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RESEARCH ARTICLE

Characterization of spider plant (*Cleome gynandra* L.) accessions from diverse agroecological regions for morphological and physiological traits associated with nitrogen deficiency tolerance

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Abstract

The selection of indigenous crops that are high-yielding with sub-optimal soil nitrogen fertilizer must be prioritized. This is crucial for attaining sustainable food production since it lessens dependency on chemical fertilizers, reduces their negative effects on the environment and enhances resource use efficiency. These efforts will boost productivity and mitigate against the adverse effects of climate change. The present study evaluated 25 spider plant genotypes for their growth, economic yield and leaf photosynthetic physiological parameters at low and optimum N levels during their vegetative and reproductive growth stages. Through principal component analysis (PCA) using nitrogen stress tolerance indices, genotype ranking using best linear unbiased prediction (BLUP), cluster analysis using dendrogram and Pearson's correlation analysis, the performance of 25 genotypes under low N stress was evaluated. Parameters such as plant height, number of leaves, number of branches, economic yield, fresh weight and leaf photosynthetic physiological indicators were considered as the evaluation criteria for N deficiency tolerance. Although genotypes have shown varying tendencies in growth, yield and leaf photosynthetic indicators under N stress, N-tolerant genotypes (NC05015, ODS15103 and ODS15044) exhibited higher levels of these activities compared to N stress-sensitive genotypes (KSI2407A, ODS15020 and TOT8926). This study offers a reliable and comprehensive method for evaluating nitrogen deficiency tolerance in spider plants and our data elucidate potential physiological adaptive mechanisms that allow spider plants to withstand N stress. Future studies should explore the genetic and molecular mechanisms underlying nitrogen deficiency tolerance, focusing on key nitrogen uptake and assimilation genes. Incorporating advanced biotechnological tools and multi-environment trials will enhance the development of resilient spider plant genotypes for production in diverse agroecological conditions.

Keywords: climate change; economic yield; nitrogen stress tolerance; spider plant

Introduction

The rising demand for food production due to the growing global population poses a significant threat, with hunger and food insecurity at the forefront of global concerns (1). The United Nations projects a global population of 10 billion by 2050, with growth particularly pronounced in developing countries. Many of these nations are grappling with economic challenges due to the escalating climate change effects, thereby amplifying the demand for sustainable agriculture (2, 3). The agricultural industry faces the dual challenge of maintaining food security for the fast-growing population while minimizing the environmental impacts of nitrogen fertilizer overuse (4). Excessive nitrogen fertilizer use has led to various environmental issues, including nitrogen runoff, soil deterioration and greenhouse gas emissions (5). These emissions, particularly those of nitrous oxide, a strong greenhouse gas, have a substantial role in climate change. Nitrous oxide traps heat in the atmosphere far more effectively than carbon dioxide, causing rising global temperatures (6). Furthermore, nitrogen runoff can cause algae blooms in aquatic systems, which produce methane, a potent greenhouse gas that exacerbates climate change (7). Therefore, optimizing nutrient management, particularly nitrogen for plant growth and development, is essential. To mitigate nitrogen-related environmental challenges and increase food production, it is necessary to identify and select crop plants that are resilient to nitrogen deficiency stress (8). Understanding their nitrogen absorption and assimilation under low soil nitrogen conditions can assist in enhancing their resilience to nitrogen deficiency and improve plant growth under low nitrogen stress.

Under low soil conditions, plants often adopt adaptation strategies to ensure reproduction and complete their life cycle (9). Their responses include changes in biochemical and physiological processes, such as increased nitrogen uptake through high-affinity transporters (HATS), nitrogen transfer from older to younger tissues, delayed growth, decreased photosynthesis and increased anthocyanin levels (10). Plant root structure plays a crucial role in determining soil-root connections and influencing water and nutrient intake (11). Reduced soil nitrogen can inhibit lateral root growth in some plants, leading to an elevated carbon-to-nitrogen ratio, lower photosynthesis and early leaf senescence. For instance, *Arabidopsis thaliana* adapts to nitrogen deficiency by employing high-affinity nitrogen transporters to optimize nitrogen intake and by improving root architecture through the activation of low -nitrogen availability regulators, enhancing nitrogen acquisition efficiency (12). Understanding these adaption methods assist in determining how genotypes differ in their tolerance to low nitrogen stress. Some genotypes may have increased tolerance because of more effective nitrogen uptake and assimilation systems or improved root architecture, whilst others may struggle to adapt, resulting in lower growth and early senescence.

Studies on spider plants have shown that increasing nitrogen fertilization improves plant height, marketable leaves, shoots and fresh yield, with larger variations observed in the late vegetative to early reproductive stage (13). However, research on lettuce indicated a decrease in the shoot-to-root ratio as soil nitrogen concentrations decrease (14). Additionally, a study on root growth in two drought-resistant wheat varieties under different nitrogen levels found that as nitrogen content increases, root development (fresh weight and root length) initially decreased significantly but then increased at the optimal level (15). This suggests that, given time, crops could enhance their root capacity for extracting soil nitrogen under low nitrogen conditions, thereby reducing the need for additional fertilizers.

In this context, the spider plant, also known as African cabbage, spider cat wasp, or morogo, appears to be a promising candidate for sustainable agriculture (16). This indigenous vegetable to Africa and Asia not only possesses high nutritional and medicinal value but also exhibits exceptional resistance to environmental stress (17). Its adaptability and tolerance to low soil nutrients and water deficits make it an excellent option for developing genotypes that thrive under low nitrogen fertilizer conditions, aligning with the broader goal of enhancing crop resilience and sustainability amid the effects of climate change (18).

This research investigates the development of spider plant genotypes optimized for low nitrogen fertilizer stress. Through comprehensive physiological and agronomic assessments, this study aims to elucidate the physio-morphological diversity of *Cleome gynandra* populations and identify specific genotypes that exhibit improved physiological growth indicators and biomass production under nitrogen-limiting soil conditions. By leveraging the natural diversity within this species, the study seeks to contribute to the development of crop varieties capable of thriving in nutrient-poor soils while providing a key source of sustenance for local communities. The findings of this study are expected to have a significant contribution to spider plant genetic resources conservation and improvement, sustainable crop production and food security.

Materials and Methods

Study area description: Plant materials and origin

Greenhouse experiments were carried out at Botswana University of Agriculture and Natural Resources (BUAN) in Gaborone, Southern Botswana (24°59’ S, 25°94’ E). Gaborone sits 1000 m above sea level, experiencing an average rainfall of 250-500 mm and a mean outdoor temperature of 20.7 °C. Two greenhouse experiments were conducted from February 2021 to August 2022. Soil from the university research field, traditionally used for growing vegetables and field crops, was used for planting. Soil analysis indicated a sandy texture with 0.62 % organic carbon and 0.0008 % nitrogen. A total of 25 different spider plant germplasm samples were used in this investigation, collected from African and Asian regions, as shown in Table 1. Twenty-one genotypes were received from the World Vegetable Centre (AVRDC) in Arusha, Tanzania, through a collaboration with the University of Namibia's Department of Crop Sciences. The AVRDC collections came from eleven different African and Asian countries. Additionally, one accession was obtained from Namibia's National Botanical Research Institute, two from Botswana's National Plant Genetic Resource Centre and one genotype was collected from the Botswana University of Agriculture and Natural Resources (BUAN) crop research field.

Treatments, experimental design and crop husbandry

Genotype selection study

Twenty-five spider plant accessions were assessed in 0.027 m^3 polyethylene planting bags (0.185 m wide and 0.363 m tall) (19). Each planting bag contained about 10 kg of air-dried sandy loam soil with physicochemical characteristics including 0.62 % organic carbon (OC), 5.8 pH ($CaCl_2$), 4.0 meq/100g CEC, 0.08% total N, 15.9 ppm P and 0.08 ppm exchangeable K. To address the crop's phosphorus requirements, a single application of superphosphate (60 kg P ha^{-1}) was applied in each bag before planting and worked into the soil. The experiment followed a factorial experiment in a completely randomized design with three replications, with nitrogen treatments, control (optimum nitrogen, ON) and low nitrogen stress (nitrogen deficiency, ND) as the main plots and 25 spider plant accessions as the sub-plots.

Table 1. Accessions and country of origin used in the study

Country of origin	Region	Accession Name
Ghana	West Africa	ODS 15121, ODS 15103
Bernini	West Africa	ODS 15037, ODS 15020 ODS 15019, ODS 15044 NC 05015
Kenya	East Africa	GA01, KSI 2407A ELG 19/07A, TOT 8926
Malawi	Southern Africa	BC-02B
Tanzania	East Africa	TOT 6426
Malaysia	Southern Asia	TOT 7196
Togo	West Africa	ODS 15061, ODS15059 ODS 15075
Lao’s Republic	Southern Asia	ODS 15045, TOT 3536
Uganda	East Africa	TOT 8887
Thailand	Southern Asia	TOT5799
Namibia	Southern Africa	LAIOGONGO
Botswana	Southern Africa	ROTHWE 1, ROTHWE 2, BUAN1
Total		**25**

Planting pots were sufficiently spaced at 0.3 m by 0.3 m to facilitate growth, easy assessments and sampling. Four seeds of each accession were sown in each pot and watered at 80% field capacity by adding 2 L of water to each pot. Thinning was done two weeks after planting (WAP), leaving two plants per pot. Nitrogen treatment was induced at 2 WAP.

Two nitrogen treatments were applied three WAP using urea (48% N) as the nitrogen source: 180 kg N ha^{-1} for optimum nitrogen (ON) and 20 kg N ha^{-1} for nitrogen deficiency stress (ND). To achieve the desired levels for the ND and ON treatments, additional applications of 4.44 kg N ha^{-1} and 164.54 kg N ha^{-1} were made, respectively, based on the soil's nitrogen concentration (15.56 kg N ha^{-1}). Each nitrogen treatment was administered in two equal portions at two and five WAP. To control aphids and whiteflies, all treatments were sprayed with 1 g/L of lambda-cyhalothrin (cypermethrin) five days after emergence, during the vegetative phase and shortly before blooming. Weeds were hand-pulled as they appeared to avoid crop-weed competition.

Leaf photosynthetic parameters study

Nine genotypes, divided into three groups of three accessions each, were used for this study. The selected genotypes are categorized as follows: drought-tolerant (NC05015, ODS15044 and ODS15103), intermediate-tolerant (LAIOGONGO, ODS15037 and ODS15045) and sensitive (KSI2407A, ODS15020 and TOT8926) based on genotype selection studies to validate the selected outcome.

The setup consisted of 54 (0.027 m^3) polyethylene planting bags (0.185 m wide and 0.363 m tall) filled with approximately 10 kg of air-dried soil. The study design, planting soil, nitrogen treatments, planting method, management practices and watering protocols used in this study followed those of the genotype selection studies.

Data collections

Harvesting occurred every two weeks from three WAP until the end of the nine-week experimental period. Economic yield fresh weight (EYFW) of leaves, tender shoots and flower buds was measured immediately after harvesting, followed by oven-drying at 60 °C for 72 hr to determine economic yield dry weight (EYDW). Growth parameters, including plant height (PH), number of leaves (NOL), number of primary branches (NPB) and chlorophyll content (SPAD values), were monitored from three weeks after planting and every two weeks thereafter. Plant height was measured from the base to the tip of the plant, NOL was counted biweekly and NPB was assessed by counting well-extended branches from the main stem. At the end of the experiment, plants were uprooted and separated into roots and shoots. Drought tolerance was evaluated using Fernandez's theory and drought tolerance indices based on EYDW yield data (20).

Physiological leaf fluorescence parameters (FvP/FmP, NPQt, PhiNPQ, Phi2), SPAD and g_s were measured every two weeks, starting at three WAP and continued throughout the nine-week experimental period. These measurements were taken using multispeQ V 2.0 (PhotosynQ INC, East Lansing, USA). Leaf stomatal conductance was assessed with an SC-1 leaf stomatal conductance porometer (Melbourne, Victoria, Australia). Economic yield, fresh and dry weight, alongside other plant growth parameters, were quantified using methods and equipment consistent with those used in the genotype selection study.

Data analysis

Data analysis was conducted using the 'doebioreseach' R package for ANOVA at a 95 % confidence level. Visualizations, including bar graphs and correlation coefficient charts for growth and yield data, were created with the 'ggplot2' and 'ggcorrplot' packages. Economic yields (Yp and Ys) were used to compute drought-tolerance indices, employing selection strategies like best linear unbiased prediction (BLUP) with Meta-R (21). Principal component analysis (PCA), genotype ranking based on indices and dendrogram clustering using the unweighted pair-group method with arithmetic average (UPGMA) were performed with the 'factoextra' and 'dendextend' packages (23). Group averages were summarized for mean separation to display observed variations.

Results

Genotype selection studies

Variations among the tested response parameters

Genotypes (A) differed significantly in terms of plant height (PH), economic yield fresh weight (EYFW) and economic yield dry weight (EYDW). All investigated parameters, except MC, were significantly impacted by soil nitrogen levels (B). The impacts of plant growth stages (C) on every metric were highly significant. For EYFW and EYDW, there were substantial interactions between genotypes and soil nitrogen levels (A × B). Significant differences were seen in the relationships between soil nitrogen levels and growth phases (B × C) for primary branches, EYFW and EYDW. However, for any parameter, no significant interactions were found between genotypes and growth stages (A × C) or between the three treatments (A, B and C) (Table S1).

Leaf chlorophyll content

Leaf chlorophyll content (SPAD values) was significantly influenced by soil nitrogen levels and growth stages (Table S1). Nitrogen-deficient genotypes showed a significantly lower chlorophyll content (31.46) than genotypes that received optimal nitrogen fertilization (41.91), as Fig. 1A illustrates. Similarly, as indicated in Fig. 1B, younger plants (4 WAP) exhibited significantly lower chlorophyll content (34.21), which increased significantly (39.17) as the plant grew older into the vegetative phase (6 WAP).

Plant height

Plant height was significantly influenced by genotypes, soil nitrogen levels and growth stages (Table S1). The plant height of different genotypes ranges between 21.66 cm (Rothwe1) and 40.62 cm (NC05015) as indicated in Fig. S1A. Nitrogen-deficient plants exhibited significantly lower plant height (28.28 cm) compared to plants that received optimal nitrogen fertilization (38.22 cm) (Fig. S1B). Younger plants (4 WAP) also exhibited significantly lower plant height (27.31 cm), which increased significantly (39.19 cm) as the plant grew older into the vegetative phase (6 WAP) (Fig. S1C).

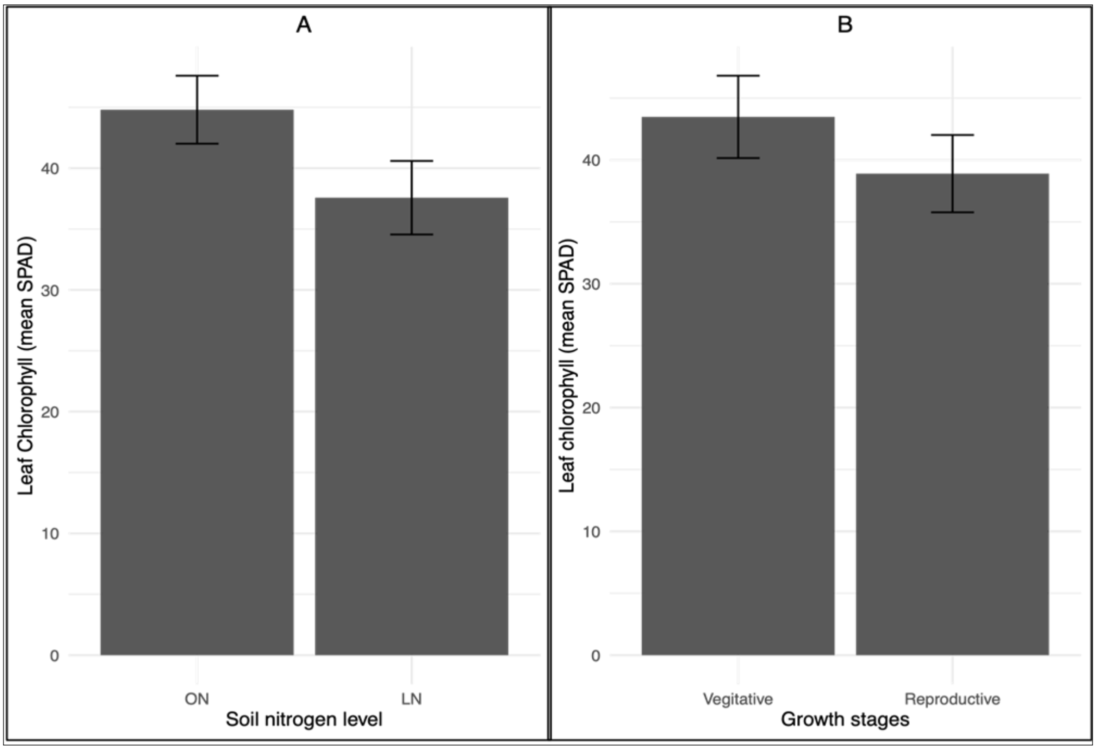

Fig. 1. The effects of soil nitrogen levels (A) and growth stages (B) on the leaf chlorophyll content (mean SPAD) of spider plants. Soil nitrogen levels are indicated by optimum nitrogen (ON) and low nitrogen (LN). The growth stages indicate the vegetative and reproductive growth stages of spider plants. All the data are expressed as mean ± SEM, n = 3, according to the MANOVA test ($P \leq 0.05$).

Number of branches and leaves

The number of branches was significantly impacted by the interactions between soil nitrogen levels and growth stages (Table S1). The interaction of nitrogen levels and growth stages showed no significant difference between deficiency and optimal nitrogen fertilized plants at 4 WAP, however, as the plant grew to 6 WAP nitrogen deficient plants had a significantly lower number of branches (14) compared to 19 branches observed on the optimum nitrogen fertilized genotypes (Fig. S2A). The variations in the number of leaves under soil nitrogen levels presented fewer leaves on nitrogen-deficient plants compared to optimum nitrogen fertilized genotypes (Fig. S2B). In addition, younger plants (4 WAP) generally produced significantly lower of leaves (12 leaves) than older plants (6 WAP), which displayed 3 leaves (Fig. S2C).

Economic yield

The interaction between nitrogen level and growth stages, as well as genotypes, was the primary cause of variations in spider plant economic yield fresh weight (EYFW) and economic yield dry weight (EYDW), as indicated in Table S1. The yield parameters (EYFW and EYDW) have generally increased in plants treated with sufficient nitrogen fertilizer. The EYFW ranged between 8.23 g/plant (BC02B) to 21.27 g/plant (TOT6426), while for EYDW varied from 1.38 g/plant (BUAN1 and Rothwe1) to 3.01 g/plant (ODS15044, ODS15059 TOT6426 and TOT7196). However, if nitrogen deficiency stress treatment is applied, these yield parameters dramatically drop in several genotypes (Fig. S3A,C). Conversely, a considerably greater economic yield (EYFW and EYDW) on optimally nitrogen-fertilized treatments in the reproductive stage compared to the nitrogen shortage stressed plants at the vegetative stage was shown by the interactive effects of soil nitrogen level and plant growth stage (Fig. S3B,D).

Correlations among the growth and economic yield parameters of spider plant

The relationship between the responses was analyzed using bivariate scatter plots with fitted lines based on Pearson's correlation coefficients to illustrate the impacts of soil nitrogen stress. showed a statistically significant positive connection with NPB ($r^2 = 0.58$), EYFW ($r^2 = 0.65$) and EYDW ($r^2 = 0.74$). Furthermore, the NPB exhibited a very negative connection ($r^2 = -0.79$) with NOL, but a positive significant correlation with the two economic yield indices, EYFW ($r^2 = 0.52$) and EYDW ($r^2 = 0.66$). With r^2 values ranging between 0.36 and 0.43, the leaf chlorophyll content (SPAD) showed a weak but positive correlation with plant height, NPB, EYFW and EYDW (Fig. 2).

Principal component analysis (PCA)

The PCA was employed to explore nitrogen deficiency tolerance variability among spider plant genotypes, focusing on key qualities contributing most to total variation using drought tolerance indices following a previous study (20). As indicated in Fig. 5, among the 11 drought stress indices tested under Yp and Ys nitrogen conditions, only the first two principal components (PCs), were significant, collectively explaining 93.3 % of total variability, with eigenvalues exceeding one each. PC1, associated with Ys, explained 57.3 % of the total variation and was positively influenced by YSI, MP, GMP, STI and HM. PC2, linked to Yp, accounted for 36 % of the total variation, with TOL, SSI, MP, GMP, STI and HM contributing significantly. Two groups of characters emerged in the biplot and correlation chart based on selection indices used: one positively correlated with both PC1 and PC2 (mean productivity-MP, geometric mean productivity-GMP, stress tolerance-STI and harmonic mean-HM), while yield stability index-YSI displayed a negative correlation with PC2. Additionally, indices within the first group were positively

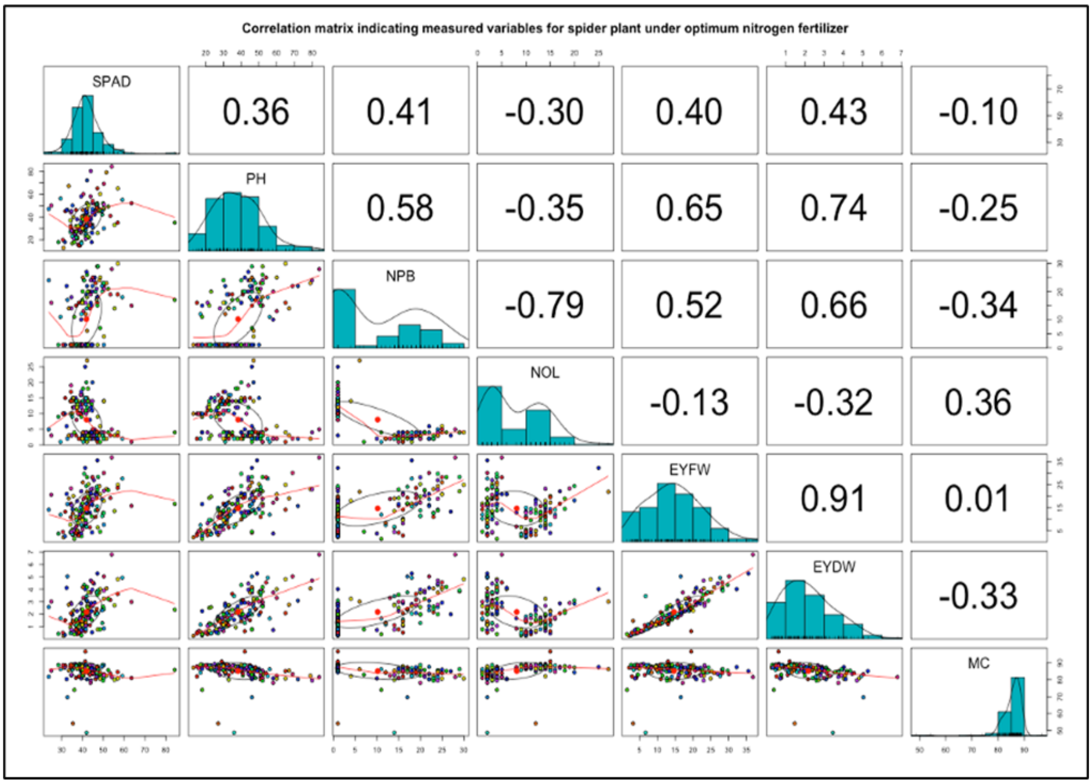

Fig. 2. The correlation matrix for the relationships between measured growth and economic yield variables of spider plant genotypes under nitrogen deficiency stress conditions. The diagonal histogram depicts the distribution of each variable in both correlation charts. A bivariate scatter plot with a fitted line and ellipse at the foot of the diagonal shows the pairwise correlation between the two relevant variables. The correlation's r^2-values are displayed above the diagonal. Variables included are PH (plant height cm), NPB (number of primary branches), NOL (number of leaves), EYFW (economic yield fresh weight), EYDW (economic yield dry weight) and EYMC (economic yield moisture content %) at $P < 0.05$.

correlated, while stress tolerance-TOL and stress susceptibility index-SSI were negatively correlated with yield stability index-YSI.

Correlation analysis among the nitrogen deficiency stress tolerance indices based on economic yield and dry weight of spider plant genotypes

In Fig. 3, optimum nitrogen fertilizer treatment yield (Yp) was positively and significantly correlated ($P<0.05$) with TOL (0.94), SSI (0.66), MP (0.66), GMP (0.82), STI (0.81) and HM (0.60) indices. Low nitrogen fertilizer treatment yield (Ys) also showed significant positive correlations ($P<0.05$) with YSI (0.51), GMP (0.76), STI (0.75) and HM (0.92) indices. STI, GMP and HM were highly positively correlated with both Ys and Yp and with each other ($P<0.05$). TOL and SSI had strong negative correlations with YSI ($P < 0.05$).

Cluster analysis

The dendrogram illustrates the arrangement of genotypes into clusters based on their similarity in response to low nitrogen stress. The genotypes are represented as endpoints on the dendrogram. The height at which two genotypes (or clusters of genotypes) are joined represents their dissimilarity. Lower heights indicate more similarity, while higher heights indicate greater dissimilarity. Using Ward's clustering method, which seeks to reduce the overall variance within clusters. The Euclidean distance metric was used to compute the straight-line distance between points in a multidimensional determining the distance between 25 spider plant genotypes grouped into four clusters. Cluster-2 and Cluster-4 were the largest, with 10 genotypes (40 %) each, followed by Cluster-1 (4 genotypes, 16 %) and Cluster-3 showed a single genotype (4 %). Notably, Cluster-1 included genotypes ODS15019, ODS15037, TOT5799 and NCO5015. Cluster-2 is comprised of ODS15020, TOT8887, ODS15061, ODS15121, BC02B, GAO1, ODS15103, ODS15059, ROTHWE2 and BUAN1 genotypes, Cluster-3 is made up of Rothwe1 and Cluster-4 is comprised of TOT8926, TOT6426, ODS15075, LAIOGONGO, ODS15044, TOT7196, KSI2407A, TOT3536, ODS15045 and ELG1907A genotypes.

Nitrogen deficiency stress indices ranking

To address the potential contradiction of relying on a single index for drought tolerance identification, genotypes were ranked based on multiple drought tolerance indices. The average sum of ranks (ASR) and standard deviation of ranks (SDR) across all indices were calculated to determine the most ideal nitrogen deficiency-tolerant genotypes. Genotypes NC05015, ODS15044, ODS15021 and TOT3536, ODS15103 emerged as the most nitrogen stress-tolerant with consistently high rankings and low variability. Conversely, Rothwe1, BUAN1, BCO2B KSI2407A and TOT8926 were identified as nitrogen deficiency-sensitive genotypes with consistently low rankings and high variability (Table 2).

Physiological studies : An additional study was conducted to assess the physiological parameters (leaf photosynthetic parameters) of selected nitrogen deficiency tolerant genotypes (NC05015, ODS15044 and ODS15103), nitrogen deficiency intermediate-tolerant genotypes (LAI-OGONGO, ODS15037 and ODS15045) and nitrogen deficiency sensitive genotypes (ODS15020, TOT8926 and KSI2407A) under both optimum and low nitrogen soil conditions. The goal was to verify the results of phenotypic selection (Experiment 1).

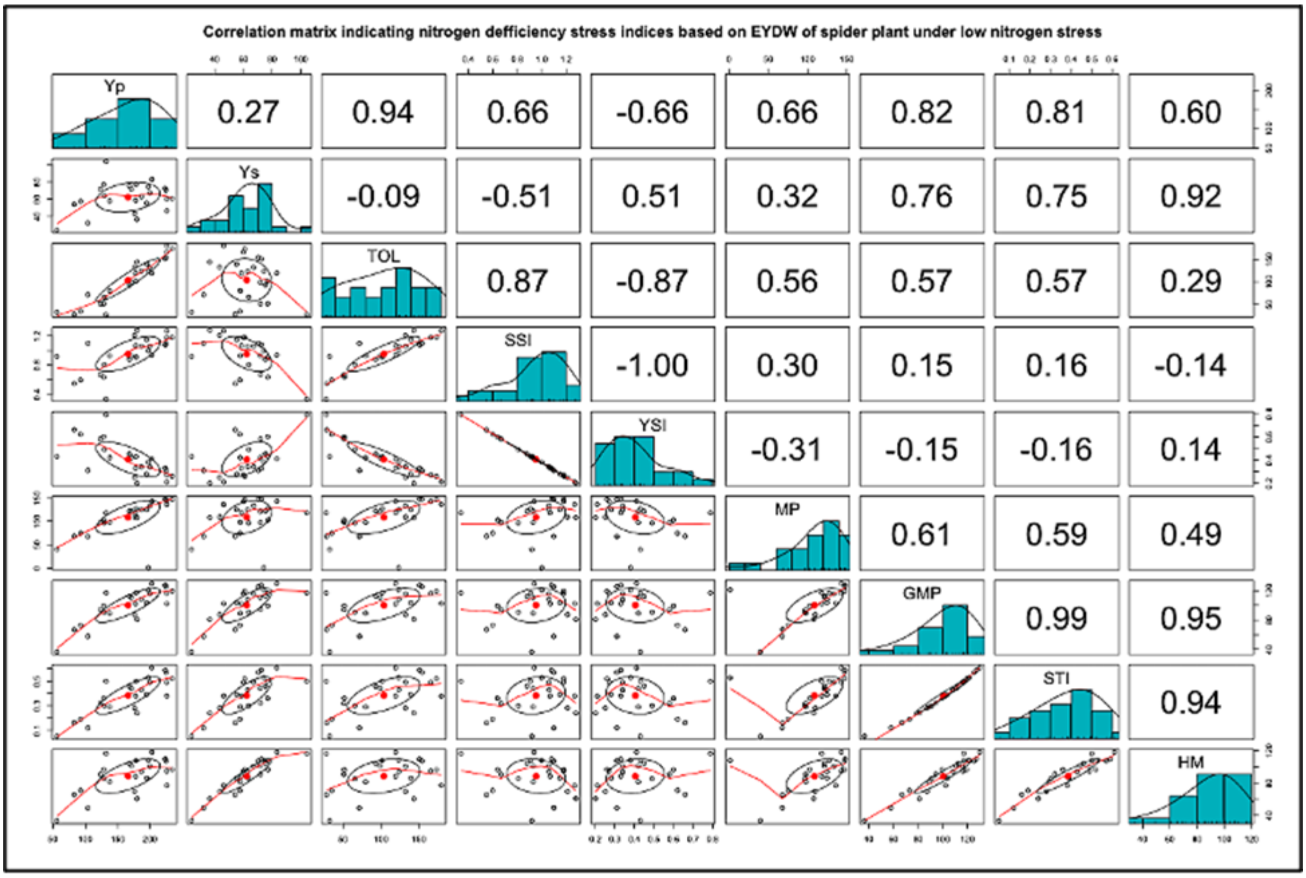

Fig. 3. Correlation matrix for the relationships between the measured stress indices of economic yield under optimum nitrogen and nitrogen deficiency stress conditions. The diagonal histogram displays each variable's distribution correlation chart. A bivariate scatter plot shows the pairwise correlation between the two relevant variables at the diagonal's foot. The correlation's r^2-values are located above the diagonal. Yp (economic dry yield under optimum nitrogen fertilizer conditions), Ys (economic dry yield under low nitrogen stress conditions), TOL (stress tolerance), SSI (stress susceptibility index), YSI (yield stability index), YI (yield index), MP (mean productivity), GMP (geometric mean productivity), STI (stress tolerance index) HM (harmonic mean) at 5 % p-values.

Table 2. Yield performance ranking of 25 spider plant genotypes under optimum nitrogen (Yp) and low nitrogen stress (Ys) conditions, along with the yield-based nitrogen tolerance and susceptibility indices

Genotypes	Yp	Ys	TOL	SSI	YSI	YI	MP	GMP	STI	HM	RSI	ASR	SDR
NC05015	6	2	12	14	12	2	4	1	1	1	12	6	5.2
ODS15044	5	7	5	9	17	7	1	2	2	3	17	7	4.7
ODS15121	4	10	4	8	18	10	3	3	3	6	18	8	4.8
TOT3536	12	4	14	18	8	4	9	6	6	5	8	9	4.7
ODS15103	18	1	25	23	1	1	15	7	7	2	1	9	9.5
ODS15059	1	14	2	4	22	14	2	5	5	10	22	9	6.9
ELG1907A	8	6	11	13	13	6	7	22	4	4	13	10	5.5
ODS15075	7	11	8	11	15	11	8	9	9	9	15	10	2.3
ODS15061	16	5	16	19	7	5	12	10	10	7	7	10	4.9
TOT 7196	3	15	6	5	21	15	5	8	8	12	21	11	5.8
LAIOGONGO	15	9	15	15	11	9	13	11	11	8	11	12	2.7
ODS15037	20	3	20	22	4	3	10	16	16	11	4	12	7.4
TOT5799	10	13	10	12	14	13	11	12	12	13	14	12	1.3
TOT 6426	2	21	1	2	24	21	6	14	14	19	24	13	8.9
TOT8887	9	19	7	6	20	19	10	13	13	16	20	14	5.2
ODS15045	21	8	21	21	5	8	20	17	17	14	5	14	6.1
ODS1519	13	16	13	10	16	16	14	15	15	15	16	14	1.9
GA01	19	12	19	20	6	12	21	18	18	17	6	15	4.7
ROTHWE 2	17	17	17	16	10	17	19	19	19	18	10	16	2.6
ODS15020	14	22	9	3	23	22	16	20	20	21	23	18	6.6
TOT8926	11	23	6	1	25	23	17	21	21	23	25	18	8.3
KSI2407A	23	18	22	23	3	18	22	22	22	20	3	18	6.0
BC02B	24	20	24	24	2	20	23	23	23	22	2	19	6.7
BUAN1	22	24	18	7	19	24	24	24	24	24	19	21	5.4
ROTHWE1	25	25	23	17	9	25	25	25	25	25	9	21	5.3

Yp (yield optimum nitrogen soil conditions), Ys (yield under nitrogen deficiency stress), TOL (tolerance index), SSI (stress susceptibility index), YSI (yield stability index), YI (yield index), MP (mean productivity), GMP (geometric mean productivity), STI (stress tolerance index), HM (harmonic mean) and RSI (relative stress index), as well as ASR and SDR.

Except for PhiNPQ and stomatal conductance, all measured physiological indicators were strongly impacted by growth stages (B). All physiological indicators examined were significantly affected by genotype (A) and soil nitrogen levels (C). Three physiological markers (FvP/FmP, NPQt and Phi2) were significantly impacted by the genotype-growth-stage interaction (AB). Interactions between genotypes and soil nitrogen level (AC) and between growth stages and soil nitrogen level (BC) had a significant effect on only qL (Table S2).

The quantum yield of PSII (Phi2, C1), non-photochemical quenching (NPQt, B1) and the quantum efficiency of PSII (FvP/FmP, A2) were all significantly impacted by the interaction of genotypes and growth stages. During the vegetative growth stages, genotypes showed increased FvP/FmP and Phi2, which decreased as they advanced into the reproductive phase (Fig. 4A2, C1). The NPQt of tolerant genotypes did not significantly vary among genotypes and growth stages (Table. 4 B1).

Moreover, leaf chlorophyll content (SPAD, E1), leaf stomatal conductance (mmol/m2/s, F1) and the quantum yield of regulated non-photochemical quenching (PhiNPQ, D1) were all significantly impacted by genotypes. The values of PhiNPQ, SPAD and stomatal conductance were significantly greater in the tolerant genotypes (NC05015, ODS15044 and ODS15103) under both growth stages. Although chlorophyll content and stomatal conductance of the intermediate genotypes were comparatively greater than those of the sensitive genotypes, these differences were not statistically significant (Fig. 4 D1, E1 & F1).

The effects of soil nitrogen levels were assessed on the quantum efficiency of PSII (FvP/FmP, A1), non-photochemical quenching (NPQt, B2), the quantum yield of PSII (Phi2, C2), the quantum yield of regulated non-photochemical quenching (PhiNPQ, D2), leaf chlorophyll content (SPAD, E3) and leaf stomatal conductance (mmol/m^2/s, F2). The physiological results indicated a significant reduction in FvP/FmP, Phi2, stomatal conductance and SPAD in plants exposed to nitrogen deficiency compared to those grown under optimum nitrogen fertilization (Fig. 4 A1, C2, F2 & E3).

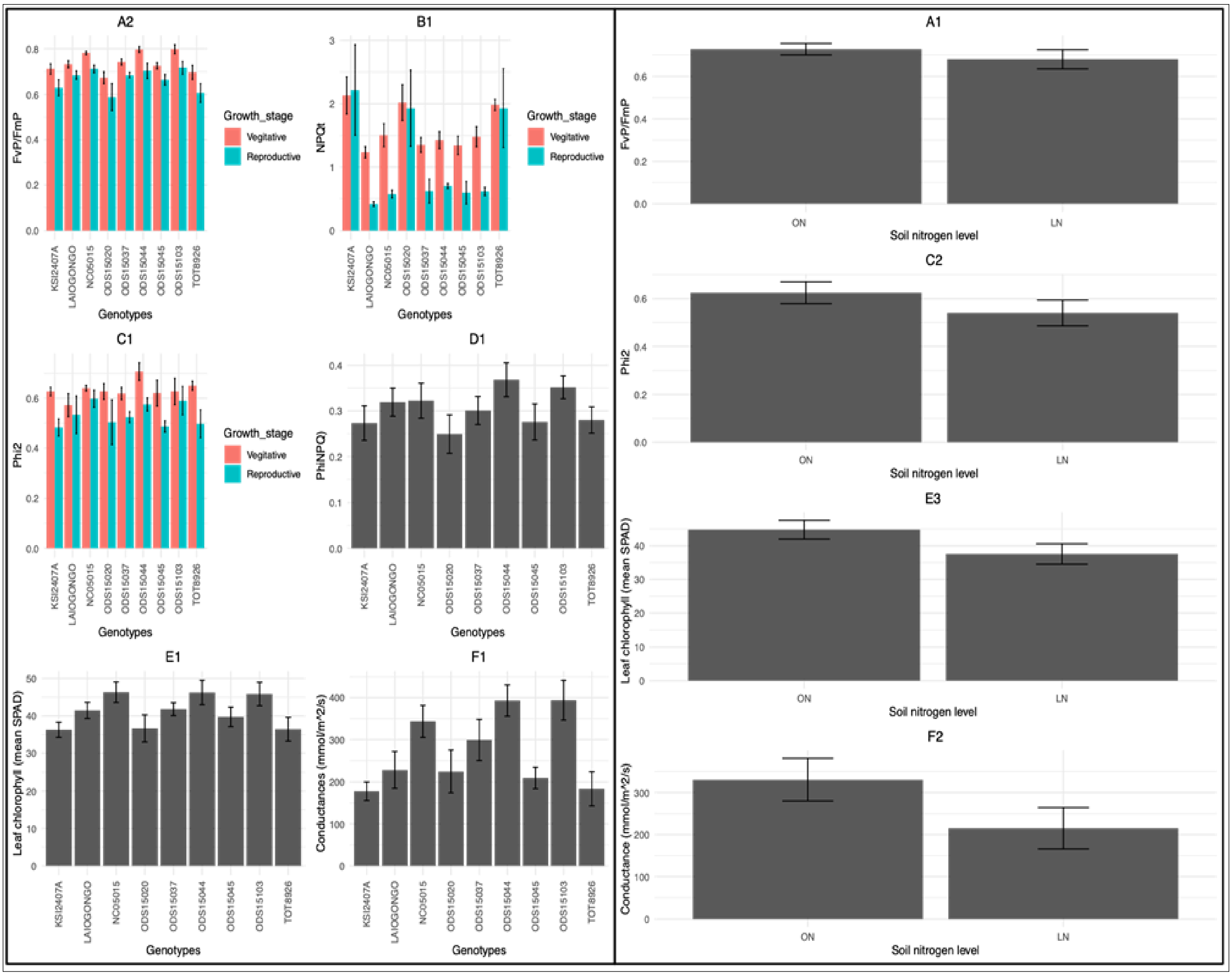

Fig. 4. The results of the physiological study on selected nitrogen deficiency-responsive spider plant genotypes grown under greenhouse conditions. The interactive effects of genotypes and growth stages were analyzed on the quantum efficiency of PSII (FvP/FmP, A2), non-photochemical quenching (NPQt, B1) and the quantum yield of PSII (Phi2, C1). The effects of genotypes were evaluated on the quantum yield of regulated non-photochemical quenching (PhiNPQ, D1), leaf chlorophyll content (SPAD, E1) and leaf stomatal conductance (mmol/m^2/s, F1). The effects of soil nitrogen level were assessed on the quantum efficiency of PSII (FvP/FmP, A1), the non-photochemical quantum yield of PSII (Phi2, C2), leaf chlorophyll content (SPAD, E3) and leaf stomatal conductance (mmol/m^2/s, F2). Soil nitrogen levels are indicated by optimum nitrogen (ON) and low nitrogen (LN), while growth stages indicate the vegetative and reproductive growth stages. All data are expressed as mean ± SEM, n = 3, according to the MANOVA test ($P \leq 0.05$).

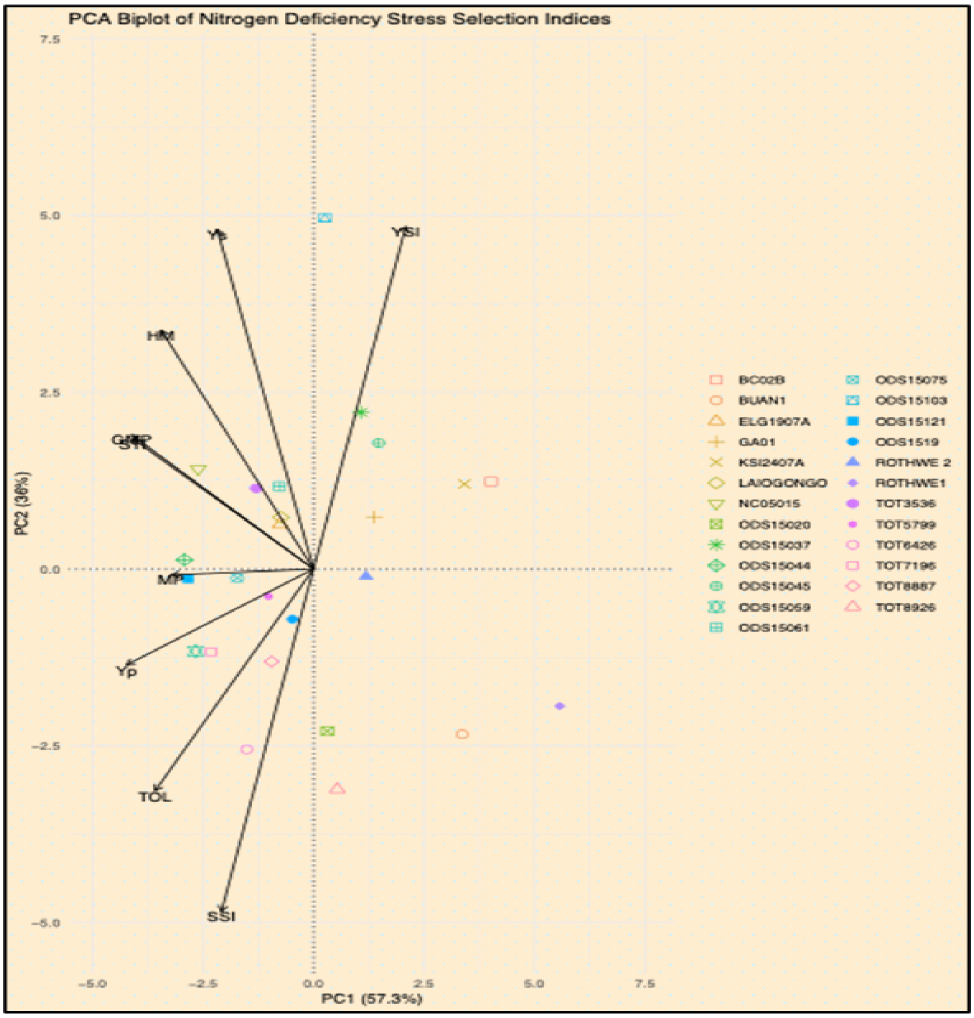

Fig. 5. A biplot of nitrogen deficiency stress tolerance indices of EYDW for 25 spider plant genotypes under both optimum nitrogen fertilizer and nitrogen deficiency stressed soil conditions, based on the first two main components Dim1 and Dim2. Variables displayed include TOL (stress tolerance), MP (mean productivity), STI (stress tolerance index), GMP (geometric mean productivity), HM (harmonic mean), Yp (economic yield under optimum nitrogen soil conditions), Ys (economic yield under nitrogen deficiency stress conditions), SSI (stress susceptibility index), YI (yield index) and YSI (yield stability index). Genotype names are represented by various shapes in the biplot.

Discussion

This study aimed to characterize 25 spider plant genotypes from various countries, focusing on growth, yield and physiological factors critical for enhancing crop resilience under soil nitrogen deficiency. Plant height, branch and leaf development are vital for stress tolerance and adaptability to nitrogen deficiency. Maintaining high economic yield is crucial for food security, especially in regions vulnerable to marginal soil fertility change, like Sub-Saharan Africa and Asia. Selection and breeding for plant tolerance improve resistance to stressors like soil nutritional deficiency (24). Techniques like BLUP, PCA and cluster analysis reveal high diversity among genotypes, offering the potential for selecting superior genotypes and varieties for discovering new recombinants for genetic enhancement (21).

Soil nitrogen levels and growth stages significantly influence leaf chlorophyll content, with nitrogen-deficient genotypes having lower content and younger genotypes showing lower content, increasing as plants grow older (Fig. 1). The chlorophyll content of genotypes that were treated with optimal nitrogen fertilization indicated a greater average SPAD value of 41.91, however, the content reduced significantly in nitrogen-deficient genotypes to an average SPAD value of 31.46. The study's results also highlighted the critical function that nitrogen plays in the synthesis of chlorophyll, suggesting that soil nitrogen levels and growth phases substantially impact the amount of pigment found in leaves. Similar results were reported on a variety of plant species that have demonstrated high correlations between chlorophyll and nitrogen contents, these crops include Amaranthus, lettuce, spinach and sorghum (4, 25-27). These variations in chlorophyll content may indicate that nitrogen plays a major role in plant growth and development by elevating light energy capture and, consequently, photosynthesis activities (28). The study shows that sufficient nitrogen supply and the developmental stage of the plant are both necessary for the plant's chlorophyll levels. Early growth phases are particularly important for nitrogen availability. The study demonstrates the intricate interaction between nutrient availability and plant development stages in influencing chlorophyll concentration, which is crucial for photosynthetic production and efficiency. The findings agree with a study conducted on the young and adult leaves of some plants (*Hibiscus rosa-sinensis, Neem Azadiracta indica, Polyalthia longifolia, Ficus benghalensis, Nerium indicum, Acacia nilotica, Bogainvillea spectabilis, Ficus religiosa)* (29). The results have indicated that adult leaves showed higher chlorophyll content in comparison to young leaves.

The study also identified genotypes, soil nitrogen levels and growth stages as the factors that significantly influenced plant height. Significant variation across different genotypes was observed, with Rothwe1 (21.2 cm) being the shortest and NC05015 (40.5 cm) being the tallest (Fig. S1 A). These differences highlight the genetic diversity among spider plant genotypes and their varying growth responses under similar conditions. Different genotypes may have different genes regulating growth hormones like auxins and gibberellins, which affect plant height and stem elongation (30). Furthermore, different genotypes respond differently to abiotic stresses such as nutrient deficiency, resulting in growth variations. This demonstrates the significance of genetic variability in regulating plant growth and the usefulness of selective breeding for desired traits. Similar results were reported on spider plants under controlled and field conditions (31). In addition, soil nitrogen levels significantly influenced plant height, with nitrogen-deficient genotypes exhibiting lower heights compared to those receiving optimal nitrogen fertilization (Fig. S1B). Nitrogen is crucial for plant growth and development, as it is a component of chlorophyll, amino acids and nucleotides. Therefore, insufficient nitrogen leads to reduced photosynthetic capacity and stunted growth, preventing genotypes from reaching their full potential. A study on tomatoes sunflowers and Amaranthus agreed with these findings (25, 32, 33). Apart from genotypes and soil nitrogen levels' effects on plant height, this study has also indicated the influence of growth stages. The variations may be attributed to physiological and developmental processes (34). Younger plants, at 4 weeks post-planting are likely to focus on root establishment and leaf growth, resulting in a lower average height of 27.31 cm. However, as plants mature into the vegetative phase, they undergo rapid cell division, elongation and increased photosynthetic activity, leading to biomass accumulation and vertical growth, resulting in an average height increase of 39.19 cm, highlighting the general natural growth trajectory in plants (35).

The significant variability revealed in branch number among spider plant genotypes was primarily influenced by soil nitrogen availability and plant developmental stages rather than genotype (Fig. S2A). This underscores the dynamic nature of plant growth and development, nitrogen nutrition and different stages play a crucial role in shaping branching patterns. The results of this study support those of a recent investigation on the impact of the developmental stages of yellowhorn (*Xanthoceras sorbifolium*), emphasizing hormonal signalling roles on a range of plant morphological characteristics and the regulation of branching patterns (36). Even though many studies highlighted positive relationships between leaves and branches in spider plants (37, 38). However, the results of this study suggest that genetic variables may influence the balance between branching and leaf growth, as evidenced by a significant negative correlation (r = -0.79). This data aligns with research on abiotically stressed wild oak trees (*Quercus suber*), which also found a negative correlation between branch and leaf counts (39). This suggests that resource allocation trade-offs within the plant favour leaf formation over branch formation.

Variations observed in leaf amount between growth phases of spider plant genotypes (Fig. S2C) can be linked to numerous physiological processes, highlighting the complexity of mechanisms regulating leaf production dynamics (40, 41). The results of this study elucidate the mechanisms that drive spider plant leaf production, indicating that plants devote energy to leaf initiation and extension in the early stages of growth, progressively reducing the leaf quantity as the canopy matures. Developmental changes, such as the commencement of reproductive growth or senescence also influence leaf production dynamics. Hormonal regulation is essential for coordinating leaf development, with differences in hormone levels and signalling pathways governing leaf initiation, growth and senescence (42). The study further reported that nitrogen deficiency stress led to a decrease in leaf count, consistent with previous research on potatoes (*Solanum tuberosum* L.), Brussels sprouts (*Brassica oleracea* L. var gemmifera DC), leek (*Allium porrum* L.) and spinach (*Spinacia oleracea* L.), which also indicated a reduction in leaf count with increasing levels of nitrogen deficiency (43). The relationship between growth stages and nitrogen deficiency stress shows how leaf output varies between vegetative and reproductive phases under drought conditions. As plant growth progresses, leaf yield declines, attributed to nitrogen deficiency stress and induces leaf shedding (24). These findings are supported by similar observations in other plant species like scarlet aubergine, where a significant decrease in leaf number occurred as plants transitioned from vegetative to reproductive growth stages under abiotic stress (44).

As presented in Fig. S3, the study further shows that soil nitrogen levels, growth phases and genotypic variances all contribute to significant changes in spider plant economic yield fresh weight (EYFW) and economic yield dry weight (EYDW). The genotypes responded differentially to nitrogen treatment, as evidenced by the considerable variance in economic production between genotypes under both optimal and low nitrogen fertilization conditions. Because of their unique genetic architecture, these variants suggest varying levels of tolerance to nitrogen deprivation. The results of this study align with the findings of amaranth spider plant sunflower and wheat (14, 25, 45, 46).

The data also indicate that some genotypes (LAIOGONGO, ODS15061, ODS15044, ODS15059, TOT3536) may be able to maintain constant individual yields even in the presence of suboptimal nitrogen levels possibly due to their better tolerance to nitrogen deficit. These genotypes may have superior capacities for nitrogen management, maximizing output and growth. On the other hand, genotypes ODS15020, ODS15021, TOT6426, TOT7196, TOT5799, TOT8889 and TOT8926 show no significant difference in economic yield between optimal and suboptimal nitrogen treatments, suggesting that these genotypes are more sensitive to increases in nitrogen availability and less tolerant of nitrogen deficiencies. Susceptibility to nitrogen deficits may be due to their genetic architecture which may not have the systems in place for effective nitrogen storage or utilization, which would lower the amount of protein synthesis, enzyme activity and overall plant development. The results agree with the findings on spinach and maize under nitrogen deficiency (47, 48). These studies suggested genetic differences in how plants use nitrogen, underscoring the significance of choosing nitrogen-efficient genotypes to ensure stable crop performance across a range of nitrogen environments.

Using indices such as MP, GMP, STI and HM, which correlated positively with both Yp and Ys, the study adopted principal component analysis (PCA) to evaluate economic yield performance under drought stress, applying Fernández's theory (20). The PCA biplot of nitrogen deficiency stress selection indices reveals significant variability in nitrogen tolerance among spider plant genotypes. PC1, accounting for 57.3 % of the variance, is primarily associated with yield under stress conditions, while PC2, accounting for 36 %, relates to yield potential under non-stress conditions (Fig. 5). Positive indices like YSI, Ys, MP, GMP, STI and HM cluster together, underscoring their importance in achieving high yields under nitrogen stress. Negative indices TOL and SSI suggest poorer performance under such conditions. Therefore, genotypes near positive indices (NCO5015, ODS15044, ODS15121 and ODS15103) are likely to perform well under nitrogen deficiency, while those appearing the opposite of the positive indices of the near negative indices (Rothwe1, BUAN1, BC02B and KSI2407A) are more sensitive and likely to yield poorly under stress. Comparable research utilizing PCA and genotype selection markers to assess environmental tolerance has been done on lentils and wheat (49, 50).

In addition to PCA, the study utilized a dendrogram of cluster analysis to cluster spider plant genotypes according to how similar they responded to low nitrogen stress. Every genotype was depicted as an endpoint and the difference between genotypes was indicated by the height at which they joined. The Euclidean distance metric was utilized to determine the straight-line distance between points in a multidimensional space and Ward's clustering method was applied to reduce variation within clusters. Four clusters were formed from the group of 25 spider plants. Cluster analysis is a technique for organizing a set of characters into clusters based on their commonalities. In genotypic clustering, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) is often utilized. This statistical strategy grouped objects so that those in the same cluster have high similarities and those in separate clusters have significant dissimilarities. Euclidean distance values between 0 and 1 imply minimal dissimilarities, whereas values greater than 1 suggest bigger dissimilarity coefficients. A minimal dissimilarity coefficient indicates that the characteristics of each genotype have limited variability. In the current study, the 25 spider plant genotypes were clustered using 6 nitrogen deficiency tolerance indices into seven separate clusters (Fig. 6). Given that distinct genes may be responsible for varying clusters' low nitrogen deficiency, these uncorrelated groups may prove valuable in the future for heterotic breeding.

Similarly, the ranking selection method was also employed to identify low nitrogen-deficient genotypes based on the average sum ranks (ASR) of all indices and standard

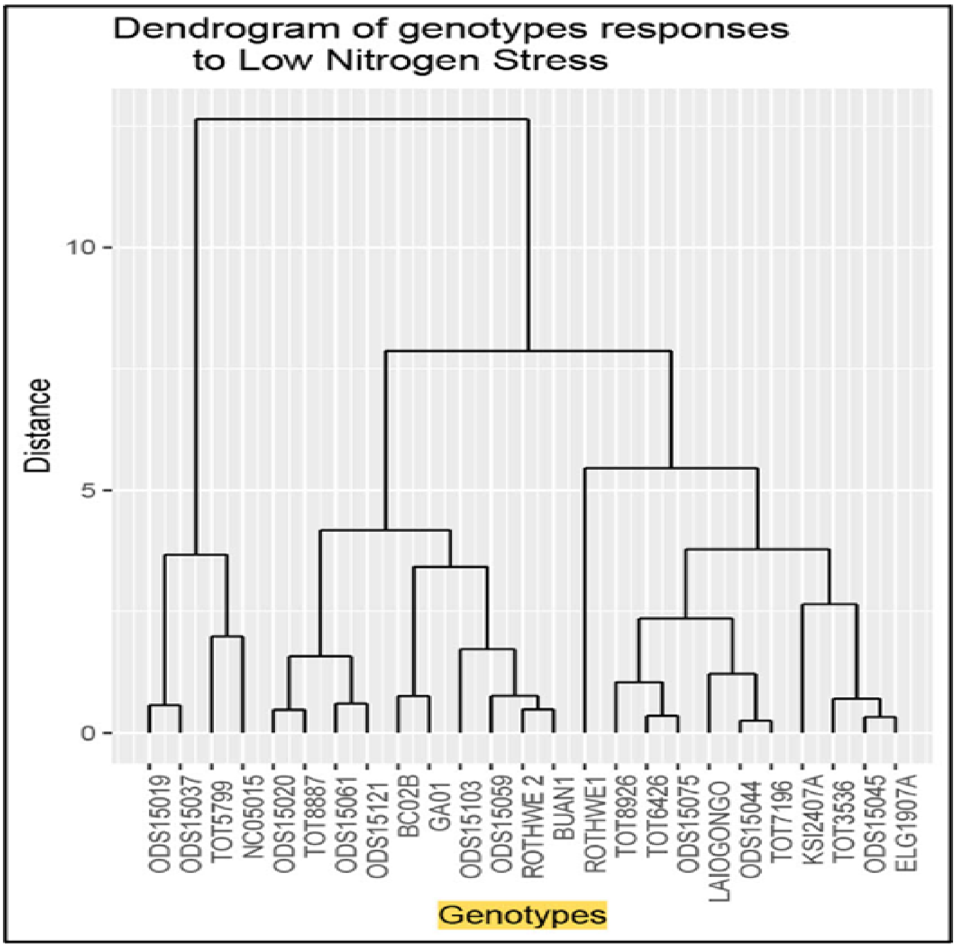

Fig. 6. Dendrogram of cluster analysis of 25 spider plant genotypes using Ward's method based on Yp, Ys and nitrogen deficiency tolerance indices. The numbers 1-4 indicate the clusters of genotypes with similar tolerance characteristics.

deviation of ranks (SDR). Genotypes were categorized as tolerant and sensitive, based on their performance. Tolerant genotypes exhibited the lowest SR and SDR, while Sensitive cultivars had the highest ASR and high standard deviation (51). Crop breeding heavily relies on economic yield characteristics to identify nitrogen-deficient tolerant genotypes (48). Therefore, economic yield parameters have been employed in all three selection techniques (ranking, PCA and cluster analysis) for nitrogen-tolerant genotypes. These methods consistently categorized similar genotypes as tolerant (NC05015, ODS15044, ODS15121, TOT3536 and ODS15103) or sensitive (ROTHWE1, BUAN1, BC02B and KSI2407A, TOT8926) to low nitrogen stress.

To validate these selections, a physiological study focused on leaf fluorescence indicators (Quantum Efficiency of PSII (FvP/FmP), Non-Photochemical Quenching (NPQt), Quantum Yield of PSII (Phi2) and Quantum Yield of Regulated Non-Photochemical Quenching (PhiNPQ), Leaf Chlorophyll Content (mean SPAD) and leaf Stomatal Conductance). This additional study was performed to confirm the results from ranking, PCA and cluster analysis and provide comprehensive validation of genotype classification. These techniques reliably classified similar genotypes as tolerant, moderate, or susceptible to nitrogen stress. A physiological study was conducted to evaluate these decisions, providing comprehensive genotype classification validation and confirming the outcomes of ranking, PCA and cluster analysis.

The observed increased FvP/FmP and Phi2, during the vegetative phase compared to the reproductive phase of tolerant and intermediate genotypes, suggests high photosynthesis efficiency compared to the rest (Fig. 4A2, C2). The results imply that these genotypes are likely to be more efficient at photosynthesis during the vegetative stage, maximizing light absorption and conversion for plant development. However, as plants enter the reproductive stage, their photosynthesis efficiency decreases due to resource reallocation towards seed production, focusing more on storage and mobilization. During the vegetative stage, plants increase their chlorophyll content, Phi2 and FvP/FmP due to their dynamic resource allocation and physiological needs. The reason for these variations suggests that during the vegetative stage, plants concentrate on growing their leaves quickly, which are the main sites of photosynthesis (52).

Higher chlorophyll content has been reported to enhance the plant's ability to capture light energy, maximizing photosynthetic efficiency and supporting robust growth. To maintain high rates of photosynthesis and energy generation, the photosynthetic equipment must operate at peak efficiency (53). As plants transition to the reproductive phase, their focus shifts from vegetative growth to developing reproductive organs like flowers, fruits and seeds. This reallocation of resources and energy results in a reduction in chlorophyll content, a drop in Phi2 and FvP/FmP values and a decline in photosystem II efficiency (54). The senescence of older leaves also contributes to these reductions. Similar results were reported on soybean (Glycine max) at different growth stages under drought and low nitrogen stress conditions (55).

Further evidence of the significant differences in nitrogen tolerance levels between these groups comes from the significantly higher PhiNPQ, chlorophyll content and stomatal conductance values observed (Fig. 4D1, E1, F1) in the tolerant genotypes (NCO5015, ODS15044 and ODS15103) as well as in the intermediate genotypes (LAI-Ogongo, ODS15037, ODS15045 and ODS15044) when compared to the sensitive genotypes (KSI2407A, ODS15020 and TOT8926).

Their significantly higher stomatal conductance allows better CO_2 uptake for enhanced photosynthesis, while elevated SPAD values reflect chlorophyll content, enhancing photosynthetic capacity and overall plant health. Increased PhiNPQ values are known for protecting the photosynthetic apparatus from photodamage, contributing to their superior performance and resilience under low-stress environmental conditions (56). These variations support the results of the genotype selection study suggesting that indeed tolerant and intermediate groups are more tolerant to nitrogen deficiency than the sensitive group. Conversely, the significantly lower values of these indicators observed in sensitive genotypes indicates that these genotypes are indeed sensitive to nitrogen stress. The results suggest that their sensitivity to nitrogen stress may stem from reduced efficiency in photosynthetic processes, limited stomatal conductance (gas exchange) and compromised chlorophyll concentration, highlighting their vulnerability to low nitrogen levels and diminished ability to sustain optimal physiological functions. The findings agree with the outcome of the study conducted on four green-leafy vegetables (*Lactuca sativa, Brassica chinensis, Spinacia oleracea* and *Brassica chinensis*) subjected to abiotic stress conditions, resulting in the reductions of the key leaf photosynthetic parameters of the sensitive genotypes compared to those that were tolerant (57).

The impact of varying soil nitrogen levels on FvP/FmP, Phi2, stomatal conductance and SPAD in combined genotypes demonstrated that low nitrogen led to reduced values of these markers in comparison to plants grown under optimal nitrogen fertilization conditions (Fig. 4A1, C1, F2, E3). The results of this study suggest that genotypes can maintain stomatal conductance, high chlorophyll content and photosynthesis, even in the face of nitrogen deficiency. Some plant genotypes can sustain vital functions under abiotic stresses due to their enhanced nitrogen use efficiency, robust photosystem II function and efficient light capture and conversion (56). They maintain higher FvP/FmP and Phi2 values, stomatal conductance, chlorophyll content, alternative metabolic pathways, stress response mechanisms and root system adaptations (53). These physiological adaptations enable growth and productivity under nitrogen-limited conditions, making them valuable for breeding programs to enhance nitrogen deficiency tolerance.

Conclusion

The study utilized various approaches, including BLUP indices ranking, PCA and cluster analysis, to evaluate spider plant genotypes for low nitrogen stress tolerance under both nitrogen-deficient and optimal nitrogen fertilization conditions. Leaf chlorophyll content, stomatal conductance and fluorescence photosynthetic analysis confirmed low nitrogen stress tolerance in genotypes NC05015, ODS15103 and ODS15044, while LAI-

Ogongo, ODS15037 and ODS15045 exhibited moderate tolerance. In contrast, KSI2407A, ODS15020 and TOT8926 were identified as susceptible. Although intermediate genotypes showed no significant difference in some tolerance indicators compared to the tolerant group, both genotype groups demonstrated superior tolerance over the sensitive genotypes. These findings highlight the promising potential for enhancing low nitrogen tolerance in spider plant genotypes, particularly in the marginal soils of Sub-Saharan Africa, to develop more resilient varieties.

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Authors' contributions

LM contributed to the conceptualization, provided resources, conducted formal analysis and investigation, performed visualization and prepared the original manuscript draft. UB contributed to the conceptualization, supervised the project, provided resources, validated the findings and reviewed and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

The study adhered to ethical standards and it was an experimental study involving crops; thus, an ethical certificate was deemed necessary.

Declaration of conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Ethical issues: None

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