Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1640556

Open Access Journal
e-ISSN: 2619 - 8991

Research Article
Volume 8 - Issue 3: 863-874 / May 2025

COMPARISON OF IN VITRO AND HYDROPONIC SCREENING METHODS FOR NITROGEN EFFICIENCY IN PEPPER (*Capsicum annuum* L.)

Alim AYDIN[1*], Hasan PINAR[2], Firdes ULAŞ[2], Halit YETİŞİR[2] Mirac ŞAHİN[2] Hamide AYDIN[2]

[1]*Kırsehir Ahi Evran University, Faculty of Agriculture, Department of Horticulture, 40100, Kırsehir, Türkiye*
[2]*Erciyes University, Faculty of Agriculture, Department of Horticulture, 38000, Kayseri, Türkiye*

Abstract: In evaluations conducted under high and low nitrogen conditions, the morphological and physiological performance of pepper genotypes was compared across tissue culture and hydroponic systems. Under low nitrogen conditions, most genotypes showed similar rankings in both systems, indicating stable and environment-independent genotypic responses. Notably, 21 H-1-1, ERÜ 462, and ERÜ 457 consistently performed well across all traits, whereas 24-H-6, 29-H-10, and ERÜ 1248 ranked among the lowest-performing genotypes. In contrast, under high nitrogen conditions, genotypic performance varied more strongly depending on the cultivation system. Genotypes grown in tissue culture showed greater biomass accumulation and nitrogen uptake. While 21 H-1-1, ERÜ 462, and ERÜ 457 were among the top performers in tissue culture, their counterparts in hydroponic culture exhibited relatively lower performance. This indicates that system-specific effects play a more prominent role under high nitrogen supply. Considering both nitrogen levels, 21 H-1-1 and ERÜ 462 emerged as promising candidates for breeding programs due to their high nitrogen use efficiency and consistent performance across multiple environments. If a low-cost selection approach is desired, preliminary selection under low nitrogen conditions using tissue culture may be sufficient and effective for genotype differentiation.

Keywords: Root volume, Leaf area, Nitrogen efficiency, Inbred line

***Corresponding author:** Kırsehir Ahi Evran University, Faculty of Agriculture, Department of Horticulture, 40100, Kırsehir, Türkiye
E mail: alim.aydin@ahievran.edu.tr (A. AYDIN)
Alim AYDIN https://orcid.org/0000-0002-9424-5556
Hasan PINAR https://orcid.org/0000-0002-0811-8228
Firdes ULAS https://orcid.org/0000-0001-6692-8424
Halit YETİSİR https://orcid.org/0000-0001-6955-9513
Mirac SAHİN https://orcid.org/0000-0002-7699-6380
Hamide AYDIN https://orcid.org/0000-0002-0588-6192

Received: February 16, 2025
Accepted: April 13, 2025
Published: May 15, 2025

Cite as: Aydın A, Pınar H, Ulaş F, Yetişir H, Şahin M, Aydın H. 2025. Comparison of in vitro and hydroponic screening methods for nitrogen efficiency in pepper (*Capsicum annuum* L.). BSJ Eng Sci, 8(3): 863-874.

1. Introduction

Chemical fertilizers are one of the most important input sources that directly affect plant growth, development and yield formation in both high-cost and low-cost agricultural systems. Since it is present in most plants in more amounts than all other mineral nutrients, nitrogen (N) is the source that is most utilized and needed in crop production in terms of quantity (Ulas et al. 2021a). N is found in the structure of many important organic compounds (amino acids, nucleic acids, proteins, enzymes, chlorophyll, ATP and ADP) (Matsuhisma et al. 2009) and the organic composition (sugar, starch, total nitrogen, cellulose) (Loudari et al. 2020; Prado, 2021; Kirkby, 2023). N affects the plant's architecture, chlorophyll formation and photosynthates allocation, flowering, and fruit development (Nawaz et al. 2017). A proper amount of N encourages root architecture, improvement in quality of fruits and regulates growth and development of plants as well as the uptake of other nutrients. In contrast, the obtainable N is often restricting plant growth and development more than any other nutrients under both high-input and low-input agriculture systems (Ulas et al. 2019). Excess N supply causes late ripening of fruits, leading to a decreased resistance to certain diseases (Bolat and Kara, 2017). On the other hand, under N deficiency there are several production problems accounting for low yield and quality of vegetable crops. As with all vegetable crops, plant growth and yield in pepper are very sensitive to N fertilizers. Tumbare and Tumbare, (2004) reported that nitrogen fertilizer increases fruit weight and yield. Madeira and De Varennes, (2005) observed that total chlorophyll content, leaf N concentration, and stems, leaves, and fruits dry weight of pepper increased with increasing N fertilization. Ulas et al. (2022) stated that leaf, and shoot fresh and dry weights, total leaf area, and leaf chlorophyll content (SPAD) of pepper plants were significantly increased with increasing N rate (3.0 mM N) than low N rate (0.3 mM N) under hydroponic conditions. Similar results were obtained in potato cultivars (Ulas et al. 2021a) and pepper inbred lines Ulas et al. (2022) under hydroponic conditions under two different N rates (Low-N: 0.5 mM N

and High-N: 3.0 mM N).

Nitrogen use efficiency (NUE) is in plants as the yield of harvestable product (dry matter)/ available soil N (or N supply) (Moll et al. 1982). Many researchers have shown that there are great differences among genotypes of the same species, especially in terms of N efficiency (N-uptake and N-use) (Schulte et al. 2007; Ulas et al. 2021a; Ulas et al. 2022). Determination of N-efficient genotypes from plant genetic resources and their exploitation in plant breeding programs is very important for sustainable crop production and N cycle. The determination of N-efficient genotypes depends on the genetic variation in genetic resources and the efficiency of the determination method. In a plant, NUE can be increased by genetic modification or plant breeding methods that can have very good N absorption from the soil and effective utilization (Hirel et al. 2007). So far, in many crop species genotypic variations have been studied such as maize (Machado and Fernandes, 2001), rice (Fageria and Baligar, 2003), tobacco (Ruiz et al. 2006), oilseed rape (Schulte et al. 2007), and pepper (Ulas et al. 2022), it was observed that developing crop varieties with improved NUE is the identification of key elements that control N assimilation processes (Ruiz et al. 2006). Because of this, investigations on N efficiency should be conducted to identify efficient, quick, accurate, and affordable screening techniques. Due to the lack of uniform nitrogen-treated fields and the labor-intensive and time-consuming nature of standard screening techniques, it is challenging to identify N-efficient genotypes. In vitro screening may be found tolerant plants and their tolerance level.

The development of tolerant plants has been the subject of numerous attempts following the success of in-vitro screening (Daneshmand et al. 2010; Soleimani et al. 2010; Homayoun et al. 2011). Murshed et al. (2015) screened nine potato cultivars under salt stress in vitro and reported that salt stress negatively affected plant growth and showed differences in the responses of the cultivars, and as a result in vitro screening could be used successfully. In addition to saving time and labor, the magnitude of the N effect depends on the plant species, variety and the N level. This study sets out to find the most effective, workable, long lasting and repeatable selection method for pepper plant nitrogen usage efficiency. Additionally, the physiological characteristics of various pepper genotypes were investigated by using high and low N administrations in tissue and hydroponic growing media.

2. Materials and Methods

2.1. Plant Material

In both Exp. 1 and 2, six pepper (*Capsicum annuum* L.) inbred lines (IL) (21-H-1-1, 29-H-10, 24-H-6, ERÜ 457, ERÜ 462, ERÜ 1248) were tested under low-N (0.3 mM N) and high-N (3.0 mM N) supply. For the comparison of results between the tissue culture and hydroponic culture environments, the data for the hydroponic culture were referenced from the study conducted by Ulas et al. (2021b).

2.2. Methods

2.2.1. Experiment I, in vitro screening

Before sowing, the pepper seeds utilized in the study underwent surface sterilization. To do this, pepper seeds were put in a glass jar with a cover, 70% ethyl alcohol was then added, and the mixture was then allowed to sit for one minute. By filtering ethyl alcohol and adding 5% commercial sodium hypochlorite, surface sterilization was carried out. Then, sterile distilled water was used to wash it three times. Pepper seeds were planted on a medium that also included Hoagland nutrient solution (Hoagland and Arnon 1950), which had low N (0.3 mM N) and high N (3.0 mM N) supplies. 330 ml glass jars were utilized for the tissue culture experiment, and an in vitro experiment with three replications and three plants in each replication was set up with the pH adjusted to 5.8. The experiment was conducted in a climate cabinet with a temperature of 25°C and 16/10-hour (light/dark) photoperiodic system for approximately six weeks.

2.2.2. Experiment II, hydroponic culture screening

The seeds were seeded in a multi-pot with a 2:1 mixture of peat (pH: 6.0-6.5) and perlite to create uniform seedlings for hydroponic growing medium. Carefully remove the seedlings with two true leaves from the peat-perlite growing media while avoiding root injury, they were then placed in 8 L plastic pots with a nutrient solution in a growth chamber. The nutritional medium was supplemented with 0.7% agar and 3% sucrose. The controlled growth chamber had an average day/night temperature of 25/22°C, a relative humidity range of 65-70%, and a photoperiod of 16/8 h of light/dark regimes that delivered roughly 350 mol m-2 s-1 of photon flux. A hydroponic experiment with four replications and three plants in each pot (replication) was set up using a totally randomized block design. The plants in the experiment received all their nutrients from an aerated hydroponic culture in 8 L plastic pots. Analytical grade (99% pure) ingredients were added to distilled water to create the nutrition solution using a modified Hoagland and Arnon, (1950). An air pump was used to continually aerate the nutrient solution in each 8 L pot. In hydroponic screenings, nitrogen was added at two different quantities (Low N: 0.3 mM, High N: 3.0 mM) using two different proportional N sources (Ca $(NO^3)^2$ at 75% and $(NH^4)2^SO^4$ at 25%) (Ulas et al. 2019). The composition of the basic nutritional solution was K^2SO^4 (500), KH^2PO^4 (250), $CaSO^4$ (1000), $MgSO^4$ (325), NaCl (50), H^3BO^3 (8), $MnSO^4$ (0.4), $ZnSO^4$ (0.4), $CuSO^4$ (0.4), $MoNa^2O^4$ (0.4), and Fe-EDDHA (80) (M). When the nutritional solution in the 3.0 mM N rate pots' N concentration dropped below 0.3 mM, as determined daily by nitrate test strips (Merck, Darmstadt, Germany) by using a NitracheckTM reflectometer, all nutrients were replenished.

2.2.3. Harvest, shoot and root dry weight and main stem length measurements

42 days after treatment (DAT) in both cases, three plants per pot were collected. Roots and shoots from harvested plants were divided. The shoot was divided into leaf, stem,

and roots to calculate the fresh and dry weight. To calculate dry weights, plant materials were dried in a forced-air oven for 48 hours at 70°C. Using a leaf-area meter (LI-3100, LI-COR. Inc. Lincoln, NE, USA), the plants' leaves were measured destructively during the harvesting process in both studies. The total leaf area was calculated from cm2 to m^2 (cm^2). Using a ruler, the main stem's length (cm) was determined.

2.2.4. Root morphological measurements

The root volume (cm^3) and total root length (m) of the plants were measured in both experiments using an Epson Expression 11000XL scanner and the specialized image analysis software WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Québec, QC G1V 1V4, Canada). Approximately 5.0 g of fresh root samples were collected from each harvested sample. The samples were positioned in the tray of the scanner. The roots were evenly distributed around the tray after water was introduced, using plastic forceps, and scanning and analysis were carried out using the WinRhizo system's user interface on a computer linked to the scanner. The ratio of the total plant root length to the total plant root volume was then calculated (Ulas et al. 2019).

2.2.5. Shoot nitrogen analysis

After grinding shoot dry materials, 200 mg from each dry plant samples were taken to analyze the shoot N concentration (mg N g^{-1} d.w.) by using Kjeldahl Nitrogen Determination Method, introduced by Johan Kjeldahl in 1883 (Kjeldahl 1883). After the determination of shoot N concentration, the value was multiplied by total shoot dry matter to calculate the total shoot N content (N uptake) of a whole plant (mg N $plant^{-1}$) (Ulas et al. 2019).

2.3. Statistical Analysis

All statistical analyses, including correlation assessments and principal component analysis (PCA), as well as the generation of graphical outputs, were conducted using Minitab Statistical Software (version 22).

3. Results

Among the pepper genotypes tested, ERÜ 462 and ERÜ 457 exhibited the highest main stem lengths under both tissue culture and hydroponic culture conditions. These genotypes consistently outperformed others under both low and high nitrogen treatments (Figure 1 a). Correlation analysis showed that the relationship between the two growing systems varied depending on nitrogen level. Under low nitrogen conditions, a moderate positive correlation was observed between tissue and hydroponic cultures (r = 0.617, Figure 1 b), whereas under high nitrogen, the correlation was stronger (r = 0.875, Figure 1 c). These results indicate that genotype responses became more parallel between the two systems when nitrogen was not limiting.

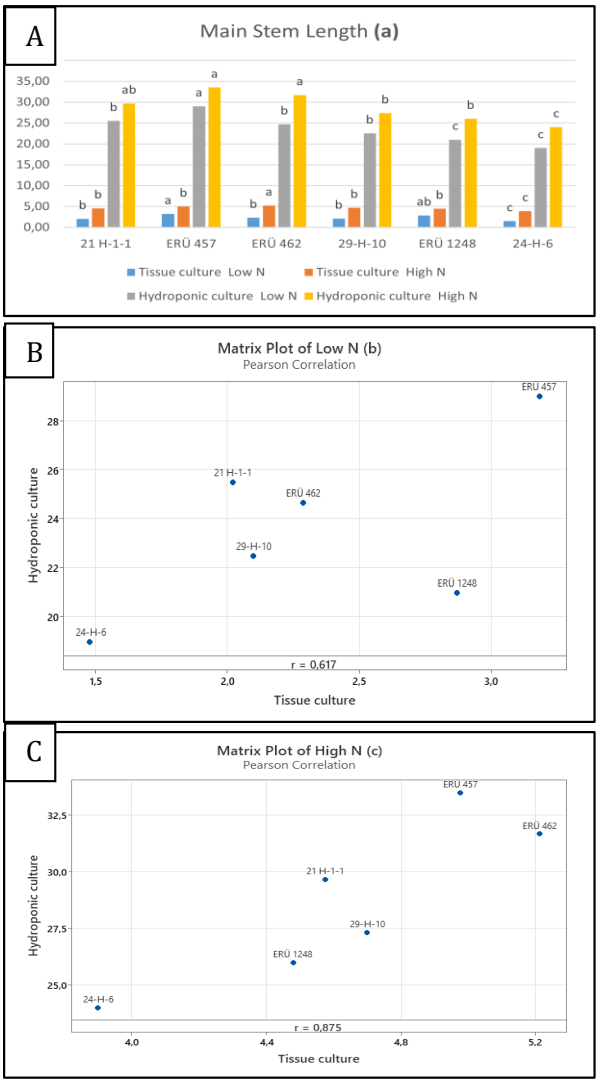

Figure 1. (a) Main stem lengths of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (cm) and hydroponic culture (cm) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

Among the pepper genotypes tested, 21 H-1-1 and 29-H-10 exhibited the highest shoot fresh weights under both tissue culture and hydroponic culture conditions. These genotypes showed superior biomass accumulation, particularly under high nitrogen conditions (Figure 2 a). Correlation analysis revealed that the relationship between the two cultivation systems varied based on nitrogen availability. Under low nitrogen conditions, a strong positive correlation was observed between tissue and hydroponic cultures (r = 0.897, Figure 2 b), indicating consistent genotype responses across systems. However, under high nitrogen conditions, the correlation weakened (r = 0.459, Figure 2 c), suggesting that genotype performance diverged more between the two systems when nitrogen was abundant. These findings indicate that shoot biomass production is more stable across growing

environments under nitrogen-limited conditions, whereas high nitrogen may reveal system-specific differences in genotype response.

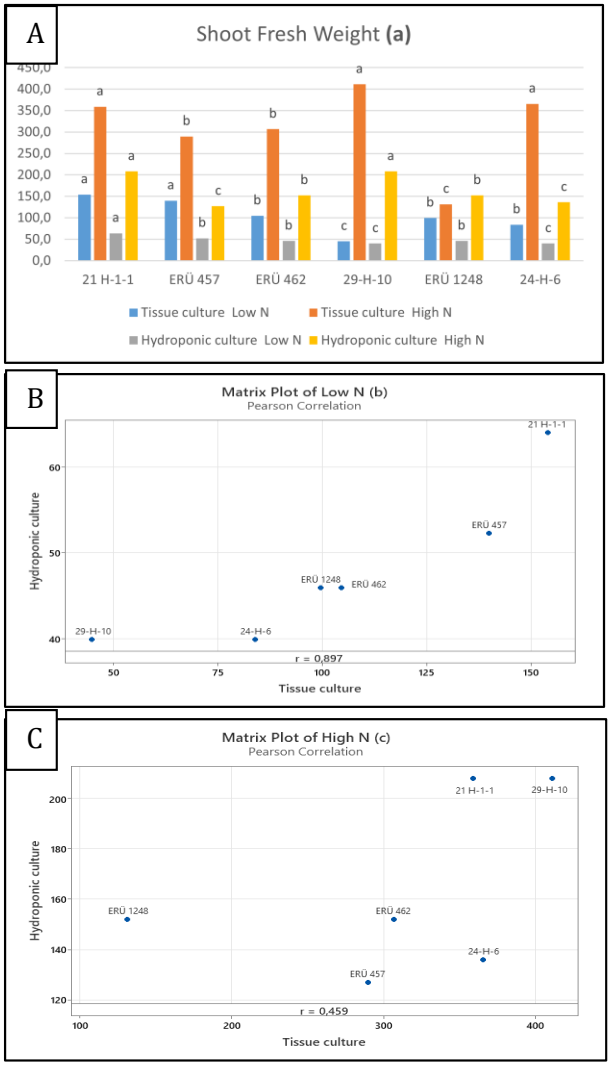

Figure 2. (a) Shoot fresh weight of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (mg) and hydroponic culture (g) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

Among the pepper genotypes tested, 21 H-1-1 and 29-H-10 exhibited the highest shoot dry weights under both tissue culture and hydroponic culture conditions (Figure 3 a). Correlation analysis between the two culture systems revealed a moderate positive relationship under low nitrogen conditions (r = 0.728, Figure 3 b), suggesting a relatively consistent genotype response across systems when nitrogen was limited. However, under high nitrogen conditions, the correlation dropped substantially (r = 0.287, Figure 3 c), indicating greater variability in genotype performance between systems when nitrogen was abundant. These results suggest that shoot dry weight is more consistently expressed across different cultivation systems under nutrient-limited conditions, while high nitrogen availability may enhance the influence of environmental or system-specific factors on genotype performance.

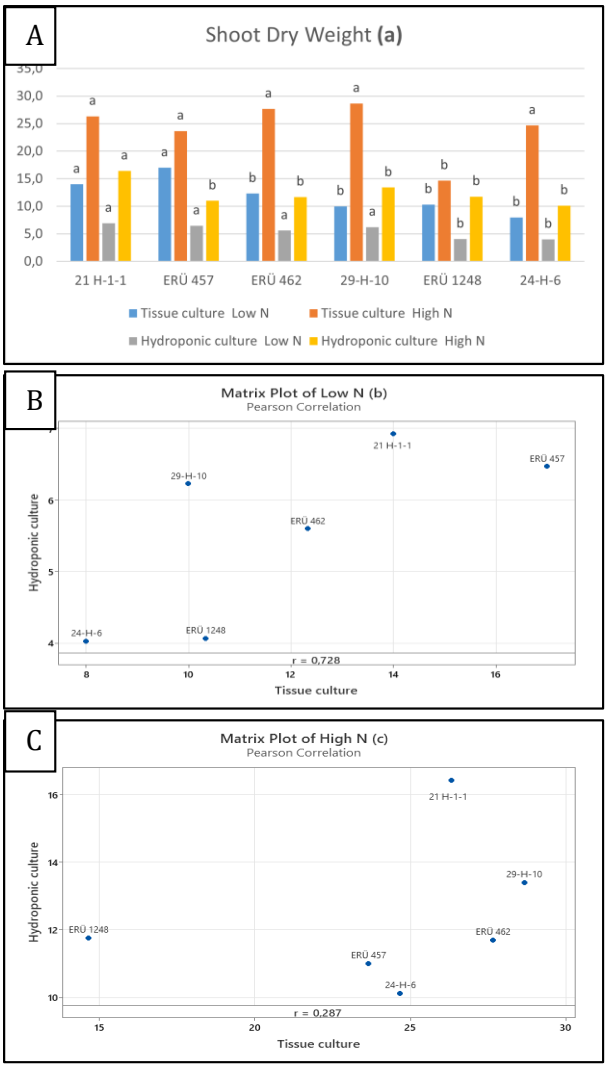

Figure 3. (a) Shoot dry weight of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (mg) and hydroponic culture (g) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

21 H-1-1 and ERÜ 462 exhibited the highest root fresh weights under both tissue culture and hydroponic culture conditions (Figure 4 a). These genotypes accumulated greater root biomass, particularly under high nitrogen conditions. Correlation analysis showed that the relationship between the two cultivation systems varied with nitrogen availability. Under low nitrogen conditions, a moderate positive correlation was observed between the systems (r = 0.552, Figure 4 b), indicating a reasonably consistent root growth response across environments.

However, under high nitrogen conditions, the correlation slightly increased to r = 0.690 (Figure 4 c), reflecting a somewhat stronger alignment of genotype performance between the systems compared to low nitrogen. These results suggest that, unlike shoot dry weight, root fresh weight responses may become more aligned between systems as nitrogen increases, possibly due to more uniform root development conditions under non-limiting nutrient availability.

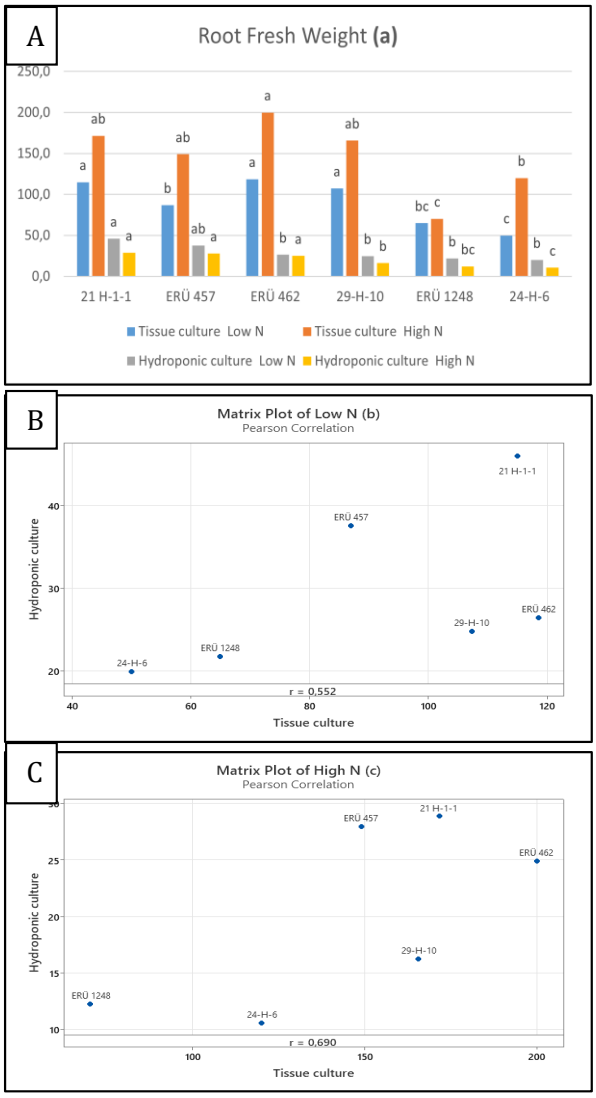

Figure 4. (a) Root fresh weight of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (mg) and hydroponic culture (g) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

21 H-1-1, ERÜ 457, and ERÜ 462 showed the highest root dry weights under both tissue culture and hydroponic culture conditions (Figure 5 a). Correlation analysis revealed that the relationship between the two growing systems varied depending on nitrogen availability. Under low nitrogen conditions, the correlation between tissue culture and hydroponic systems was very weak (r = 0.207, Figure 5 b), indicating inconsistent genotype performance across systems when nitrogen was limited. However, under high nitrogen conditions, the correlation increased notably to r = 0.809 (Figure 5 c), reflecting a stronger alignment between the two systems and suggesting that genotype responses became more comparable when nitrogen was not a limiting factor. These results imply that root dry weight accumulation is more variable between systems under nutrient-limited conditions, while under sufficient nitrogen availability, genotype performance becomes more stable and parallel between tissue and hydroponic culture environments.

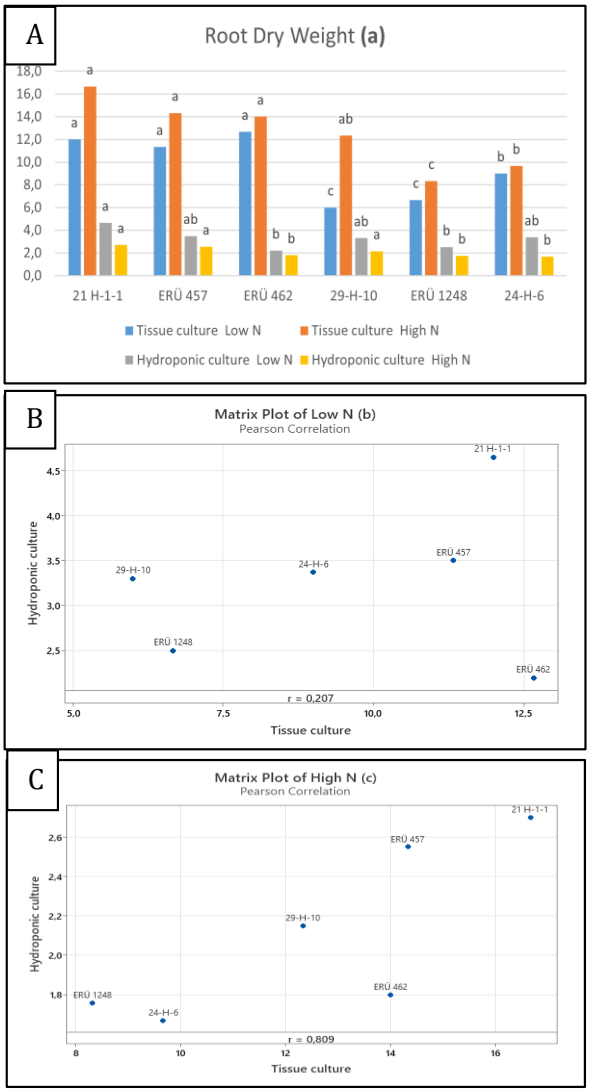

Figure 5. (a) Root dry weight of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (mg) and hydroponic culture (g) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

Among the pepper genotypes tested, 21 H-1-1 and ERÜ 457 displayed the highest leaf area values under both tissue culture and hydroponic culture conditions (Figure 6 a). Particularly under high nitrogen conditions. Correlation analysis between the two growing systems revealed that the relationship strengthened under high nitrogen availability. Under low nitrogen conditions, the correlation between tissue culture and hydroponic systems was weak (r = 0.379, Figure 6 b), indicating variable genotype performance between the systems when nitrogen was limited. In contrast, under high nitrogen conditions, the correlation increased to r = 0.843 (Figure 6 c), suggesting a strong and consistent response across both systems when nitrogen was sufficiently available.

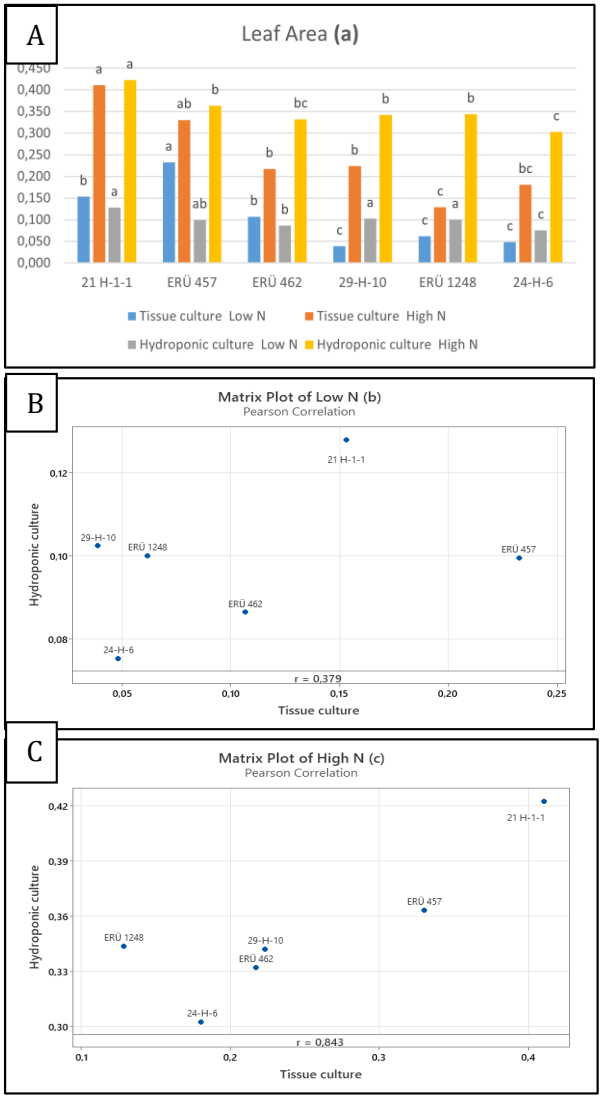

Figure 6. (a) Leaf area of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (cm^2) and hydroponic culture (m^2) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

Among the pepper genotypes evaluated, 21 H-1-1, ERÜ 457, and ERÜ 462 exhibited the greatest root lengths under both tissue culture and hydroponic culture conditions (Figure 7 a). Correlation analysis showed that the relationship between the two growing systems was stronger under low nitrogen conditions (r = 0.758, Figure 7 b) compared to high nitrogen conditions (r = 0.610, Figure 7 c). This indicates that genotype performance in terms of root elongation was more consistent between the two systems when nitrogen availability was limited, whereas increased nitrogen levels may have introduced system-specific variability. These findings suggest that root length is a relatively stable trait across different cultivation systems, particularly under nutrient-deficient conditions. However, under high nitrogen availability, environmental or system-specific factors may play a more pronounced role in determining genotype performance.

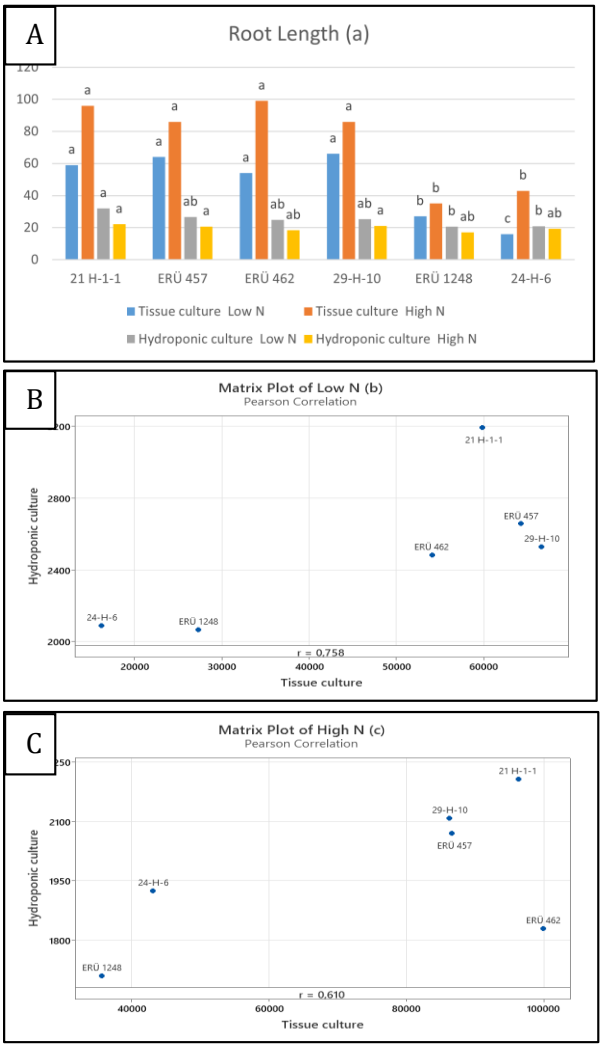

Figure 7. (a) Root lenght of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (cm) and hydroponic culture (m) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

Root diameter measurements showed relatively small variation across genotypes and nitrogen levels in both tissue and hydroponic culture systems (Figure 8 a). Although genotypes such as 21 H-1-1, ERÜ 457, and ERÜ 462 tended to have slightly greater root diameters. Correlation analysis revealed a moderate positive relationship between the two systems under low nitrogen conditions (r = 0.682, Figure 8 b), suggesting some consistency in genotype responses for this trait when nitrogen was limited. However, under high nitrogen conditions, the correlation weakened significantly (r = 0.356, Figure 8 c), indicating increased variability in root diameter across systems in response to nitrogen enrichment.

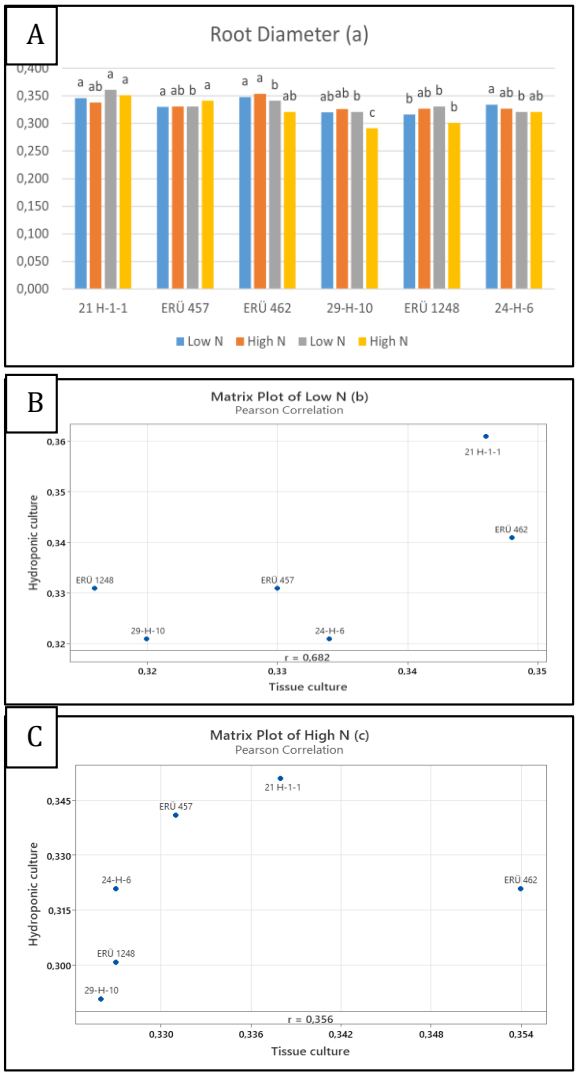

Figure 8. (a) Root diamater of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (mm) and hydroponic culture (mm) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

Root volume varied considerably between genotypes and cultivation systems, with notably higher values recorded in hydroponic culture compared to tissue culture under both nitrogen conditions (Figure 9 a). Correlation analysis showed a moderate positive relationship between tissue culture and hydroponic culture under low nitrogen conditions (r = 0.641, Figure 9 b), suggesting some consistency in genotype responses. However, the correlation became stronger under high nitrogen conditions (r = 0.890, Figure 9 c), indicating that root volume responded more similarly across systems when nitrogen was not a limiting factor.

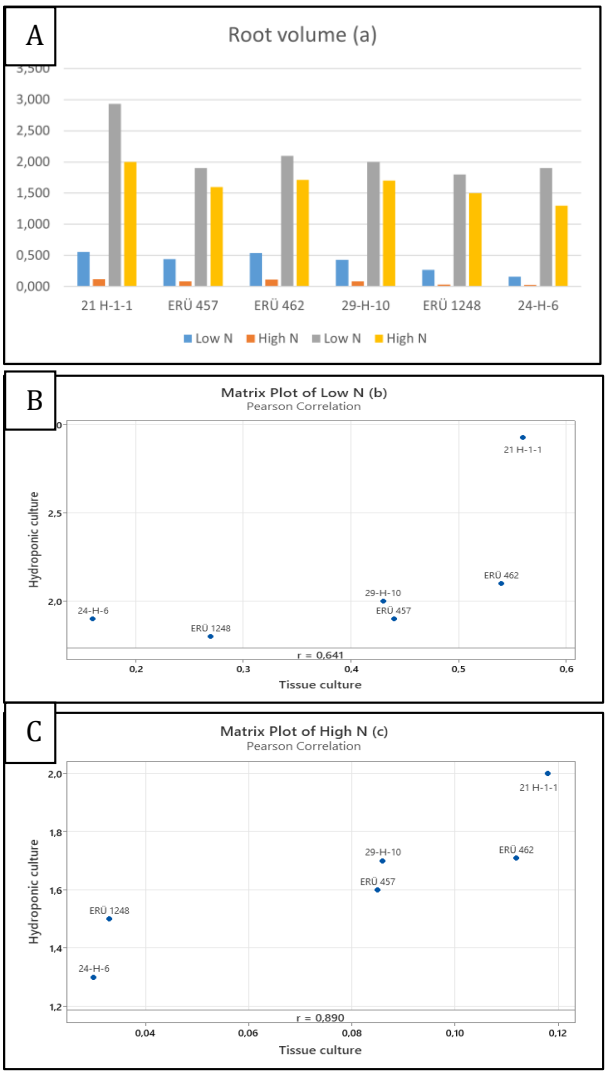

Figure 9. (a) Root volüme of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (m^3x100) and hydroponic culture (m^3) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

Nitrogen (N) concentration in pepper plants showed clear differences between nitrogen treatments and cultivation systems (Figure 10 a). Higher nitrogen concentrations

were observed under high N conditions across all genotypes, with especially elevated levels in the hydroponic system. 21 H-1-1, ERÜ 457, and ERÜ 462 tended to accumulate more nitrogen. Correlation analysis revealed a strong positive relationship between tissue and hydroponic culture systems under low nitrogen conditions (r = 0.814, Figure 10 b), suggesting consistent nitrogen uptake behavior across systems when nitrogen was limited. However, under high nitrogen conditions, this correlation decreased sharply to r = -0.115 (Figure 10 c), indicating that genotype responses to nitrogen availability diverged between systems when nitrogen was abundant.

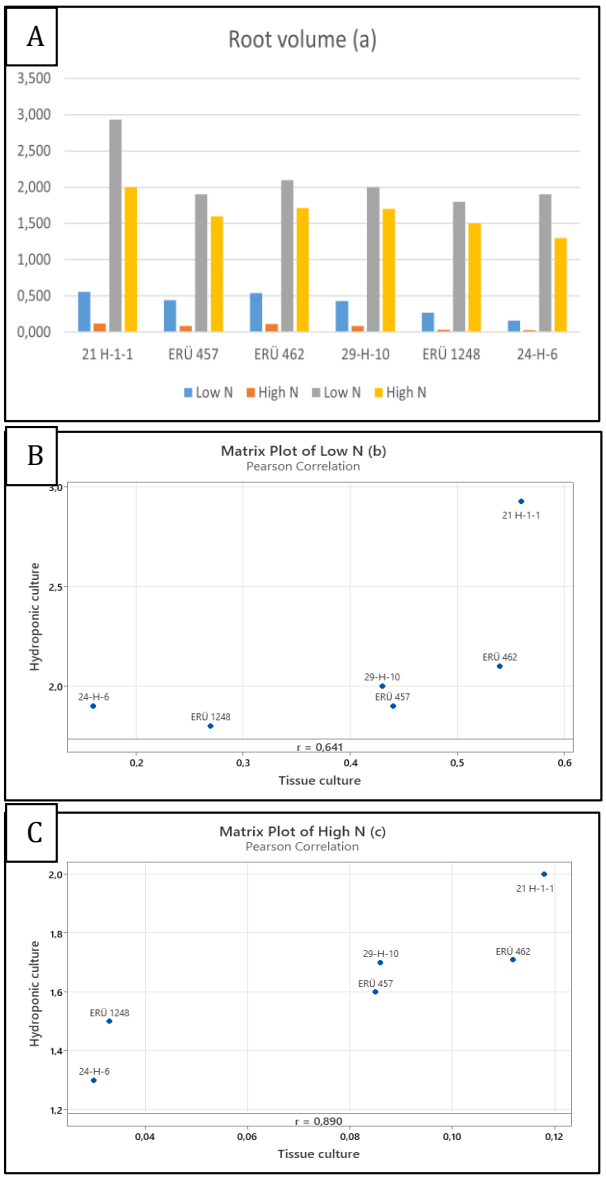

Figure 9. (a) Root volüme of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (m^3x100) and hydroponic culture (m^3) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

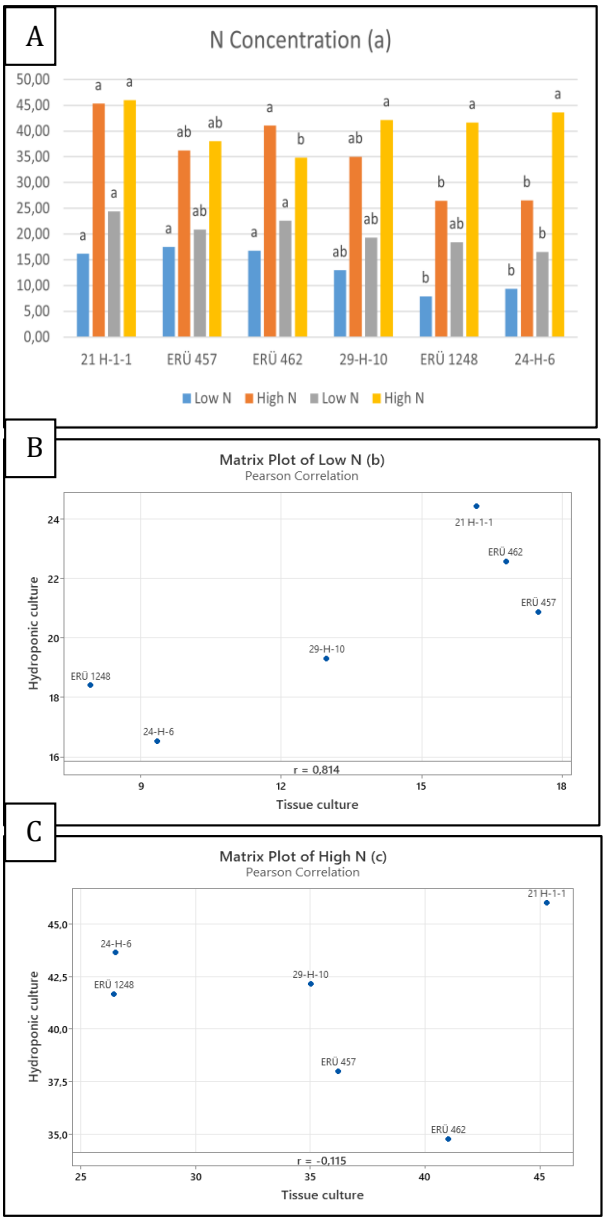

Figure 10. (a) N concentration of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (mg g^{-1} DW) and hydroponic culture (mg g^{-1} DW) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

Total nitrogen (N) content varied notably across genotypes and growing systems, with significantly higher values recorded under high nitrogen supply in both tissue culture and hydroponic culture conditions (Figure 11 a). 21 H-1-1, ERÜ 457, and ERÜ 462 exhibited the highest total N accumulation, particularly under high nitrogen levels. Correlation analysis showed a moderate positive relationship between tissue and hydroponic systems under low nitrogen conditions (r = 0.669, Figure 11 b), indicating a fair level of consistency in total N accumulation across environments when nitrogen was limited. However, under high nitrogen conditions, the

correlation weakened (r = 0.349, Figure 11 c), suggesting increased variation in genotype responses depending on the cultivation system.

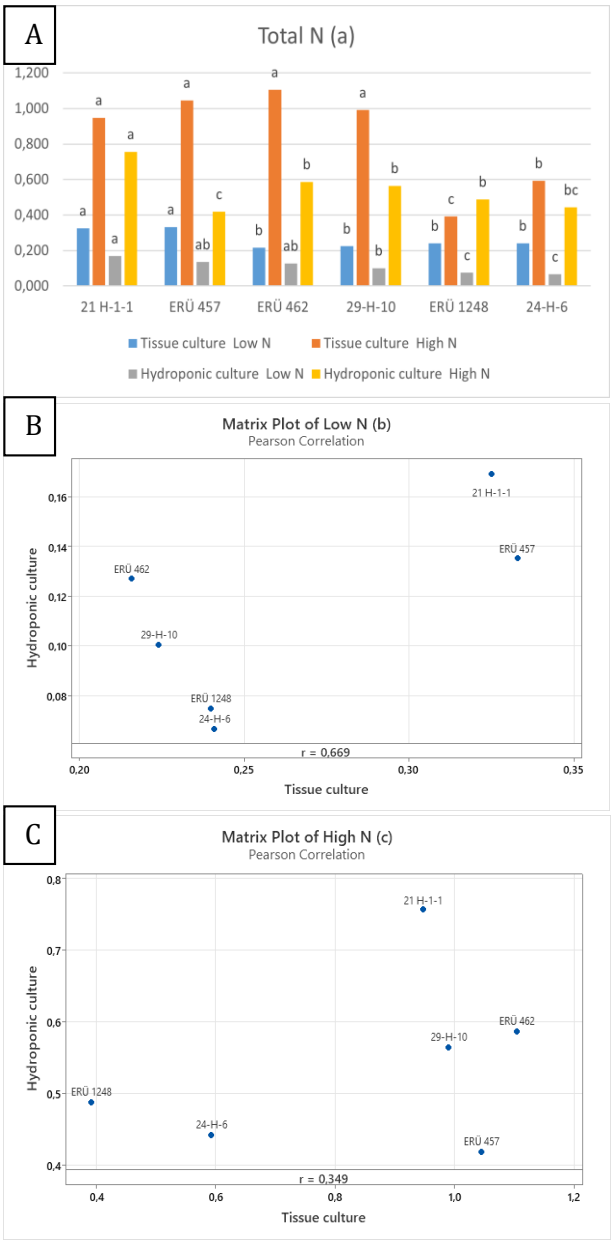

Figure 11. (a) Total N of pepper genotypes grown under different nitrogen levels (low and high N) in tissue culture (mg g^{-1} DW) and hydroponic culture (mg g^{-1} DW) conditions; (b) correlation between tissue culture and hydroponic culture under low nitrogen conditions, and (c) correlation between the two culture systems under high nitrogen conditions. Means that do not share a letter are significantly different.

3.1. Evaluation of Genotypic Consistency Across Growing Systems (Under Low Nitrogen Conditions)

Under low nitrogen conditions, a comparison of all measured traits—including main stem length, shoot/root fresh and dry weight, root length, leaf area, root volume, nitrogen concentration, and total nitrogen—revealed that genotypes with high and low values generally showed similar rankings in both tissue culture and hydroponic systems. This indicates that genotypic responses to low nitrogen availability were stable and largely independent of the cultivation environment. Notably, 21 H-1-1, ERÜ 462, and ERÜ 457 consistently demonstrated high performance across multiple parameters in both systems under low nitrogen. Conversely, 24-H-6, 29-H-10 and ERÜ 1248 exhibited limited development, ranking among the lowest-performing genotypes in both conditions. These findings suggest that, under nitrogen-deficient conditions, the influence of environmental factors on genotype selection is limited, and that high-performing genotypes can maintain consistent performance across different production systems. Therefore, these genotypes can be considered as promising candidates for use in breeding programs and nitrogen management studies, particularly in systems where multi-environment stability is essential (Figure 12).

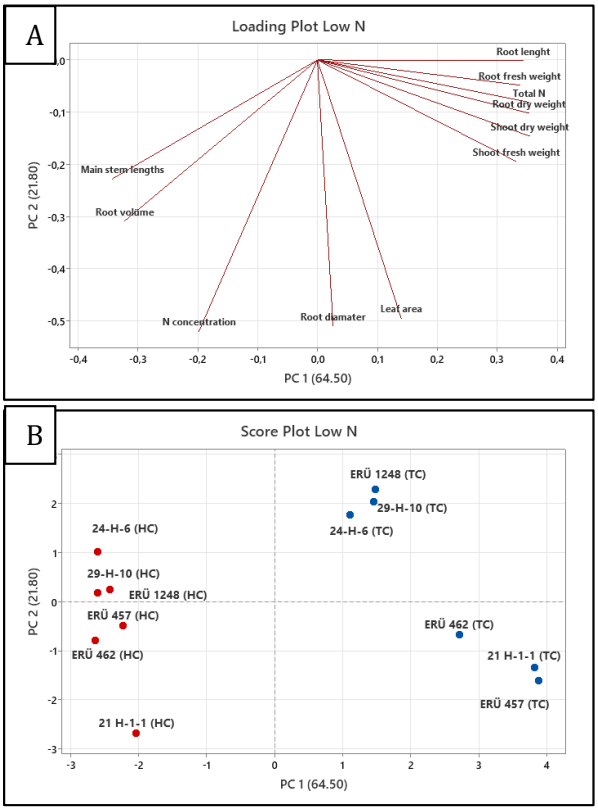

Figure 12. Principal Component Analysis (PCA) of pepper genotypes under low nitrogen conditions. (a) Loading plot showing the contribution of morphological and physiological traits to PC1 and PC2; (b) score plot showing the distribution of genotypes grown in tissue culture (TC) and hydroponic culture (HC) systems based on trait expression.

3.2. Evaluation of Genotypic Performance Consistency Across Growing Systems (Under High Nitrogen Conditions)

Under high nitrogen conditions, the evaluation of all measured traits—including main stem length, shoot/root fresh and dry weight, root length, leaf area, root volume,

nitrogen concentration, and total nitrogen—revealed a more differentiated performance among genotypes across tissue culture and hydroponic systems. While some genotypes showed similar rankings in both environments, others responded differently depending on the cultivation system. This indicates that under high nitrogen availability, system-specific factors may have a greater influence on genotype performance. Notably, 21 H-1-1, ERÜ 462, and ERÜ 457 demonstrated strong and consistent performance across multiple traits in the tissue culture system, ranking among the top-performing genotypes under high nitrogen conditions. In contrast, the same genotypes grown in hydroponic culture exhibited relatively lower performance for similar traits, indicating divergence in genotypic expression between the two systems. These findings suggest that genotype performance under high nitrogen supply may be more strongly influenced by the growing system. Therefore, when selecting genotypes for production environments with high nitrogen availability, system-specific responses should be considered. Nevertheless, genotypes such as 21 H-1-1 and ERÜ 462, which performed well under tissue culture conditions, may still be regarded as promising candidates in breeding programs aiming to improve nitrogen use efficiency (Figure 13).

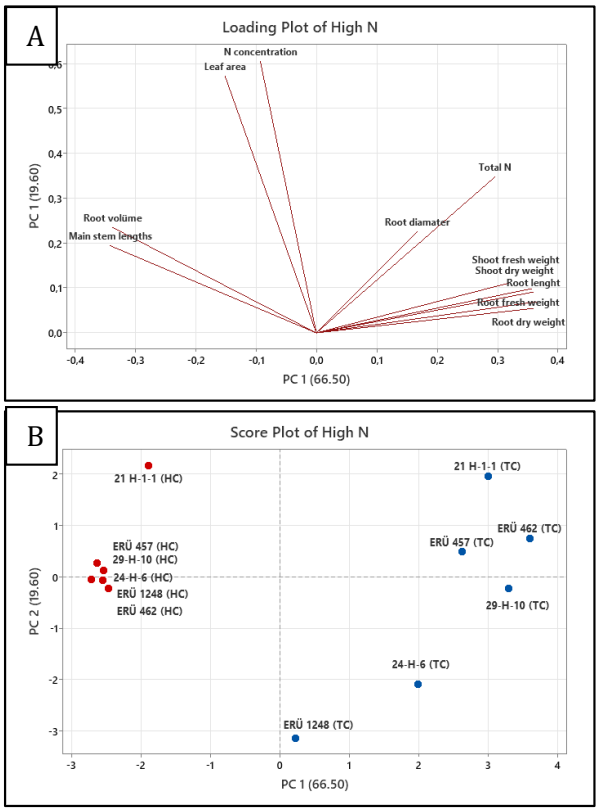

Figure 13. Principal Component Analysis (PCA) of pepper genotypes under high nitrogen conditions. (a) Loading plot showing the contribution of morphological and physiological traits to PC1 and PC2; (b) score plot showing the distribution of genotypes grown in tissue culture (TC) and hydroponic culture (HC) systems based on trait expression.

4. Discussion

Nitrogen is a key macronutrient influencing plant growth and development, especially under controlled cultivation systems. The results of this study demonstrated that nitrogen availability and the type of cultivation system (tissue culture vs. hydroponic culture) significantly affected the morphological and physiological traits of pepper genotypes. Under low nitrogen conditions, genotypes exhibited relatively stable and environment-independent performance, showing similar rankings in both systems. This suggests that low nitrogen environments, particularly in combination with tissue culture, may offer a cost-effective and reliable platform for early-stage genotype screening, especially in breeding programs with limited resources.

In contrast, under high nitrogen conditions, genotypic responses were more divergent between tissue culture and hydroponic systems, indicating that system-specific physiological factors such as nutrient uptake dynamics and root architecture may influence phenotypic expression. PCA analysis further supported these findings by clearly separating genotypes and traits based on nitrogen levels and cultivation systems. Traits related to biomass production such as shoot dry weight, root dry weight, and total nitrogen content were the primary contributors to variation, particularly in tissue culture under high nitrogen. Genotypes such as 21 H-1-1, ERÜ 462, and ERÜ 457 consistently performed well across environments and traits, highlighting their potential as stable and nitrogen-efficient candidates in breeding programs. These findings align with previous studies reporting that nitrogen positively influences biomass accumulation and vegetative growth (Ulas et al. 2019, 2021a, 2022; Jay et al. 2017; Zhang et al. 2020a, 2020b). Moreover, morphological root traits and physiological parameters such as root length, root diameter, and leaf chlorophyll content have been shown to play key roles in nitrogen uptake efficiency under stress conditions (Graciano et al. 2009). Our results also suggest that root diameter is a less nitrogen-sensitive trait compared to others and may be more influenced by the cultivation system, as supported by Barber and Silberbush (2015) and Föhse et al. (1988).

In addition to nitrogen stress, salt stress is another major limiting factor in crop productivity, particularly for sensitive crops like pepper. Therefore, there is an increasing need for practical and low-cost in vitro methods to identify salt-tolerant genotypes in a short time. Recent studies have shown that in vitro salt screening is a simple and inexpensive method that can be used to obtain reliable results rapidly (Pınar et al., 2024). For instance, the addition of ascorbic acid (AA) to in vitro cultures has been found to enhance salt tolerance by promoting callus growth under saline conditions, as observed in alfalfa (*Medicago sativa* L.) (Arab et al., 2006). Taken together, these results emphasize the importance of considering both abiotic stress factors and cultivation systems in plant breeding strategies. Tissue culture

systems, when used under controlled low-input stress conditions such as limited nitrogen or salt, can serve as a powerful selection platform for identifying genotypes with improved nutrient use efficiency and stress tolerance.

5. Conclusion

This study demonstrated that pepper genotypes responded differently to nitrogen availability and cultivation systems across a range of morphological and physiological traits. Under low nitrogen conditions, genotypes generally showed stable performance across both tissue culture and hydroponic systems, indicating environment-independent responses. In particular, 21 H-1-1, ERÜ 462, and ERÜ 457 consistently exhibited high performance, while genotypes such as 24-H-6 and ERÜ 1248 performed poorly under both conditions. These results suggest that selection under low nitrogen stress can effectively identify genotypes with stable and efficient nitrogen use across systems. In contrast, under high nitrogen conditions, genotypic performance was more influenced by the cultivation system. Tissue culture-grown plants generally showed greater biomass accumulation and nitrogen uptake compared to hydroponically grown plants. This highlights the importance of considering system-specific responses when selecting genotypes for environments with abundant nitrogen. Overall, 21 H-1-1 and ERÜ 462 were identified as promising candidates for future breeding efforts aimed at improving nitrogen use efficiency. Moreover, if cost-effective selection is a priority, early-stage screening under low nitrogen using tissue culture may provide a practical and reliable approach.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	A.A.	H.P.	F.U.	H.Y.	M.S	H.A.
C	20	20	15	15	15	15
D	20	20	15	15	15	15
S	20	20	15	15	15	15
DCP	20	20	15	15	15	15
DAI	20	20	15	15	15	15
L	20	20	15	15	15	15
W	20	20	15	15	15	15
CR	20	20	15	15	15	15
SR	20	20	15	15	15	15
PM	20	20	15	15	15	15
FA	20	20	15	15	15	15

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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