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Characterization and diversity analysis of total oxalate content among tomato wild relatives

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Abstract

Wild tomato relatives constitute an important source of genetic variation for improving nutritional and physiological traits in cultivated tomato. However, information on natural variation in leaf oxalate content among wild tomato germplasm remains limited. The present study aimed to characterize total oxalate content and assess biochemical diversity among sixteen wild tomato accessions and one cultivated genotype. The experiment was conducted during two growing seasons using a randomized complete block design with two biological replications. Total oxalate content was quantified by high-performance liquid chromatography (HPLC), and the data were analyzed using analysis of variance, descriptive statistics, hierarchical cluster analysis, and cluster validation. Analysis of variance revealed highly significant genotypic differences for total oxalate content ($P < 0.001$), whereas season and replication effects were non-significant, indicating that oxalate accumulation was primarily under genetic control and remained stable across seasons. Total oxalate concentration ranged from 1.45 to 3.53 g 100 g⁻¹ DW with an overall mean of 2.43 g 100 g⁻¹ DW. Hierarchical clustering classified the accessions into three distinct groups representing low, intermediate, and high oxalate accumulators. Cluster validation demonstrated satisfactory clustering reliability with a cophenetic correlation coefficient of 0.799 and an average silhouette width of 0.520. Accessions LA1409, LA2172, and LA2695 exhibited the highest oxalate concentrations, whereas LA1994, LA1777, and LA1910 recorded the lowest values. The observed genetic diversity provides valuable germplasm for investigating oxalate metabolism and offers potential donor parents for breeding tomato cultivars with improved nutritional quality and mineral utilization.

Keywords: Total oxalate content, wild accessions

Introduction

Tomato (*Solanum lycopersicum* L.) is among the most important vegetable crops cultivated throughout the world because of its nutritional richness, economic value, and widespread utilization in fresh and processed products. Tomato fruits contain appreciable amounts of vitamins, carotenoids, minerals, organic acids, flavonoids, and antioxidant compounds that contribute to human health and nutrition (Raiola *et al.*, 2014) [7]. Despite significant advances in tomato improvement, cultivated tomato has undergone severe genetic erosion during domestication and modern breeding, resulting in reduced variability for many physiological and biochemical traits (Bai and Lindhout, 2007) [1]. Consequently, wild tomato relatives have emerged as valuable genetic resources for broadening the genetic base and improving crop performance. Wild tomato species exhibit remarkable diversity in morphology, physiology, biochemical composition, and environmental adaptation owing to their evolution under contrasting ecological conditions in South America (Peralta *et al.*, 2008) [6]. These species have been extensively exploited for improving disease resistance, abiotic stress tolerance, fruit quality, and adaptation in cultivated tomato. In recent years, increasing attention has been directed towards the biochemical characterization of wild germplasm because metabolite diversity often reflects important physiological and genetic differences that are not evident through morphological evaluation alone.

Among naturally occurring plant metabolites, oxalic acid represents one of the most important organic acids influencing both plant physiology and nutritional quality. Oxalates occur either as soluble salts or as insoluble calcium oxalate crystals and are synthesized through several metabolic pathways involving glyoxylate, glycolate, oxaloacetate, and ascorbate metabolism (Franceschi and Nakata, 2005) [12]. Calcium oxalate crystals are widely distributed in higher plants and play important physiological roles including regulation of intracellular calcium concentration, maintenance of ionic balance, detoxification of excess metal ions, storage of calcium, structural support, and protection against oxidative stress. The accumulation of oxalates varies considerably among plant species, organs, developmental stages, and environmental conditions, suggesting strong genetic regulation of oxalate metabolism. While oxalates contribute to normal physiological processes, excessive accumulation in edible plant tissues is considered undesirable because oxalic acid readily binds calcium to form insoluble calcium oxalate complexes, thereby reducing calcium bioavailability and increasing the risk of kidney stone formation in susceptible individuals (Noonan and Savage, 1999) [15]. Consequently, total oxalate content has become an important nutritional quality parameter in several vegetable crops. In tomato, research has primarily focused on fruit quality traits such as carotenoids, lycopene, sugars, organic acids, vitamin C, antioxidant activity, and phenolic compounds. Comparatively fewer studies have examined the extent of variation in oxalate content among wild tomato species, particularly in leaf tissues. Since oxalate metabolism is closely associated with calcium homeostasis and mineral nutrition, characterization of naturally occurring variation among wild germplasm may provide important information for understanding physiological diversity and identifying accessions with favourable biochemical characteristics. Evaluation of total oxalate content among genetically diverse wild relatives may also facilitate the identification of donor parents possessing desirable oxalate profiles for future breeding programmes aimed at improving nutritional quality and mineral metabolism. Furthermore, diversity analysis of oxalate accumulation may contribute to a better understanding of the evolutionary variation present within the tomato gene pool. Therefore, the present study was undertaken to characterize tomato wild relatives for total oxalate content and assess the extent of biochemical diversity among different accessions. The findings are expected to provide valuable information on natural variation in oxalate accumulation and contribute to the effective utilization of wild germplasm in tomato improvement programmes.

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: Finely ground leaf powder (200 mg) was taken in a 50 ml volumetric flask. 30 ml of 2N HCl was added to the flask and the flask was heated in a shaker water bath for 20 minutes at boiling temperature. The flask was made up to the mark with 2N HCl and shaken well. 10 ml of the extract was filtered in to a test tube through a filter paper. The extract was again filtered through 0.22 µm PTFE syringe filter (Himedia, 13 mm, cat No:SF17-2X75NO). The extract was further purified through SPE before injecting into HPLC.

Solid Phase Extraction (SPE)

Sample cleanup was performed with Strata C 18-E SPE cartridges (500 mg/3 ml, Phenomenex, USA, cat No.8B-S001-HBJ). Initially, the cartridges were conditioned with 3 mL methanol then equilibrated with 3 mL distilled water. The sample extract (1mL) was loaded on to the column. Then, the cartridge was eluted with 2 ml of 2N HCl. The eluants during loading of the sample and elution were collected in a 5 ml graduated tube. The solution was made up to 3 ml with 2N HCl and injected in to HPLC.

HPLC analysis: Oxalate content in the samples was estimated as per the method reported by Jham et.al (2002), 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, autosampler (SIL-20A HT) and controlled by LC solutions software. The column used was supelcogel C-610 H, 300 x 8.7 mm, ion exchange column (Supelco, USA) with a guard column. The injection volume was 10 µl. The column and guard column were thermostatically controlled at 30 °C. The flow rate was 0.5 ml/min and mobile phase consisted of 0.008 N sulphuric acid. The instrument was run in an isocratic mode. The detection was monitored at 215 nm. Oxalic acid was used as external standard. Total oxalate concentration was expressed as g 100 g⁻¹ dry weight (DW). RT of oxalic acid appeared at 9.8 minutes.

Results

Analysis of Variance

Analysis of variance revealed highly significant differences among wild tomato accessions for total oxalate content (P = 0.0018), indicating substantial genetic variation for this trait

(Table 1). In contrast, neither season nor replication significantly affected total oxalate concentration ($p > 0.05$), demonstrating that oxalate accumulation remained relatively

stable across seasons and that the experimental precision was satisfactory (Table 1.).

Table 1: Analysis of variance for total oxalate content.

Source	df	Mean square	P-value
Genotype	16	1.305	<0.001
Season	1	0.007	0.899
Replication	1	0.046	0.748
Error	49	0.440	-

Descriptive Statistics

The total oxalate concentration among the seventeen wild tomato accessions ranged from 1.45 to 3.53 g 100 g⁻¹ DW,

with an overall mean of 2.43±0.57 g 100 g⁻¹ DW. (Table 2). The distribution exhibited slight negative skewness (−0.29) and negative kurtosis (−0.77), as presented in Table 2.

Table 2: Mean total oxalate content (g 100 g⁻¹ DW) of tomato wild relatives and descriptive statistics across seasons.

Genotype	Overall Mean
LA0372	2.79
LA2172	3.03
LA1777	1.54
LA1970	2.22
LA2934	2.64
LA1955	2.31
LA2695	2.87
LA0462	1.83
LA1994	1.45
LA1718	2.67
IIHR 38-7	2.49
LA1940	2.7
LA2808	2.41
LA0735	2.82
LA1910	1.54
LA0530	2.58
LA1409	3.53
Number of accessions	17
Mean	2.432
Median	2.575
Standard deviation	0.571
Variance	0.326
Range	1.446-3.526

Hierarchical Cluster Analysis

Hierarchical cluster analysis using Euclidean distance and Ward's minimum variance method classified the seventeen wild tomato accessions into three distinct clusters (Figure X). Cluster I contained eight accessions with intermediate

oxalate concentrations, Cluster II consisted of five accessions characterized by relatively high total oxalate concentrations (g 100 g⁻¹ DW), whereas Cluster III comprised four accessions with consistently low oxalate concentrations as presented in Table 3 and Fig1.

Table 3: Cluster composition based on total oxalate content.

Cluster	Number of accessions	Accessions
I	8	IIHR 38-7, LA0530, LA1718, LA1940, LA1955, LA1970, LA2808, LA2934
II	5	LA0372, LA0735, LA1409, LA2172, LA2695
III	4	LA0462, LA1777, LA1910, LA1994

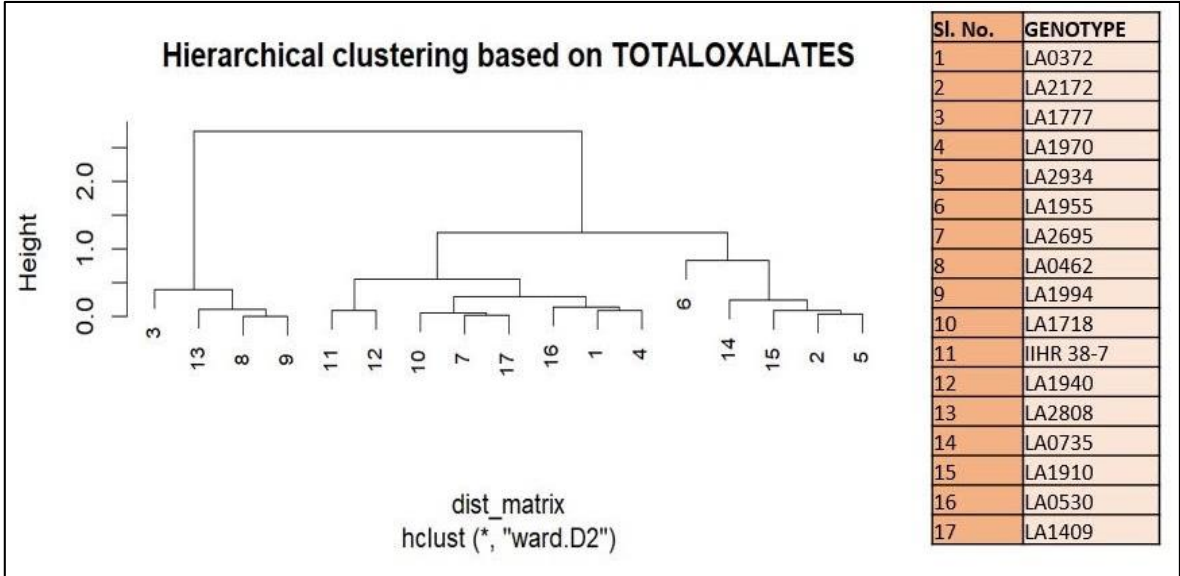


Fig 1: Dendrogram Showing Phenotypic Diversity for Total Oxalates Content (g 100 g⁻¹ DW) in Wild Tomato Accessions

Cluster Characteristics

Distinct differences were observed among the three clusters for total oxalate content (Table 4). Cluster II recorded the highest mean total oxalate concentration (3.00), whereas

Cluster III exhibited the lowest mean concentration (1.58). Cluster I displayed intermediate oxalate levels with relatively low within-cluster variation.

Table 4: Cluster statistics for total oxalate content (g 100 g⁻¹ DW).

Cluster	Number of accessions	Mean total oxalates	Minimum	Maximum	SD
I	8	2.50	2.22	2.69	0.175
II	5	3.00	2.78	3.53	0.307
III	4	1.58	1.45	1.83	0.168

Cluster Validation

The reliability of the clustering was evaluated using the cophenetic correlation coefficient and silhouette analysis. The cophenetic correlation coefficient was 0.799, indicating that the dendrogram adequately represented the original

Euclidean distance matrix. Furthermore, the average silhouette width was 0.520, suggesting moderate but acceptable separation among the three identified clusters as presented in Fig 2.

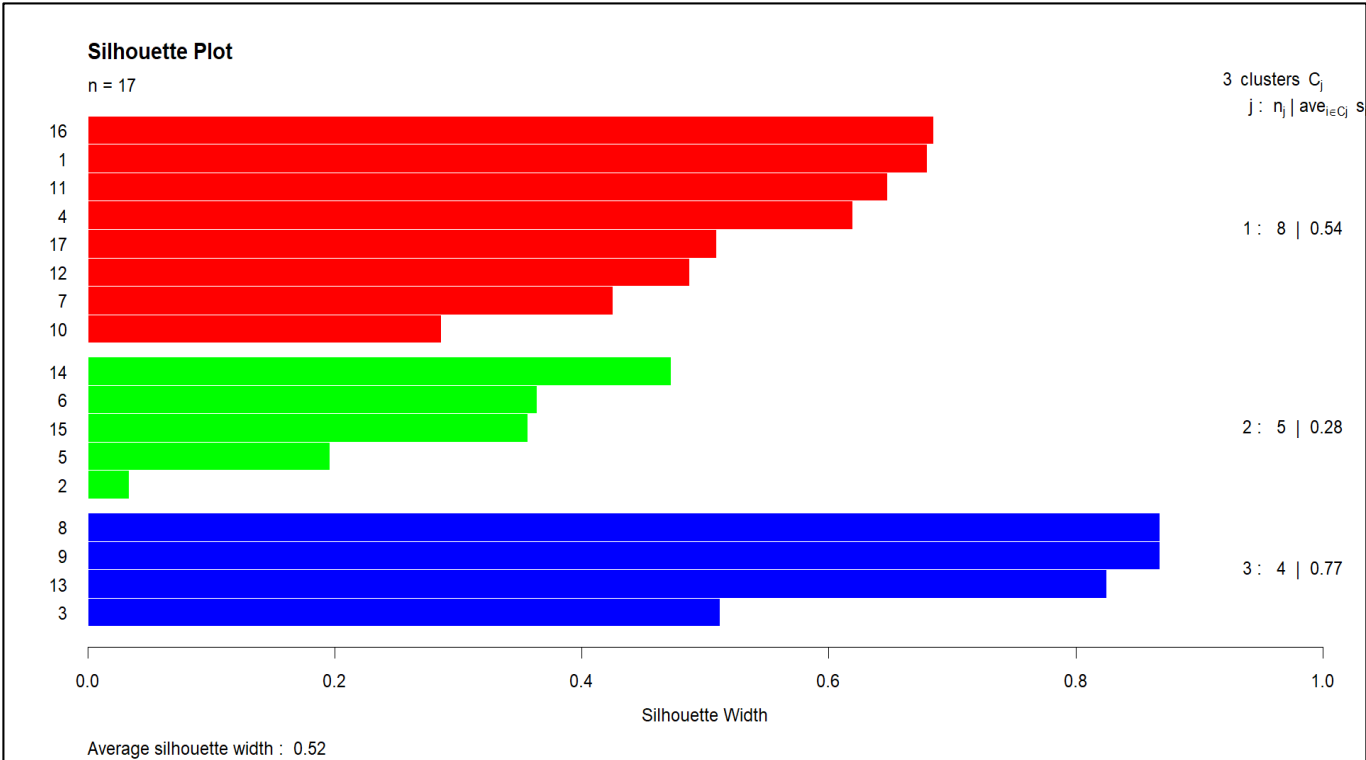


Fig 2: Silhouette Plot for Hierarchical Clustering Based on Total Oxalate Content

Ranking of Wild Tomato Accessions

Marked variation in total oxalate concentration was observed among the evaluated wild tomato accessions. The lowest total oxalate content was recorded in LA1994 (1.45 g 100 g⁻¹ DW), followed by LA1777 (1.53 g 100 g⁻¹ DW) and LA1910 (1.53 g 100 g⁻¹ DW). Conversely, the highest total oxalate concentration was observed in LA1409 (3.53 g 100 g⁻¹ DW), followed by LA2172 (3.02 g 100 g⁻¹ DW) and LA2695 (2.87 g 100 g⁻¹ DW).

Discussion

The present study revealed significant genotypic variation for total oxalate content, whereas seasonal effects were non-significant, indicating that oxalate accumulation is a relatively stable trait predominantly governed by genetic factors. Oxalate biosynthesis is regulated by multiple metabolic pathways and is closely associated with calcium homeostasis, ion balance, and plant defence mechanisms (Franceschi & Nakata, 2005; Noonan & Savage, 1999) ^[2, 5]. The stability of oxalate concentration across seasons suggests that the evaluated accessions maintain consistent oxalate accumulation under different growing conditions. The wide range in total oxalate content (1.45-3.53 g 100 g⁻¹ DW) demonstrates substantial biochemical diversity among the evaluated wild tomato accessions. Such diversity is expected in wild germplasm because these species have evolved under contrasting ecological conditions and retain allelic variation that has largely been lost during tomato domestication (Bai & Lindhout, 2007; Peralta *et al.*, 2008) ^[1, 6]. This variation represents an important resource for improving nutritional quality and understanding oxalate metabolism. Hierarchical clustering further separated the germplasm into three distinct groups corresponding to low, intermediate, and high oxalate accumulators, while the cophenetic correlation coefficient (0.799) and silhouette width (0.520) confirmed acceptable clustering reliability. Similar clustering approaches have been widely used to characterize biochemical diversity and identify genetically divergent parents for breeding programmes (Mohammadi & Prasanna, 2003) ^[4]. The identification of LA1994, LA1777, and LA1910 as low oxalate accessions is particularly valuable because reduced oxalate levels may improve calcium bioavailability and nutritional quality in tomato-derived foods (Noonan & Savage, 1999) ^[5]. Conversely, high-oxalate accessions such as LA1409, LA2172, and LA2695 provide useful material for investigating the genetic regulation of oxalate biosynthesis. Future integration of biochemical phenotyping with genomic and transcriptomic approaches could facilitate the identification of genes controlling oxalate metabolism and support the development of tomato cultivars with improved nutritional attributes.

Conclusion

This study demonstrated substantial genetic diversity for total oxalate content among wild tomato relatives, with genotype exerting a significant influence while seasonal effects remained negligible. Distinct clustering of accessions and the identification of contrasting high-and low-oxalate genotypes highlight the potential of wild tomato germplasm as a valuable resource for improving nutritional quality. In particular, LA1994, LA1777, and LA1910 may serve as promising donor parents for developing low-oxalate cultivars, whereas LA1409, LA2172, and LA2695 provide useful material for studying oxalate metabolism. These

findings establish a foundation for future physiological, genetic, and molecular studies aimed at enhancing mineral nutrition and nutritional quality in tomato breeding programmes.

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