



Genotypic variation in nitrogen use efficiency trait in 35 cucumber inbred lines

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Abstract

Nitrogen is an essential nutrient for plant growth and yield, however excess nitrogen fertilizer application can lead to detrimental environmental impacts. Identifying cucumber genotypes with high nitrogen use efficiency (NUE) could facilitate sustainable crop production through optimized nitrogen management. This study was conducted in March 2021 at the research greenhouse of Department of Horticultural Sciences, University of Guilan, Iran. The objective of this study was to assess growth, yield components, nitrogen accumulation, NUE, and nitrate reductase activity in 60 cucumber genotypes under sufficient (NS) and deficient (ND) nitrogen conditions. Nitrogen deficiency caused reductions in growth and yield across all genotypes, although the severity varied. Nitrogen content was higher in all plant organs under NS compared to ND. NUE and its components nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE) were significantly higher under ND versus NS. Line 57 exhibited the best NUE and yield under both NS and ND, and could be considered a nitrogen-efficient genotype. Based on NUE performance, lines were categorized as efficient, semi-efficient, low-efficient, and inefficient. Nitrate reductase activity was higher in leaves versus roots, and positively correlated with NUE, suggesting a relationship between enzyme activity and nitrogen uptake efficiency in roots. This study demonstrates genotype-specific responses to nitrogen availability in cucumber. The identification of genotypes efficient in NUE, such as line 57, can inform breeding efforts to improve nitrogen responsiveness. Characterizing nitrogen responsiveness across genotypes also allows for optimized, genotype-specific nitrogen management recommendations to be made, facilitating sustainable crop production.

Keywords Nitrate reductase · Nitrogen uptake efficiency · Nitrogen utilization efficiency · Screening · Yield

Introduction

The seed production industry, along with the manufacturing of fertilizers, crop protection products, and other agricultural inputs, plays a vital role in initiating the food-agriculture chain. In addition to adhering to its laws and regulations, this industry is influenced by various other factors, including consumer needs, environmental concerns, and economic issues. When developing new varieties or introducing new traits to existing ones, researchers in this field should take

these requirements into consideration (Persley and Anthony 2017).

Enhancing the nitrogen use efficiency (NUE) of agricultural products is essential for reducing environmental pollution and increasing farmers' profits by optimizing the application of mineral nitrogen fertilizers (Andrade et al. 2020). Factors such as soil conditions, climate, and management practices influence the NUE characteristics of plants. Worldwide, it is common to report nitrogen recovery in agriculture a less than 50% in nitrogen use management. A low nitrogen recovery rate leads to higher agricultural product prices and contributes to environmental pollution (Shillito et al. 2009). Studies have shown that a one percent increase in the NUE of agricultural products can yield annual savings of approximately 1.1 billion USD (Kant et al. 2011). Hence, enhancing NUE brings benefits such as increased agricultural yields, reduced production costs, and environmental protection. Currently, there are two practical methods for enhancing nitrogen consumption deficiency; one method

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is modifying agricultural practices to reduce nitrogen loss through leaching and volatilization, and the other method is utilizing nitrogen-efficient cultivars. In recent years, the selection of nitrogen-efficient products has been considered as an effective way to reduce nitrogen fertilizer inputs and minimize waste on agricultural lands (Wang et al. 2005).

There are several approaches for measuring NUE. The central question surrounding NUE is whether plants with higher NUE also perform better. More specifically, what part of the plant may utilize the absorbed nitrogen (Lammerts van Bueren and Struik 2017). NUE varies among plant species and even between cultivars. Consequently, plants seem to have developed distinct mechanisms for nitrogen absorption and transportation. Leveraging the genetic diversity within a particular species is a crucial aspect of enhancement, although it remains challenging due to extensive genotypes within the species (Mifflin and Habash 2002).

Genotypic differences among plants in the NUE may result from variations in the two principal components of NUE: nitrogen productivity efficiency (the production of dry matter per absorbed nitrogen), and the nitrogen uptake efficiency of the roots as is influenced by a combination of diverse and interconnected characteristics (Xing et al. 2019). Several of these characteristics include root size and structure, nitrogen uptake per unit of root length, nitrogen assimilation, nitrogen transport within the plant, leaf senescence, nitrogen remobilization within the plant, and nitrogen harvest index. Proper root growth and the development of a dense and extensive root system enhance nitrogen uptake, and significantly impact nitrogen uptake efficiency (DoVale et al. 2012). Luo et al. (2015) reported that nitrogen deficiency stimulates root expansion. Bahrman et al. (2005) also observed an increase in the root-to-stem ratio at low nitrate levels.

Numerous factors influence the variations in nitrogen assimilation between genotypes. Enzymes play a key role in transferring energy or electrons during the nitrogen assimilation process. Several essential enzymes involved in nitrogen assimilation are nitrate reductase, nitrite reductase, nitrogenase, glutamine synthetase, glutamate synthase, and glutamate dehydrogenase (Reda et al. 2022). The nitrate reductase enzyme produces NO_2^- and H_2O molecules by consuming two H^+ ions and providing two electrons. This process takes place in the cytoplasm of root and leaf cells. Identifying various nitrogen uptake factors can potentially enhance NUE (Iqbal et al. 2020).

Variation of NUE is also reported in several other plants, including rice (Namai et al. 2009), corn (Gallais and Coque 2005), wheat (Gaju et al. 2011), and sugarcane (Robinson et al. 2007). Due to the expensive and time-consuming nature of the experiments, only a small number of cultivars were examined in these studies. In addition, introducing cultivars with high NUE has not been practical for many crops because NUE is a

complex trait that is also affected by soil conditions, making its control challenging (Dan et al. 2022). The Simultaneous utilization of multiple genotypes is the optimal approach for introducing cultivars with a higher NUE. This approach allows us to enhance our understanding of the mechanism behind this trait more efficiently and expedite the introduction of cultivars with improved NUE. To achieve this, it is preferable to conduct the experiments in a controlled environment, such as the pot experiment, to minimize the impact of environment factors (Andrade et al. 2020).

Various investigations on developing breeding strategies to improve NUE in important agricultural crops such as wheat, corn, soybean, and rapeseed have been conducted to date. However, the NUE has garnered less interest in vegetable crops (Lammerts van Bueren and Struik 2017). Hence, enhancing nitrogen consumption is rapidly growing and resource-intensive plants, such as cucumber, is crucial. Several researchers have explored the modification of agricultural practices to improve NUE in cucumber plants (Lee et al. 2020). They have determined that if nitrogen intake and fertilizer application are correctly regulated, enhanced cucumber NUE would be achievable (Ming et al. 2021). Furthermore, irrigation water management may significantly reduce the amount of nitrogen lost through nitrate leaching. Despite studies on modifying agricultural operations to improve NUE in cucumber, suitable cultivars of cucumber with high NUE are still limited (Wang et al. 2019).

While NUE is critical for productivity and sustainability in cucumber production, there remain key knowledge gaps in understanding NUE variability among cucumber genotypes. Our study seeks to address this research gap and further understand NUE variability in cucumber genotypes, as well as provide insights to improve sustainable cucumber production. First, we will systematically classify and evaluate 35 cucumber lines for NUE performance to establish a framework of NUE trait variation. This will allow us to identify genotypes with high or low NUE. Second, we will comprehensively analyze NUE components and their response to varying nitrogen conditions, from deficiency to sufficiency. By uncovering how NUE dynamically responds to nitrogen levels, we can elucidate adaptive traits key for optimizing NUE in cucumbers. Ultimately, these genotype evaluations and component analyses will provide valuable insights to guide genetic improvements and agronomic practices for enhancing NUE and sustainability of cucumber crops.

Materials and methods

Plant materials and experimental details

This study was conducted in March 2021 at the research greenhouse of Department of Horticultural Sciences,

University of Guilan. The greenhouse which is situated at 49° 36' East longitude, 37° 16' North latitude, and an elevation of 7 m above sea level. In this experiment, 35 inbred cucumber lines from the Germplasm Bank of the University of Guilan were examined.

A drip irrigation system precisely delivered water and nutrients to individual cucumber plants housed in pots. Each pot contained a 1:1 (w/w) mixture of coco peat and perlite. Pots measured 25 inches wide by 15 inches deep. Seedlings were planted until the second set of true leaves, and then transferred. The system included a centralized nutrient reservoir with an air pump. A circulation pump cycled the aerated nutrient solution through tubing connected to each pot. Within tubing, emitters dripped the solution directly into each pot's growing media. The nutrient solution composition is in Table 1. After root establishment, this solution was used daily for irrigation. In N-deficient and N-sufficient treatments, all nutrients were similar except for nitrogen. N-deficient plots lacked nitrogen, while N-sufficient plots contained 8.5 meq/L nitrate in the form of potassium nitrate (3.2 meq/L), calcium nitrate (5.2 meq/L), and ammonium nitrate (0.1 meq/L) (Table 1). To prevent the accumulation of salts, leaching was performed every 7–10 days. The multiple harvest method was employed to assess the fruit yield of the lines in each treatment. After the first marketable fruits were observed 45 days after transplanting, the harvesting process was initiated following the method described by Wehner and Lower (1996), which involved conducting six harvests at three-day intervals. These harvests typically took place when the fruits were slightly immature, nearing full size but before their seeds fully enlarged and hardened. The required samples were collected and transported to the laboratory, where characteristics such as fresh weight (FW), dry matter (DM), total nitrogen in various plant parts, nitrate reductase enzyme activity, and NUE, and its components were measured.

Plant analysis and nitrogen use efficiency indexes

After 30 days of sowing, the cucumber plants were completely removed from the container. The roots were separated from the shoots and washed with deionized water. Subsequently, the roots and shoots were dried in an oven at 70 °C for 48 h to obtain their dry weight. The ground samples were analyzed to determine nitrogen content following the methods described by Marschner (1995). The nitrogen content of each sample was determined using the Velp NDA 701 Dumas nitrogen analyzer and the Dumas method as outlined by Lequeue et al. (2016). Depending on the type of final product, there are various definitions for NUE in crops. This study considered some nitrogen use indices (Hawkesford 2017). Nitrogen use efficiency (NUE) is the amount of crop that can be harvested (yield) per unit of available nitrogen

for the plant. NUtE measures a plant's harvested product (yield) per unit of absorbed nitrogen. The NUpE measures the amount of nitrogen absorbed per unit of total nitrogen in the soil. NHI% quantifies the proportion of nitrogen in the harvestable portion of the plant to the total nitrogen in biological yield (Lammerts van Bueren and Struik 2017). The following equations represent the nitrogen indices:

1. $NUE (g \text{ FW } g^{-1} N) = \text{fresh yield (g)}/N \text{ application (g)}$.
2. $NUtE (g \text{ FW } mg^{-1} N \text{ g DM}) = \text{fresh yield (g)}/N \text{ content (mg N } g^{-1} \text{ DM)}$.
3. $NUpE (mg \text{ N } g^{-1} \text{ DM } g^{-1} N) = N \text{ Content (mg N } g^{-1} \text{ DM)}/N \text{ application (g)}$.
4. $NHI (\%) = (N \text{ yield (mg N } g^{-1} \text{ DM)}/N \text{ Content (mg N } g^{-1} \text{ DM)}) \times 100$.

Nitrate reductase assay

The samples were placed in ice water for a few minutes after 200 mg of desired plant tissue (root and leaf) from each plant. Once cooled, the samples were transferred in microfuge tubes. Before uniformly crushing the samples, 600 mL of cold buffer (0.2 M sodium phosphate buffer with 5 mM Na₂EDTA or phosphate buffer with a pH of 7.5) was added to the microfuge tubes. The samples were then centrifuged at 4°C for 15 min., and the supernatant for each sample was extracted.

For enzyme activity measurement, 5 mL vials were filled with the following components: 1.6 mL of a nitrate buffer solution with a pH of 7.5 (25 mM sodium phosphate, 12.5 mM potassium nitrate, 0.25 mM Na₂EDTA, 0.1 M hydrochloric acid), 0.15 mL of distilled water, 0.2 mL of a 3.5 mM NADH solution, and 50 µL of an enzyme extract. After adding all the ingredients, the samples were kept at laboratory temperature for 15 min. after adding all ingredients. Subsequently, 2 mL of an indicator (1:1 ratio of 1% sulfanilamide in 1.5% normal HCl and 0.02% *N*-(1-naphthyl) ethylenediamine dihydrogen chloride in distilled water) was added. The appearance of a pink color, indicating the formation of nitrite, was observed, and the color intensity was measured at 540 nm using a spectrophotometer (T80 + UV/VIS PG Instrument) (Tabatabaei 2006). Nitrate reductase activity was reported as the amount of nitrite produced per hour per unit of leaf weight for each treatment, expressed in micromoles of NO₂⁻ per gram of FW per hour (µmol NO₂⁻ g FW⁻¹ h⁻¹).

Statistical analysis

Our methodology featured a factorial experiment, examining cucumber lines and two nitrogen (N) levels. We employed ANOVA to assess group distinctions, performed LSD for specific comparisons, ensured data normality, and applied

Table 1 Mean comparisons of cucumber plant growth and yield components under two nitrogen conditions, sufficient nitrogen (NS) and nitrogen deprivation (ND)

line	Plant height (cm)		Leaf area (cm ²)		Plant dry matter (g)		Root volume (cm ³)		Root weight (g)		Fruit number		Fruit weight (g)	
	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND
L21	154.5 ^{mn}	125.7 ^{hi}	477.60 ^a	367.14 ^a	68.41 ^{f-h}	39.76 ^b	18.3 ^{kl}	16.8 ^{f-i}	25.21 ^{h-j}	21.88 ^e	5.5 ^{g-i}	2.2 ^{-j}	427.667 ^{m-p}	135.77 ^{ij}
L22	187.3 ^{e-e}	137.8 ^g	369.28 ^{f-l}	210.79 ^{d-i}	70.85 ^{ef}	26.81 ^{k-n}	19.6 ^{h-k}	14.8 ^{j-l}	29.61 ^e	18.20 ^{f-h}	4.4 ^{-l}	1.6 ^{b-l}	415.778 ^{n-p}	136.33 ^{ij}
L24	187.3 ^{e-e}	140.1 ^{fg}	406.67 ^{c-f}	240.48 ^{b-h}	70.56 ^{e-g}	28.62 ^{-m}	17.8 ^{kl}	14.5 ^{kl}	17.33 ⁿ	14.02 ^{j-m}	3.6 ^{mn}	1.4 ^{-l}	331.649 ^s	94.55 ^{mn}
L25	184.8 ^{d-f}	108.1 ^{m-o}	444.19 ^{a-e}	262.77 ^{b-e}	61.45 ^{kl}	27.79 ^{-m}	32.8 ^a	20.7 ^c	35.94 ^{bc}	28.21 ^c	5.3 ^{g-i}	2.6 ^{-g}	590.517 ^g	169.56 ^{gh}
L26	161.4 ^{j-m}	96.1 ^q	384.75 ^{e-j}	235.25 ^{c-i}	51.51 ^{qr}	23.51 ^{n-p}	26.5 ^{bc}	18.4 ^{d-f}	34.33 ^{cd}	28.14 ^c	7.4 ^{cd}	3.5 ^{bc}	529.889 ^{hi}	198.18 ^{ef}
L27	147.6 ^{no}	114.0 ^{kl}	322.78 ^{k-m}	246.21 ^{b-g}	60.16 ^{lm}	26.17 ⁻ⁿ	19.7 ^{h-k}	16.0 ^{h-k}	24.11 ^{jk}	18.78 ^{fg}	5.8 ^{f-h}	2.1 ^{g-k}	679.882 ^{ef}	145.35 ^{h-j}
L28	189.2 ^{cd}	126.5 ^h	455.01 ^{a-d}	238.17 ^{b-i}	52.37 ^{pq}	28.15 ^{-m}	12.8 ^m	11.2 ^{m-o}	20.04 ^m	14.18 ^{-l}	4.4 ^{-l}	1.4 ^{-l}	447.944 ⁻ⁿ	93.05 ^{nm}
L29	172.3 ^{hi}	144.6 ^{ef}	399.07 ^{d-h}	214.34 ^{d-i}	55.90 ^{np}	24.16 ^{no}	13.1 ^m	10.3 ^{n-q}	13.16 ^{p-r}	12.12 ⁻ⁿ	3.7 ⁻ⁿ	1.4 ^{-l}	369.293 ^{qr}	104.82 ^{k-m}
L30	203.8 ^b	181.7 ^a	469.97 ^{ab}	248.54 ^{b-h}	66.88 ^{g-i}	39.31 ^b	10.7 ⁿ	8.6 ^{qr}	13.78 ^{o-q}	12.01 ^{mn}	3.8 ⁻ⁿ	1.4 ^{-l}	431.121 ^{m-o}	126.73 ^{-l}
L32	177.5 ^{e-h}	96.4 ^q	390.04 ^{e-i}	216.43 ^{d-i}	64.41 ^{i-k}	28.03 ^{-m}	23 ^{d-f}	19.0 ^{c-e}	33.65 ^d	14.33 ^{jk}	6.5 ^{ef}	2.5 ^{l-h}	724.161 ^d	182.96 ^{fg}
L33	184.3 ^{d-g}	139.8 ^{fg}	314.36 ^{lm}	251.32 ^{b-h}	59.46 ⁻ⁿ	31.23 ^{g-j}	18.6 ^k	15.0 ^{i-k}	18.00 ⁿ	14.22 ^{-l}	4 ^{k-n}	1.3 ^{-l}	450.689 ^{lm}	101.22 ^{lm}
L34	175.8 ⁻ⁱ	154.8 ^{cd}	377.33 ^{f-k}	285.51 ^{bc}	56.74 ^{m-o}	25.61 ⁻ⁿ	12.6 ^{mn}	9.4 ^{o-r}	14.68 ^{op}	12.81 ^{k-n}	5.7 ^{-h}	2.2 ^{-j}	527.247 ^{h-j}	158.93 ^{g-i}
L35	156.6 ^{k-n}	134.5 ^g	416.47 ^{b-f}	251.18 ^{b-h}	42.71 ^u	23.91 ^{no}	24.8 ^{cd}	19.7 ^{cd}	34.94 ^{cd}	29.90 ^c	1.7 ^o	1 ^{-l}	157.711 ^t	56.88 ^p
L36	113.6 ^f	95.1 ^q	442.81 ^{a-e}	239.00 ⁻ⁱ	48.13 ^{rs}	20.20 ^{pq}	13.1 ^m	9.7 ^{n-q}	15.09 ^o	10.98 ^{n-p}	3.7 ⁻ⁿ	1.2 ^{kl}	375.302 ^{qr}	93.98 ^{nm}
L37	103.4 ^f	84.5 ^r	332.47 ^{j-m}	202.01 ^{hi}	46.54 st	21.13 ^{op}	24.7 ^{-e}	17.6 ^{-h}	34.68 ^{cd}	25.64 ^d	5.2 ^{h-j}	2.4 ^{d-h}	395.711 ^{pq}	147.26 ^{h-j}
L39	128.6 ^q	120.4 ^{ij}	325.09 ^{-m}	204.45 ⁻ⁱ	42.78 ^{tu}	26.13 ⁻ⁿ	13.8 ^m	9.8 ^{n-q}	14.36 ^{o-q}	1092 ^{n-p}	5.2 ^{h-j}	3.2 ^{-e}	451.06 ^{lm}	234.04 ^{cd}
L41	140.3 ^{op}	103.6 ^{op}	363.02 ^{f-l}	259.13 ^{b-f}	54.36 ^{o-q}	25.06 ^{mn}	12.1 ^{mn}	7.5 ^r	12.7 ^{qr}	9.73 ^{op}	4.7 ^{-k}	1.44 ^{-l}	400.722 ^{o-q}	93.63 ^{nm}
L42	170.5 ^{h-j}	107.2 ^{no}	351.44 ^{g-l}	219.18 ^{d-i}	74.32 ^{de}	32.84 ^{e-h}	13.8 ^m	8.8 ^{p-r}	13.50 ^{o-r}	11.78 ^{no}	5.7 ^{-h}	3.33 ^{cd}	659.922 ^f	258.59 ^c
L43	172.1 ^{hi}	107.1 ^{no}	314.35 ^{lm}	207.45 ⁻ⁱ	61.99 ^{-l}	34.32 ^{d-g}	13.4 ^m	9.0 ^{p-r}	15.06 ^o	11.15 ^{n-p}	1.8 ^o	1.1 ^{-l}	203.269 ^t	68.28 ^{n-p}
L44	138.4 ^{o-q}	84.5 ^r	365.74 ^{f-l}	253.54 ^{b-h}	65.55 ^{h-j}	29.00 ^{-l}	19.0 ^{jk}	16.1 ^{g-k}	20.84 ^{lm}	19.48 ^f	4.3 ^{k-m}	2.4 ^{d-h}	477.606 ^{kl}	162.47 ^{g-i}
L45	155.8 ^{mn}	125.8 ^{cd}	358.39 ^{g-l}	279.64 ^{bc}	68.27 ^{-h}	35.33 ^{c-f}	27.8 ^b	25.4 ^b	35.73 ^{bc}	32.48 ^f	3.5 ^{mn}	1.1 ^{-l}	353.333 ^{rs}	58.54 ^{op}
L46	157.5 ^{k-n}	122.3 ^{-j}	314.82 ^{lm}	236.28 ⁻ⁱ	79.68 ^{bc}	37.97 ^{bc}	22.7 ^{e-g}	13.0 ^{lm}	28 ^{ef}	18.60 ^{f-h}	6.1 ^{e-g}	2.7 ^{-g}	616.322 ^g	217.74 ^{de}
L47	141.4 ^{op}	199.5 ^{pq}	352.84 ^{g-l}	209.82 ⁻ⁱ	82.76 ^b	34.60 ^{c-g}	27.7 ^b	25.4 ^b	37.38 ^b	34.59 ^b	7.8 ^{bc}	2.5 ^{d-h}	757.021 ^c	210.85 ^{de}
L48	173.7 ^{g-i}	160.7 ^b	388.85 ⁻ⁱ	275.22 ^{bc}	73.91 ^{de}	35.61 ^{c-e}	26 ^{bc}	17.2 ^{e-h}	26.66 ^{f-i}	15.99 ^{ij}	2.1 ^o	1 ^{-l}	234.371 ^t	84.42 ^{m-o}
L49	154.3 ^{mn}	119.8 ^j	328.48 ^{-m}	258.05 ^{b-g}	59.74 ^{lm}	29.88 ^{h-k}	16.4 ^l	11.0 ^{no}	17.71 ⁿ	11.70 ^{no}	8.2 ^{bc}	3.1 ^{c-f}	704.606 ^{de}	181.77 ^{fg}
L50	154.3 ^{mn}	125.1 ^{hij}	336.08 ^{-i-m}	235.33 ⁻ⁱ	75.47 ^d	30.00 ^{h-k}	17.8 ^{kl}	11.4 ^{mn}	26.92 ^{f-h}	17.75 ^{f-i}	8.4 ^b	4.4 ^b	870.108 ^b	325.51 ^b
L51	165.7 ^{-l}	125.7 ^{hi}	364.72 ^{-l}	282.86 ^{bc}	80.47 ^{bc}	34.67 ^{c-g}	21.2 ⁻ⁱ	16.0 ^{h-k}	27.23 ^{fg}	17.70 ^{f-i}	4.4 ^{-l}	2.4 ^{d-h}	495.338 ^{jk}	216.83 ^{de}
L52	158.7 ^{k-m}	113.7 ^{k-m}	365.49 ^{-l}	262.08 ^{b-e}	67.22 ⁻ⁱ	28.34 ^{-m}	19.3 ^{-k}	14.6 ^{-l}	20.11 ^m	17.16 ^{g-i}	8.2 ^{bc}	2.4 ^{d-h}	766.044 ^c	153.59 ^{h-j}
L53	171.2 ^{h-j}	134.5 ^g	343.68 ^{b-m}	185.93 ⁱ	75.38 ^d	17.34 ^d	13.0 ^m	9.3 ^{o-r}	11.72 ^r	9.30 ^p	6.7 ^{de}	2.6 ^{-g}	521.671 ^{ij}	135.76 ^{ij}
L54	166.5 ^{-k}	119.5 ^{kl}	342.71 ^{b-m}	267.92 ^{bc}	74.75 ^d	33.37 ^{e-h}	20.7 ^{g-j}	10.6 ^{n-p}	17.13 ⁿ	14.23 ^{-l}	7.4 ^{cd}	2.3 ⁻ⁱ	857.864 ^b	182.32 ^{fg}
L55	135.1 ^{pq}	110.4 ⁻ⁿ	366.1 ^{-l}	208.59 ⁻ⁱ	77.57 ^{cd}	44.86 ^a	22.4 ^{fg}	16.5 ^{-j}	24.92 ^{ij}	16.59 ^{hi}	5.4 ^{g-i}	1.6 ^{-l}	619.344 ^g	129.53 ^{jk}
L56	158.8 ^{k-m}	138.7 ^g	289.23 ^m	262.22 ^{b-e}	62.24 ^{-l}	37.41 ^{b-d}	25.1 ^c	18.0 ^{d-g}	36.86 ^b	34.62 ^b	3.4 ⁿ	1.2 ^{kl}	325.246 ^s	101.79 ^{lm}
L57	276.8 ^a	125.2 ^{h-j}	462.20 ^{a-c}	264.00 ^{b-d}	103.49 ^a	41.02 ^b	31.8 ^a	28.0 ^a	40.09 ^a	37.14 ^a	11.6 ^a	6.4 ^a	1371.711 ^a	547.82 ^a
L58	141.4 ^{op}	155.8 ^{bc}	333.28 ^{g-m}	291.13 ^b	65.20 ^{b-k}	31.81 ^{f-i}	21.5 ^{-h}	16.8 ⁻ⁱ	22.65 ^{kl}	14.33 ^{jk}	4.3 ^{k-m}	1.1 ^{-l}	379.584 ^{qr}	64.80 ^{op}

Table 1 (continued)

Line	Plant height (cm)		Leaf area (cm ²)		Plant dry matter (g)		Root volume (cm ³)		Root weight (g)		Fruit number		Fruit weight (g)	
	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND	NS	ND
159	195.8 ^{bc}	149.5 ^{de}	322.2 ^{6k-m}	257.0 ^{3b-g}	68.23 ^{f-h}	28.48 ^{i-m}	22.4 ^{fg}	15.1 ^{i-k}	25.63 ^{g-j}	16.91 ^{e-i}	5.2 ^{h-j}	1.2 ^k	554.8 ^h	68.68 ^{n-p}

In each column, the means with at least a similar letter are not significantly different at a 0.05 level of probability

PCA for comprehensive analysis. SAS 9.4 was used for data analysis.

Results and discussion

Growth and yield components in cucumber genotypes

As demonstrated by the mean comparisons of growth and yield components (plant height, leaf area, dry biomass weight, root volume, root weight, fruit amount, and fruit weight) in Table 1, the amount of nitrogen (N) has a varying effects on the growth and yield components across different cucumber lines. Consistent with our findings, Zhang et al. (2020) reported that the response mechanisms to nitrogen deficiency depend on genotype. The results indicate that plant height, regardless of N treatment, varied between 84 and 276 cm, with line 57 in the nitrogen-sufficient (NS) condition and line 30 in the nitrogen-deficient (ND) condition being the highest. Figure 1 provides a visual comparison of two treatment conditions, nutrient deficiency (ND) and normal fertilization (NF), over a defined time period.

Environmental conditions, including environmental stress, have been shown to significantly affect the height of cucumber plants (Douh et al. 2013). Since leaf size and its durability are directly influenced by nitrogen (N), N deficiency would reduce the light-harvesting surfaces or light use efficiency, resulting in a decrease in photosynthetic resources and enzyme activities. Consequently, this reduction leads to a decrease in product yield (Muchow and Sinclair 1994; Paris et al. 2018). However, different plant species utilize different mechanisms in response to varying levels of N nutrition, and cultivars with greater NUE can be introduced through meticulous examination. The leaf surface area of the examined lines varied from 185.9 to 477.60 cm² regardless of N treatment. In both the sufficient N and deficient N treatments, line 21 demonstrated the greatest leaf area value. Growth reduction in plants induced by nitrogen stress has two components: reduction of leaf size and subsequent reduction of dry weight accumulation rate (Chapin et al. 1988).

The plant dry weight showed significant differences ($p < 0.01$) between the two nitrogen treatments. The plant dry weight values were 109.49 g for line 57 in the NS treatment and 44.86 g for line 55 in the nitrogen-deprivation (ND) treatment. The average reduction in DM production for root, stem, leaf, and fruit due to N deficiency was 24, 49, 51, and 43%, respectively. The mean comparisons revealed that line 57 exhibited the highest root weight and volume characteristics in both the NS and ND conditions. When a nutrient, such as nitrogen, is the limiting factor, more biomass is allocated to the roots than to the shoots (Poorter et al. 2012).



Fig. 1 Visual symptoms of chlorosis, characterized by yellowing of leaves, and reduced root growth were observed in line 57, indicative of nitrogen deficiency

Research has indicated that certain root features are essential for absorption nitrogen. Cultivars with high NUE (NUE) typically exhibit higher root absorption, length, surface area, and volume compared to cultivars with low nitrogen efficiency. This suggests that these traits enhance the capacity for DM production under low nitrogen stress (Zhang et al. 2015). Line 57 demonstrated the highest fruit yield and weight under both NS and ND conditions. Maintaining an acceptable yield range under ND conditions is a crucial aspect of increasing NUE and introducing genotypes with higher NUE (Todeschini et al. 2016; Andrade et al. 2020).

In summary, our study demonstrates the intricate relationship between nitrogen availability and growth and yield components in cucumber plants, with a focus on

genotype-specific responses and the significance of root traits for NUE. This research highlights the potential for improving crop performance under ND conditions and calls for further investigation into the genetic and physiological mechanisms governing these responses. Ultimately, this knowledge may lead to the development of cucumber cultivars with enhanced NUE, offering valuable insights for sustainable and high-yielding crop production.

Nitrogen content

Regardless of the plant organ, nitrogen content was higher in all genotypes under the treatment with sufficient nitrogen supply. Regardless of genotypes, nitrogen accumulation in plant organs followed the order: leaf > fruit > stem > root.

According to Table 2, in the ND condition, the nitrogen content of the organs in the studied lines ranged from 0.006 mg N (L42) to 0.258 mg N (L21) in the roots, from 0.431 mg N (L53) to 0.149 mg N (L45) in the stem, from 0.309 mg N (L35) to 1.74 mg N (L39) in the fruit, and from 0.151 mg N (L26) to 0.799 mg N (L55) in the leaf. However, under the NS condition, the range varied from 0.009 mg N (L41) to 0.047 mg N (L21) in the roots, from 0.113 mg N (L39) to 0.475 mg N (L57) in the stem, from 0.163 mg N (L35) to 1.08 mg N (L57) in the fruit, and from 0.819 mg N (L26) to

2.166 mg N (L57) in the leaf. Nitrogen deficiency inhibited growth and reduced yield in all genotypes.

The efficiency with which a plant utilizes supplied nitrogen is an important indicator of its production and market value. The application of nitrogen fertilizer can significantly increase agricultural productivity. Understanding the response of NUE in plants is crucial for breeding species with high NUE. The ideal genotype possesses a high genetic NUE and N reactivity (Han et al. 2015). NUE and its constituents, NUpE and nitrogen utilization efficiency (NUE),

Table 2 Mean comparisons of the nitrogen content (N accumulation) under two nitrogen conditions, sufficient nitrogen (NS) and nitrogen deprivation (ND)

Lines	N content (mg g ⁻¹) plant ⁻¹							
	Roots		Stem		Fruit		Leaves	
	NS	ND	NS	ND	NS	ND	NS	ND
L21	0.047 ^{a*}	0.025 ^a	0.212 ^{kl}	0.1212 ^{b-d}	0.287 ^{l-n}	0.178 ^{c-f}	1.111 ^k	0.4465 ^{cd}
L22	0.039 ^b	0.016 ^{c-e}	0.148 ^{mn}	0.0469 ^o	0.502 ^f	0.136 ^{c-i}	1.31 ^h	0.4141 ^{de}
L24	0.016 ^{i-o}	0.012 ^{e-k}	0.31 ^{d-f}	0.0883 ^{e-k}	0.338 ^{j-m}	0.083 ^{e-i}	1.247 ^{h-j}	0.310 ^{f-i}
L25	0.016 ^{i-o}	0.013 ^{c-i}	0.213 ^{j-l}	0.0825 ^{g-l}	0.666 ^d	0.085 ^{d-i}	1.1 ^k	0.2384 ^{j-m}
L26	0.02 ^{g-j}	0.010 ^{j-o}	0.242 ^{h-k}	0.0673 ^{i-o}	0.5 ^f	0.103 ^{d-i}	0.819 ⁿ	0.1553 ^o
L27	0.025 ^{e-g}	0.0137 ^{c-h}	0.263 ^{g-i}	0.0769 ^{h-n}	0.637 ^{de}	0.056 ^{g-i}	0.841 ^{mn}	0.3008 ^{g-i}
L28	0.012 ^{n-p}	0.0161 ^{c-f}	0.229 ^{i-l}	0.0920 ^{e-i}	0.339 ^{j-m}	0.038 ⁱ	1.027 ^{kl}	0.2615 ^{i-k}
L29	0.017 ^{i-o}	0.0095 ^{k-p}	0.313 ^{d-f}	0.0639 ^{k-o}	0.326 ^{k-n}	0.046 ⁱ	1.136 ^{jk}	0.2671 ^{ij}
L30	0.014 ^{j-p}	0.0096 ^{k-p}	0.299 ^{e-g}	0.1081 ^{c-g}	0.311 ^{k-n}	0.068 ^{f-i}	1.363 ^h	0.4138 ^{de}
L32	0.018 ^{i-m}	0.0101 ⁱ⁻ⁿ	0.288 ^{e-g}	0.0680 ^{i-o}	0.636 ^e	0.153 ^{c-h}	1.315 ^h	0.3046 ^{g-i}
L33	0.018 ^{i-m}	0.0115 ^{g-l}	0.353 ^{cd}	0.0908 ^{e-j}	0.396 ^{h-j}	0.058 ^{g-i}	1.131 ^{jk}	0.2767 ^{h-j}
L34	0.013 ^{k-p}	0.0097 ^{j-p}	0.268 ^{f-i}	0.0608 ^{l-o}	0.428 ^{g-i}	0.150 ^{c-h}	0.95 ^{lm}	0.1700 ^{no}
L35	0.011 ^{op}	0.164 ^{cd}	0.272 ^{f-i}	0.0666 ^{i-o}	0.163 ^o	0.030 ⁱ	1.148 ^{i-k}	0.2346 ^{j-m}
L36	0.013 ^{k-p}	0.0104 ^{h-n}	0.211 ^{kl}	0.0788 ^{h-n}	0.266 ⁿ	0.082 ^{e-i}	1.06 ^{kl}	0.1972 ^{m-o}
L37	0.009 ^p	0.0201 ^b	0.215 ^{j-l}	0.0553 ^{m-o}	0.285 ^{mn}	0.066 ^{g-i}	1.027 ^{kl}	0.2023 ^{k-o}
L39	0.015 ^{j-p}	0.0079 ^{m-p}	0.113 ⁿ	0.0972 ^{d-h}	0.341 ^{j-m}	1.739 ^a	0.947 ^{lm}	0.1998 ^{l-o}
L41	0.009 ^p	0.0067 ^{op}	0.184 ^{lm}	0.1146 ^{c-e}	0.365 ^{i-k}	0.077 ^{e-i}	1.284 ^h	0.3483 ^{fg}
L42	0.013 ^{l-p}	0.0064 ^{op}	0.291 ^{e-g}	0.0758 ^{h-n}	0.706 ^{cd}	0.162 ^{c-g}	1.515 ^g	0.3057 ^{f-i}
L43	0.013 ^{m-p}	0.0079 ^{m-p}	0.283 ^{e-h}	0.1082 ^{c-g}	0.191 ^o	0.053 ^{g-i}	1.814 ^{de}	0.6097 ^b
L 44	0.017 ⁱ⁻ⁿ	0.0224 ^{ab}	0.352 ^{cd}	0.1096 ^{c-f}	0.344 ^{j-m}	0.065 ^{g-i}	1.674 ^f	0.3266 ^{f-h}
L45	0.02 ^{g-j}	0.0104 ^{h-n}	0.324 ^{de}	0.1494 ^a	0.356 ^{j-l}	0.068 ^{f-i}	1.671 ^f	0.7869 ^a
L46	0.015 ^{j-p}	0.0115 ^{g-l}	0.387 ^{bc}	0.1299 ^{a-c}	0.469 ^{fg}	0.136 ^{c-i}	1.918 ^{b-d}	0.4220 ^{c-e}
L47	0.019 ^{h-k}	0.0071 ^{n-p}	0.272 ^{f-i}	0.0961 ^{d-h}	0.583 ^e	0.185 ^{c-e}	1.983 ^{bc}	0.4510 ^{cd}
L48	0.028 ^{d-f}	0.011 ^{g-l}	0.414 ^b	0.1429 ^{ab}	0.306 ^{k-n}	0.115 ^{d-i}	1.876 ^{cd}	0.5604 ^b
L49	0.013 ^{l-p}	0.0121 ^{g-l}	0.203 ^{kl}	0.0867 ^{f-l}	0.751 ^c	0.133 ^{c-i}	1.294 ^h	0.4784 ^c
L50	0.015 ^{j-p}	0.0099 ^{j-o}	0.232 ^{i-k}	0.0467 ^o	1.036 ^a	0.194 ^{cd}	1.262 ^{hi}	0.2216 ^{j-n}
L51	0.031 ^{cd}	0.0130 ^{d-j}	0.301 ^{e-g}	0.0645 ^{j-o}	0.372 ^{i-k}	0.234 ^c	2.009 ^b	0.3352 ^{f-h}
L52	0.021 ^{g-i}	0.0143 ^{c-g}	0.258 ^{g-j}	0.0535 ^{no}	0.581 ^e	0.0958 ^{d-i}	1.65 ^f	0.3446 ^{ef}
L53	0.012 ^{n-p}	0.0080 ^{m-p}	0.371 ^{bc}	0.0431 ^o	0.484 ^{fg}	0.099 ^{d-i}	1.81 ^{de}	0.2588 ^{i-l}
L54	0.021 ^{g-i}	0.0103 ^{h-n}	0.233 ^{i-k}	0.0808 ^{h-m}	0.823 ^b	0.131 ^{c-i}	1.324 ^h	0.4181 ^{de}
L55	0.019 ^{i-l}	0.0137 ^{c-h}	0.351 ^{cd}	0.1089 ^{c-g}	0.589 ^c	0.119 ^{d-i}	1.647 ^f	0.7990 ^a
L56	0.031 ^{c-e}	0.0075 ^{n-p}	0.348 ^{cd}	0.1475 ^{ab}	0.277 ^{mn}	0.074 ^{f-i}	1.705 ^{ef}	0.7636 ^a
L57	0.036 ^{bc}	0.0165 ^c	0.475 ^a	0.1430 ^{ab}	1.079 ^a	0.432 ^b	2.166 ^a	0.4176 ^{de}
L58	0.025 ^{f-h}	0.0124 ^{g-l}	0.2 ^{kl}	0.0905 ^{e-k}	0.455 ^{f-h}	0.046 ^{hi}	1.876 ^{cd}	0.4466 ^{cd}
L59	0.019 ^{i-l}	0.00921 ^p	0.37 ^{6-c}	0.1348 ^{a-c}	0.466 ^{fg}	0.374 ^b	1.099 ^k	0.5947 ^b

In each column, the means with at least a similar letter are not significantly different at a 0.05 level of probability

had significantly higher values in ND, regardless of genotype. Under the NS condition, NUE trait values ranged from 23.73 g FW g⁻¹ N (L35) to 206.4 g FW g⁻¹ N (L57), and under the ND condition, they ranged from 25.18 g FW g⁻¹ N (L25) to 824.3 g FW g⁻¹ N (L57). A decrease can explain the decline in NUE and the rise in nitrogen supply in any component, including NUpE and NUtE (Kubota et al. 2018).

According to Serret et al. (2008), as supplied nitrogen decreased, both major components of NUE, namely NUpE and NUtE, increased, resulting in NUE increase across various genotypes. Line 57 (0.565 mg N g DM g⁻¹ N) in the (NS condition and line 39 (1.538 mg N g DM g⁻¹ N) in the ND condition had the highest NUpE values (Table 3). However, in the NS and ND conditions, line 27 (385.882 g FW mg⁻¹ N g DM) and line 50 (1498.46 g FW mg⁻¹ N g DM) had significantly higher NUtE values, respectively. The NHI is calculated by dividing the nitrogen accumulation in the plant's final yield by the nitrogen that the plant absorbed. In other words, it represents the amount of nitrogen allocated by plants to the final product. The lines with the highest NHI values were 57 and 50 for the NS and ND conditions, respectively. Higher NHI values indicate that these genotypes are more effective at transferring nitrogen from the roots to the final product. Therefore, the NHI values provide useful information about the nitrogen transferred to the final product (Fan et al. 2018), which can be utilized to manage nitrogen nutrition.

The study highlights the importance of nitrogen supply in cucumber plants, with nitrogen content being highest in leaves, followed by fruits, stems, and roots. Nitrogen deficiency inhibits growth and reduces yield. Higher NUE values indicate better nitrogen utilization. The NHI reflects nitrogen allocation to the final product. Understanding these factors is crucial for optimizing nitrogen management and developing nitrogen-efficient cucumber varieties.

Nitrate reductase enzyme

Most seed plants are capable of reducing nitrate (NO₃⁻) in both their roots and shoots (Marschner 1995). However, nitrate is more efficiently reduced in leaves than in roots, primarily due to the high activity of nitrate reductase enzyme in leaves (Chen et al. 2004). (Chen et al. 2004). There was a significant difference ($p < 0.01$) in nitrate reductase enzyme activity between leaves and roots, regardless of genotype. Under NS condition, the activity of the nitrate reductase enzyme in leaves (15.71 $\mu\text{mole NO}_2^- [\text{g FW h}]^{-1}$) was significantly higher ($p < 0.01$) than in roots (1.72 $\mu\text{mole NO}_2^- [\text{g FW h}]^{-1}$) (Table 4). Similarly, under the ND condition, the mean activity of this enzyme was 3.44 $\mu\text{mole NO}_2^- [\text{g FW h}]^{-1}$ in the leaf and 1.30 $\mu\text{mole NO}_2^- [\text{g FW h}]^{-1}$ in the root. Lines 47 and 41 exhibited the highest nitrate reductase enzyme activity in their leaves under the NS and

ND conditions, respectively. In addition, the lines with the highest nitrate reductase enzyme activity in their roots were lines 21, 37, 47, and 57 under NS condition and lines 45 and 57 under ND condition. Nitrate induces the nitrate reductase enzyme, which is a limiting factor for nitrate uptake in seed plants (Caba et al. 1995; Bussi et al. 1997). Therefore, nitrate reductase enzyme activity and nitrate concentration in plants are closely related (Skrdleta et al. 1979). Very small amounts of nitrate are sufficient to induce the nitrate reductase enzyme (Sherrard and Dalling 1979). Moreover, in some plant genotypes, there is a correlation between the rise in NUE and the increase in nitrate reductase enzyme activity (Nawaz et al. 2017; Guilherme et al. 2019). Our biochemical experiments also indicated a positive correlation between nitrate reductase enzyme activity and NUE, suggesting that the increase in NUE due to the increase in NUpE in the roots is associated with the increased activity of the nitrate reductase enzyme. In this study, differences in nitrate reductase enzyme activity among the investigated lines indicate that nitrate reduction in cucumber plants in response to nitrogen deficiency is genotype-dependent.

In conclusion, our study highlights the genotype-dependent nature of nitrate reduction in cucumber plants, shedding light on the varying nitrate reductase enzyme activities in both roots and leaves across different genotypes. Notably, nitrate is more efficiently reduced in leaves, primarily due to the higher activity of the nitrate reductase enzyme. This enzyme's activity is closely linked to nitrate concentration, and our findings suggest a positive correlation between nitrate reductase enzyme activity and NUE in certain plant genotypes. The increase in NUE due to enhanced NUpE in the roots is associated with elevated nitrate reductase enzyme activity. These insights underscore the importance of genotype-specific responses in understanding nitrogen utilization and may hold potential for optimizing plant performance under varying nitrogen conditions.

Grouping cucumber genotypes based on nitrogen use efficiency

In this study, genotypes were categorized as efficient, semi-efficient, low-efficient, or inefficient in NUE (Fig. 2). To categorize them, the mean values of NUE and plant yield (fruit weight) for each genotype were scattered on an x - y scale. The genotypes in the first, second, third, and fourth quadrants were categorized as efficient, semi-efficient, low-efficient, and inefficient, respectively. Line 57 was identified as the efficient line, line 50 is introduced as the semi-efficient line, lines 21, 28, 33, 37, 44, 46, 51, 52, 53, and 59 are introduced as low-efficient lines, and the remaining lines are inefficient lines. Consistent with our findings, DoVale et al. (2012) observed that only 5% of genotypes exhibit a high potential for NUE under low nitrogen availability.

Table 3 Mean comparisons of nitrogen use efficiency (NUE), nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUE), and nitrogen harvest index (NHI) under two conditions, sufficient nitrogen (NS), and nitrogen deprivation (ND)

Lines	NUE (g FW g ⁻¹ N)		NUpE (mg N g ⁻¹ DM g ⁻¹ N)		NUE (g FW mg ⁻¹ N g DM)		NHI (%)	
	NS	ND	NS	ND	NS	ND	NS	ND
L21	64.34 ^{m-p*}	204.30 ^{ij}	0.249 ^{m-o}	0.580 ^{c-e}	248.41 ^{ij}	323.00 ^{l-p}	16.09 ^{m-p}	47.84 ^{f-h}
L22	62.56 ^{n-p}	205.13 ^{ij}	0.301 ^k	0.461 ^{f-i}	200.40 ^{l-n}	500.00 ^{i-k}	25.05 ^{gh}	44.02 ^{g-i}
L24	49.90 ^s	142.27 ^m	0.287 ^{kl}	0.372 ^{i-o}	186.90 ⁿ	260.46 ^{m-q}	17.67 ^{l-o}	23.84 ^{n-s}
L25	88.85 ^g	25.18 ^{gh}	0.3 ^k	0.315 ^{l-q}	304.04 ^{ef}	854.63 ^{de}	33.20 ^{cd}	40.17 ^{h-j}
L26	79.73 ^{hi}	298.19 ^{ef}	0.238 ^{op}	0.252 ^q	342.15 ^{cd}	1215.71 ^b	31.55 ^d	59.59 ^e
L27	102.29 ^{ef}	218.70 ^{h-j}	0.266 ^{l-n}	0.337 ^{k-q}	385.88 ^a	590.97 ^{g-i}	36.52 ^b	24.17 ^{m-s}
L28	67.40 ^{l-n}	140.88 ^{mn}	0.242 ^{no}	0.306 ^{n-q}	278.44 ^{gh}	361.49 ^{lm}	21.04 ^{ik}	18.68 ^{q-v}
L29	55.56 ^{qr}	157.72 ^{k-m}	0.27 ^{lm}	0.291 ^{o-q}	206.00 ^{l-n}	408.63 ^{j-l}	17.33 ^{m-o}	22.76 ^t
L30	64.86 ^{m-o}	190.68 ^{j-l}	0.299 ^k	0.451 ^{g-j}	217.62 ^{k-m}	394.89 ^{kl}	15.64 ^{n-q}	23.64 ^{n-s}
L32	108.96 ^d	275.29 ^{fg}	0.34 ^j	0.403 ^{h-m}	321.20 ^{de}	754.60 ^{ef}	28.13 ^{ef}	59.24 ^e
L33	67.81 ^{lm}	152.31 ^{lm}	0.286 ^{kl}	0.329 ^{k-q}	237.58 ^{jk}	403.48 ^{j-l}	20.84 ^{ik}	26.93 ^{l-q}
L34	79.33 ^{h-j}	239.14 ^{g-i}	0.25 ^{m-o}	0.293 ^{o-q}	317.44 ^{de}	778.16 ^{ef}	25.75 ^{f-h}	72.79 ^{cd}
L35	23.73 ^u	85.59 ^p	0.24 ^o	0.262 ^{pq}	115.43 ^p	326.15 ^{l-o}	12.11 ^r	17.72 ^v
L36	56.47 ^{qr}	141.41 ^{mn}	0.233 ^{op}	0.277 ^{o-q}	241.75 ^{i-k}	384.05 ^{kl}	17.15 ^{m-o}	32.0 ^{j-n}
L37	59.54 ^{pq}	221.58 ^{h-j}	0.231 ^{op}	0.258 ^q	257.74 ^{h-j}	958.72 ^d	18.28 ^{lm}	34.44 ^{j-l}
L39	67.86 ^{lm}	352.16 ^{cd}	0.213 ^p	1.538 ^a	318.44 ^{de}	231.48 ^{n-r}	24.07 ^{g-i}	69.62 ^d
L41	60.29 ^{o-q}	140.88 ^{mn}	0.277 ^{kl}	0.411 ^{h-l}	218.10 ^{k-m}	337.97 ^{l-n}	20.06 ^{kl}	19.56 ^{p-u}
L42	99.29 ^f	389.09 ^c	0.38 ^{e-i}	0.414 ^{h-k}	261.26 ^{g-j}	939.61 ^d	26.20 ^{fg}	54.76 ^{ef}
L43	30.58 ^t	102.74 ^{n-p}	0.346 ^j	0.586 ^{cd}	88.51 ^q	175.80 ^{qr}	7.71 ^s	10.22 ^v
L 44	71.86 ^{kl}	244.46 ^{g-i}	0.359 ^{h-j}	0.396 ^{h-n}	199.96 ^{l-n}	623.26 ^{gh}	15.56 ^{o-q}	25.23 ^{m-r}
L45	53.16 ^{rs}	88.09 ^{op}	0.357 ^{ij}	0.764 ^b	148.90 ^o	115.43 ^r	14.62 ^{pq}	13.56 ^{uv}
L46	92.73 ^g	327.63 ^{de}	0.42 ^{bc}	0.526 ^{d-g}	220.98 ^{kl}	621.93 ^{gh}	16.07 ^{m-p}	38.06 ^{i-k}
L47	113.90 ^c	317.26 ^{de}	0.43 ^b	0.556 ^{c-f}	265.26 ^{g-i}	566.61 ^{g-i}	20.78 ^{jk}	55.94 ^{ef}
L48	35.26 ^t	127.02 ^{m-o}	0.395 ^{c-f}	0.624 ^c	89.45 ^q	204.12 ^{p-r}	11.66 ^r	27.83 ^{l-p}
L49	106.01 ^{de}	273.50 ^{fg}	0.34 ^j	0.534 ^{c-g}	311.77 ^e	516.26 ^{h-j}	32.78 ^{cd}	30.07 ^{k-o}
L50	130.92 ^b	530.41 ^b	0.383 ^{e-h}	0.356 ^{j-p}	342.16 ^{b-d}	1498.46 ^a	40.66 ^a	82.94 ^b
L51	74.53 ^{jk}	326.27 ^{de}	0.408 ^{b-d}	0.486 ^{e-h}	182.37 ⁿ	672.82 ^{fg}	13.61 ^{qr}	73.93 ^{cd}
L52	115.26 ^c	231.10 ^{g-i}	0.378 ^{f-i}	0.397 ^{h-n}	305.49 ^{ef}	577.64 ^{g-i}	25.25 ^{gh}	32.79 ^{j-m}
L53	78.49 ^{ij}	204.27 ^{ij}	0.403 ^{c-e}	0.308 ^{m-q}	194.81 ^{mn}	660.47 ^{fg}	18.06 ^{l-n}	49.99 ^{fg}
L54	129.08 ^b	274.33 ^{fg}	0.361 ^{g-j}	0.482 ^{f-h}	358.20 ^{bc}	569.99 ^{g-i}	34.62 ^{bc}	44.27 ^{g-i}
L55	93.19 ^g	194.90 ^{jk}	0.392 ^{d-f}	0.783 ^b	237.65 ^{jk}	247.94 ^{m-q}	22.58 ^{ij}	21.76 ^{o-u}
L56	48.93 ^s	153.16 ^{lm}	0.355 ^j	0.746 ^b	137.70 ^{op}	204.09 ^{p-r}	11.62 ^r	14.69 ^{t-v}
L57	206.39 ^a	724.29 ^a	0.565 ^a	0.759 ^b	366.92 ^{ab}	1092.94 ^c	28.65 ^e	92.03 ^a
L58	57.11 ^{qr}	97.50 ^{op}	0.385 ^{d-g}	0.448 ^{g-j}	148.95 ^o	217.75 ^{o-r}	17.84 ^{k-o}	15.53 ^v
L59	83.47 ^h	103.35 ^{n-p}	0.295 ^k	0.460 ^{f-i}	283.05 ^{fg}	226.98 ^{n-r}	23.73 ^{hi}	81.36 ^{bc}

In each column, the means with at least a similar letter are not significantly different at a 0.05 level of probability

The response of different cucumber lines to nitrogen supply varied significantly. This demonstrates that even members of the same genetic group may exhibit distinct characteristics in response to nitrogen supply. Consequently, N fertilizer management should ideally be tailored to each cultivated genotype (Andrade et al. 2020). Overall, the categorization of genotypes based on NUE provides valuable information for breeders, researchers, and growers to identify lines with improved NUE and guide future

efforts towards developing more sustainable and productive cucumber cultivars.

Conclusion

The findings of this study revealed significant genotypic variations in cucumber plant responses to different nitrogen supply conditions. Line 57 consistently showed the highest NUE,

Table 4 Mean comparison of the nitrate reductase enzyme activity in leaves and roots under two conditions, sufficient nitrogen (NS), and nitrogen deprivation (ND)

Lines	$\mu\text{mole NO}_2^- (\text{g FW h})^{-1}$			
	Leaves		Roots	
	NS	ND	NS	ND
L21	15.818 ^{gh*}	3.12 ^{g-k}	2.395 ^a	1.14 ^{h-l}
L22	14.23 ^{j-l}	3.15 ^{g-k}	1.46 ^{g-o}	1.38 ^{f-j}
L24	9.552 ^{pq}	1.54 ⁿ	1.09 ^{no}	0.80 ^{no}
L25	18.276 ^e	3.38 ^{f-j}	1.749 ^{c-l}	1.44 ^{e-i}
L26	20.852 ^{bc}	3.85 ^{c-h}	2.114 ^{a-e}	0.87 ^{l-o}
L27	11.361 ^{no}	2.63 ^{i-l}	1.485 ^{f-n}	1.60 ^{d-f}
L28	17.735 ^e	3.52 ^{e-i}	1.654 ^{e-m}	1.22 ^{g-k}
L29	15.14 ^{h-j}	1.831 ⁿ	1.351 ^{i-o}	0.90 ^{l-o}
L30	9.71 ^{pq}	4.08 ^{b-f}	0.902 ^o	0.82 ^{m-o}
L32	21.303 ^{bc}	4.36 ^{b-e}	1.963 ^{a-h}	1.49 ^{e-g}
L33	15.422 ^{hi}	3.48 ^{f-j}	1.549 ^{f-n}	1.45 ^{e-h}
L34	19.531 ^d	2.45 ^{k-m}	2.027 ^{a-f}	1.08 ^{j-n}
L35	19.43 ^d	4.55 ^{bc}	2.218 ^{a-d}	2.27 ^a
L36	11.894 ⁿ	3.58 ^{d-h}	1.447 ^{h-o}	0.96 ^{k-o}
L37	21.336 ^{bc}	4.49 ^{bc}	2.337 ^{ab}	1.97 ^{a-c}
L39	13.513 ^{kl}	4.56 ^{bc}	1.394 ^{i-o}	0.95 ^{k-o}
L41	16.552 ^{fg}	5.48 ^a	1.139 ^{m-o}	0.97 ^{k-o}
L42	15.914 ^{gh}	4.51 ^{bc}	1.817 ^{b-i}	1.09 ^{j-n}
L43	15.522 ^{g-i}	3.01 ^{h-k}	2.026 ^{a-f}	1.06 ^{j-o}
L 44	14.854 ^{h-j}	3.60 ^{d-h}	2.015 ^{a-g}	1.55 ^{d-f}
L45	21.555 ^b	3.39 ^{f-j}	2.298 ^{a-c}	2.02 ^{ab}
L46	14.575 ^{i-k}	2.69 ^{b-d}	1.719 ^{d-l}	0.93 ^{k-o}
L47	23.495 ^a	4.44 ^{b-d}	2.314 ^{ab}	1.86 ^{b-d}
L48	19.509 ^d	4.40 ^{b-d}	1.798 ^{b-j}	1.70 ^{c-e}
L49	12.072 ⁿ	1.71 mn	1.191 ^{l-o}	0.76 ^o
L50	8.739 ^q	1.60 ^{mn}	1.24 ^{j-o}	0.93 ^{k-o}
L51	12.283 ^{mn}	2.68 i-l	1.201 ^{k-o}	0.78 ^{no}
L52	17.386 ^{ef}	3.44 f-j	1.66 ^{d-m}	1.13 ^{i-m}
L53	8.65 ^q	3.98 b-g	1.351 ^{i-o}	1.47 ^{e-g}
L54	13.297 ^{lm}	3.46 f-j	1.714 ^{d-l}	1.35 ^{f-j}
L55	14.491 ^{i-k}	3.19 ^{g-k}	1.824 ^{b-i}	1.46 ^{e-h}
L56	20.273 ^{cd}	3.02 ^{h-k}	2.3 ^{a-c}	2.06 ^{ab}
L57	20.752 ^{bc}	4.75 ^{ab}	2.343 ^{ab}	2.23 ^a
L58	14.472 ^{i-k}	3.35 ^{f-j}	1.751 ^{c-k}	0.95 ^{k-o}
L59	10.392 ^{op}	3.02 ^{g-k}	1.452 ^{h-o}	0.86 ^{l-o}

In each column, the means with at least a similar letter are not significantly different at a 0.05 level of probability

yielding well even under nitrogen deficiency. It exhibited optimal growth, nitrogen accumulation, and nitrogen partitioning traits. In contrast, some genotypes demonstrated insufficient nitrogen transfer stability or low nitrogen use. This research on 35 cucumber genotypes provides valuable insights into genetic variations in nitrogen use patterns. The data generated establishes the foundation for deeper mechanistic investigations

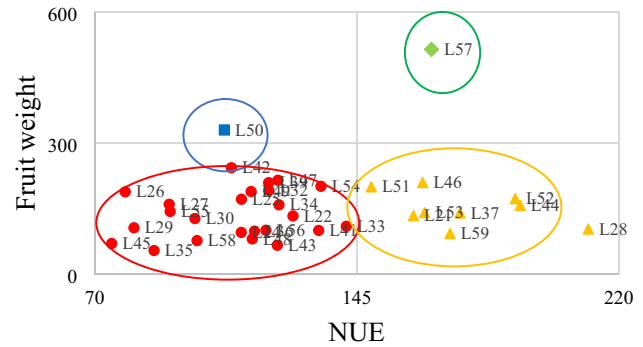


Fig. 2 Grouping 35 cucumber plant lines based on nitrogen use efficiency and yield (fruit weight). Efficient lines are illustrated in the first quadrant (diamond), semi-efficient lines in the second (square), low-efficient lines in the third (triangle), and inefficient lines in the fourth (circle)

into traits controlling NUE at physiological and molecular levels. Future breeding efforts could leverage this information to develop cultivars precisely tailored to local fertilization practices. Reducing external nitrogen inputs while maintaining productivity would protect the environment and food safety. Key genotypes like Line 57 warrant closer examination to understand the genetic and physiological basis of their high NUE. Comparing efficient versus inefficient lines may uncover genes influencing nitrogen acquisition and utilization. Elucidating these mechanisms could aid in marker-assisted selection and genetic engineering of new cucumber varieties with optimized nitrogen homeostasis and use. Ultimately, this may lead to more sustainable agricultural systems with lessened nitrogen dependence and footprint.

Author contribution statement AM: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft preparation. MHA: conceptualization, methodology, project administration, writing—review and editing, resources, supervision, validation. J-AO: formal analysis, investigation, software, validation. MBF: methodology, Writing—review and editing, investigation, validation.

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Declarations

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.

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