

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(7): 993-997
www.biochemjournal.com
Received: 16-05-2026
Accepted: 19-06-2026

Jayanth KV
(1) Department of Vegetable Science,
College of Horticulture, Bengaluru,
Karnataka, India
(2) ICAR-Indian Institute of
Horticultural Research, Bengaluru,
Karnataka, India

HC Prasanna
ICAR-Indian Institute of Horticultural
Research, Bengaluru, Karnataka, India

CN Hanchinamani
Department of Vegetable Science, College
of Horticulture, Mysuru, Karnataka,
India

Rao VK
ICAR-Indian Institute of Horticultural
Research, Bengaluru, Karnataka, India

Shivanand Hongal
Krishi Vigyan Kendra, Kolar,
Karnataka, India

V Sridhar
ICAR-Indian Institute of Horticultural
Research, Bengaluru, Karnataka, India

G Basavaraj
Department of Agricultural Economics,
UHS, Dharwad, Karnataka, India

H Amarananjundeswara
Department of Vegetable Science, College
of Horticulture, Bengaluru, Karnataka,
India

Abhishek V Karadagi
(1) ICAR-Indian Institute of
Horticultural Research, Bengaluru,
Karnataka, India
(2) Department of Genetics and Plant
Breeding, UHS, Dharwad, Karnataka,
India

Darshan D
ICAR-Indian Institute of Horticultural
Research, Bengaluru, Karnataka, India

Siddu CB
Central Tasar Research & Training
Institute, Jharkhand, India

Nikhil G
ICAR-Indian Institute of Horticultural
Research, Bengaluru, Karnataka, India

Hrudya Biji
ICAR-Indian Institute of Horticultural
Research, Bengaluru, Karnataka, India

Ayesha Sheikh
ICAR-Indian Institute of Horticultural
Research, Bengaluru, Karnataka, India

Corresponding Author:
Jayanth KV
(1) Department of Vegetable Science,
College of Horticulture, Bengaluru,
Karnataka, India
(2) ICAR-Indian Institute of
Horticultural Research, Bengaluru,
Karnataka, India

Characterization and diversity analysis of leaf nitrate content among tomato wild relatives

Jayanth KV, HC Prasanna, CN Hanchinamani, Rao VK, Shivanand Hongal, V Sridhar, G Basavaraj, H Amarananjundeswara, Abhishek V Karadagi, Darshan D, Siddu CB, Nikhil G, Hrudya Biji and Ayesha Sheikh

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7SI.9167>

Abstract

Wild tomato relatives represent an important reservoir of genetic diversity for improving physiological traits associated with nitrogen use efficiency. However, information on natural variation in leaf nitrate accumulation among wild tomato germplasm remains limited. The present study aimed to characterize leaf nitrate content and assess biochemical diversity among sixteen wild tomato accessions and one cultivated genotype. The experiment was conducted during two growing seasons in a randomized complete block design with two biological replications. Leaf nitrate concentration was quantified using high-performance liquid chromatography (HPLC), and the resulting data were subjected to analysis of variance, descriptive statistics, hierarchical cluster analysis, and cluster validation. Analysis of variance revealed that season significantly influenced leaf nitrate concentration ($p < 0.001$), whereas genotype effects were not significant, indicating that environmental conditions had a greater influence on nitrate accumulation than stable genetic differences. Nevertheless, substantial phenotypic variation was observed, with nitrate concentration ranging from $0.252 \text{ g } 100 \text{ g}^{-1} \text{ DW}$ to $0.930 \text{ g } 100 \text{ g}^{-1} \text{ DW}$ and a mean of $0.459 \text{ g } 100 \text{ g}^{-1} \text{ DW}$. Hierarchical clustering classified the accessions into three distinct groups representing low, intermediate, and high nitrate accumulators. Cluster validation statistics, including a cophenetic correlation coefficient of 0.871 and an average silhouette width of 0.660, confirmed the robustness of the clustering pattern. Accessions LA0735, LA1994, and LA1910 exhibited the highest nitrate concentrations, whereas LA0530, LA0462, and LA0372 recorded the lowest values. Although nitrate accumulation was strongly influenced by seasonal conditions, the observed phenotypic diversity among wild tomato accessions provides valuable germplasm for future physiological studies and breeding programmes targeting improved nitrogen use efficiency and sustainable tomato production.

Keywords: Nitrate content, wild accessions

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops worldwide because of its high nutritional value, economic importance, and extensive utilization in both fresh and processed forms (FAOSTAT, 2024; Raiola *et al.*, 2014) [4, 13]. It is an excellent source of vitamins, minerals, carotenoids, phenolic compounds, flavonoids, and dietary antioxidants that contribute significantly to human health (Raiola *et al.*, 2014; Klee and Tieman, 2013) [13, 7]. In addition to its nutritional importance, tomato serves as a model species for studies in genetics, physiology, and genomics owing to its relatively small genome and well-established genetic resources (The Tomato Genome Consortium, 2012) [16]. Continuous breeding efforts have resulted in substantial improvements in yield, fruit quality, and resistance to various biotic and abiotic stresses (Foolad, 2007) [5]. However, domestication and intensive selection have considerably narrowed the genetic diversity of cultivated tomato, thereby limiting the availability of novel alleles governing physiological efficiency and nutrient metabolism (Miller and Tanksley, 1990; Blanca *et al.*, 2015) [10, 1]. Wild tomato relatives belonging to the *Solanum* section *Lycopersicon* constitute an invaluable reservoir of genetic diversity for crop improvement. Species such as *Solanum habrochaites*, *S. pennellii*, *S. chilense*, *S. peruvianum*, and *S. pimpinellifolium* exhibit remarkable variation in morphology, physiology, adaptation, and biochemical composition owing to their evolution under diverse ecological environments (Peralta *et al.*, 2008) [12].

These species have contributed numerous genes for disease resistance, abiotic stress tolerance, improved fruit quality, and enhanced adaptation to cultivated tomato (Rick, 1973)^[14]. Beyond these agronomically important traits, wild relatives also possess substantial diversity in nutrient metabolism, making them potential sources of improved nutrient use efficiency.

Nitrogen is one of the most important macronutrients required for plant growth and productivity because it forms the structural basis of amino acids, proteins, nucleic acids, chlorophyll, enzymes, and numerous secondary metabolites. Among the different forms of nitrogen available in soil, nitrate (NO_3^-) is the predominant source absorbed by higher plants under aerobic conditions. Following uptake by root nitrate transporters, nitrate is translocated to aerial tissues where it is reduced sequentially by nitrate reductase and nitrite reductase before being assimilated into amino acids through the glutamine synthetase-glutamate synthase pathway (Marschner, 2012)^[8]. The efficiency of nitrate uptake and assimilation directly influences photosynthesis, biomass accumulation, crop productivity, and nitrogen use efficiency. Leaf nitrate concentration reflects the balance between nitrate uptake, transport, reduction, and assimilation within plant tissues and is therefore considered an important physiological indicator of nitrogen metabolism. Significant variation in nitrate accumulation has been reported among plant species and genotypes due to differences in nitrate transporter activity, nitrate reductase activity, root architecture, environmental adaptation, and genetic regulation of nitrogen metabolism (Smart and Bloom, 1988)^[15]. Understanding this natural variation is particularly important because improving nitrogen use efficiency has become a major objective in sustainable agriculture to reduce fertilizer inputs and minimize environmental pollution caused by excessive nitrogen application.

Although numerous studies have investigated nitrogen metabolism in cultivated tomato, information regarding natural variation in leaf nitrate accumulation among tomato wild relatives remains limited. Most previous investigations have focused on fruit quality attributes, disease resistance, or molecular diversity, while biochemical characterization of wild germplasm for nitrate content has received comparatively little attention. Characterizing leaf nitrate content in diverse wild accessions may therefore provide valuable insights into physiological diversity and facilitate the identification of genetically superior accessions possessing efficient nitrogen metabolism. The present investigation was undertaken to characterize tomato wild relatives for leaf nitrate content and to assess the extent of biochemical diversity among different accessions. The study is expected to identify promising donor genotypes with desirable nitrate profiles that can be utilized in future breeding programmes aimed at improving nitrogen use efficiency and physiological performance in cultivated tomato.

Materials and Methods

Plant materials

Seventeen tomato genotypes comprising sixteen wild tomato accessions and one cultivated genotype were used in the present investigation. The wild accessions included LA2172 (*Solanum arcanum*), LA1409 (*S. cheesmaniae*), LA1970 (*S. chilense*), LA2695 (*S. chmielewskii*), LA1910 (*S.*

corneliomulleri), LA0530 (*S. galapagense*), LA1777 and LA1718 (*S. habrochaites*), LA2808 (*S. huaylasense*), LA0735 (*S. neorickii*), LA1940 (*S. pennellii*), LA0372, LA0462 and LA1955 (*S. peruvianum*), LA2934 (*S. pimpinellifolium*), and LA1994 (*S. lycopersicum* var. *cerasiforme*). The cultivated tomato genotype IHR38-7 (*S. lycopersicum*) was included as the cultivated check. The experiment was conducted during two growing seasons, March and August, following a randomized complete block design with two biological replications. For each genotype, fully expanded healthy leaves were collected from ten randomly selected plants during each season. To obtain representative biological replicates, leaves from five plants were pooled to constitute the first replication, while leaves from the remaining five plants were pooled to constitute the second replication. The pooled leaf samples were immediately transported to the laboratory, washed thoroughly with distilled water to remove surface contaminants, blotted dry, and processed for biochemical analyses. For nitrate and total oxalate estimation, the samples were oven-dried at 60 °C until constant weight, ground into a fine powder using a laboratory grinder, and stored in airtight containers until further analysis.

Extraction of Sample

Freshly harvested plants were washed with tap water followed by distilled water and spread on the blotting paper. Then the leaves were taken in brown paper bags and kept in oven for drying. They were dried at 50 °C for 2 days and then powdered using a mixer mill. Sample powder (200 mg) was taken in 100mL volumetric flask, to which 50 mL of distilled water was added and heated in a waterbath at 80 °C for 20 minutes with occasional shaking. Then the volumetric flask was made up to the volume with distilled water. The above solution was filtered through 0.2µm nylon filter membrane and injected in to HPLC.

HPLC analysis

Nitrates were analyzed by adopting the method of Chou *et al* (2003)^[2] with minor modifications. The HPLC analyses were carried out on a Shimadzu Series LC-10A system (Shimadzu, Kyoto, Japan) consisting of a liquid chromatograph connected to a UV-VIS detector (10 A), binary pump and controlled by LC solution Workstation software. The column used was Synergy Hydro RP, 250 x 4.6 mm, 4 µm (Phenomenex, USA) with security guard cartridge (cat no: AJO 7511, Phenomenex, CA, USA). Samples were injected using an autosampler (SIL-20A HT, Shimadzu). The column and guard column were thermostatically controlled at 32 °C. The flow rate was 0.8 ml/min and mobile phase consisted of 0.01 M Octylammonium phosphate and methanol (70:30). The instrument was run in an isocratic mode. The detection was monitored at 213 nm. The mobile phase solution was allowed to pass through the HPLC column for 2-3 hours until a stable baseline signal was obtained. When the injections of the standard solution gave reproducible retention times and peak areas, each sample solution were then injected for analysis. The amount of nitrate in the test solution was calculated from the peak areas by using linear regression equations of sodium nitrate standard curves. Leaf nitrate concentration was expressed as g 100 g⁻¹ dry weight (DW). The injection volume was 20µL.

Recovery Test

A series of various concentrations of 1,10,50 and 100 µg/mL standard solutions containing nitrate was spiked into Amaranth samples. Each concentration spiked was analyzed in triplicate, including a blank test to evaluate the average recoveries. The average recoveries were in the range of 97 to 101%.

Results

Analysis of Variance

Analysis of variance revealed that season had a significant effect on nitrate concentration ($p < 0.001$), whereas genotype effects were not significant ($P = 0.095$), indicating that nitrate accumulation was influenced predominantly by seasonal variation rather than stable genetic differences among the evaluated wild tomato accessions (Table 1).

Table 1: Analysis of variance for nitrate concentration.

Source	df	Mean square	P-value
Genotype	16	0.154	0.095
Season	1	10.949	<0.001
Replication	1	0.050	0.469
Error	49	0.094	-

Descriptive Statistics

The mean leaf nitrate concentration of the seventeen wild tomato accessions was 0.459 g 100 g⁻¹ DW, with values ranging from 0.252 to 0.930 g 100 g⁻¹ DW. The standard deviation was 0.196 for nitrate accumulation among the evaluated germplasm. The frequency distribution exhibited slight positive skewness (1.00) with near-normal kurtosis (-0.01), suggesting moderate variability without extreme departures from normality as presented in Table 2.

Table 2: Descriptive statistics for mean leaf nitrate concentration (g 100 g⁻¹ DW) across seasons.

Genotype	Mean
LA0372	0.266
LA2172	0.485
LA1777	0.283
LA1970	0.382
LA2934	0.519
LA1955	0.347
LA2695	0.474
LA0462	0.26
LA1994	0.817
LA1718	0.454
IIHR 38-7	0.52
LA1940	0.461
LA2808	0.356
LA0735	0.93
LA1910	0.692
LA0530	0.252
LA1409	0.311
Number of accessions	17
Mean	0.459
Median	0.454
Standard deviation	0.196
Variance	0.038
Range	0.252-0.930

Hierarchical Cluster Analysis

Hierarchical clustering based on Euclidean distance using Ward's minimum variance method classified the seventeen wild tomato accessions into three distinct clusters according to nitrate concentration (g 100 g⁻¹ DW). Cluster I comprised six accessions with intermediate nitrate concentrations,

Cluster II contained eight accessions characterized by comparatively low nitrate concentrations, whereas Cluster III included three accessions exhibiting the highest nitrate concentrations. The clustering pattern demonstrated clear phenotypic diversity among the evaluated wild tomato germplasm as presented in Table 3 and Fig 1.

Table 3: Cluster composition of wild tomato accessions based on nitrate concentration.

Cluster	Number of accessions	Accessions
I	6	IIHR 38-7, LA1718, LA1940, LA2172, LA2695, LA2934
II	8	LA0372, LA0462, LA0530, LA1409, LA1777, LA1955, LA1970, LA2808
III	3	LA0735, LA1910, LA1994

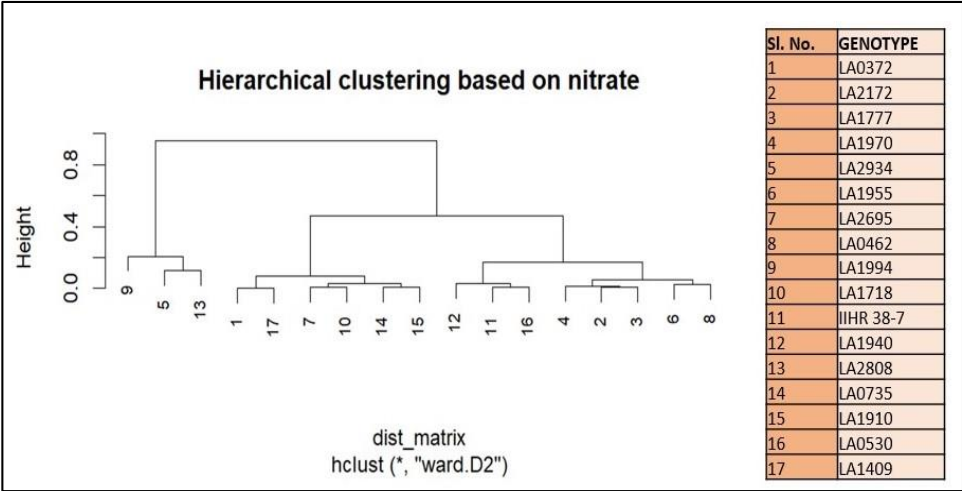


Fig 1: Dendrogram Showing Phenotypic Diversity for Nitrate Content (g 100 g⁻¹ DW) in Wild Tomato Accessions

Cluster Characteristics

Distinct differences in nitrate concentration were observed among the three clusters. Cluster II recorded the lowest mean nitrate concentration (0.307 g 100 g⁻¹ DW), whereas Cluster III exhibited the highest mean nitrate concentration (0.813 g 100 g⁻¹ DW). Cluster I showed intermediate nitrate values with a comparatively narrow range of variation (Table 4.).

Table 4: Cluster statistics for nitrate concentration.

Cluster	Number of accessions	Mean nitrate (g 100 g ⁻¹ DW)	Minimum	Maximum	SD
I	6	0.485	0.454	0.520	0.028
II	8	0.307	0.252	0.382	0.049
III	3	0.813	0.692	0.930	0.119

Cluster Validation

The reliability of the clustering pattern was evaluated using

the cophenetic correlation coefficient and silhouette analysis. The cophenetic correlation coefficient was 0.871, indicating that the dendrogram accurately represented the original Euclidean distance matrix. Furthermore, the average silhouette width of 0.660 suggested good separation among the three clusters, supporting the robustness of the clustering pattern as presented in Fig 2.

Ranking of Wild Tomato Accessions

Marked variation in nitrate concentration was observed among the wild tomato accessions. The lowest nitrate concentration was recorded in LA0530 (0.252 g 100 g⁻¹ DW), followed by LA0462 (0.260 g 100 g⁻¹ DW) and LA0372 (0.266 g 100 g⁻¹ DW). Conversely, the highest nitrate concentrations were observed in LA0735 (0.930 g 100 g⁻¹ DW), LA1994 (0.816 g 100 g⁻¹ DW) and LA1910 (0.692 g 100 g⁻¹ DW), identifying these accessions as the most contrasting for nitrate accumulation.

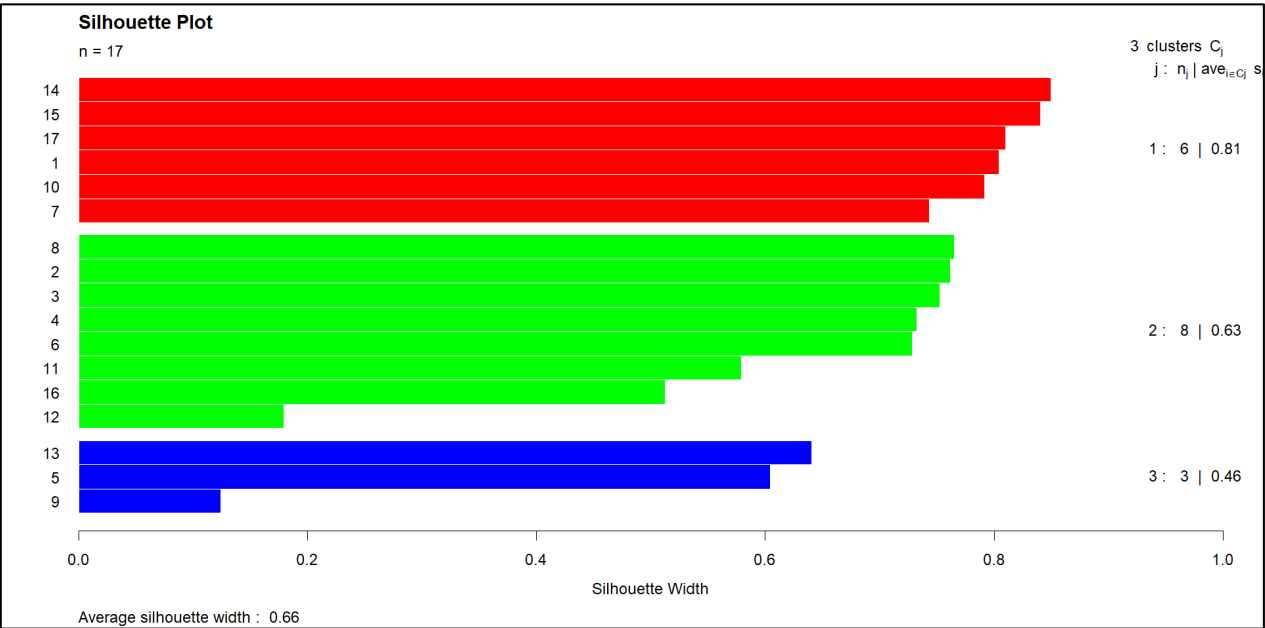


Fig 2: Silhouette Plot for Hierarchical Clustering Based on Total Nitrate (g 100 g⁻¹ DW)

Discussion

Leaf nitrate concentration was influenced predominantly by seasonal environmental conditions, whereas genotype effects were not statistically significant, indicating that

environmental factors exert a stronger influence on nitrate accumulation than stable genetic differences among the evaluated accessions. Nitrate uptake and assimilation are highly responsive to temperature, light intensity, soil

moisture and nitrogen availability, all of which regulate nitrate transporter activity and nitrate reductase-mediated assimilation (Marschner, 2012; Crawford and Glass, 1998) [8, 3]. Similar environmental regulation of nitrate accumulation has been reported in tomato and other crop species (Smart and Bloom, 1988) [15]. Despite the non-significant genotype effect, the evaluated germplasm exhibited nearly a four-fold variation in nitrate concentration (0.252-0.930 g 100 g⁻¹ DW), suggesting the presence of useful phenotypic diversity. Such variability is expected for physiological traits governed by multiple genes and influenced by environmental conditions (Hirel *et al.*, 2007) [6]. The positive skewness further indicates that only a few accessions accumulated high nitrate levels, providing valuable contrasting material for future physiological and genetic studies. Hierarchical clustering grouped the accessions into three distinct clusters with low, intermediate, and high nitrate concentrations, demonstrating meaningful biochemical diversity among the wild tomato germplasm. The high cophenetic correlation coefficient (0.871) and average silhouette width (0.660) confirmed the reliability of the clustering pattern, indicating good agreement between the dendrogram and the original distance matrix. Such clustering facilitates the identification of genetically contrasting accessions for parent selection and germplasm utilization (Mohammadi and Prasanna, 2003) [11]. The identification of LA0735, LA1994, and LA1910 as high nitrate accumulators and LA0530, LA0462, and LA0372 as low accumulators provides promising genetic resources for investigating nitrogen metabolism. However, leaf nitrate concentration alone does not necessarily indicate superior nitrogen use efficiency because high nitrate levels may result from enhanced uptake or reduced nitrate assimilation. Therefore, complementary evaluation of nitrate reductase activity, total nitrogen, and related physiological traits would provide a more comprehensive understanding of nitrogen metabolism (Xu *et al.*, 2012; Masclaux-Daubresse *et al.*, 2010) [17, 9]. Overall, the present study demonstrates that environmental conditions largely determine leaf nitrate accumulation, while the observed phenotypic diversity among wild tomato accessions offers valuable material for future breeding and physiological studies aimed at improving nitrogen use efficiency.

Conclusion

The present study demonstrated that leaf nitrate accumulation in tomato is influenced primarily by seasonal environmental conditions, while appreciable phenotypic variation exists among wild tomato relatives. Despite the non-significant genotype effect, hierarchical clustering identified distinct groups of low, intermediate, and high nitrate accumulators, highlighting the biochemical diversity present within the evaluated germplasm. Accessions LA0735, LA1994, and LA1910 were identified as high nitrate accumulators, whereas LA0530, LA0462, and LA0372 exhibited consistently low nitrate concentrations, making them valuable contrasting genetic resources. These findings provide a useful foundation for future investigations integrating physiological, biochemical, and molecular approaches to elucidate the genetic basis of nitrogen metabolism and to develop tomato cultivars with improved nitrogen use efficiency and reduced fertilizer requirements.

Reference

1. Blanca J, Cañizares J, Roig C, Ziaresolo P, Nuez F. Variation revealed by SNP genotyping and morphology provides insight into the origin of the tomato. *PLoS ONE*. 2015;10(10):e0139937.
2. Chou SS, Chung JC, Hwang DF. A high performance liquid chromatography method for determining nitrate and nitrite levels in vegetables. *Journal of Food and Drug Analysis*. 2003;11(3):233-238.
3. Crawford NM, Glass ADM. Molecular and physiological aspects of nitrate uptake in plants. *Trends in Plant Science*. 1998;3(10):389-395.
4. Food and Agriculture Organization of the United Nations. FAOSTAT statistical database. Rome: Food and Agriculture Organization of the United Nations; 2024.
5. Foolad MR. Current status of breeding tomatoes for salt and drought tolerance. *Plant Breeding*. 2007;126(1):1-11.
6. Hirel B, Le Gouis J, Ney B, Gallais A. The challenge of improving nitrogen use efficiency in crop plants. *Journal of Experimental Botany*. 2007;58(9):2369-2387.
7. Klee HJ, Tieman DM. Genetic challenges of flavor improvement in tomato. *Trends in Genetics*. 2013;29(4):257-262.
8. Marschner P, editor. Marschner's Mineral Nutrition of Higher Plants. 3rd ed. London: Academic Press; 2012.
9. Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany*. 2010;105(7):1141-1157.
10. Miller JC, Tanksley SD. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theoretical and Applied Genetics*. 1990;80(4):437-448.
11. Mohammadi SA, Prasanna BM. Analysis of genetic diversity in crop plants: Salient statistical tools and considerations. *Crop Science*. 2003;43(4):1235-1248.
12. Peralta IE, Spooner DM, Knapp S. Taxonomy of wild tomatoes and their relatives (*Solanum* section *Lycopersicoides*, section *Juglandifolia*, section *Lycopersicon*). *Systematic Botany Monographs* No. 84. St. Louis: American Society of Plant Taxonomists; 2008.
13. Raiola A, Rigano MM, Calafiore R, Frusciante L, Barone A. Enhancing the health-promoting effects of tomato fruit for biofortified food. *Frontiers in Plant Science*. 2014;5:283.
14. Rick CM. Potential genetic resources in tomato species: Clues from observations in native habitats. *Annual Review of Genetics*. 1973;7:253-269.
15. Smart DR, Bloom AJ. Kinetics of nitrate and ammonium uptake among wild and cultivated tomatoes. *Oecologia*. 1988;76(3):336-340.
16. The Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*. 2012;485(7400):635-641.
17. Xu G, Fan X, Miller AJ. Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*. 2012;63:153-182.