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Nitrogen use efficiency, growth and physiological parameters in different tomato genotypes under high and low N fertilisation conditions

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A R T I C L E I N F O

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A B S T R A C T

Identification of novel genotypes with enhanced nitrogen use efficiency (NUE) is a key challenge for a sustainable tomato production. In this respect, the performance of a panel of thirty tomato accessions were evaluated under high (HN; 5 mM N) and low (LN; 0.5 mM N) nitrogen irrigation solutions. For each treatment, when 50% of plants reached the first flower bud stage, plant growth and biomass traits, chlorophyll, flavonol and anthocyanin indexes, nitrogen balance index (NBI), C:N ratio in leaves, stems, and roots, and NUE were evaluated. Significant ($p < 0.05$) effects were observed for accession, N treatment, and their interaction across all the traits. Under LN, plants showed a delayed development (40 days for HN vs. 65 days for LN) and reduced growth and biomass. On average, LN condition led to 41.8% decrease in nitrogen uptake efficiency (NUpE) but also 189.0% increase in NUtE, resulting in 62.2% overall increase in NUE. A broad range of variation among accessions was observed under both HN and LN conditions. Under LN conditions, chlorophyll index and NBI decreased, while flavonol and anthocyanin indexes increased. Leaf C:N ratio was positively correlated with nitrogen utilisation efficiency (NUtE) in both N treatments. Multi-trait analyses identified top-performing accessions under each condition, allowing to identify one accession among top performers under both conditions. Correlation analysis revealed that high root biomass and leaf C:N ratio are useful markers for selecting high NUE accessions. These findings offer valuable insights for improving tomato NUE under varying nitrogen fertilization conditions and for breeding high-NUE cultivars.

1. Introduction

Meeting the demand of food by a growing global population requires increasing crop yields, which, in recent decades, have been achieved with excessive fertilisers application (Maja and Ayano, 2021; Stewart and Roberts, 2012). Indeed, N fertilisation has significantly boosted worldwide food production (Eickhout et al., 2006; Guo et al., 2022), causing concurrently detrimental effects on the environment, leading to soil acidification, leaching of N into groundwater, and increasing greenhouse gas emissions (Sainju et al., 2019). In particular, both these last aspects are critical also for human health and well-being (Abascal et al., 2022). Concerning soil acidification, the N fertilisers application has caused a global reduction in soil pH by 0.26 units (Tian and Niu, 2015), which alters ecosystems and becomes a limiting factor for crop yields (Goulding, 2016). Also, excessive N fertilisers have contributed to climate change through the emission of greenhouse gases as a result of the Haber-Bosch process for the synthesis of ammonia from atmospheric N (Cecílio Filho et al., 2022; Xuejun and Fusuo, 2011).

The application of N fertilisers to crops has been and will continue to be a critical factor in food production. Therefore, it is crucial to improve nitrogen use efficiency (NUE), thereby reducing environmental impact while maintaining production (Conant et al., 2013). NUE is a complex trait that can divided in two components: nitrogen uptake efficiency (NUpE), which represents the ability of the plant to absorb N from the N source, and nitrogen utilisation efficiency (NUtE), which refers to the ability of the plant to produce biomass per unit of N taken up (Anas et al., 2020; Hawkesford, 2011; Moll et al., 1982). To improve NUE, several physiological and agronomic mechanisms, should be considered,

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Table 1
Accessions used, institution of provenance (Universitat Politècnica de València, UPV; Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environment, INRAE; University Mediterranea of Reggio Calabria (UNIRC; Italy), species (SL= *Solanum lycopersicum*; SLC= *Solanum lycopersicum. var cerasiforme*) and country origin.

Accession	Institution	Species	Origin
TRBA0420	UPV	SL	Spain
TRBA0720	UPV	SL	Spain
TRBA1580	UPV	SL	Spain
TRBA1830	UPV	SL	Spain
TRCA0390	UPV	SL	Spain
TRCA0570	UPV	SL	Spain
TRCA1280	UPV	SL	Spain
TRPO0010	UPV	SL	Italy
TRPO0040	UPV	SL	Italy
TRVA0030	UPV	SL	Spain
TRVA1480	UPV	SL	Spain
TRVA1730	UPV	SL	Spain
TRVA2240	UPV	SL	Spain
TRVI0040	UPV	SL	Italy
TRVI0130	UPV	SL	Italy
TRVI0460	UPV	SL	Italy
TRVI1060	UPV	SL	Italy
TRVI1870	UPV	SL	Italy
Ferum	INRAE	SL	France
Cervil	INRAE	SLC	France
Levovil	INRAE	SL	France
Plovdiv	INRAE	SLC	France
LA1420	INRAE	SLC	Ecuador
LA0147	INRAE	SL	Honduras
Criollo	INRAE	SLC	France
SPR	INRAE	SL	France
Regina Ostuni	UNIRC	SL	Italy
Linosa	UNIRC	SL	Italy
UC82	UNIRC	SL	USA
Piriddu	UNIRC	SL	Italy

Table 2
Macronutrient and micronutrient concentrations for the high N (HN) and low N (LN) solutions for the evaluation of 30 tomato accessions.

Nutrients	High nitrogen (HN)	Low nitrogen (LN)
Macronutrients		
K_2SO_4	2.5 mM	2.5 mM
$MgSO_4$	2 mM	2 mM
KH_2PO_4	1 mM	1 mM
$Ca(NO_3)_2$	5 mM	0.5 mM
$CaSO_4$	0 mM	4.5 mM
Micronutrients		
H_3BO_3	46 μM	46 μM
$MnCl_2$	9 μM	9 μM
$ZnSO_4$	0.76 μM	0.76 μM
$CuSO_4$	0.32 μM	0.32 μM
Na_2MoO_4	0.11 μM	0.11 μM
Fe-EDTA	20 μM	20 μM

such as leaf photosynthetic and N uptake ability, as well as high root biomass (Lammerts van Bueren and Struik, 2017). Improving NUE in crops is a challenging task for breeders due to its polygenic genetic control. However, significant progress has been made through the identification of major and minor quantitative trait loci (QTLs) and the use of transgenic and genetically edited plants (Karunarathne et al., 2022; Ranjan and Yadav, 2019).

Highly productive horticulture requires a significant application of N fertilisers, which poses a risk of contamination to adjacent ecosystems (Cameira and Mota, 2017). Among the most important horticultural crops, tomato (*Solanum lycopersicum* L.) requires large amounts of N fertiliser (up to 220–300 kg/ha) to achieve optimal yields (Zotarelli et al., 2009). Therefore, N fertilisation reduction and NUE improvement in tomato could make a relevant contribution to a more sustainable agriculture. In order to select and breed higher NUE tomato genotypes

Table 3
Contribution of the effect of accession, N treatment, and accession × N treatment interaction factors to the total sums of squares (%) resulting from an analysis of variance for the traits evaluated in thirty tomato accessions evaluated under high and low N fertilization. Numbers are percentages of the total sums of squares.

Trait	Accession	N Treatment	Accession × N Treatment	Residual
d.f.	29	1	29	223
Leaf number	37.0***	2.1***	16.1***	44.7
Stem length	45.0***	2.6***	15.3***	37.0
Total dry weight	14.3***	44.1***	14.2***	27.4
Leaf dry weight	17.4***	37.6***	15.5***	29.5
Stem dry weight	21.6***	19.4***	16.3***	42.8
Root dry weight	13.1***	55.1***	10.4***	21.4
Chlorophyll index	24.6***	7.3***	14.9***	53.2
Flavonol index	5.9***	82.6***	4.0***	7.5
Anthocyanin index	11.8***	62.1***	8.9***	17.3
Nitrogen Balance Index (NBI)	5.9***	79.9***	4.8***	9.4
C:N ratio in leaf	3.9***	81.3***	3.5***	11.2
C:N ratio in stem	5.4**	68.9***	6.0***	19.6
C:N ratio in root	2.6*	79.1***	6.4***	11.9
Nitrogen Uptake Efficiency (NUpE)	5.3***	81.6***	5.3***	7.9
Nitrogen Utilisation Efficiency (NUtE)	12.8***	34.8***	13.6***	38.8
Nitrogen Use Efficiency (NUE)	12.8***	37.8***	13.6***	38.8

*, **, *** indicate significant at $p < 0.05$, <0.01 and < 0.001, respectively.

more adapted to limited N fertiliser supply rates, a screening of plant genetic resources together with an identification of suitable parents for breeding programs are essential (Han et al., 2015). Abenavoli et al. (2016) found that high NUE tomato genotypes were characterized by increased root length and thickness and that NUtE may play a greater role than NUpE for improving NUE. More recently, the same authors confirmed these results and found that the increased expression of N transporters in both root and shoot was correlated with higher NUE (Aci et al., 2021). Among traditional tomato varieties, the Mediterranean region traditional 'De penjar'/'Da serbo' tomato types, which encompass a large genetic diversity type, show high resilience (Conesa et al., 2020; Esposito et al., 2020) and are able to maintain yields with low N inputs, maintaining a high chlorophyll content and fruit quality traits (Rosa-Martínez et al., 2021). On the other hand, tomato mutants with low sensitivity to auxin were shown to adapt better to N-deficient conditions despite lower growth (Santos et al., 2020).

The objective of this study was to evaluate the performance during the vegetative phase of a set of thirty different tomato accessions enriched in 'De penjar'/'Da serbo' materials under contrasting N conditions in order to analyse their responses in terms of growth, pigment content, C:N ratio and NUE. The aim was to identify tomato accessions with high NUE, particularly under low N fertilisation supply.

2. Materials and methods

2.1. Plant material

The plant material used in this study included eighteen accessions of the highly resilient 'De penjar'/'Da serbo' type from Spain and Italy provided by the germplasm bank of the Universitat Politècnica de València (UPV; Spain). In addition, eight accessions corresponding to the parental lines of a Multi-parent Advanced Generation InterCross (MAGIC) population (Pascual et al., 2015) were obtained from the Institut National de Recherche pour l'Alimentation, l'Alimentation et l'Environment (INRAE, France). Four of these eight MAGIC parents belong to the weedy *S. lycopersicum* var. *cerasiforme* (SLC). Finally, four accessions were provided by the University Mediterranea of Reggio Calabria (UNIRC; Italy) already evaluated for NUE in hydroponics

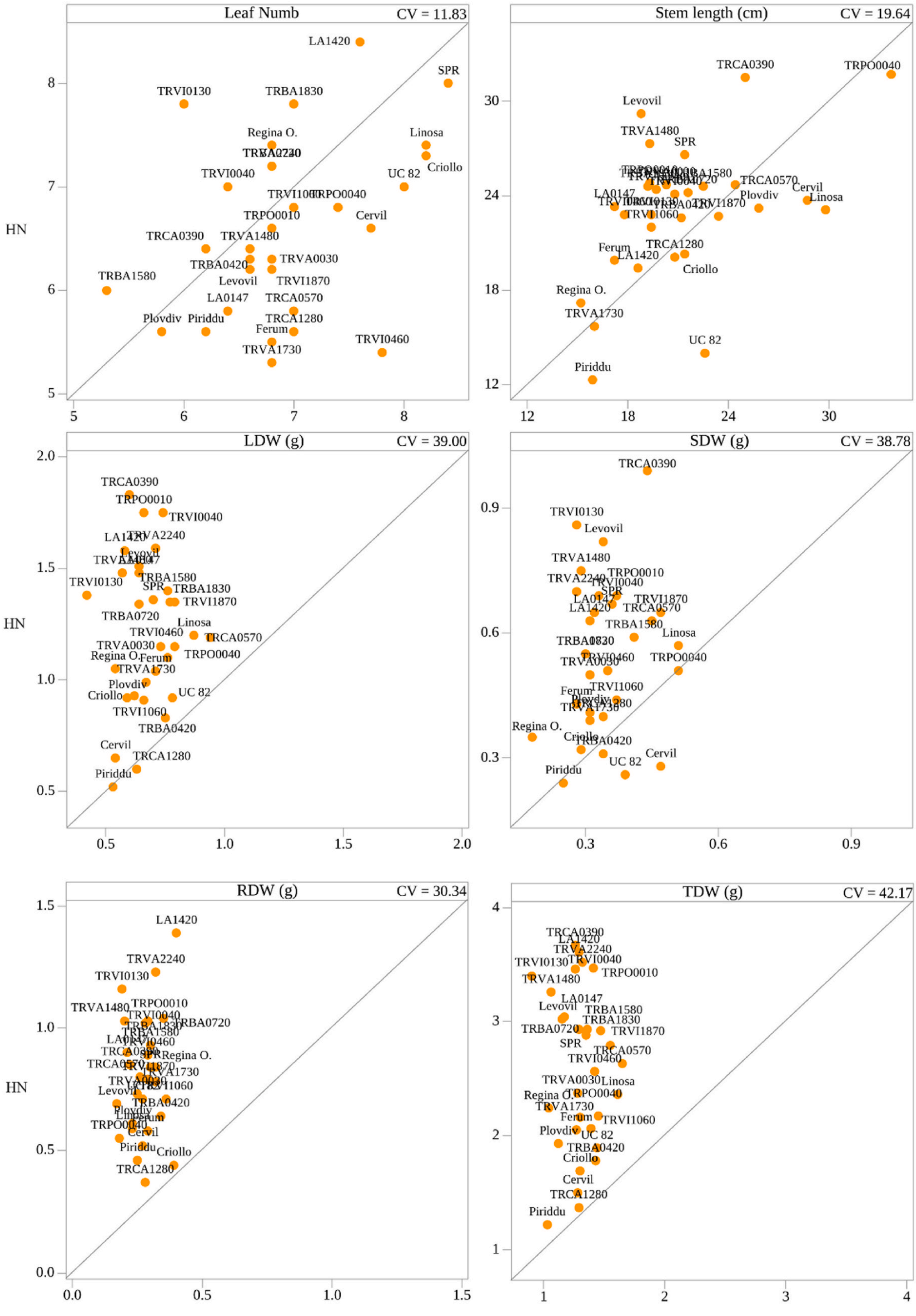

Fig. 1. Variation in leaf number, stem length, leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW) and total plant dry weight (TDW) of 30 tomato accessions exposed to low (LN; x-axis) and high (HN; y-axis) N supply. The coefficient of variation (CV) for each trait was also calculated.

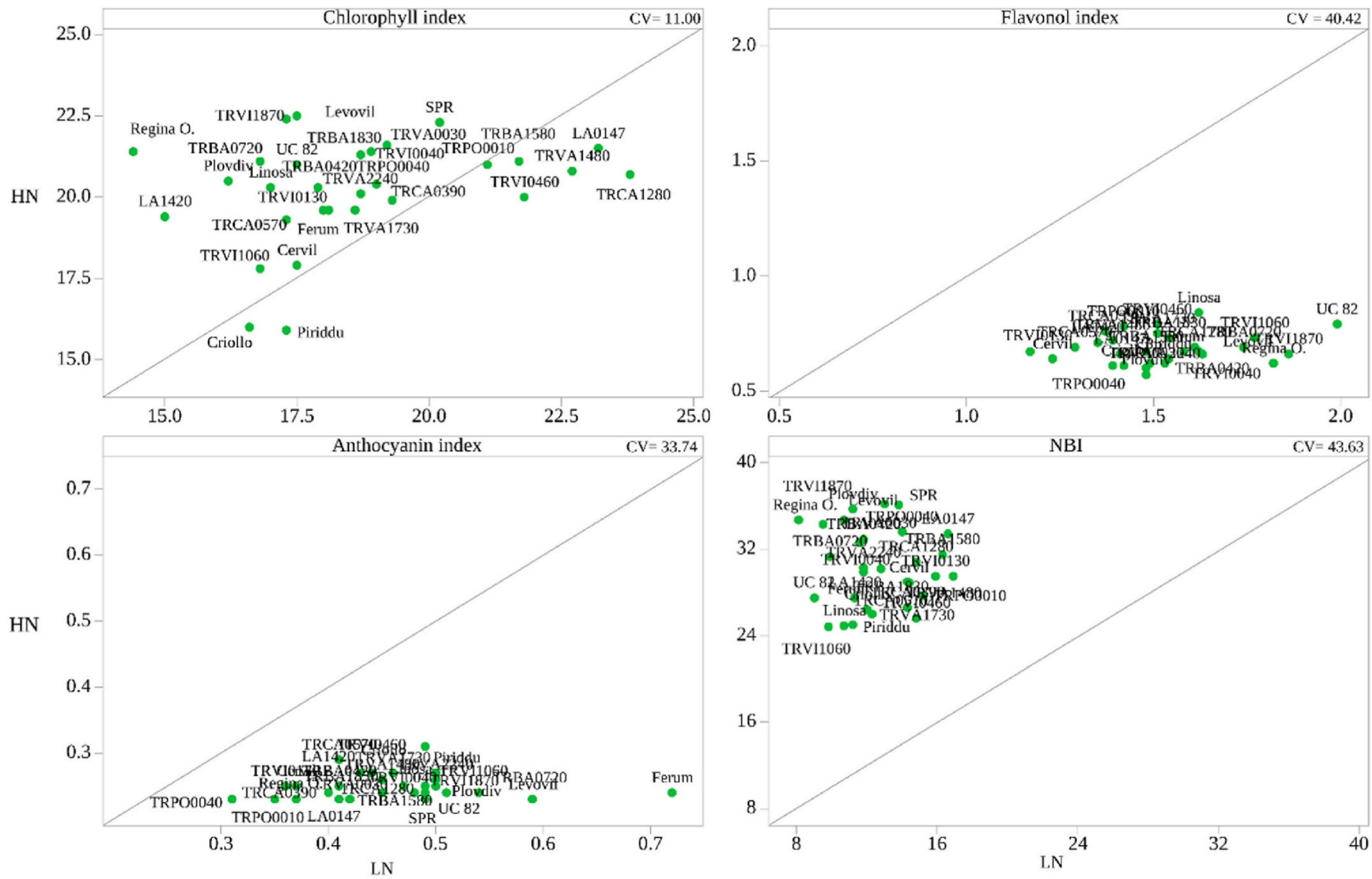

Fig. 2. Variation in chlorophyll, flavonoids and anthocyanins indexes as well as the nitrogen balance index (NBI) of 30 tomato accessions exposed to low (LN; x-axis) and high (HN; y-axis) N supply. The coefficient of variation (CV) for each trait was also calculated.

(Abenavoli et al., 2016). The list of accessions, species, and their country of origin is reported in Table 1.

2.2. Growing conditions and experimental design

Seeds from thirty tomato accessions were germinated in Petri dishes (seven days) and the germinated seedlings were transplanted into 770 ml pots containing a substrate composed of coconut fibre and perlite in a 3:1 ratio (v:v). Two different N treatments, consisting of a high (HN) and a low N (LN) concentration (5 and 0.5 mM, respectively) watering solution, were adopted (Table 2). The pH of the solutions was adjusted to 5.8 using diluted hydrochloric acid (HCl). The electrical conductivity of two solutions was similar (2.48 μS/cm for HN and 2.46 μS/cm for LN), despite their differing ion concentrations. Specifically, the HN solution had 4.5 mM more $Ca(NO_3)_2$, while the LN solution had 4.5 mM more $CaSO_4$. This led us to conclude that the difference in ion concentration did not significantly impact our results. Two or three times a week, depending on the evapotranspiration rates resulting from the prevailing climatic conditions, each pot was irrigated with 150 ml of the corresponding nutrient solution.

Plants were arranged according to a completely randomised experimental design with five replicates for each accession and N treatment condition, resulting in 150 experimental units for each fertilization treatment (HN and LN). The trials were carried out in a climatised greenhouse (maximum of 30 °C and minimum of 15 °C) at the Universitat Politècnica de València (GPS coordinates: 39°29′00.4″N 0°20′27.1″W).

2.3. Phenotypic trait evaluation

For each treatment, the experiment was finished when 50% of the plants had reached the first flower bud stage. This coincided with 40 and 65 days after transplantation (DAT) for HN and LN treatment, respectively. At the end of the experiment, the Dualex Scientific® optical sensor (Force-A, Orsay, France) was used to non-destructively measure chlorophyll index, flavonol index, anthocyanin index and the nitrogen balanced index (NBI) (Cerovic et al., 2005). Data were collected from both the adaxial and abaxial sides of three young leaves from the top of the plant and a mean was obtained for each experimental unit.

After the Dualex measurements, the number of leaves was counted, and the stem length measured. Later, the plants were cut at substrate level and leaves were first separated from the stem, then the soil substrate was gently removed from the roots by hand without washing with water. Small remains of the substrate were cleared away with a fine brush. The different plant tissues were then dried in oven at 65 °C for 72 h to determine the dry weight of each plant organ (leaf, LDW; stem, SDW; and root, RDW). Then, LDW, SDW and RDW were summed to obtain the total dry weight (TDW). Nitrogen (N) and carbon (C) content of leaves, stem and roots were determined by grinding 0.5 g of dry plant tissues (leaves, stems, and roots) using a mortar and pestle. N content was assessed with the Dumas method, which involves combusting the sample with oxygen at 950 °C, using a TruSpec CN elemental analyser (Leco, MI, USA). C content was estimated by measuring the carbon dioxide (CO_2) with an infrared detector (Gazulla et al., 2012). Certified reference standards with varying N and C concentrations, were utilized for the measurements.

NUE was calculated based on the total dry biomass produced. NUpE was calculated by dividing the N content of plants by the total N supplied

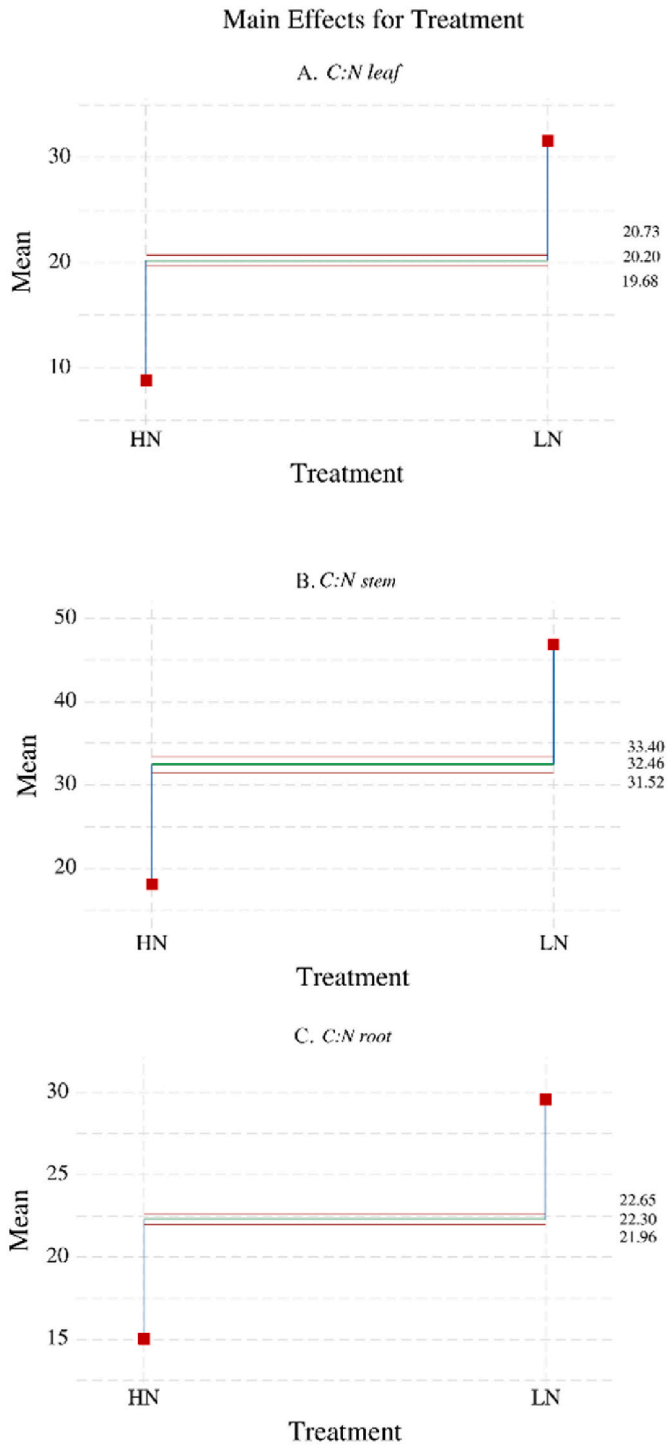

Fig. 3. Analysis of means (ANOM) for C:N ratio in leaf (A), stem (B) and root (C) in thirty tomato accessions at high (HN) and low (LN) N conditions. The red lines represent the upper and lower limits for significance (α = 0.05).

with the irrigation solution throughout the trial. NUtE was calculated by dividing total dry weight biomass by the N content of plants. Then, NUE was also obtained by multiplying NUpE with NUtE (Anas et al., 2020; Moll et al., 1982).

2.4. Statistical analysis

A two-way (accession x N treatment) analysis of variance (ANOVA) was performed with the Infostat software version 2020 (Di Renzo et al., 2020). Differences between treatments, as well as their interaction, were considered as significant when $p < 0.05$ For the C:N ratio, a two-way normal analysis of means was performed to analyse the effect of treatment, genotype and interaction at a significance level of $\alpha = 0.05$. Analysis of Mean (ANOM), which is a test for the equality between the means of the different factors, useful to verify the equality of the means with the overall means of the entire population, was performed with the Minitab® 21 software. The ANOM graphs show the average of each factor, the overall average and the decision limits (α = 0.05).

Pairwise Pearson correlations were carried out between analysed parameters within each N fertilization level (HN and LN). Correlations were calculated for each treatment using the statistical programme R-Studio (R Core Team, 2022), and their statistical significance was evaluated using the Bonferroni correction at $p = 0.001$, using the R packages psych (Revelle, 2023) and corrplot (Wei and Simko, 2021). To assess the relationship between measured parameters as well as among N treatments and accessions, a principal component analysis was also performed by using the R package stats (R Core Team, 2022) and ggplot2 (Wickham, 2016). A multi-trait genotype-ideotype distance index (Olivoto and Nardino, 2021) using total dry weight, NUE, NUpE and NUtE as desirable traits was used to select accessions with the better performance under HN and LN conditions. To identify the accessions with the best performances under HN or LN conditions, a selection pressure of 23.3% (7 out of 30 accessions) was applied.

3. Results

3.1. Analysis of variance

The analysis of variance revealed significant differences ($p < 0.05$) for all the traits considering the accessions, N treatments, and their interaction. Indeed, p-values were <0.001 in all the cases, except for the C:N ratio in stem and root among accessions, which was significant at p-values of 0.01 and 0.05, respectively (Table 3). The accession factor was the greatest contributor to the sum of squares in the ANOVA for stem length, while N treatments represented the highest contribution to the sums of squares of the total dry weight, leaf dry weight, root dry weight, flavonol index, anthocyanin index, NBI, C:N ratio in leaf, stem and root, NUE, NUpE, and NUtE. The interaction (accession × N treatment) showed a limited contribution to the sums of squares. Finally, the residual was the greatest contributor to the sums of squares for leaf number, stem dry weight and chlorophyll index (Table 3).

3.2. Growth analysis

Plants of the HN treatment grew and developed faster than those of LN, therefore, the HN experimental units reached the phenological stage of 50% of the plants at the first flower bud stage much earlier (40 DAT) than the plants under LN treatment (65 DAT). A biplot analysis allowed us to explore which trait was mostly involved in response to NO_3^- supply. Indeed, the genetic variation among genotypes was explained by their distribution along the diagonal bisector of biplots. The proximity of accessions to the diagonal means that the variable showed a similar value at both high and low N, indicating the inferior and the superior genotypes, from the left to the right parts of the figure; by contrast, the distance from the bisector indicates the responses to the treatment of each genotype with the best genotypes located to the right of the bisector (Fig. 1). The coefficient of variation (CV) and the average values of growth and biomass traits of tomato accessions at both high (HN) and low (LN) nitrogen conditions were also calculated (Fig. 1; Table S1). The reduced N availability (LN) resulted in an increase in the average number of leaves and a decrease in the average stem length. However, the mean differences for these traits were not consistent, with a 4.5% decrease in leaf number (CV 11.83%) and a 7.4% increase in stem length (CV 19.64%) (Fig. 1; Table S1). At HN, the number of leaves ranged from 5.3 (TRVA1730) to 8.4 (LA1420), while at LN, ranged from 5.3

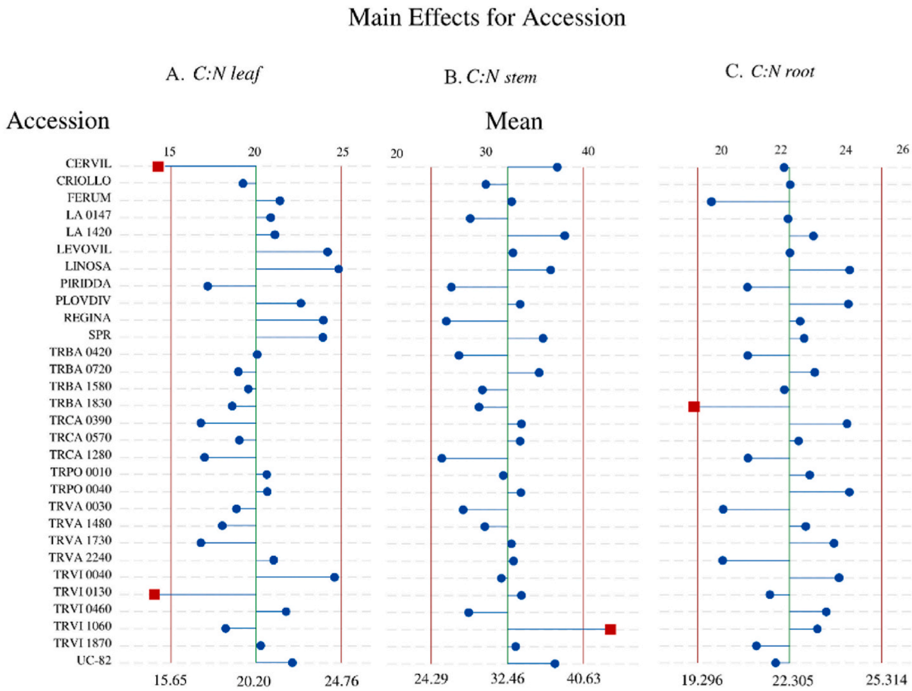

Fig. 4. Analysis of means (ANOM) relative to C:N ratios in leaf (A), stem (B) and root (C). The red lines represent the upper and lower limits for significance ($\alpha = 0.05$). The main effects for accession revealed four genotypes (red square) showing C:N ratios significantly different from the overall mean.

(TRBA1580) to 8.4 (SPR) (Table S1). Similarly, at HN, stem length ranged from 12.30 cm (Piriddu) to 31.7 cm (TRPO0040), while at LN, from 15.2 cm (Regina Ostuni) to 33.7 cm (TRPO0040) (Fig. 1; Table S1).

A wide range of variation (CV) was observed among accessions under each N condition for TDW and its components (LDW, SDW and RDW) (Fig. 1). At LN, the biomass dry weight on average was significantly lower compared to HN, with a decrease of 49.2%, 43.8%, 35.2% and 65.4% for TDW, LDW, SDW and RDW, respectively (Table S1). TDW ranged from 1.22 g (Piriddu) to 3.67 g (TRCA0390) at HN and from 0.9 g (TRVI0130) to 1.65 g (TRCA0570) at LN (Fig. 1; Table S1). At HN, the accessions exhibiting the lowest and highest TDW (Piriddu and TRCA0390), registered also the lowest and highest LDW and SDW. Regarding RDW it ranged from 0.37 g (TRCA1280) to 1.39 g (LA1420) under HN conditions (Fig. 1, Table S1). At LN, the lowest and highest LDW values were measured in TRVI0130 (0.42 g) and TRCA0570 (0.94 g), as already observed for TDW. Finally, the SDW values ranged from 0.18 g (Regina Ostuni) to 0.51 g (TRVA0030 and Linosa), while the RDW ranged from 0.17 g (Levovil) to 0.40 g (LA1420) (Fig. 1; Table S1).

3.3. Pigments and nitrogen balance index

An average 7.9% reduction in the chlorophyll index was observed at LN compared to HN, with a low CV value (11%) was observed (Fig. 2; Table S2). Significant variation among the accessions was observed under both N treatments, with a similar range of chlorophyll index, with the values at HN ranging from 15.9 (Piriddu) to 22.5 (TRVI1870), while at LN they ranged from 14.4 (Regina Ostuni) to 23.8 (TRCA1280) (Fig. 2; Table S2).

By contrast, the flavonol and anthocyanin indexes consistently increased at LN compared to HN, with average increases of 125.0% and 84.0%, respectively. All the accessions showed a significant increase in flavonol and anthocyanin indexes when grown at LN, determining high CV of 40.42% and 33.72%, respectively (Fig. 2; Table S2). At HN, the flavonol index ranged from 0.57 (Plovdiv) to 0.84 (Linosa), while at LN it ranged from 1.17 (TRVI0130) to 1.99 (UC82). At HN, the anthocyanin index ranged from 0.23 (TRPO0010, TRPO0040, TRVA0030, Levovil, LA0147, SPR, and Regina O.) to 0.31 (Criollo) while at LN ranged from 0.31 (TRPO0040) to 0.72 (Ferum) (Fig. 2; Table S2).

The NBI was greatly affected by the N rate reduction, with a 57.9% decrease on average under LN compared to HN (Table S2). In all the accessions the NBI was lower at LN. However, significant differences among accessions in NBI values were displayed, ranging from 24.8 (TRVI1060) to 36.2 (TRVA0030) at HN and from 8.1 (Regina Ostuni) to 16.9 (TRVA1480) at LN (Fig. 2; Table S2).

3.4. C:N ratio and nitrogen use efficiency

The C:N ratio in the leaf ranged from 6.75 (Cervil) to 10.91 (Levovil) at HN and from 20.82 (TRVI0130) to 39.59 (Regina Ostuni) at LN. In the stem the C:N ratio ranged from 8.98 (Cervil) to 30.99 (TRVI1060) at HN and from 36.32 (Regina Ostuni) to 66.64 (Cervil) under LN, finally, in the root the C:N ratio ranged from 6.24 (Ferum) to 20.40 (TRVA1730) at HN and from 23.89 (TRBA1830) to 35.37 (Plovdiv) at LN (Table S3).

C:N ratio values were on average higher in the stem than in the root and leaf (Fig. 3). The treatment had a significant effect on all the plant organs, indeed, an increase of 257.8%, 157.23% and 96.36% at LN compared to HN on leaf, stem and root, respectively, was observed. Within the accessions, significant differences were observed, Cervil and TRVI0130 exhibited significantly lower leaf C:N values than the others accessions, TRVI1060 showed a significantly higher stem C:N value, while TRBA1830 evidenced a significantly lower root C:N value than the rest of accessions (Fig. 3).

The analysis of means (ANOM) for main effects for accession showed that CERVIL (14.98) and TRVI0130 (14.75) leaf C:N ratio deviated significantly from the overall mean ($\alpha = 0.05$) below the lower decisional limit (15.65) (Fig. 4A). The accession TRVI1060 deviated significantly from the overall mean for C:N ratio in stem (43.51) compared to the decisional limit at 40.63 (Fig. 4B), and the accession TRBA1830 resulted significantly different from the mean of the sampled population for C:N ratio in root with a value of 19.18 (Fig. 4C).

To test whether there is an interaction effect between Accession and Treatment, the ANOM analysis was used. The analysis showed significant interaction Accession × Treatment for C:N ratio in leaf in the accessions TRCA0390 and TRVI0130, C:N ratio in stem in the accession

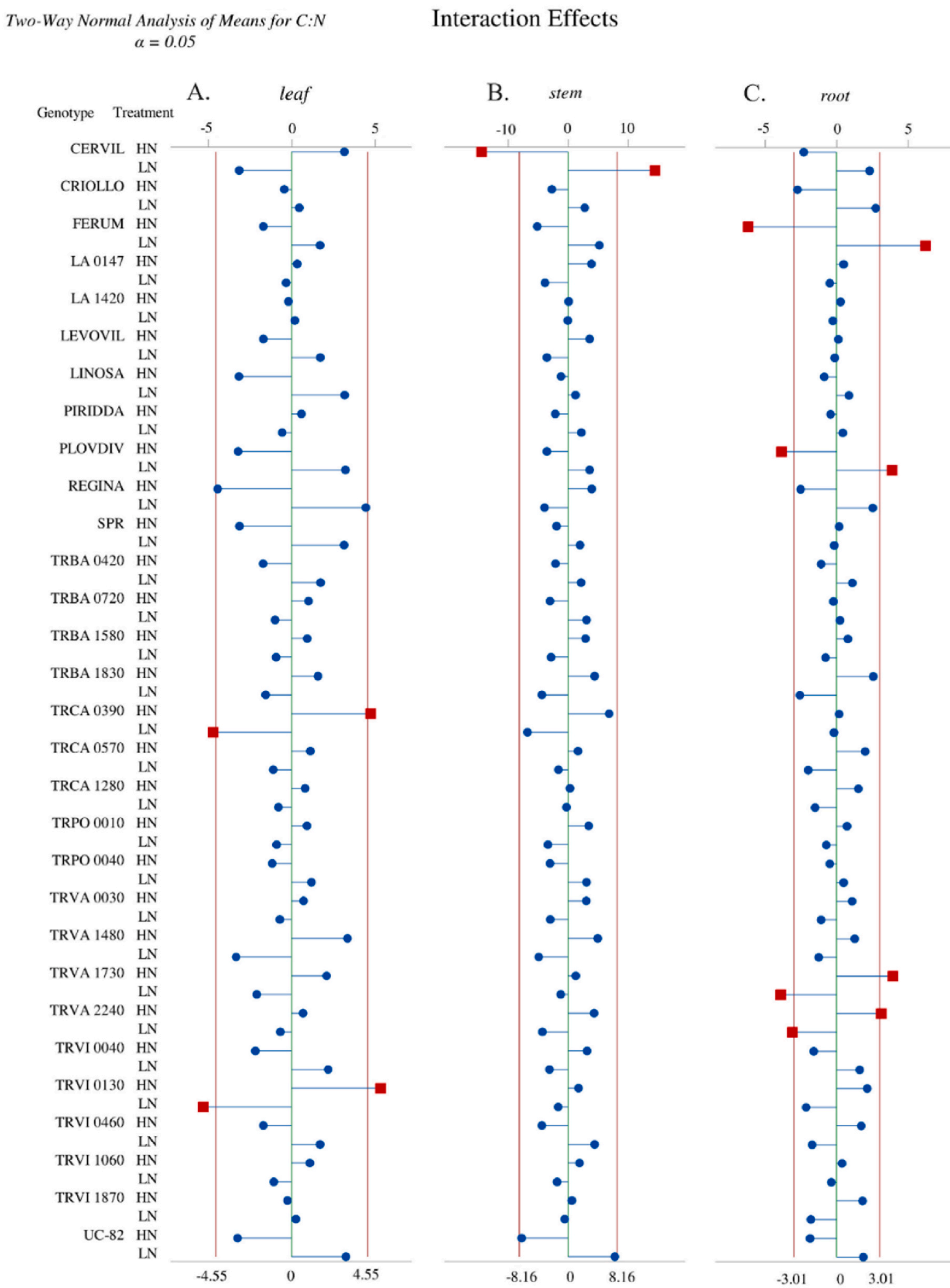

Fig. 5. Analysis of means (ANOM) in leaf (A), stem (B) and root (C) C:N ratios in thirty tomato accessions exposed at high (HN) and low (LN) N conditions was adopted to test the Accession × Treatment interaction. The red lines represent the upper and lower limits for significance (α = 0.05). In red square the C:N ratios significantly different from the overall mean (α = 0.05).

Cervil, and C; N ratio in root in the accessions Ferum, Plovdiv, TRVA1730 and TRVA2240 (Fig. 5). In detail, the accessions TRCA0390 and TRVI0130 at both N conditions exceeded the thresholds of the decisional limits (±4.55) with values of ±4.71 and ± 5.32 respectively (Fig. 5A). Further, for the stem C:N ratio only the accession Cervil was affected by treatment (±14.44) compared to overall mean (Fig. 5B). Finally, in root C:N ratio 4 accessions, Ferum (±6.22), Plovdiv (±3.87), TRVA1730 (±3.93) and TRVA2240 (±3.11), exceeded the decisional

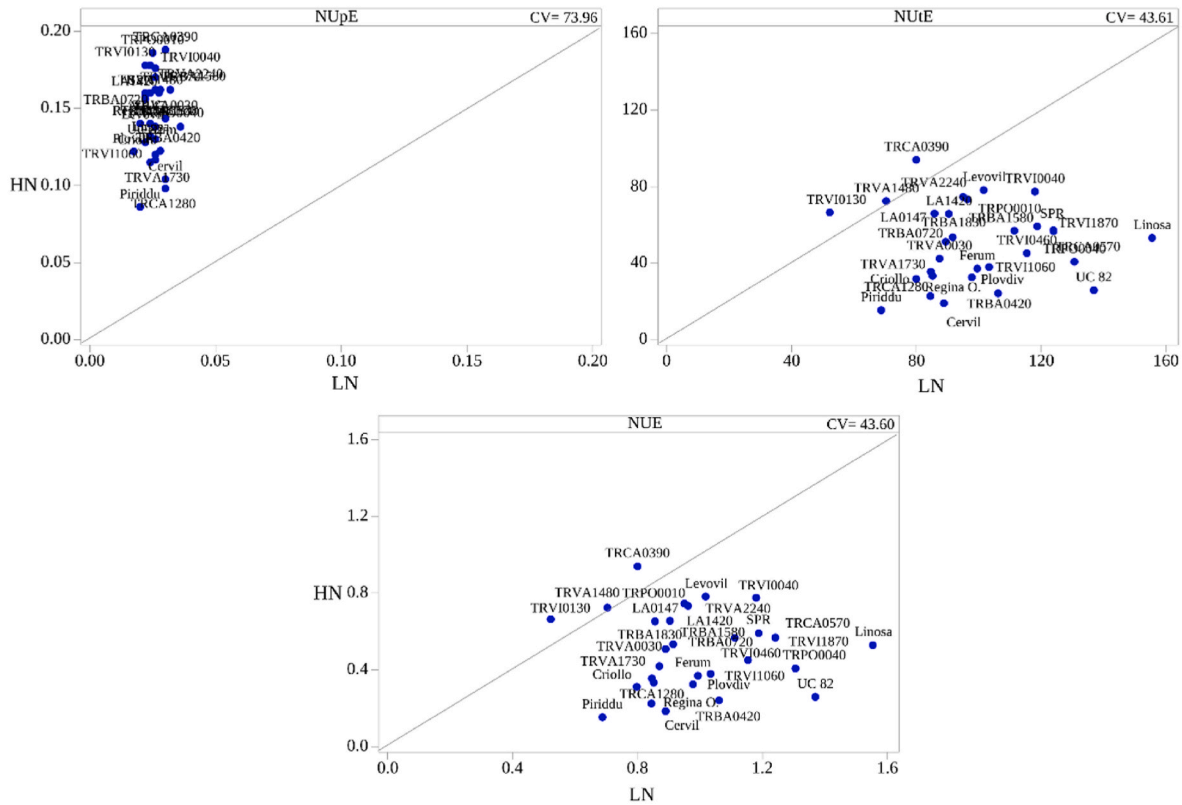

Fig. 6. Variation in NUE and its components, NUpE and NUtE of 30 tomato accessions exposed to low (LN; x-axis) and high (HN; y-axis) N supply. The coefficient of variation (CV) for each trait was also calculated.

thresholds (Fig. 5C).

Finally, the nitrogen use efficiency (NUE) and its components, NUpE and NUtE, their variation in the tomato accessions exposed to low and high N supply as well as the coefficient of variation (CV) were calculated (Fig. 6; Table S4). The LN treatment resulted in an increase of NUE in all the accessions when compared to HN values, except the accession TRVI0130 and significant differences among accessions within each treatment were found (Fig. 6; Table S4). At LN, the NUpE component decreased on average by 41.8% when compared to HN condition, ranged from 0.40 (Piriddu) to 0.86 (LA1420) at HN and from 0.27 (Regina Ostuni) to 0.52 (TRCA0570) at LN (Table S4). The CV of NUpE resulted high (73.96%), indicating that the ability of plants to absorb N from the soil varies significantly among the accessions only at HN (Fig. 6). By contrast, the NUtE component increased on average by 188.4% at LN compared to HN, ranging from 24.3 (Cervil) to 35.9 (TRVA2240) at HN and from 73.0 (TRVI0130) to 106.6 (Linosa) at LN (Table S3). NUtE showed a CV lower (43.61%) then NUpE, but the significant variation among the accessions at both experimental conditions (mainly at LN) can result useful to identify genotypes more able to utilize the N absorbed from the soil at both N conditions (Fig. 6).

Finally, NUE also increased on average by 62.3% at LN compared to HN, ranged from 10.1 (Piriddu) to 30.4 (TRCA0390) at HN and from 23.7 (TRVI0130) to 43.6 (TRCA0570) at LN (Table S4). The CV of NUE (43.60%) and the variation among accessions at both experimental conditions mirrored its component NUtE, indicating that tomatoes more efficient to convert N absorbed from the soil into biomass can be identified at both LN and HN (Fig. 6).

3.5. Correlations and principal component analysis

The correlation analysis carried out at both HN and LN conditions revealed a higher number of significant correlations at HN compared to LN (Fig. 7). At HN, positive correlations between NUE, NUpE and NUtE with morphological traits such as stem length, number of leaves and total dry weight of leaves, stems, and roots were observed. At LN, NUE, NUpE and NUtE also showed correlations with dry weight, but compared to the HN condition, NUtE had lower correlation values and was not significant for the root dry weight. At HN, C:N ratio displayed high positive correlation with NUE as well as the growth traits, therefore at LN conditions, the correlations between C:N ratio and the same traits were confirmed with lower values and only for some growth traits (Fig. 7). At HN, chlorophyll index was positively correlated with biomass traits and NUE, while anthocyanin index was negatively correlated with the same traits. Conversely, at LN the correlations between the same traits became not-significant (Fig. 7). At both N conditions, NBI showed significant correlations only with chlorophyll index, as expected, and negatively correlated to flavonol and anthocyanin indexes (Fig. 7).

The first two components of the principal component analysis (PCA) accounted for 81.4% of the variation observed, with the first (PC1) and second (PC2) principal components accounting for 65.3% and 16.1% of the variation, respectively (Fig. 8). In the loading plot, PC1 displayed high positive correlations (>0.5) with flavonol and anthocyanin indexes, leaf, stem, and root C:N ratios, NUtE and NUE, while it showed high negative correlations with biomass traits (total, leaf, stem, and root dry weights), chlorophyll index, NBI and NUpE. Otherwise, PC2 displayed high positive correlations with stem length, stem dry weight and NUE (Fig. 8). In the score plot, in the left part the accessions with negative values for traits correlated to PC1 under HN condition, in the right part were plotted the accessions with positive values for traits correlated to PC1 under LN condition (Fig. 8). The accessions treated at HN displayed a wider range of values for traits correlated to PC2 compared to LN treatment. At both N conditions, the accessions did not cluster for the country or institution of origin (Fig. 8).

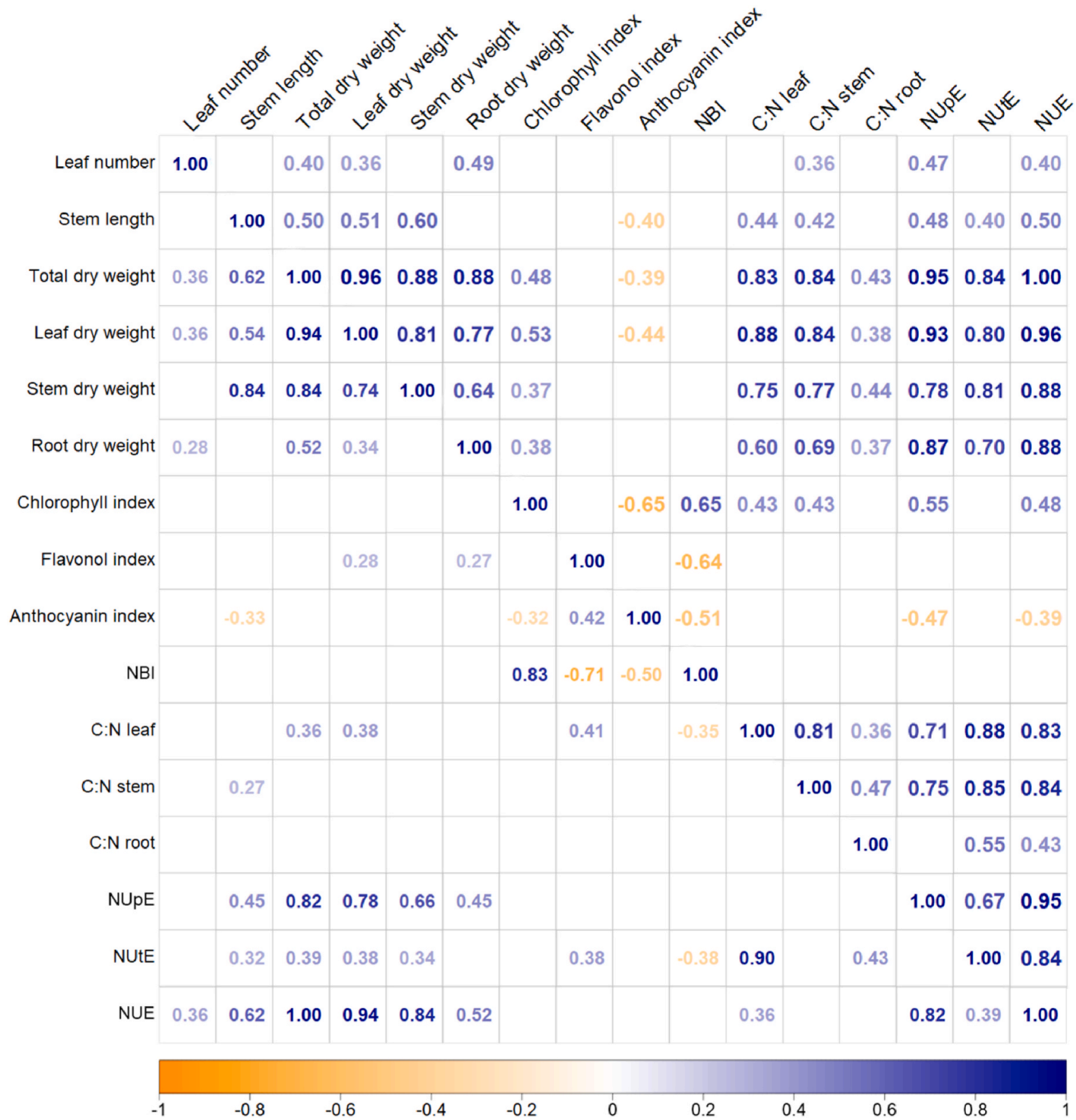

Fig. 7. Correlation matrix coefficients for high (HN; upper right diagonal) and low nitrogen (LN; lower left diagonal) conditions. Only significant correlations according to the Bonferroni test at a significance level of $p < 0.05$ are displayed. Values in bold are significant at p values < 0.001.

3.6. Selection of tomato accessions

The multi-trait genotype-ideotype distance index was used to select accessions with the best performances under HN and LN conditions. Accessions were selected based on their high total dry matter, NUpE, NUtE, and NUE. Among the accessions, TRCA0390, LA1420, TRVA2240, TRVI0040, TRPO0010, TRVI0030 and TRVA1480 were selected at HN, while TRVI1870, Linosa, TRPO0040, TRVI0460, TRCA0570, TRBA0420, TRVI0040 were selected at LN conditions (Fig. 9). Interestingly, TRVI0040 was selected at both N conditions.

4. Discussion

The identification of genes and traits that can be used to breed new improved varieties requires the evaluation and selection of plant genetic resources (Parry and Hawkesford, 2012). The excessive use of N fertilizers has important negative impacts on the environment, but at the same time, N limitation in the soil or growing substrate represents a major constraint for maximazing yield in tomato. Therefore, the evaluation of tomato germplasm for NUE to select for this complex trait is becoming necessary (Machado et al., 2022). In agreement with previous studies (Hasnain et al., 2020; Ronga et al., 2019), all the accessions showed a decrease in biomass under low N conditions. Furthermore, also the days to the appearance of the first flower buds decreased by 25 days at LN compared to HN. The effect of N rate on crop phenology is variable, indeed, higher N applications can increase (Shrestha et al., 2018) but also decrease (Ma et al., 2023; Shen et al., 2022) the days to flowering in different plants.

A significant variation among the accessions for all the traits indicated that the genotypic selection for better performance at both high and/or low N supply is feasible. The accessions with higher growth performances at high N were different from those at low N, indicating that NUE is a complex trait strongly influenced by the environment, such as N availability (Hawkesford and Riche, 2020). The different responses of the genotypes to the two treatments can partially be attributed to the fact that biomass production is associated with NUE only under

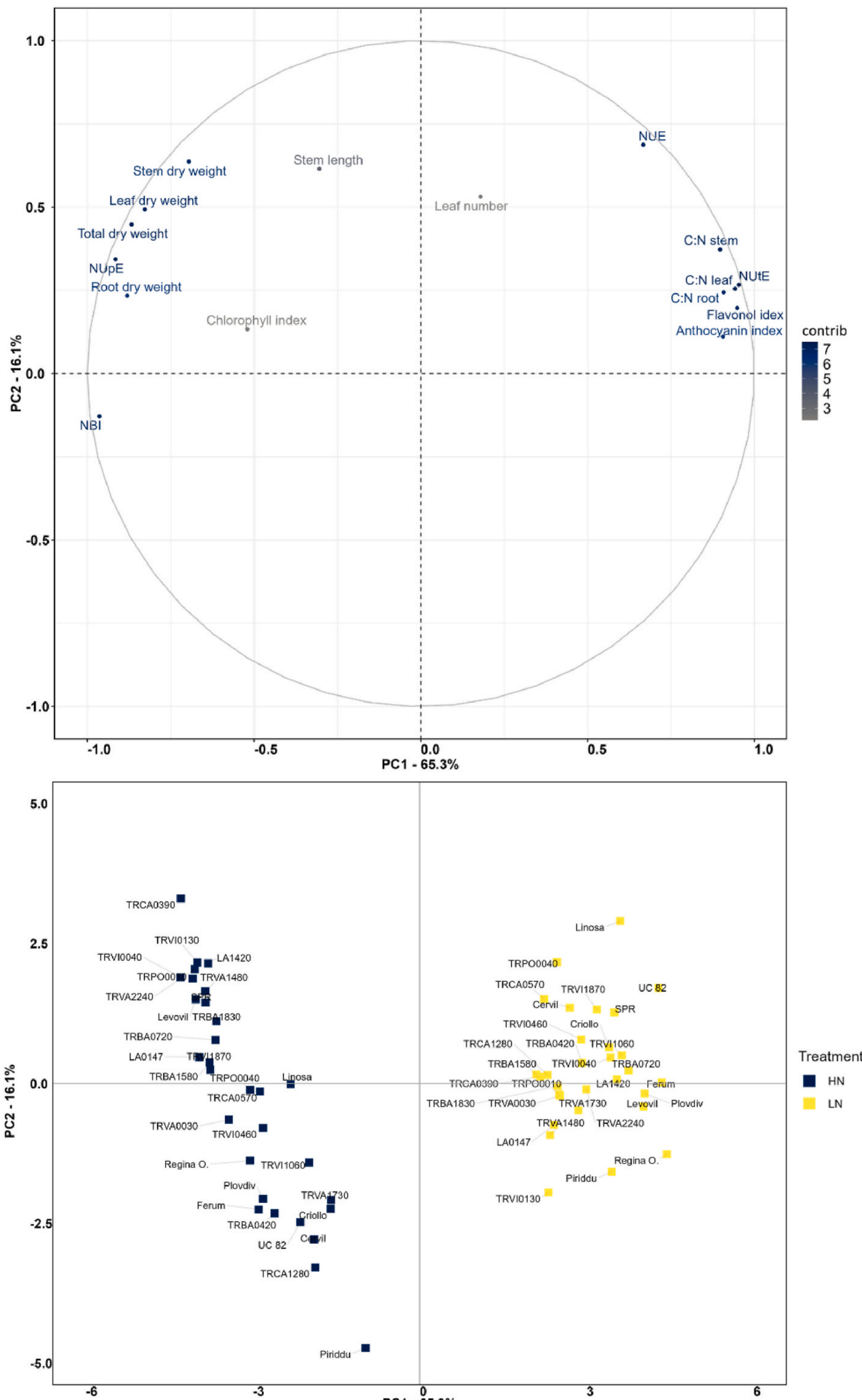

Fig. 8. Loading plot (above) and score plot (below) of the principal component analysis was performed on thirty tomato accessions grown under low (LN) and high (HN) nitrogen conditions. The first and second principal components (PC1 and PC2) accounted for 65.3% and 16.1% of the total variation, respectively.

conditions of sub-optimal internal nitrogen concentrations (Santa-María et al., 2015). Indeed, the genotypes, including a 'De penjar'/'Da serbo' collection from Italy and Spain and the eight parents of a MAGIC population from France (Pascual et al., 2015), showed a wide variation in their responses to N supply rates. In the PCA loading plot, a positive correlation among flavonol and anthocyanin indexes, C:N ratios, NUtE and NUE, as well as a negative correlation among biomass, NBI and NUpE were highlighted. These results appeared in agreement with the main role of NUtE to improve NUE in tomato and the limited role played by NUpE previously described (Abenavoli et al., 2016; Aci et al., 2021). The PCA score plot showed two clusters relative to N conditions; by contrast, subclusters for the origin or type of the accessions could not be identified, as already reported (Mercati et al., 2015).

According to Bijay-Singh & Ali (2020), the higher N fertilization lead to greater chlorophyll concentration contributing to a higher biomass production. The increase in flavonols and anthocyanins was favoured by

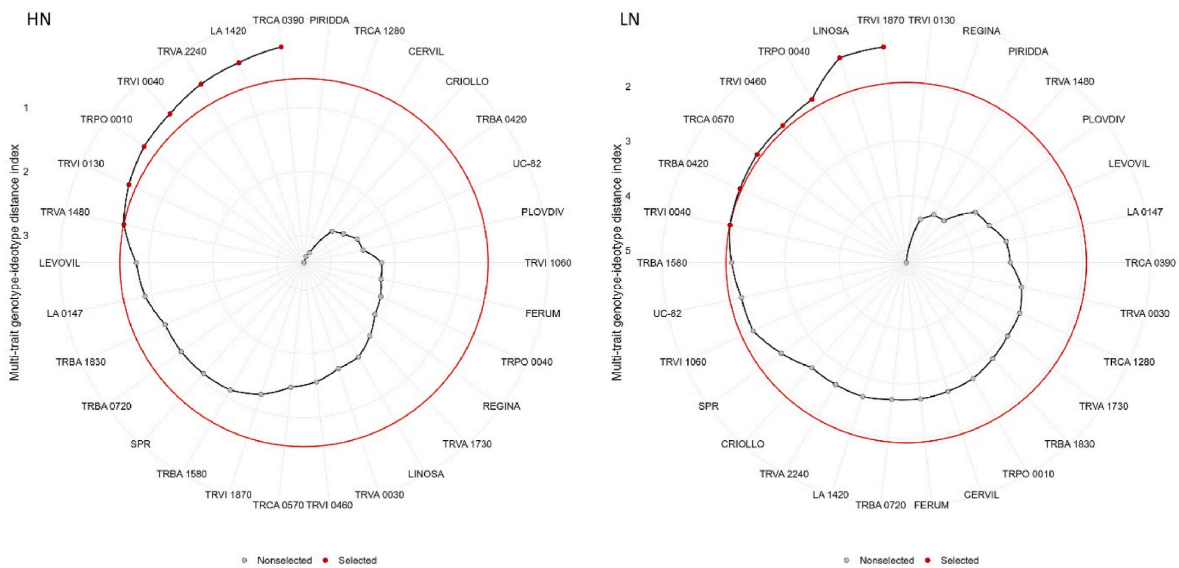

Fig. 9. Multi-trait genotype-ideotype distance index for the selection of accessions with higher total dry weight, nitrogen uptake efficiency (NUpE), nitrogen utilisation efficiency (NUtE) and nitrogen use efficiency (NUE) at high (HN) and low (LN) nitrogen conditions. The red circle represents the cut-off point according to the selection pressure adopted (23.3%; 7 out of 30 accessions).

low N fertilization, because their high content may sustain higher plant growth under N deficiency (Li et al., 2021; Peng et al., 2008; K. Zhang et al., 2020). However, the lack of significant correlations between pigments content and biomass suggested that these compounds could be not suitable indicators for selecting well-adapted accessions at low N. Otherwise, another parameter here evaluated was the NBI, an indicator of plant N status, by measuring the ratio of chlorophyll and flavonols. In general, plants with high N content synthesize more chlorophyll, leading to an increase of NBI, while plants with low N synthesize more flavonols, resulting in a decrease in the NBI (Cerovic et al., 2015). Accordingly, in our study plants under high N showed on average a higher NBI than those under low N condition. However, as already observed in eggplant by Mauceri et al. (2020), NBI values did not correlate with plant growth traits within each N treatment, suggesting that the selection for high NBI in tomato under HN or LN conditions in the vegetative stages did not necessarily result in higher biomass.

The C:N ratio is another indicator of the plant nutritional status that decreases with higher N inputs. This effect was confirmed by our results, where the accessions exhibited on average a higher C:N ratio under LN. Previous research highlighted an increase of C:N ratio under N-limited rates resulting in higher NUE, thereby guaranteeing plant survival (Zhang et al., 2020). Moreover, an interesting correlation among the C:N ratio, growth parameters and NUE was observed, mainly at HN. However, at LN, the correlations between C:N ratio and NUE were moderate, making C:N ratio an important trait to be considered in selecting genotypes suitable mostly for HN conditions.

NUE is defined as the product or biomass produced per N supplied and it is obtained by multiplying their components NUpE and NUtE (Moll et al., 1982; Weih et al., 2018). NUpE is the root ability to absorb N from the soil, while NUtE is the plant ability to produce biomass per unit of N taken up involving several physiological traits related to plant N storage and remobilisation (Moll et al., 1982; Han et al., 2015). In our conditions, the tomato accessions with higher NUpE showed a high positive correlation with higher root biomass in HN and a moderate positive correlation in LN, while NUtE was positively correlated to the leaf C:N ratio at both HN and LN. These results suggest that the selection of tomatoes with high root biomass and leaf C:N ratio could lead to an increase in NUpE and NUtE, respectively, resulting in a higher NUE.

The variation observed in NUE and its components were mainly caused by N treatment effects, as expected. Indeed, NUpE and NUtE should increase under low N, as already observed by other authors (Ierna and Mauromicale, 2019; Maltas et al., 2018; Moitzi et al., 2020; Torres et al., 2018). By contrast, we observed a NUpE decrease at LN, probably due to the lower N concentration available in the substrate (i.e., the capacity to incorporate N per unit of root surface and per unit of time) in addition to the effect of reduced root growth and to a potential higher competition between plant roots and soil microorganisms, which are highly efficient at low N conditions (Kuzyakov and Xu, 2013). Moreover, although a high correlation between NUE and its components was observed, the average value of NUpE was lower than that of NUtE, making this last component the key factor to improve NUE, as already demonstrated in both tomato and eggplant (Abenavoli et al., 2016; Aci et al., 2021; Mauceri et al., 2020, 2021). Overall, our results indicated that both components should be taken into account for selecting accessions with higher NUE at HN, while at LN, NUpE resulted inefficient to identify N use efficient genotypes. Among the genotypes selected by multi-trait genotype-ideotype distance index at both N conditions, only the accession TRVI0040 showed higher performances in both conditions, resulting very interesting due to the high NUE complexity depending on GxE interaction (Cormier et al., 2013). Eleven out of thirteen (85%) accessions selected either at LN and HN, belonged to the 'De penjar'/'Da serbo' collection, highlighting the importance of this traditional varieties for improving NUE, as recently reported (Rosa-Martínez et al., 2021).

5. Conclusions

The analysis of thirty tomato accessions at both HN and LN revealed a significant effect of N treatment on plant development rate, growth, biomass, physiological traits as well as NUE and its components. N inputs mainly affected plant development. In this way, plants at LN required more time to reach the same stage compared to HN and plant biomass at this same developmental stage was lower at LN. Increased N availability resulted in higher chlorophyll content but reduced flavonol and anthocyanin accumulation, leading to a higher NBI. However, these parameters did not appear to be directly correlated with the best performant accessions at each condition. The C:N ratio significantly decreased with higher N input and it was highly correlated to NUtE at both N conditions. Seven accessions excelled for multi-traits including biomass and NUE at each N condition, leading to 13 selected accessions, one of which (TRVI0040) was selected under both N conditions. Overall, the results revealed significant variability among accessions in plant growth, NUE and its components, enabling the selection of genotypes with superior NUE under different N conditions. In this respect, some of the 'De penjar'/'Da serbo' types appeared to be not only more tolerant than other genotypes to low N, but also displaying higher NUE values under low N conditions, and could be considered useful breeding lines for the improvement of this complex trait.

CRediT authorship contribution statement

Martín Flores-Saavedra: Formal analysis, Investigation, Methodology, Writing – original draft. **Gloria Villanueva:** Investigation, Methodology, Writing – review & editing. **Pietro Gramazio:** Data curation, Formal analysis, Software, Writing – review & editing. **Santiago Vilanova:** Conceptualization, Investigation, Resources, Supervision, Writing – review & editing. **Antonio Mauceri:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Maria Rosa Abenavoli:** Methodology, Supervision, Writing – review & editing. **Francesco Sunseri:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. **Jaime Prohens:** Conceptualization, Formal analysis, Project administration, Resources, Writing – original draft. **Mariola Plazas:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft.

Declaration of competing 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.108447.

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