B value'. Therefore, the isotopic composition of the legume would be expected to be similar to that of atmospheric N₂. A legume's δ¹⁵N 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 estimating soil N uptake and the B-value of -1.988 ‰ (Unkovich et al., 2008) was used for the calculations.

Percent N derived from fixation (%Nd_{fa})

The proportion of N derived from atmospheric N₂ fixation (% Nd_{fa}) was estimated using the equations described by (Shearer and Kohl, 1986; Unkovich et al., 2008):

$$\%Nd_{fa} = \frac{\delta^{15}N_{ref} - \delta^{15}N_{leg}}{\delta^{15}N_{ref} - B_{value}} \times 100$$

where δ¹⁵N_{ref} is the ¹⁵N natural abundance of non-N₂-fixing reference plant, δ¹⁵N_{leg} is the ¹⁵N natural abundance of the legume, and B-value is the ¹⁵N natural abundance of common bean plants deriving all their N nutrition from N₂ fixation. The B value replaces the value of atmospheric N₂ as it incorporates the isotopic fraction in relation to N₂ fixation (Unkovich et al., 2008). The combined mean δ¹⁵N value of all the reference plant species sampled was +6.58.

Shoot N content

Shoot %N was obtained directly from the mass spectrometer, and the shoot N content was calculated as the product of %N and shoot dry matter, as described by Pausch et al. (1996):

$$N_{\text{content}} = \%N_{\text{shoot}} \times \text{dry mass}_{\text{shoot}}$$

Amount of N-fixed

The amount of N-fixed by the common bean plants was calculated as (Unkovich et al., 2008):

$$N - \text{fixed} = \%Nd_{fa} \times \text{legume biomass N}$$

where legume biomass N is the N content of common bean shoots.

Soil N uptake

The total soil N uptake by common bean genotypes was determined as the difference between the total N content of legumes and the amount of N-fixed.

¹³C/¹²C isotopic analysis

The ¹³C/¹²C isotopic analysis of bean shoots was performed at the Stable Light Isotope Laboratory, Department of Archaeology at the University of Cape Town, South Africa, as described for the ¹⁵N/¹⁴N isotopic analysis. Common bean shoot samples were analyzed to determine the ratio of ¹³C/¹²C and the C concentration (%C). The ¹³C natural abundance (or δ¹³C) was calculated as (Farquhar et al., 1989):

$$\delta^{13}C = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

where, R_{sample} is the ¹³C/¹²C ratio of the sample and R_{standard} is the ¹³C/¹²C isotopic ratio of PDB, a universally accepted standard from the Belemnite Pee Dee limestone formation. The %C of each plant sample was obtained directly from the mass spectrometric analysis.

C content

The C content of common bean shoots was calculated as the product of C concentration and shoot weight, where C concentration (%C) was obtained directly from the mass spectrometer:

$$C \text{ content} = \% C_{\text{plant organ}} \times \text{dry matter}_{\text{plant organ}}$$

C/N ratio

The C/N value was calculated as the ratio of C to N concentration in plant shoots.

Grain yield determination

At physiological maturity, all plants were harvested from two middle rows of each plot. Pods were detached, air-dried to 15% moisture content and weighed to determine grain yield per hectare. The weight of hundred seeds was also determined.

Statistical analysis

All data collected were subjected to a normality test before being subjected to analysis of variance (ANOVA) using Statistical software version 10.1. A 1-way ANOVA was used to compare grain yield, symbiotic performance, shoot dry matter, C concentration, C content, C/N ratio and water use-efficiency among the 63 genotypes, and to determine the mean differences. Duncan's multiple range test at $p \leq 0.05$ level was used to separate means that showed significant difference. Correlation analyses were performed using python software version 3 to assess the relationship between measured parameters.

Results

Nodulation

There were significant differences ($p \leq 0.05$) in nodulation among the 63 common bean genotypes. As shown in Table 4, genotype MCA 98 produced the highest number of nodules (59 nodules per plant), followed by CIM-RM01-97-8 (31 nodules per plant) and then KAB06F2.8-51 (18 nodules per plant). Genotypes NUA 35, NUA 735, NUA 743, DAB 470, NAIN DEKYONDO, CZ-104-13, G5207 and AND 277 produced the least number of nodules per plant (Table 4).

Shoot N concentration and content

The 63 bean genotypes differed in shoot N concentration and content. The shoot N concentration was 2% or more in 28 genotypes (Table 4), with MCA 98 (2.69%) showing the highest concentration, followed by KAB 10 F2.8-84 (2.62%), CIM-RK06-

ALS-S1-2 (2.61%) and CIM-RM01-97-8 (2.58%). The lowest shoot N concentration was recorded by genotype DAB 411 (1%), followed by CIM-KHAK02-14-1 (1.17%) then KG 98-42 (1.20%). Shoot N content also differed among the bean genotypes, with genotype NUC 451 recording the highest N content (1.039 g plant⁻¹), followed by NUC 219 (0.831 g plant⁻¹), and genotype MCA 78 (0.703 g plant⁻¹) because of their greater shoot dry matter. In contrast, genotypes CIM-KHAK02-20-1 (0.039 g plant⁻¹), DAB 370 and KG 98-42 (0.053 g plant⁻¹) exhibited the lowest N content due to their small shoot dry matter yield (Tables 4, 5).

$\delta^{15}\text{N}$ values of reference plants

Several plant species were collected from Malkerns fields and used as reference plants. The shoot $\delta^{15}\text{N}$ of reference plants ranged from +5.34‰ in *Tagetes minuta* to +8.91‰ for *Solanum nigrum*. The combined mean $\delta^{15}\text{N}$ of the reference plants was used to estimate the percent N derived from atmospheric N₂ fixation by the 63 common bean genotypes +6.58‰ (Table 4).

$\delta^{15}\text{N}$, %Nd_{fa} and N-fixed

The shoot $\delta^{15}\text{N}$ values differed markedly between and among the 63 common bean genotypes, with a range of +0.87‰ for CIM-RM01-92-3 up to +4.90‰ for NUA 743 (Table 5). The genotype with the next lowest $\delta^{15}\text{N}$ value after CIM-RM01-92-3 was DAB 174 (+0.91‰), while genotype NUC 461 had the next higher value (+4.87‰) after NUA 743. Estimates of N derived from atmospheric N₂ fixation (%Nd_{fa}) showed marked genotypic differences among the genotypes. Of the 63-test common bean genotypes, only 21 recorded %Nd_{fa} values above 50% (Table 5), indicating a low dependency of the bean genotypes on symbiotic N₂ fixation for their N-nutrition. Quite expectedly, the genotypes with lower shoot $\delta^{15}\text{N}$ showed higher %Nd_{fa}, and these included genotypes CIM-RM01-92-3, DAB 174, DAB 559, DAB 447, CZ 113-15 (SAB 630) and DAB 411. Conversely, the genotypes with higher $\delta^{15}\text{N}$ values such as NUA 743, NUC 461 and KAB 10 F2.8-84 derived less N from fixation (Table 5). As a result of the differences in $\delta^{15}\text{N}$ and %Nd_{fa}, the amount of N-fixed by the 63 common bean genotypes also varied significantly, ranging from 2.7 kg ha⁻¹ for NUA 35 to 66.8 kg ha⁻¹ for NUC 219. Genotypes NUC 219, NUC 451, MCA 78 and MCA 98 fixed significantly greater amounts of N, while genotypes NUA 35, CIM-KHAK02-20-1, KG-98-36 and DAB 411 contributed the least symbiotic N (Table 5). Only genotypes NUC 219 and MCA 78 out of 63 contributed over 50 kg N ha⁻¹.

Soil N uptake

There were significant differences in the level of soil N uptake by the bean genotypes (Table 5) at $p < 0.05$. Out of the 63 genotypes, 40 obtained more N from soil than symbiosis. Soil N uptake by the common bean genotypes ranged from 3.78 to 54.01 kg ha⁻¹ for

TABLE 5 Yield and symbiotic parameters of 63 common bean genotypes planted at Malkerns Research Station, Eswatini, during the 2017/2018 cropping season.

Genotype	Nodule number	N	N content	$\delta^{15}\text{N}$	Ndfa	N fixed	Soil N uptake	Grain yield	Shoot DM
	plant ⁻¹	%	g plant ⁻¹	‰	%	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	g plant ⁻¹
CIM-RM02-79-1	5 ± 0.5ijk	1.98 ± 0.2b-m	0.17 ± 0.0m-s	3.16 ± 0.3d-m	39.97 ± 3.1k-t	11.22 ± 0.4m-v	15.45 ± 1.8o-v	3618.75 ± 17.6a	8.72 ± 0.4n-q
NAIN DEKYONDO	1 ± 0.3qr	1.34 ± 0.2o-u	0.14 ± 0.0p-v	1.79 ± 0.3n-v	55.87 ± 4.0b-j	13.02 ± 0.1k-r	12.41 ± 1.4r-y	3383.61 ± 99.2b	15.47 ± 1.9f
CIM-RM00-7-1	5 ± 0.5ijk	2.40 ± 0.5a-f	0.15 ± 0.0p-u	2.73 ± 0.2g-p	44.96 ± 1.7h-q	11.29 ± 2.4m-v	12.93 ± 1.1r-x	3322.50 ± 11.5b	6.23 ± 0.2rst
CAL 96	5 ± 0.3pqr	1.70 ± 0.0h-t	0.21 ± 0.0l-p	3.56 ± 0.3c-j	35.24 ± 3.4n-u	12.46 ± 1.3k-t	18.52 ± 0.6i-r	3085.83 ± 76.9c	12.41 ± 0.2ghi
NUC 219	5 ± 0.4hij	1.99 ± 0.1b-m	0.83 ± 0.0b	2.45 ± 0.0j-r	48.21 ± 0.4f-n	66.82 ± 3.4a	27.22 ± 1.8e-h	3093.75 ± 52.6c	41.52 ± 0.6a
CIM-RM02-20-3	3 ± 0.6-q	1.87 ± 0.0e-r	0.22 ± 0.0k-p	3.66 ± 0.2b-i	34.08 ± 1.7o-v	12.45 ± 0.8k-t	19.15 ± 0.3i-q	2996.25 ± 54.5c	11.73 ± 0.4g-j
CIM-RK06-ALS-S1-2	2 ± 0.4o-r	2.61 ± 0.0a	0.21 ± 0.0l-p	3.79 ± 0.5a-h	32.61 ± 5.2p-w	11.22 ± 1.7m-v	18.44 ± 0.6i-r	2956.67 ± 98.0c	7.94 ± 0.0o-r
CIM-RM02-76-1	7 ± 0.7gh	1.76 ± 0.1g-t	0.27 ± 0.0i-l	3.60 ± 0.1b-j	34.82 ± 1.1n-v	15.67 ± 0.5i-o	22.80 ± 0.7g-m	2892.92 ± 11.9c	15.41 ± 0.5f
MCA 78	4 ± 0.5j-o	1.92 ± 0.1c-q	0.70 ± 0.0c	2.89 ± 0.1f-o	43.07 ± 1.3i-s	50.59 ± 2.9b	34.57 ± 1.2cd	2690.00 ± 47.1d	37.05 ± 0.5b
MCA 98*	59 ± 1.7a	2.69 ± 0.2a	0.62 ± 0.1d	3.30 ± 0.1c-k	38.31 ± 1.2m-u	39.22 ± 3.1c	37.04 ± 1.8c	2648.75 ± 11.8de	24.32 ± 1.6c
DAB 190	9 ± 0.5ef	1.63 ± 0.0j-t	0.11 ± 0.0r-y	1.54 ± 0.1q-v	58.78 ± 1.2b-g	10.67 ± 0.2n-w	9.73 ± 0.1v-B	2620.42 ± 79.4de	6.86 ± 0.2qrs
DAB 411	3 ± 0.5k-p	1.00 ± 0.0u	0.05 ± 0.0xy	1.10 ± 0.1s-v	63.99 ± 0.9b-e	4.98 ± 0.1wx	4.44 ± 0.1A	2616.67 ± 18.0de	5.06 ± 0.3s-v
CZ 104-13	1 ± 0.3qr	1.98 ± 0.0b-n	0.19 ± 0.0m-q	3.87 ± 0.0a-g	31.57 ± 0.4q-w	10.07 ± 0.4o-w	17.22 ± 0.8k-t	2541.67 ± 52.9def	9.68 ± 0.3k-o
NUA 720	3 ± 0.3m-r	1.87 ± 0.1e-r	0.22 ± 0.0l-p	4.05 ± 0.3a-f	29.49 ± 3.6r-w	10.66 ± 1.5n-w	19.28 ± 0.8i-q	2485.42 ± 12.1def	11.56 ± 0.2g-k
NCB 281	2 ± 0.4o-r	1.61 ± 0.1k-t	0.09 ± 0.0t-y	3.11 ± 0.2d-m	40.53 ± 2.4k-t	6.27 ± 0.4u-x	8.88 ± 1.1w-B	2436.25 ± 10.6ef	5.83 ± 0.3stu
DAB 369	10 ± 0.7e	2.32 ± 0.4a-h	0.29 ± 0.1hij	3.12 ± 0.1d-m	40.43 ± 1.7k-t	19.25 ± 2.4g-j	23.18 ± 3.2g-m	2365.83 ± 53.1fg	12.60 ± 0.3ghi
CODMLB 03	2 ± 0.3n-r	2.42 ± 0.0a-f	0.12 ± 0.0q-x	3.60 ± 0.6b-j	34.80 ± 7.1n-v	7.24 ± 1.1r-x	11.13 ± 0.9t-A	2338.75 ± 11.1fg	4.99 ± 0.5tuv
NUA 735	1 ± 0.3qr	1.30 ± 0.1r-u	0.12 ± 0.0q-x	1.79 ± 0.1n-v	55.90 ± 1.1b-j	11.22 ± 0.5m-v	10.71 ± 0.5u-A	2192.92 ± 77.80gh	9.58 ± 0.2l-p
DAB 363	7 ± 0.4fgh	1.90 ± 0.1c-r	0.21 ± 0.0l-p	2.24 ± 0.5k-s	50.63 ± 5.5e-m	17.26 ± 1.7i-m	16.99 ± 0.5m-t	2120.83 ± 30.19hi	10.85 ± 0.2h-l
DAB 429	8 ± 0.7fg	1.33 ± 0.3p-u	0.16 ± 0.0p-t	2.06 ± 0.4l-u	52.78 ± 4.6c-l	13.08 ± 1.7k-r	13.47 ± 2.5q-w	2090.83 ± 43.6hi	11.75 ± 0.3g-j
KAB 06 F2.8-51*	18 ± 0.6c	1.80 ± 0.0f-s	0.41 ± 0.0ef	2.49 ± 0.1i-r	47.69 ± 0.8f-o	32.47 ± 1.7d	27.48 ± 0.7efg	2075.00 ± 94.4hij	22.62 ± 0.8d
NUC 451	3 ± 0.4l-q	2.50 ± 0.1a-d	1.04 ± 0.0a	4.14 ± 0.4a-d	28.52 ± 4.2t-w	48.59 ± 5.5b	54.01 ± 7.56a	2054.17 ± 43.2hij	41.04 ± 0.8a
DAB 381	3 ± 0.5m-q	1.62 ± 0.4k-t	0.13 ± 0.0q-w	2.74 ± 0.5g-p	44.85 ± 6.1h-q	8.55 ± 0.3p-x	11.53 ± 2.6s-z	2037.22 ± 63.3hij	7.67 ± 0.4pqr
DAB 410	3 ± 0.5k-p	1.54 ± 0.1l-u	0.07 ± 0.0v-y	1.33 ± 0.2r-v	61.28 ± 2.3b-f	7.18 ± 0.9-x	6.46 ± 0.5y-B	2005.83 ± 96.0h-k	4.54 ± 0.2t-w
CIM-RM02-73-1	3 ± 0.3m-r	2.33 ± 0.1a-h	0.3 ± 0.0h-k	3.17 ± 0.1d-l	39.85 ± 0.6l-t	19.26 ± 0.6g-j	23.40 ± 0.4f-k	2005.42 ± 10.4h-k	12.46 ± 0.2ghi
CIM-SUG-03-09-04	2 ± 0.4o-r	2.46 ± 0.2a-e	0.25 ± 0.0j-n	2.97 ± 0.4d-n	42.15 ± 4.1j-t	16.81 ± 0.9i-m	20.41 ± 2.1i-p	1986.67 ± 57.6h-l	9.99 ± 0.7j-o

(Continued)

TABLE 5 Continued

Genotype	Nodule number	N	N content	$\delta^{15}\text{N}$	Ndfa	N fixed	Soil N uptake	Grain yield	Shoot DM
	plant ⁻¹	%	g plant ⁻¹	‰	%	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	g plant ⁻¹
CIM-KHAK02-14-1	3 ± 0.7m-r	1.17 ± 0.1tu	0.16 ± 0.0p-t	1.27 ± 0.2s-v	61.95 ± 2.8b-e	15.80 ± 0.6i-o	13.00 ± 0.9r-x	1965.00 ± 44.9h-l	13.20 ± 0.2g
KG 98-36	2 ± 0.4o-r	1.40 ± 0.1m-u	0.06 ± 0.0wxy	3.37 ± 0.1c-k	37.43 ± 0.6m-u	3.97 ± 0.4x	6.13 ± 0.6zAB	1912.50 ± 83.4i-m	4.59 ± 0.5t-w
DAB 207	10 ± 0.7e	1.50 ± 0.1l-u	0.08 ± 0.0u-y	2.76 ± 0.9g-p	44.54 ± 10.9h-q	6.05 ± 1.9u-x	7.17 ± 0.5x-B	1857.08 ± 31.4j-m	5.13 ± 0.5s-v
KG 98-42	2 ± 0.3pqr	1.20 ± 0.0stu	0.05 ± 0.0wxy	1.67 ± 0.1p-v	57.27 ± 1.2b-h	5.09 ± 0.1wx	5.07 ± 0.1AB	1847.08 ± 105.4j-m	4.45 ± 0.1t-w
CZ 113-15 (SAB 630)	2 ± 0.3pqr	1.98 ± 0.1b-m	0.1 ± 0.0s-y	1.07 ± 0.2tuv	64.35 ± 2.0bcd	11.28 ± 0.8m-v	9.28 ± 0.3v-B	1791.67 ± 55.5k-n	16.64 ± 0.1f
CIM-RM00-141	3 ± 0.5m-q	2.33 ± 0.3a-h	0.25 ± 0.0j-n	2.80 ± 0.8g-p	44.15 ± 8.9h-q	17.13 ± 1.1i-m	20.69 ± 2.6i-p	1774.58 ± 54.5lmn	10.81 ± 0.4i-m
G 5207	3 ± 0.3qr	1.92 ± 0.2c-q	0.21 ± 0.0l-p	2.25 ± 0.6k-s	50.55 ± 6.7e-n	16.66 ± 1.5i-n	17.07 ± 1.9l-t	1602.92 ± 79.8no	10.68 ± 0.7i-m
KAB 77 F7.2-52	5 ± 0.6ijk	2.39 ± 0.1a-g	0.19 ± 0.0m-q	4.39 ± 0.3abc	25.56 ± 3.5uvw	8.35 ± 1.7p-x	17.53 ± 1.1j-s	1513.33 ± 135.8op	8.02 ± 0.4o-r
DAB 559	2 ± 0.4o-r	2.40 ± 0.2a-f	0.12 ± 0.0q-x	0.99 ± 0.1uv	88.41 ± 1.1a	17.72 ± 1.6i-l	9.87 ± 0.8v-B	1508.02 ± 53.3op	15.71 ± 1.7f
KAB 10 F2.8-84	3 ± 0.3m-r	2.62 ± 0.2a	0.58 ± 0.0d	4.73 ± 0.2ab	21.65 ± 2.4vw	21.28 ± 3.3f-i	45.44 ± 1.7b	1492.08 ± 48.7op	22.32 ± 0.8de
DAB 174	3 ± 0.3m-r	1.60 ± 0.1k-t	0.35 ± 0.0fgh	0.91 ± 0.1uv	66.22 ± 1.6bc	38.83 ± 3.2c	21.23 ± 0.4i-o	1306.25 ± 11.2pq	22.40 ± 0.7de
CIM-RM01-97-8*	31 ± 0.9b	2.58 ± 0.4ab	0.21 ± 0.0l-p	2.45 ± 0.7j-r	48.15 ± 7.9f-n	15.62 ± 0.7i-o	17.55 ± 2.3j-s	1200.00 ± 96.5qr	8.08 ± 0.2n-r
AND 277	2 ± 0.3qr	2.08 ± 0.1a-l	0.17 ± 0.0n-t	2.69 ± 0.5g-q	45.43 ± 5.2g-q	12.74 ± 1.4k-s	14.87 ± 1.1p-w	1186.25 ± 22.2qrs	8.18 ± 0.4n-q
DAB 387	3 ± 0.5m-q	2.34 ± 0.1a-g	0.11 ± 0.0s-y	1.40 ± 0.5r-v	60.42 ± 5.9b-f	10.62 ± 1.2n-w	9.38 ± 0.2v-B	1141.67 ± 7.7q-t	4.50 ± 0.1t-w
DAB 155	3 ± 0.5fg	1.58 ± 0.2k-t	0.15 ± 0.0p-u	1.74 ± 0.4o-v	56.49 ± 5.1b-i	13.65 ± 0.6j-q	12.90 ± 1.5r-x	1132.50 ± 125.0q-t	9.49 ± 0.4l-p
CIM-RM-03-03-45	3 ± 0.3k-p	2.53 ± 0.1abc	0.27 ± 0.0i-l	3.80 ± 0.1a-h	32.47 ± 2.0p-w	14.35 ± 0.2j-p	23.01 ± 1.6g-m	1130.56 ± 44.1q-t	12.75 ± 0.9gh
CIM-RM02-76-4	2 ± 0.5pqr	2.24 ± 0.0a-j	0.17 ± 0.0m-s	2.83 ± 0.1g-p	43.80 ± 1.3h-q	12.59 ± 1.2k-s	15.06 ± 1.3p-w	1106.25 ± 35.6q-u	7.69 ± 0.7pqr
DAB 470	1 ± 0.5r	2.32 ± 0.1a-h	0.31 ± 0.0hij	1.96 ± 0.2m-v	53.91 ± 2.6b-k	27.91 ± 0.5de	22.53 ± 1.0g-m	1087.50 ± 47.8q-u	13.50 ± 0.1g
NUA 743	1 ± 0.3qr	2.19 ± 0.3a-k	0.46 ± 0.1e	4.90 ± 0.4a	19.64 ± 4.7w	13.64 ± 1.8j-q	39.60 ± 5.7c	1044.58 ± 43.9r-v	20.84 ± 0.7e
DFA 73	2 ± 0.3n-r	1.95 ± 0.1c-o	0.17 ± 0.0o-t	3.04 ± 0.5d-m	41.37 ± 6.3k-t	11.66 ± 2.0m-u	14.88 ± 0.8p-w	1017.50 ± 55.6r-w	8.65 ± 0.5n-q
DAB 386	3 ± 0.3m-r	1.92 ± 0.5c-q	0.07 ± 0.0v-y	1.53 ± 0.4q-v	58.89 ± 4.6b-g	6.46 ± 1.5t-x	6.51 ± 1.8y-B	1015.42 ± 10.1r-w	3.59 ± 0.1vw
SMC 17	2 ± 0.3pqr	2.30 ± 0.2a-h	0.11 ± 0.0r-y	4.07 ± 0.1a-e	29.24 ± 0.8r-w	5.58 ± 0.5vwx	10.78 ± 0.7u-A	983.33 ± 64.9r-x	4.98 ± 0.2tuv
CIM-RM01-92-3	6 ± 0.4ghi	2.08 ± 0.3a-l	0.07 ± 0.0v-y	0.87 ± 0.5v	66.65 ± 5.3b	7.78 ± 1.5q-x	6.31 ± 0.7zAB	953.75 ± 33.5s-x	3.42 ± 0.4vw
DAB 447	2 ± 0.5n-r	1.67 ± 0.1i-t	0.06 ± 0.0wxy	0.97 ± 0.1uv	65.43 ± 0.1bc	6.81 ± 0.4-x	5.82 ± 0.3zAB	941.67 ± 17.4t-y	3.73 ± 0.0vw
CIM-RM00-27-4	3 ± 0.3n-r	2.43 ± 0.1a-f	0.40 ± 0.0ef	3.34 ± 0.2c-k	37.78 ± 1.7m-u	25.06 ± 1.9ef	29.69 ± 0.7de	886.67 ± 64.0u-Z	16.39 ± 0.6f
CIM-RM07-ALS-71-2*	15 ± 0.1d	2.44 ± 0.2a-e	0.31 ± 0.0hij	2.60 ± 0.2h-q	46.47 ± 1.9g-p	24.25 ± 3.9efg	22.93 ± 1.7g-m	846.67 ± 35.8v-A	12.56 ± 0.4ghi

(Continued)

TABLE 5 Continued

Genotype	Nodule number	N	N content	$\delta^{15}\text{N}$	Ndfa	N fixed	Soil N uptake	Grain yield	Shoot DM
	plant ⁻¹	%	g plant ⁻¹	‰	%	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	g plant ⁻¹
CIM-RM02-46-1	2 ± 0.3pqr	2.18 ± 0.0a-k	0.24 ± 0.0j-o	4.43 ± 0.1abc	25.11 ± 0.9uvw	10.15 ± 0.4o-w	21.86 ± 0.1g-n	846.25 ± 92.6v-A	11.15 ± 0.4h-l
DAB 378	4 ± 0.4j-n	1.94 ± 0.3c-p	0.30 ± 0.1hij	2.89 ± 0.3e-o	43.07 ± 3.2i-s	20.85 ± 2.4f-i	23.51 ± 3.5f-j	815.00 ± 7.6v-A	15.32 ± 0.6f
CIM-RM00-109*	15 ± 0.5d	2.25 ± 0.1a-j	0.30 ± 0.0hij	2.57 ± 0.2i-q	46.80 ± 2.2g-o	23.35 ± 0.8e-h	23.22 ± 1.7g-l	812.08 ± 48.9v-A	13.44 ± 0.7g
CIM-RM05-ALS-103	4 ± 0.3j-m	1.88 ± 0.1d-r	0.19 ± 0.0m-r	3.31 ± 0.3c-k	38.13 ± 3.6m-u	11.99 ± 2.0l-u	16.18 ± 0.9n-u	788.75 ± 19.7w-A	9.81 ± 0.4k-o
DAB 370	3 ± 0.7m-r	1.32 ± 0.1q-u	0.05 ± 0.0wxy	1.41 ± 0.2r-v	60.36 ± 3.3b-f	5.27 ± 0.2vwx	4.98 ± 0.1AB	752.50 ± 7.7x-B	4.02 ± 0.2uvw
NUA 35	1 ± 0.3qr	1.52 ± 0.1l-u	0.06 ± 0.0wxy	4.09 ± 0.0a-d	29.02 ± 0.5t-w	2.74 ± 0.1x	5.51 ± 0.2zAB	735.00 ± 52.5mn	3.73 ± 0.1vw
DAB 270	5 ± 0.6hij	1.66 ± 0.1i-t	0.15 ± 0.0p-u	2.18 ± 0.8k-t	51.41 ± 9.5d-m	12.83 ± 2.7k-s	12.90 ± 0.6r-x	721.25 ± 15.1y-B	8.94 ± 0.2m-q
CIM-KHAK02-20-1	3 ± 0.3m-r	1.36 ± 0.1n-u	0.04 ± 0.0y	2.88 ± 0.2f-o	43.20 ± 2.3i-s	2.77 ± 0.3x	3.78 ± 0.5B	707.92 ± 44.4Zab	2.85 ± 0.4w
CIM-RM02-62-1	3 ± 0.3m-q	2.48 ± 0.1a-e	0.33 ± 0.0ghi	2.67 ± 0.1h-q	45.68 ± 1.5g-p	25.13 ± 1.9ef	24.56 ± 0.9e-i	623.75 ± 27.6BC	13.28 ± 0.6g
CIM-RM00-21	5 ± 0.5i-l	2.28 ± 0.2a-i	0.39 ± 0.0efg	3.17 ± 0.3d-l	39.78 ± 2.9l-t	25.38 ± 0.7ef	29.03 ± 2.3ef	562.92 ± 33.1BC	17.07 ± 0.6f
NUC 461	2 ± 0.4o-r	2.19 ± 0.1a-k	0.55 ± 0.0d	4.87 ± 0.1a	19.99 ± 0.9w	18.42 ± 0.9h-k	45.11 ± 1.2b	420.42 ± 3.1C	25.27 ± 0.4c
F-Statistics	258.69**	5.89**	74.76**	11.29**	11.29**	47.08**	35.05**	138.52**	202.65**

Values (means ± SE; n=4) with dissimilar letters in a column are significantly different at **p ≤ 0.01, DM, dry matter; N, nitrogen; Ndfa, nitrogen derived from the atmosphere), * =Nematodes tolerant genotypes.

CIM-KHAK02-20-1 and NUC 451, respectively. Soil N uptake was highest for genotypes NUC 451 (54.01 kg ha⁻¹), NUC 461 and NUA 743 (39.60 kg ha⁻¹) in contrast with genotypes CIM-KHAK02-20-1 (3.78 kg ha⁻¹), DAB 411 (4.44 kg ha⁻¹) and DAB 370 (4.98 kg ha⁻¹), which recorded the least soil N uptake.

Grain yield

The grain yield of the 63 common bean genotypes ranged from 420.4 kg ha⁻¹ for NUC 461 to 3618.8 kg ha⁻¹ for CIM-RM02-79-1. Of the 63 bean genotypes, 24 produced grain yield above 2000 kg ha⁻¹, and of these 24, five genotypes could produce over 3000 kg ha⁻¹ grain yield. Genotype CIM-RM02-79-1 produced grain yields above 3000 kg ha⁻¹, followed by genotypes NAIN DEKYONDO, CIM-RM00-7-1, NUC 219 and CAL 96, which produced grain yields of 3 384, 3 323, 3–094 and 3–086 kg ha⁻¹, respectively. Sixteen genotypes produced grain yield below 1000 kg ha⁻¹, with values ranging from 420.4 to 983.3 kg ha⁻¹ (Table 5).

Shoot dry matter

Shoot dry matter differed significantly between the 63 test genotypes (Table 5) with genotypes NUC 219 (41.52 g plant⁻¹), NUC 451 (41.04 g plant⁻¹) and MCA 78 (37.05 g plant⁻¹) recording the highest shoot biomass, and genotypes CIM-KHAK02-20-1 (2.85 g plant⁻¹) and CIM-RM01-92-3 (3.42 g plant⁻¹) producing the least shoot dry matter (Table 5). Thirty one out of the 63 genotypes had shoot biomass above 10 g plant⁻¹.

Shoot C concentration (%C) and C content

Shoot C concentration revealed significant differences between and among the 63 bean genotypes (Table 6), with genotype DAB 174 exhibiting the highest shoot C concentration (43.68%), followed by DAB 447 (42.97%) and then CIM-RM02-62-1 (42.31%). In contrast, the lowest C concentration was recorded by genotype KHAK02-20-1 (35.91%), followed by CIM-RM-03-03-45 (36.70%), and DAB 369 (36.73%). About 40 genotypes recorded shoot C concentration above 40% (Table 5). The C content of common bean shoots reflected the shoot dry matter and ranged from 1.03 to 17.49 g plant⁻¹ (Table 6). The shoot C content was much higher in genotype NUC 219 (17.49 g plant⁻¹), followed by NUC 451 (16.19 g plant⁻¹), and MCA 78 (14.89 g plant⁻¹) due to their greater shoot biomass. In contrast, genotypes CIM-KHAK02-20-1 (1.03 g plant⁻¹), CIM-RM01-92-3 (1.41 g plant⁻¹), and DAB 386 (1.50 g plant⁻¹) recorded the lowest C content due to their lower shoot dry matter (Table 6).

Shoot $\delta^{13}\text{C}$

The one-way ANOVA revealed significant difference in the shoot $\delta^{13}\text{C}$ values of the 63 genotypes. The genotypes AND 277, CIM-RM00-

27-4 and CIM-RM01-97-8 showed much greater $\delta^{13}\text{C}$ values (high water use efficiency) of -26.58‰, -26.78‰ and -26.90‰, respectively, with several other genotypes exhibiting statistically similar values for the parameter (Table 6). The lowest shoot $\delta^{13}\text{C}$ values were recorded in the common bean genotypes KG 98-36, DAB 410 and DAB 411 with values of -30.11‰, -29.58‰ and -29.44‰, respectively (Table 6).

C/N ratio

The C/N ratio is a measure of the C to N relationship in plants and is an estimate of photosynthetic N-use efficiency. The C/N ratio of the common bean shoots differed significantly between the genotypes (Table 6) and ranged from 14.72 to 42.21 g g⁻¹. The shoot C/N values were much greater for genotype DAB 411 (42.21 g g⁻¹), followed by CIM-KHAK02-14-1 (35.53 g g⁻¹) and then DAB 429 (32.63 g g⁻¹) (Table 6), as a result of their lower shoot N (Table 5). In contrast, genotypes CIM-RM-03-03-45 (14.72 g g⁻¹), NUC 451 (15.63 g g⁻¹) and MCA 98 (15.64 g g⁻¹) showed the least C/N ratios because of their greater shoot N (Table 5).

Correlation analysis

Several correlations between measured parameters were significant, although weak. For example, there was a significant correlation between the number of nodules per plant and %N ($r = 0.21^{**}$), N content ($r = 0.23^{***}$), N-fixed ($r = 0.28^{***}$), as well as soil N uptake ($r = 0.28^{***}$) (Table 7, Figure 1, and S1). Also, there was a strong negative correlation between %Ndfa and $\delta^{15}\text{N}$ ($r = -1.000^{***}$), while a strong positive correlation was observed between N-fixed and shoot dry matter ($r = 0.88^{***}$). Shoot N content correlated significantly with shoot $\delta^{13}\text{C}$ ($r = 0.25^{***}$). Similarly, shoot biomass also correlated significantly with $\delta^{13}\text{C}$ ($r = 0.17^{**}$) and, as expected, shoot dry matter was significantly correlated with shoot C content ($r = 0.99^{***}$). The strong relationship among the measured parameters has been reinforced in Table 7, Figure 1 and Supplementary Figure S1.

Discussion

Symbiotic N nutrition by common bean in Malkerns, Eswatini

The application of chemical N fertilizers has been reported to significantly improve crop yields in nutrient-poor soils, especially those with low N levels. However, the high costs of these fertilizers to resource-poor farmers and their negative impact on the environment are well documented (Tadesse et al., 2013), highlighting the need to seek friendly alternatives.

In this study, 63 common bean genotypes were evaluated for plant growth and symbiotic parameters including grain yield. The tested genotypes differed markedly in root nodulation, with nodule numbers ranging from an average of 1 to 59 nodules per plant for genotypes DAB 470 and MCA 98 respectively (Table 5). Although

TABLE 6 Plant growth, C concentration and shoot $\delta^{13}\text{C}$ (WUE) of 63 common bean genotypes planted at Malkerns Research station, Eswatini, during 2017/2018 cropping season.

Genotype	C	C Content	$\delta^{13}\text{C}$	C/N ratio
	%	g plant ⁻¹	‰	g g ⁻¹
AND 277	40.76 ± 0.8a-i	3.33 ± 0.1tuv	-26.58 ± 0.1a	19.73 ± 1.1j-q
CIM-RM00-27-4	41.46 ± 0.4a-h	6.80 ± 0.3gh	-26.78 ± 0.2ab	17.19 ± 0.9opq
CIM-RM01-97-8	40.94 ± 0.5a-h	3.31 ± 0.1tuv	-26.90 ± 0.2abc	16.80 ± 2.3opq
KAB 77 F7.2-52	40.42 ± 0.1b-k	3.25 ± 0.2uv	-27.11 ± 0.4b-d	17.00 ± 0.6opq
DFA 73	39.60 ± 0.7c-l	3.43 ± 0.2s-v	-27.13 ± 0.2b-d	20.35 ± 0.8i-q
CIM-KHAK02-14-1	39.71 ± 0.4c-l	5.24 ± 0.1klm	-27.20 ± 0.4a-e	34.53 ± 2.7b
CIM-RM02-62-1	42.31 ± 0.9a-d	5.63 ± 0.4jk	-27.21 ± 0.2a-e	17.16 ± 1.0opq
CIM-RM02-76-1	40.74 ± 1.2a-i	6.27 ± 0.2hi	-27.28 ± 0.2a-f	23.31 ± 1.4e-p
CIM-RM02-76-4	41.87 ± 0.6a-f	3.22 ± 0.3uv	-27.39 ± 0.2a-f	18.71 ± 0.5l-q
CIM-RM-03-03-45	36.70 ± 1.3lm	3.89 ± 0.2q-u	-27.41 ± 0.3a-f	14.72 ± 1.3q
NAIN DEKYONDO	38.85 ± 0.9f-m	4.13 ± 0.1o-s	-27.45 ± 0.1a-f	30.32 ± 3.1b-e
CIM-RM00-7-1	40.35 ± 0.5b-k	2.51 ± 0.1wx	-27.47 ± 0.4a-f	18.93 ± 3.7l-q
CIM-RM00-21	40.67 ± 1.2a-i	6.93 ± 0.2g	-27.49 ± 0.2a-g	18.14 ± 1.5m-q
KAB 06 F2.8-51	42.05 ± 0.6a-e	9.52 ± 0.4e	-27.53 ± 0.2b-g	23.35 ± 0.6e-p
NUA 743	40.66 ± 0.2a-i	8.47 ± 0.3f	-27.55 ± 0.2b-g	19.52 ± 2.6k-q
NUC 219	41.88 ± 0.4a-f	17.49 ± 0.2a	-27.59 ± 0.08b-h	21.19 ± 1.0g-q
CIM-SUG-03-09-04	38.83 ± 0.3f-m	3.88 ± 0.3q-u	-27.60 ± 0.55b-h	16.01 ± 1.0opq
CIM-RM02-79-1	38.38 ± 0.8h-m	3.35 ± 0.2tuv	-27.61 ± 0.16b-h	19.60 ± 1.1k-q
CIM-RK06-ALS-S1-2	41.07 ± 0.9a-h	3.26 ± 0.1uv	-27.71 ± 0.31b-i	15.73 ± 0.5pq
DAB 470	40.98 ± 0.4a-h	5.53 ± 0.1jkl	-27.73 ± 0.54b-i	17.84 ± 1.0m-q
DAB 381	40.19 ± 0.3b-k	3.18 ± 0.1uv	-27.74 ± 0.77b-i	28.56 ± 4.1b-g
CIM-RM00-109	41.17 ± 0.9a-h	5.53 ± 0.3jkl	-27.74 ± 0.1b-i	18.38 ± 0.7l-q
CIM-RM02-20-3	39.74 ± 0.2c-l	4.66 ± 0.2m-p	-27.76 ± 0.1c-i	21.29 ± 0.4g-q
CODMLB 03	39.74 ± 0.9c-l	1.97 ± 0.2xy	-27.77 ± 0.2c-i	16.45 ± 0.4opq
NUC 451	38.99 ± 1.4e-l	16.19 ± 0.6b	-27.78 ± 0.5c-i	15.63 ± 0.6pq
CIM-RM07-ALS-71-2	39.36 ± 1.6d-l	4.95 ± 0.3lmn	-27.81 ± 0.0c-j	16.64 ± 1.8opq
MCA 98	41.06 ± 0.2a-h	9.41 ± 0.2e	-27.81 ± 0.3c-j	15.64 ± 1.5pq
NUA 720	41.26 ± 0.5a-h	4.77 ± 0.1mno	-27.83 ± 0.1c-k	22.19 ± 0.9f-q
CIM-RM02-73-1	39.65 ± 0.6c-l	4.94 ± 0.2lmn	-27.93 ± 0.1d-l	17.05 ± 0.4opq
DAB 559	40.52 ± 0.5b-j	2.03 ± 0.1xy	-27.97 ± 0.2d-l	17.09 ± 1.1opq
DAB 370	40.95 ± 0.2a-h	1.65 ± 0.1yz	-27.98 ± 0.5d-l	31.42 ± 1.7bcd
CZ 113-15 (SAB 630)	38.59 ± 1.1g-m	2.04 ± 0.1xy	-28.03 ± 0.1d-l	19.51 ± 0.6k-q
CIM-RM02-46-1	40.69 ± 0.6a-i	4.54 ± 0.2n-q	-28.06 ± 0.0d-m	18.68 ± 0.4l-q
CIM-RM00-141	39.54 ± 0.1c-l	4.27 ± 0.1n-r	-28.10 ± 0.5d-m	17.89 ± 2.2m-q
CIM-RM01-92-3	41.09 ± 1.0a-h	1.41 ± 0.2yz	-28.15 ± 0.4e-n	21.13 ± 3.2g-q
KAB 10 F2.8-84	41.36 ± 0.7a-h	9.22 ± 0.3e	-28.17 ± 0.3e-n	16.07 ± 1.2opq
KG 98-42	37.36 ± 1.0klm	1.66 ± 0.1yz	-28.19 ± 0.2e-n	31.13 ± 0.9bcd

(Continued)

TABLE 6 Continued

Genotype	C	C Content	$\delta^{13}\text{C}$	C/N ratio
	%	g plant ⁻¹	‰	g g ⁻¹
DAB 378	40.12 ± 0.4b-k	6.15 ± 0.3ij	-28.25 ± 0.2f-o	22.13 ± 3.4f-q
DAB 386	41.69 ± 0.4b-k	1.50 ± 0.0yz	-28.26 ± 0.2f-p	27.97 ± 7.9b-h
DAB 387	40.30 ± 0.2b-k	1.81 ± 0.0xy	-28.27 ± 0.3f-p	17.27 ± 0.4opq
DAB 155	40.41 ± 0.1b-k	3.83 ± 0.2q-u	-28.48 ± 0.1g-q	26.65 ± 3.1c-k
SMC 17	37.45 ± 0.8j-m	1.86 ± 0.1xy	-28.48 ± 0.1g-q	16.43 ± 0.8opq
DAB 369	36.73 ± 0.8lm	4.63 ± 0.2m-p	-28.55 ± 0.2h-q	17.46 ± 3.2n-q
CZ 104-13	39.42 ± 0.3d-l	3.82 ± 0.2q-u	-28.69 ± 0.0i-r	19.95 ± 0.2j-q
MCA 78	40.71 ± 0.5a-i	14.89 ± 0.3c	-28.70 ± 0.1i-r	21.20 ± 0.5g-q
DAB 174	43.68 ± 2.3a	9.55 ± 0.6e	-28.77 ± 0.5j-r	27.47 ± 1.8b-i
DAB 447	42.97 ± 1.9ab	1.60 ± 0.1yz	-28.79 ± 0.1k-r	25.71 ± 0.8c-k
CAL 96	38.49 ± 0.9h-m	4.78 ± 0.2mno	-28.85 ± 0.4l-r	22.61 ± 0.8f-p
NUA 35	41.08 ± 0.6a-h	1.53 ± 0.1yz	-28.87 ± 0.1l-r	27.14 ± 1.2c-j
G 5207	37.74 ± 0.9i-m	4.02 ± 0.2p-t	-28.88 ± 0.3l-r	20.12 ± 1.6i-q
DAB 429	38.67 ± 1.0g-m	4.54 ± 0.2n-q	-28.89 ± 0.2l-r	32.63 ± 6.3bc
CIM-RM05-ALS-103	39.20 ± 0.8d-l	3.85 ± 0.2q-u	-28.90 ± 0.3l-r	21.01 ± 0.1h-q
NUC 461	42.69 ± 2.4abc	10.80 ± 0.7d	-29.04 ± 0.3m-r	19.56 ± 1.3k-q
DAB 363	40.85 ± 0.6a-i	4.42 ± 0.1n-q	-29.11 ± 0.1n-r	21.59 ± 0.9g-q
DAB 270	41.22 ± 0.4a-h	3.68 ± 0.1r-u	-29.17 ± 0.4o-r	24.98 ± 1.1d-n
NCB 281	36.98 ± 0.9lm	2.15 ± 0.1wxy	-29.17 ± 0.7o-r	23.50 ± 2.3e-o
DAB 190	41.01 ± 0.5a-h	2.74 ± 0.1vw	-29.23 ± 0.0p-s	25.13 ± 0.5d-m
NUA 735	41.26 ± 0.5a-h	3.84 ± 0.0q-u	-29.30 ± 0.0rs	32.02 ± 1.5bcd
CIM-KHAK02-20-1	35.96 ± 1.3m	1.03 ± 0.1z	-29.41 ± 0.1qrs	26.75 ± 2.1c-k
DAB 207	40.32 ± 0.4b-k	2.07 ± 0.2xy	-29.43 ± 0.2qrs	27.17 ± 1.7b
DAB 411	42.30 ± 0.4a-d	1.97 ± 0.0xy	-29.44 ± 0.2qrs	42.21 ± 1.1a
DAB 410	42.13 ± 0.7a-e	1.91 ± 0.1xy	-29.58 ± 0.2rs	27.80 ± 1.7b-h
KG 98-36	40.61 ± 1.2a-i	1.85 ± 0.2xy	-30.11 ± 0.1s	29.39 ± 1.1b-f
F-Statistics	3.30***	251.17**	7.90**	7.17**

Values (means ± SE; n=4) with dissimilar letters in a column are significantly different at **p ≤ 0.01, ***p ≤ 0.001, DM, dry matter; C, carbon; N, nitrogen.

the experiment was conducted at one location, growth and root nodulation can also be altered by micro-site variation through differences in mineral nutrient concentration and/or soil rhizobial populations. In this instance, however, the observed variation in root nodulation were statistically significant and could be attributed to genetic variation among the tested common bean genotypes. It is worth noting that, the experimental field was largely nematode infested, a factor that might have inhibited nodulation in some genotypes, but not in others (Table 5). Whereas nematode infestation was not considered as an experimental factor in this study, the bean genotypes that produced many effective root nodules possess resistant traits, while those that were susceptible showed no nodulation (i.e., zero nodules per plant). It will therefore

be worthwhile to test these hypotheses under controlled conditions to ascertain bean genotypes with resistance to nematodes, an effort that can contribute to breeding efforts to improve the crop's yield under both biotic and abiotic constraints in the field.

Shoot N content showed a marked variation between and among the bean genotypes, highlighting differences in both N₂ fixation and soil N uptake (Table 5). Nevertheless, the observed variation in shoot N accumulation were found to be dependent on plant growth measured as shoot biomass. Among the common bean genotypes tested, the highest N concentration was found in genotype MCA 98, followed by KAB 10 F2.8-84, CIM-RM01-97-8, CIM-RM-03-03-45 and NUC 451; however, the total N accumulated in shoots (N content) was not necessarily high in those genotypes. These observations suggests that

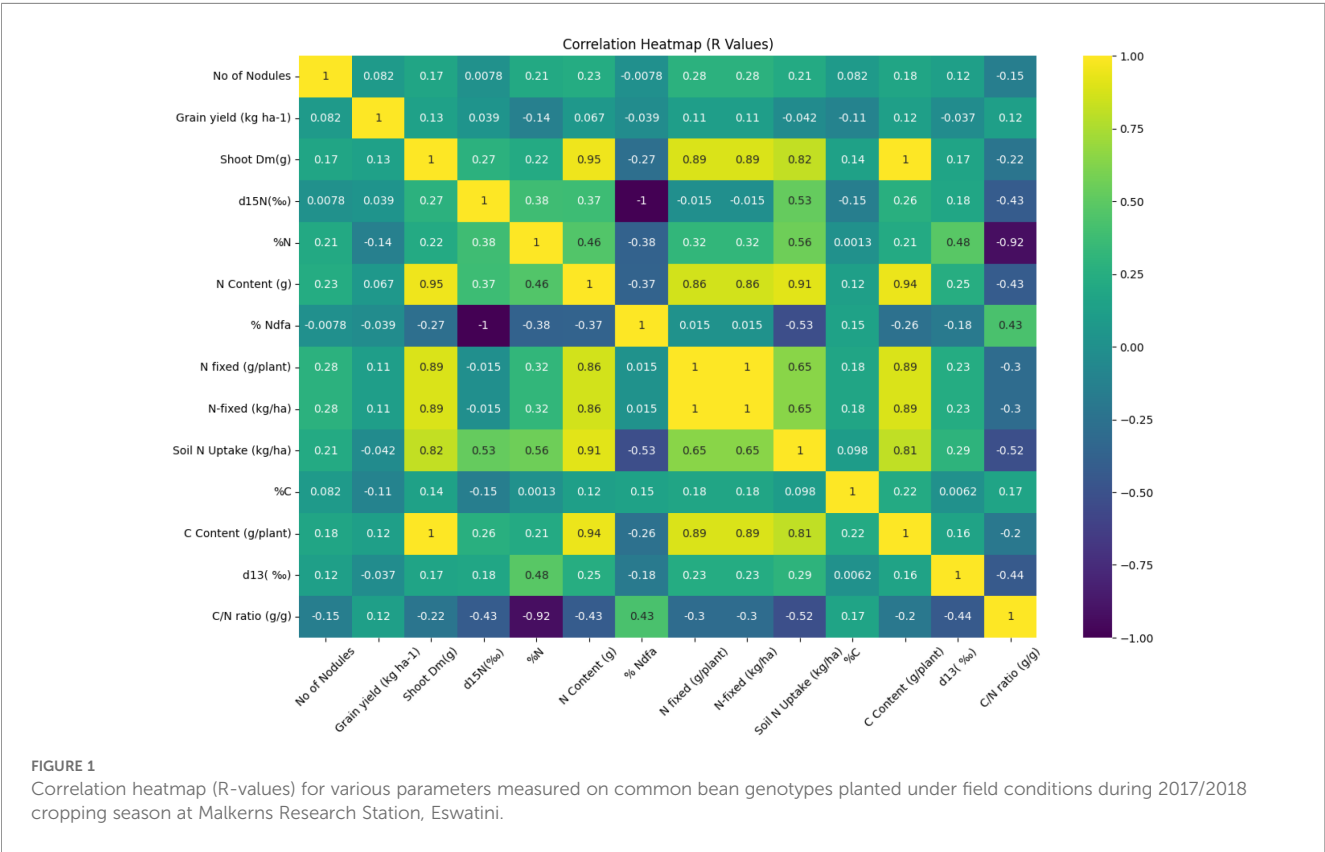
TABLE 7 Correlation analysis of common bean genotypes planted under field conditions during 2017/2018 cropping season at Malkerns Research Station, Eswatini.

Parameter	R-value	P-value
%N vs Nodule number	0.2064	0.001
N content vs Nodule number	0.2337	0.0002
Soil N uptake vs Nodule number	0.2119	0.0007
N-fixed vs Nodule number	0.2765	0.00001
$\delta^{15}\text{N}$ vs %Ndfa	-1.000	0.00001
Shoot DM vs N-fixed	0.8805	0.00001
N content vs $\delta^{13}\text{C}$	0.2495	0.00006
Shoot DM vs $\delta^{13}\text{C}$	0.1711	0.0065
% N vs Amount of N-fixed	0.3191	0.0001
$\delta^{13}\text{C}$ vs Grain yield	-0.0366	0.054
%Ndfa vs soil N uptake	-0.5305	0.001

some genotypes may exhibit high N use efficiency, thereby accumulating greater shoot biomass in the presence of limited amount of N. Moreover, the fact that shoot N concentration did not mirror its $\delta^{15}\text{N}$ or %Ndfa values in most instances is an indication that the plants exhibited a significant dependence on soil N uptake for their N nutrition and hence, growth. Due to the high energy requirement for symbiotic N_2 fixation, legumes are known to show preference endogenous soil N uptake (Vance, 1998). Nevertheless, shoot $\delta^{15}\text{N}$

values differed markedly among the common bean genotypes and ranged from +0.87 to +4.90‰ for CIM-RM01-92-3 and NUA 743, respectively (Table 5). In accordance with earlier studies (Unkovich et al., 2008; Mohale et al., 2014; Manandhar et al., 2024), the genotypes with low shoot $\delta^{15}\text{N}$ were found exhibit a higher %Ndfa, and vice versa. In this study, 21 genotypes with relatively lower shoot $\delta^{15}\text{N}$ values recorded 50% and more %Ndfa values, indicating a relatively higher dependence on N_2 fixation for their N nutrition when compared to soil N uptake. As shown in Table 5, common bean genotypes with greater shoot $\delta^{15}\text{N}$ values (e.g., NUA 743, NUC 461, and KAB 10 F2.8-84) were found to derive very small of their N requirements from symbiotic fixation (Table 5).

Because of the differences in shoot $\delta^{15}\text{N}$ values, %Ndfa and shoot dry matter yield, the amounts of N-fixed also varied significantly between and among the 63 bean genotypes and ranged from 2.7 kg N ha⁻¹ to 66.8 kg N ha⁻¹ for NUA 35 and NUC 219, respectively (Table 5). Symbiotic N contribution by the common bean genotypes was therefore generally low, an observation consistent with earlier reports that the species is a low N_2 -fixer (Mnasri et al., 2007; Peoples et al., 2009). Despite the low N_2 fixation by most genotypes, genotypes NUC 219 and MCA 78 contributed over 50 kg N ha⁻¹, suggesting that they could withstand low N soils. High N_2 -fixing food legumes such as cowpea, groundnut and Bambara groundnut are reported to have huge rotation benefits when followed by cereal crops (Lengwati et al., 2020). Given the importance of the common bean in Eastern and Southern Africa, field studies are needed to assess the rotation benefit of this species. Consistent with their low levels of N_2 fixation,



most of the genotypes depended on soil N to meet their N requirements, soil N uptake values ranging from a low 3.8 kg ha⁻¹ for genotype CIM-KHAK02-20-1 to 54.0 kg ha⁻¹ for genotype NUC 451 (Table 5). Taken together, 40 out of the 63 genotypes tested obtained more N from soil than from symbiosis, suggesting a need for more efforts to improve the efficiency of the common bean/rhizobia symbiosis for improved yields.

Despite this heavy reliance on soil N by the tested common beans, there was a weak but statistically significant correlation between nodule numbers per plant and shoot N concentration (%) ($r=0.21^{**}$), N content ($r=0.23^{***}$) and soil N uptake ($r=0.21^{***}$). These significant correlations can have different interpretations. For example, the relationship between nodule number and soil N uptake could mean that soil N uptake at small levels stimulated nodule formation which mimics the effect of starter N on root nodulation in low N soils (Tsai et al., 1993); consequently, shoot N concentration and content also correlate positively with nodule number per plant. However, the significant correlation between nodule number and amount of N-fixed could also suggest that effective nodulation led to greater N₂ fixation and fixed-N accumulation in bean plants (Kontopoulou et al., 2015; Habinshuti et al., 2021; Kebede, 2021). It is more likely that increased soil N uptake by the plants complemented N₂ fixation in meeting the N requirements of the plants. Greater shoot $\delta^{15}\text{N}$ values in nodulated legumes tend to reflect reduced percent N derived from nitrogen fixation as illustrated in Table 7, Figure 1, and Supplementary Figure S1. On the other hand, an increase in N₂ fixation often results in increased plant growth, measured as shoot biomass (Belane and Dakora, 2010), and shows up as a significant correlation between amount of N-fixed and shoot dry matter and/or grain yield. In this study, the 63 bean genotypes revealed marked differences in grain yield, with the high yielding genotype CIM-RM02-79-1 producing up to 3619 kg ha⁻¹ of grain yield and the lowest yielding genotype NUC 461 producing only 420 kg ha⁻¹ of seed (Table 5). Often, an increase in grain yield could be linked to enhanced N nutrition from symbiosis, soil N uptake, or both (Govindasamy et al., 2023).

However, the highest yielding genotype CIM-RM02-79-1 was not the highest in plant growth and water-use efficiency (Table 5 and 6), nodulation and N₂ fixation (Table 5). Therefore, its marked reproductive performance (grain yield) must be an intrinsic trait linked to factors such as efficient mineral uptake, translocation, and exchange at the xylem/phloem interface. About 24 bean genotypes produced grain yield above 2000 kg ha⁻¹, out of which five produced more than 3000 kg ha⁻¹ of grain (Table 5). These observed wide variability in yield traits of the test genotypes offers opportunity for selection and further manipulation in breeding programs to produced more desirable varieties.

Carbon assimilation, C/N and water use efficiency in common bean

Shoot C accumulation, C/N ratio and $\delta^{13}\text{C}$ were assessed in the 63 bean genotypes grown under field conditions. The data revealed a marked variation in shoot C content, C concentration, C/N ratio and

$\delta^{13}\text{C}$ among the 63 genotypes (Table 6). Carbon accumulation in plants is a direct measure of photosynthetic activity (Maseko and Dakora, 2016). Shoot C concentration of the 63 common bean genotypes ranged from 36.98 to 43.68%, values similar to those reported in previous studies for Bambara groundnut by Mohale et al. (2014) and groundnut (Muhaba and Dakora, 2020). Of the genotypes tested, forty (40) recorded shoot C concentrations higher than 40%. Post et al. (2007) demonstrated that shoot C concentration above 40% is associated with elevated lipid distribution. Shoot C content also varied among the bean genotypes and was more related to the amount of shoot biomass and is highlighted by a direct relationship between biomass and C content ($r=0.99^{***}$) (Table 7; Figure 1).

Differences in C assimilation between the test genotypes could be attributed to genotypic variation, as photosynthetic C accumulation is genetically controlled (Roberts et al., 2007; Maseko and Dakora, 2016). Photosynthetic C assimilation is also linked to N₂ fixation in symbiotic legumes such that, greater the N₂ fixation in legumes may stimulate higher photosynthetic rate as earlier reported for cowpeas (Belane and Dakora, 2010).

Leaf photosynthetic C accumulation in plants is regulated by N nutrition (Maseko and Dakora, 2016). The C/N ratio is therefore an excellent indicator of the N nutrition status in plants and serves as a metabolic link between C and N in shoots (Mohale et al., 2014). Nitrogen-fixing legumes often have a C/N ratio of less than 24 g g⁻¹, whereas non-legumes have a C/N ratio greater than 24 g g⁻¹ (Mohale et al., 2014; Maseko and Dakora, 2016). In this study, the C/N ratios varied from 14.71 to 42.21 g g⁻¹, with genotype DAB 411 recording the highest C/N ratio (42.21 g g⁻¹), which indicates elevated photosynthetic C accumulation. Of the 63 common bean genotypes tested, 19 recorded C/N ratios above 24 g g⁻¹, thus showing a reduced tissue N concentration.

WUE in C3 plants is linked to carbon isotope discrimination during photosynthesis, measured using ^{13}C natural abundance (Makoi et al., 2010; Maseko and Dakora, 2016). In general, lower (more negative) $\delta^{13}\text{C}$ values indicate low WUE, while greater (less negative) shoot $\delta^{13}\text{C}$ values indicate high WUE. In that regard, genotype KG 98-36 exhibited higher ^{13}C discrimination during photosynthesis (i.e., lower $\delta^{13}\text{C}$) and therefore showed the lowest WUE, followed by genotypes DAB 410 and DAB 411 with $\delta^{13}\text{C}$ values of -30.11 ‰, -29.58 ‰ and -29.44 ‰, respectively). In contrast, the shoot of genotype AND 277 recorded the lowest ^{13}C discrimination (i.e., higher $\delta^{13}\text{C}$), and thus higher WUE (Table 5). These water-use efficient genotypes identified in Eswatini are ideal candidates for increased food security in a changing climate. The exact mechanisms of water use efficiency by the genotypes were not determined; nevertheless, plant traits related to root morphology or root architecture often play key roles in the adaptation of plants to water stressed environments (Huang, 2000). Moreover, differences in the levels of metabolites (e.g., lumichrome and riboflavin) released by rhizobia can alter plant water relations (Matiru and Dakora, 2005). Furthermore, N nutrition can also affect the water-use efficiency of the bean genotypes, as shown by the significant correlation between shoot N content and $\delta^{13}\text{C}$ in this study (Table 1; Figure 1).

In conclusion, the 63 common bean genotypes differed in plant growth, root nodulation, N nutrition via soil uptake and/or

symbiosis, shoot C concentration, C content, $\delta^{13}\text{C}$ and C/N ratio and ultimately in grain yield. The best performing genotype produced 3619 kg ha⁻¹ of grain yield, while the least produced only 420 kg ha⁻¹ of grain. The high yield in genotype CIM-RM02-79-1 was not linked to any obvious trait such as enhanced N nutrition via soil uptake or symbiosis (indicating that there is window to further improve the yield of this genotype by optimizing its nitrogen nutrition, particularly via symbiosis), shoot accumulation of minerals or better water-use efficiency, and must therefore be explored further at the level of gene expression for metabolite loading in the xylem and phloem and/or efficiency in translocation between sources and sink in shoot organs. Shoot biomass was greater in genotype NUC 219, followed by NUC 451 and MCA 78; generally, the genotypes with larger shoot biomass recorded greater C content. Genotype AND 277 showed a greater shoot $\delta^{13}\text{C}$, followed by CIM-RM00-27-4 and CIM-RM01-97-8, all of which have the potential for inclusion in breeding programs aimed at developing varieties that can thrive under drought conditions. In contrast, genotype KG 98-36 recorded the least shoot $\delta^{13}\text{C}$ and will therefore require significant improvement for cultivation in this era of climate change. Water-use efficiency measured as $\delta^{13}\text{C}$ from isotopic analysis was useful tool in identifying drought tolerant genotypes. Screening more common bean material could permit the selection of new genotypes with better plant growth, symbiotic N₂ fixation and WUE.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

RG: Data curation, Methodology, Validation, Writing – original draft, Writing – review & editing. TYN: Writing – review & editing. FD: Funding acquisition, Supervision, Writing – review & editing. MM: Writing – review & editing, Supervision.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work was supported with

funds from the National Research Foundation (NRF) grant number MND200709542372. The Tshwane University of Technology is grateful acknowledged for financial support of the research.

Acknowledgments

The authors also appreciate support from Mr. Manana and all staff of the Malkerns Research Station in Eswatini for helping with field experiments to obtain plant samples.

Conflict of interest

The author(s) declared that this work 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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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2025.1713315/full#supplementary-material>

References

- Belane, A. K., and Dakora, F. D. (2010). Symbiotic N₂ fixation in 30 field-grown cowpea (*Vigna unguiculata* L. Walp.) genotypes in the Upper West Region of Ghana measured using ¹⁵N natural abundance. *Biol. Fertil.* 46, 191–198. doi: 10.1007/s00374-009-0415-6
- Blum, A. (2009). Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Res.* 112, 119–123. doi: 10.1016/j.fcr.2009.03.009
- Bray, R. H., and Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* 59, 39–46. doi: 10.1097/00010694-194501000-00006
- Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analyses of soils 1. *Agron. J.* 54 (5), 464–465.
- Broughton, W. J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., and Vanderleyden, J. (2003). Beans (*Phaseolus* spp.)—model food legumes. *Plant Soil* 252, 55–128. doi: 10.1023/A:1024146710611

- Condon, A. G., Richards, R. A., Rebetzke, G. J., and Farquhar, G. D. (2004). Breeding for high water-use efficiency. *J. Exp. Bot.* 55, 2447–2460. doi: 10.1093/jxb/erh277
- Dlamini, S. T., Jaiswal, S. K., Mohammed, M., and Dakora, F. D. (2021). Studies of phylogeny, symbiotic functioning and ecological traits of indigenous microsymbionts nodulating Bambara groundnut (*Vigna subterranea* L. Verdc) in Eswatini. *Microb. Ecol.* 1, 1–6. doi: 10.1007/s00248-021-01684-0
- Du plessis, R. F., and Burger, R. D. T. (1965). A comparison of chemical extraction methods for the evaluation of phosphate availability of topsoil. *S. Afr. J. Sci.* 8, 1113–1122. Available online at: https://hdl.handle.net/10520/AJA05858860_414 (Accessed July 21, 2025).
- Edje, O. T., and Ossom, E. M. (2009). *Crop science handbook* (Manzini, Swaziland: Blue Moon Publishers).
- Farid, M., Earl, H. J., and Navabi, A. (2016). Yield stability of dry bean genotypes across nitrogen-fixation-dependent and fertilizer-dependent management systems. *Crop Sci.* 56, 173–182. doi: 10.2135/cropsci2015.06.0343
- Farid, M., and Navabi, A. (2015). N₂ Fixation ability of different dry bean genotypes. *Can. J. Plant Sci.* 95, 1243–1257. doi: 10.4141/cjps-2015-084
- Farooq, M., Wahid, A., Kobayashi, N. S. M. A., Fujita, D. B. S. M. A., and Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. In: *Sustainable Agriculture*, eds. E. Lichtfouse, M. Navarrete, P. Debaeke, V. Souchere and C. Alberola (Dordrecht Heidelberg London New York: Springer), 153–188. doi: 10.1007/978-90-481-2666-8
- Farquhar, G. D., Ehleringer, J. R., and Hubick, K. T. (1989). Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 503–537. doi: 10.1146/annurev.pp.40.060189.002443
- Farquhar, G. D., and Richards, R. A. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Funct. Plant Biol.* 11, 539–552. doi: 10.1071/PP9840539
- Ferrio, J. P., Florit, A., Vega, A., Serrano, L., and Voltas, J. (2003). $\Delta^{13}C$ and tree-ring width reflect different drought responses in *Quercus ilex* and *Pinus halepensis*. *Oecologia* 137, 512–518. doi: 10.1007/s00442-003-1372-7
- Good, A. G., and Beatty, P. H. (2011). Fertilizing nature: a tragedy of excess in the commons. *PLoS Biol.* 9, 1001124. doi: 10.1371/journal.pbio.1001124
- Govindasamy, P., Muthusamy, S. K., Bagavathiannan, M., Mowrer, J., Jagannadham, P. T. K., Maity, A., et al. (2023). Nitrogen use efficiency—a key to enhance crop productivity under a changing climate. *Front. Plant Sci.* 14, 1121073. doi: 10.3389/fpls.2023.1121073
- Habinshuti, S. J., Maseko, S. T., and Dakora, F. D. (2021). Inhibition of N₂ fixation by N fertilization of common bean (*Phaseolus vulgaris* L.) plants grown on fields of farmers in the eastern cape of South Africa, measured Using ¹⁵N natural abundance and tissue ureide analysis. *Front. Agron.* 3, 692933. doi: 10.3389/fagro.2021.692933
- Huang, B. (2000). “Role of root morphological and physiological characteristics in drought resistance of plants,” in *Plant-Environ. Interact* (CRC Press), 39–64.
- Kamfwa, K., Cichy, K. A., and Kelly, J. D. (2015). Genome-wide association analysis of symbiotic nitrogen fixation in common bean. *Theor. Appl. Genet.* 128, 1999–2017. doi: 10.1007/s00122-015-2562-5
- Kebede, E. (2021). Contribution, utilization, and improvement of legumes-driven biological nitrogen fixation in agricultural systems. *Front. Sustain. Food Syst.* 5, 767998. doi: 10.3389/fsufs.2021.767998
- Kontopoulou, C. K., Bilalis, D., Pappa, V. A., Rees, R. M., and Savvas, D. (2015). Effects of organic farming practices and salinity on yield and greenhouse gas emissions from a common bean crop. *Sci. Hortic.* 183, 48–57. doi: 10.1016/j.scienta.2014.12.012
- Kunene, T. R., Masarirambi, M. T., Wahome, P. K., and Oseni, T. O. (2019). Influence of kraal manure, chicken manure and inorganic fertilizer on growth, yield and post-harvest quality of pepper (*Capsicum annuum* L.) in a Sub-tropical environment. *Asian J. Adv. Agric. Res.* 11, 1–11. doi: 10.9734/AJAAR/2019/v11i130043
- Lengwati, D. M., Mathews, C., and Dakora, F. D. (2020). Rotation benefits from N₂-fixing grain legumes to cereals: From increases in seed yield and quality to greater household cash-income by a following maize crop. *Front. Sustain. Food Syst.* 4, 10.3389/fsufs.2020.00094
- Makoi, J. H., Chimphango, S. B., and Dakora, F. D. (2010). Photosynthesis, water-use efficiency and $\delta^{13}C$ of five cowpea genotypes grown in mixed culture and at different densities with sorghum. *Photosynthetica* 48, 143–155. doi: 10.1007/s11099-010-0019-2
- Manandhar, A., Pichaco, J., and McAdam, S. A. (2024). Absciscic acid increase correlates with the soil water threshold of transpiration decline during drought. *Plant Cell. Environ.* 47, 5067–5075. doi: 10.1111/pce.15087
- Manavalan, L. P., Guttikonda, S. K., Phan Tran, L. S., and Nguyen, H. T. (2009). Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiol.* 50, 1260–1276. doi: 10.1093/pcp/pcp082
- Maseko, S. T., and Dakora, F. D. (2016). Nitrogen nutrition, carbon accumulation and $\delta^{13}C$ of *Cyclopia* and *Aspalathus* species in different settings of the Cape fynbos, South Africa. *J. Plant Ecol.* 9, 586–595. doi: 10.1093/jpe/rtv078
- Mnasri, B., Aouani, M. E., and Mhamdi, R. (2007). Nodulation and growth of common bean (*Phaseolus vulgaris*) under water deficiency. *Soil Biol. Biochem.* 39, 1744–1750. doi: 10.1016/j.soilbio.2007.01.030
- Mnasri, B., Liu, T. Y., Saidi, S., Chen, W. F., Chen, W. X., Zhang, X. X., et al. (2014). *Rhizobium azibense* sp. novel, a nitrogen fixing bacterium isolated from root-nodules of *Phaseolus vulgaris*. *Int. J. Syst. Evol. Microbiol.* 64, 1501–1506. doi: 10.1099/ijls.0.058651-0
- Mohale, K. C., Belane, A. K., and Dakora, F. D. (2014). Symbiotic N nutrition, C assimilation, and plant water use efficiency in Bambara groundnut (*Vigna subterranea* L. Verdc) grown in farmers’ fields in South Africa, measured using ¹⁵N and ¹³C natural abundance. *Biol. Fertil. 50*, 307–319. doi: 10.1007/s00374-013-0841-3
- Muhaba, S. K., and Dakora, F. D. (2020). 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. *Front. Agric. Sci. Eng.* 7, 455–466. doi: 10.15302/J-FASE-2020354
- Mwenda, G. M., O’hara, G. W., De meyer, S. E., Howieson, J. G., and Terpolilli, J. J. (2018). Genetic diversity and symbiotic effectiveness of *Phaseolus vulgaris*-nodulating rhizobia in Kenya. *Syst. Appl. Microbiol.* 41, 291–299. doi: 10.1016/j.syapm.2018.02.001
- Pausch, R. C., Mulchi, C. L., Lee, E. H., and Meisinger, J. J. (1996). Use of ¹³C and ¹⁵N isotopes to investigate O₃ effects on C and N metabolism in soybeans. Part II. Nitrogen uptake, fixation, and partitioning. *Agric. Ecosyst. Environmen.* 60, 61–69. doi: 10.1016/S0167-8809(96)01062-6
- Peoples, M. B., Brockwell, J., Herridge, D. F., Rochester, I. J., Alves, B. J. R., Urquiaga, S., et al. (2009). The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis* 48, 1–17. doi: 10.1007/BF03179980
- Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., and Montana, C. G. (2007). Getting to the fat of the matter: models, methods, and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152, 179–189. doi: 10.1007/s00442-006-0630-x
- Reinprecht, Y., Schram, L., Marsolais, F., Smith, T. H., Hill, B., and Pauls, K. P. (2020). Effects of nitrogen application on nitrogen fixation in common bean production. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.01172
- Sanyal, D., Goos, R. J., and Chatterjee, A. (2018). Determining symbiotic nitrogen fixation in dry bean cultivars using ureide method and isotope dilution techniques. *Commun. Soil. Sci. Plan.* 49, 2042–2052. doi: 10.1080/00103624.2018.1495727
- Shearer, G., and Kohl, D. H. (1986). N₂-fixation in field settings: estimations based on natural ¹⁵N abundance. *Funct. Plant Biol.* 13, 699–756. doi: 10.1071/PP9860699
- Shiferaw, B., Tesfaye, K., Kassie, M., Abate, T., Prasanna, B. M., and Menkir, A. (2014). Managing vulnerability to drought and enhancing livelihood resilience in sub-Saharan Africa: Technological, institutional and policy options. *Weather Climate extremes* 3, 67–79. doi: 10.1016/j.wace.2014.04.004
- Singh, S. P. (1999). Production and utilization. In *Common bean improvement in the twenty-first century*. 1–24. Dordrecht. *Springer Netherlands*. 10, 199–224. doi: 10.1007/978-94-015-9211-6
- Stevens, C. J., Dise, N. B., Mountford, J. O., and Gowing, D. J. (2004). Impact of nitrogen deposition on the species richness of grasslands. *Science* 303, 1876–1879. doi: 10.1126/science.1094678
- Tadesse, T., Dechassa, N., Bayu, W., and Gebeyehu, S. (2013). Effects of farmyard manure and inorganic fertilizer application on soil physico-chemical properties and nutrient balance in rain-fed lowland rice ecosystem. *Am. J. Plant Sci.* 4, 8. doi: 10.4236/ajps.2013.42041
- Thilakarathna, M. S., and Raizada, M. N. (2018). Challenges in using precision agriculture to optimize symbiotic nitrogen fixation in legumes: Progress, limitations, and future improvements needed in diagnostic testing. *Agronomy* 8, 78. doi: 10.3390/agronomy8050078
- Tsai, S. M., Bonetti, R., Agbala, S. M., and Rossetto, R. (1993). Minimizing the effect of mineral nitrogen on biological nitrogen fixation in common bean by increasing nutrient levels. *Plant Soil* 152, 131–138. doi: 10.1007/BF00016342
- Unkovich, M. J. (2008). Measuring of symbiotic nitrogen fixation in Australian agriculture. *Soil Biol. Biochem.* 40, 2915–2921. doi: 10.1016/j.soilbio.2008.08.021
- Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, B., Giller, K., et al. (2008). Measuring plant-associated nitrogen fixation in agricultural systems. *ACIAR*. doi: 10.1071/AR9940119
- Unkovich, M. J., and Pate, J. S. (2000). An appraisal of recent field measurements of symbiotic N₂ fixation by annual legumes. *Field Crops Res.* 65, 211–228. doi: 10.1016/S0378-4290(99)00088-X
- Vance, C. P. (1998). “Legume symbiotic nitrogen fixation: agronomic aspects,” in *The Rhizobiaceae: molecular biology of model plant-associated bacteria* (Springer Netherlands, Dordrecht), 509–530.
- Van der Velde, M., Folberth, C., Balković, J., Ciais, P., Fritz, S., Janssens, I. A., et al. (2014). African crop yield reductions due to increasingly unbalanced Nitrogen and Phosphorus consumption. *Global Change Biol.* 20, 1278–1288. doi: 10.1111/gcb.12481
- Van kessel, C., and Hartley, C. (2000). Management of grain legumes: has it led to an increase in nitrogen fixation field. *Res. Crop* 65, 165–181. doi: 10.1016/S0378-4290(99)00085-4
- Wang, X., Shen, J., and Liao, H. (2010). Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops. *Plant Sci.* 179, 302–306. doi: 10.1016/j.plantsci.2010.06.007
- Wilker, J., Navabi, A., Rajcan, I., Marsolais, F., Hill, B., Torkamaneh, D., et al. (2019). Agronomic performance and nitrogen fixation of heirloom and conventional dry bean varieties under low-nitrogen field conditions. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00952