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COMPARATIVE ANATOMICAL STRUCTURE AND PHYTOCHEMICAL COMPOSITION OF THE ROOT IN THREE *CYNARA SCOLYMUS* L. VARIETIES GROWN ON MODERATELY SALINE SOILS OF THE BUKHARA REGION, UZBEKISTAN

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ABSTRACT

Root anatomy and chemical composition may differ among plant genotypes cultivated in the same environment. We compared three globe artichoke (Cynara scolymus L.) varieties—Green Gold, Imperial Star and Violetto—grown on moderately saline soil in the Bukhara Region of Uzbekistan. We evaluated transverse root sections by light microscopy; determined nitrogen and protein by the Kjeldahl method, flavonoids by HPLC, and elemental concentrations by ICP-MS. The varieties had the same principal anatomical regions (periderm, cortical parenchyma and vascular cylinder), but their measurements differed. Imperial Star had the largest cortical parenchyma cells (60.93 ± 0.68 µm) and secretory channels (149.95 ± 1.51 µm), whereas Green Gold had the widest xylem vessels (64.35 ± 0.71 µm). Neither nitrogen nor total protein differed significantly among varieties (P>0.05). Green Gold had the greatest rutin concentration (0.75 mg/g) and was the only variety in which dihydroquercetin was detected (1.2 mg/g). Imperial Star and Violetto had higher quercetin than Green Gold. Violetto had the highest concentrations of several measured mineral elements. These results document cultivar-associated differences in root structure and chemistry at one moderately saline field site. They provide comparative baseline information but do not establish differences in salinity tolerance, which would require controlled salinity treatments.

Keywords: *Cynara scolymus*, Globe artichoke, Salinity, Root anatomy, Phytochemicals, HPLC; ICP-MS.

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1. INTRODUCTION

Even in dry regions such as the Bukhara Region in Uzbekistan, salinity is a serious problem, as irrigation and evaporative demand can lead to salt buildup in cultivated soils. The reactions to these conditions can include reorganization of tissue, uptake of minerals and secondary metabolism. In artichoke, potassium chloride exposure is associated with changes in dry mass of leaves, mineral balance, hormone-related pathways and phenolic composition (Lucini et al., 2016). Comparisons have also been made between phenolic and flavonoid accumulation and leaf quality at different growth phases, depending on the salt used in the studies (Borgognone et al., 2014; Khair et al., 2026). These observations support investigating plant structure alongside chemical properties, but may not apply to roots.

Cynara cardunculus has been found to be sensitive to salinity, and shoot and root length were measured, being reduced with the increase of the salinity; moreover, the total phenolic content and antioxidant activity were increased at increasing salinity (Pappalardo et al., 2020). Although salinity tolerance mechanisms are best known for halophytic genera, e.g., Aster, they can provide information on general adaptive strategies, and only indirect evidence about the morphometric and phytochemical reactions to salinity stress in the genus Cynara itself (Wiszniewska et al., 2021). These wider perspectives on salt-tolerance mechanisms and beneficial soil microorganisms that enhance plant performance under salinity have furthered the knowledge of plant adaptive strategies and are yet to incorporate vegetative organ morphometric in Cynara (Hanin et al., 2016). This overview has been expanded through extensive studies of how plants react to several abiotic stresses. Nevertheless, the anatomical and phytochemical evaluation of Cynara vegetative organs in saline field is not yet fully investigated (Boscaiu & Fita, 2020). Studies on photosynthesis limitations under soil salinity and growth impairment (Zahra et al., 2021) and salinity- and root-specific anatomical

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and phytochemical traits (Pan et al., 2021) have shown the range of physiological responses reported across the family, but neither study considered them separately. The accumulation of bioactive compounds in cardoon has been shown to be significantly affected by growth stage and salinity stress. Despite this, there is still a lack of detailed evidence on the relationship between soil salinity and the morphometric changes of the vegetative organs, and a comprehensive phytochemical evaluation (Petropoulos et al., 2018). The "Carciofo di Procida" cultivar has been characterized both chemically and biologically, showing high antioxidant and cytotoxic activity, highlighting the pharmacological potential of the genus, without taking into account the variability induced by salinity that can be detected in the anatomical variability of the plants (Tommonaro et al., 2025). As a comparison, foliar application of potassium silicate in the related species (Cichorium intybus) had a positive effect on the stress tolerance and physiological performance under salinity. However, similar study associating morphometric and phytochemical attributes of Cynara in saline soil is limited (Mohammadi et al., 2024). In recent years, the leaves of Cynara cardunculus have been investigated with various phytochemicals, which confirmed their high antioxidant activity and a wide range of bioactive components, confirming the medicinal value of the genus. Yet, their work focused on non-structural responses of vegetative organs to saline soils did not assess (Cerulli et al., 2024). Again, without any anatomical part, chemical composition of the related PGI "Carciofo di Paestum" variety revealed many caffeoylquinic acid derivatives, phenolics, flavonoids and terpenoids in leaf extracts (Cerulli et al., 2022). Analogous metabolomic and phenolic-profiling studies of other Italian landraces of Cynara and other edible sprouts have corroborated the high chemical diversity found among Cynara accessions without considering their anatomical and ionomic traits at root level (Blanco et al., 2022; Ferioli & D'Antuono, 2022), and genotype screening of disease resistance has also highlighted the variability of the Cynara germplasm from Turkey (Aydogdu et al., 2022). A halophytic factor was also reported to influence vegetative organ structure in other genera associated with salt in arid Central Asian areas, including Glycyrrhiza and Meristotropis (Shamsuvalieva, 1999), underscoring the general importance of a halophytic factor to vegetative organ structure. A globe artichoke (Cynara cardunculus L. var. scolymus (L.)) heads the list.The globe artichoke, Cynara cardunculus L. var. scolymus (L.), heads the list. Cardoon (Cynara scolymus L.) is a perennial plant originating from the southern Mediterranean and North Africa region; it was domesticated from wild cardoon in Roman times and today is used throughout the world as a vegetable crop and for its antioxidant and anti-inflammatory properties, which are believed to be mainly due to its phenolic acid and flavonoid content (Sonnante et al., 2007; Keramati et al., 2022; Arnaboldi et al., 2022; Rotondo et al., 2022). The varieties of Green Gold, Imperial Star, and Violetto studied here are perennial herbs that are 0.5-2 m tall with erect, moss-like, dense indumentum stems and large, densely indumentum basal leaves, and flowering takes place in May-June (Lishchuk, 1991). In Uzbekistan, adaptive anatomical characteristics of vegetative organs under irrigation and non-irrigated conditions have been studied, as well as the influence of mineral fertilizers on the uptake of microelements (Abzalov et al., 2013), and the elemental and bioactive composition of leaves (Mirrakhimova & Yunuskhojaev, 2014). Our group's recent studies have helped to understand the anatomical features of the Uzbekistan artichoke varieties. Isomov et al. (2024) studied the vegetative anatomy of the 'Green Gold' variety grown at moderate salinity in the Bukhara Region, making an initial description of vegetative anatomy at moderate salinity. The study was, however, limited to just one cultivar and did not consider its phytochemical profile. Isomov et al. (2025) continued this research field and added to the existing body of knowledge on growing artichoke in local environmental conditions. They then described adaptive anatomical features of vegetative organs under various soil salinity levels in different varieties of Cynara, but they only described anatomy with no phytochemical characterization. The present study is based on the previous research and consists of comparative assessment of the root anatomy of three cultivars of artichoke: 'Green Gold', 'Imperial Star' and 'Violetto' grown in the same moderately saline conditions of the same soil under the same cultivation conditions in the Bukhara Region, Uzbekistan. Besides the anatomical characteristics of the roots, the study incorporates also phytochemical analysis of protein and nitrogen content, content of flavonoids and macro- and microelements. The integrated approach provides a more comprehensive overview of the structural and biochemical features of the three cultivars under saline growing conditions. The focus on this organ is based on the evidence that bioactive compound concentrations in Cynara can differ significantly across plant organs and also across varieties, with the receptacle and non-leaf organs sometimes being superior in specific bioactivities (Engin et al., 2020) highlighting the importance of studies based on the root rather than extrapolation from work with leaves. We hypothesized that there would be measurable differences among the three varieties in root tissue size and in the nutritional components of the root tissue as a reflection of different adaptive and metabolic strategies by the different varieties in the same saline-soil environment. For this reason, detailed characterization and comparison of the root anatomical structure and phytochemical composition of the three varieties of artichoke (Green Gold, Imperial Star and Violetto) was designed and used to collect new baseline data that can be useful for future artichoke breeding and plants selection for cultivation in moderately saline soils of arid regions like Bukhara.

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2. MATERIALS AND METHODS

Root anatomy was studied on the Green Gold, Imperial Star and Violetto varieties. Sample was stored in 70 % of ethanol and transverse sections were made of the selected parts of roots in accordance with the botanical microtechniques (Barykina et al., 2004). We used the B1-220A microscope from Motic to observe the sections and took photos using a computer-attached micrometer and a Canon A123 digital camera. Fieldwork took place in 2021–2023 in Vobkent District, Bukhara Region, Uzbekistan (40.017072° N, 64.561123° E). It is an arid continental region with hot dry summers, relatively humid springs and variable winters. Irrigated sierozem-type soils are sandy loam to medium loam and have the following reported properties: Humus content 1·2-1·8%, total phosphorus 0·09-0·11%, total potassium 1·4-2·0%, and carbonates 3-6%. Of all the irrigated land in the district, 65.5% is classified as weakly saline, 18.4% is moderately saline, and 1.5% is strongly saline. Experimental plots were in the moderately saline class (Fig. 1). Cultivation of all three varieties was done in parallel and without salinity imposed experiments. The 3 plants for every variety were biological replicates and samples were taken from 10 points on each plant.

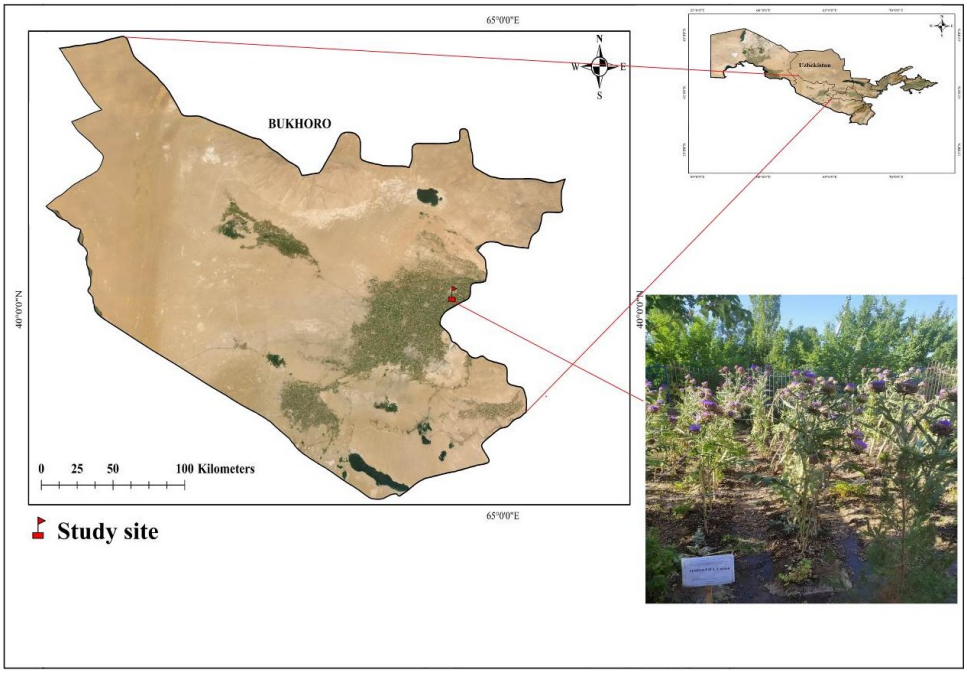


Fig. 1: Study-area location map showing Uzbekistan, Bukhara Region, Vobkent District, and the experimental site (40.017072° N, 64.561123° E), with scale bar, north arrow, and projection/source information.

2.1. Chemical Composition Analysis

- Determination of Total Protein and Nitrogen Content

The chemical analyses were performed by the Laboratory of Protein and Peptide Chemistry, Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan. The total nitrogen was estimated by Kjeldahl method and crude protein was estimated by multiplying the nitrogen content by 6.38. This technique involves the concentrated sulfuric acid dissolving the organic matrix and converting the nitrogen to ammonium sulfate. The following chemical reactions are summarized.

Nitrogen-containing organic substances + H_2SO_4 → $(NH_4)_2SO_4$ + CO_2 + H_2O

Following digestion, sodium hydroxide was added to the resulting solution to release ammonia from ammonium sulfate:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2NH_3\uparrow + 2H_2O$$

Alkali was added after the digestion to liberate ammonia, which was trapped in the sulfuric acid. The amount of acid remaining was determined by titration with a standardized alkaline solution; the difference in the titrations of sample and blank was used to estimate the nitrogen.

Plant material was ground prior to analysis. A weighed aliquot, taken with a weighing error of less than 0.1%, was placed in the digestion flask, and digested according to the Kjeldahl method used in the laboratory.

$$X = \frac{(V_0 - V_1) \times C \times 0.014 \times 100}{m}$$

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The mass fraction of nitrogen (X) was calculated from the titration data obtained from the titration of the sample and the blank determination and was expressed as a percentage of the original sample mass. In the Blank experiment, 0.1 mol L−1 sodium hydroxide was used, the volume consumed was written as (V_0). Give the full calculation formula including all variables, sample mass, in line with the laboratory protocol.

2.2. Extraction and HPLC Analysis of Flavonoids and Steroids

The targeted flavonoids and steroids were characterized by high performance liquid chromatography (HPLC). Weighed 5-10g of plant material into 300ml round bottom flask containing 70% ethanol. It was refluxed for 1 h in the presence of magnetic stirring at 70–80°C and then stirred for 2 h at ambient temperature.

The extract was allowed to settle and filtered. Residue was further extracted twice with 25 mL of 70% ethanol. The combined filtrates were made up to 100 mL using the same solvent. The supernatant was saved for HPLC analysis after centrifugation at 6000-8000 rpm for 20-30 min (Rabotnov et al., 1960).

An Agilent 1200 system with an autosampler and diode-array detector was used with a phosphate-buffer/acetonitrile mobile phase. An Eclipse XDB-C18 column (5 µm, 4.6 × 250 mm) was maintained at 30°C. The flow rate was 1.0 mL/min, injection volume was 10 µL and absorbance was measured at 247nm, 254nm and 276nm.

The mobile-phase composition was programmed as follows: 95:5 (phosphate buffer) from 0–5 min, 70:30 from 6–12 min, 50:50 from 12–13 min, and 95:5 from 13–15 min. Working standard solutions were injected prior to the prepared sample extracts, to aid chromatographic identification and analysis.

2.3. Determination of Macro- and Microelements

The elemental composition of the root samples was analysed by inductively coupled plasma mass spectrometry (ICP-MS). The results tables did not indicate any other elements as analytical targets.

The homogenized material (0.0500–0.500 g) was added to a purified concentrated nitric acid and hydrogen peroxide solution in a Teflon vessel to be digested by the acid. All sealed samples were digested in a Berghof MWS-3+ microwave digestion system using a program specific to the sample matrix.

After mineralization, the digests were quantitatively transferred into 50- or 100-mL volumetric flasks, mixed with 0.5% nitric acid and measured instrumentally. All the elements were measured by ICP-MS or another ICP argon plasma emission spectrometer.

The following equipment was used in the above analyses: ICP-MS NexION-2000 or similar mass spectrometer; microwave digestion systems (Germany) or Teflon autoclaves of different sizes; volumetric flasks of different sizes.

Reagents: Multielement standard No. 3 (29 elements for MS), mercury, nitric acid, hydrogen peroxide, bidistilled water and argon (gas purity 99.995%) (Boscaiu & Fita, 2020). Compilation of quantitative anatomical and phytochemical data were done in MS Excel, and statistically analyzed using SPSS and Statistica software, and selected analyses were cross-checked each other checked in R. A one-way analysis of variance (ANOVA) was used to analyze differences between the three varieties of artichoke (Green Gold, Imperial Star, and Violetto) for each measured parameter with the variety as the fixed effect. The ANOVA showed significant differences among treatments ($P<0.05$), followed by Tukey's honestly significant difference (HSD) test. Before analysis, we checked normality and Homogeneity of variance. The results are presented as mean \u00b1 standard deviation (SD) of n replicate measurements per variety as shown in the table captions.

3. RESULTS

In transverse sections, the Green Gold cultivar root had a circular outline and non-fascicular organization, and no lignification staining was observed. The root tissues of all cultivars examined could be separated into three main anatomical regions: the outer periderm, the cortical parenchyma and the central vascular cylinder (stele) (Fig. 2–3). The outermost protective layer of the root is called periderm, made up of three parts, phellem, phellogen and phelloderm. The phellogen is composed of radially elongated, rectangular cells which produce living cells phelloderm and dead cells containing suberin phellem towards the outside and the inside, respectively. The phelloderm cells are relatively large and rectangular and thus easily distinguished from the adjacent cortical parenchyma. The cortical region is made up of thin walled parenchymatous cells with rounded, oval or approximately isodiametric outline. These cells in Green Gold had a mean diameter of 33.33 ± 0.41 µm which was less than the size observed in the Imperial Star cultivar. The cortical parenchyma made up about 30% of the total diameter of the roots and consisted of tightly packed cells. The vascular cambium is also involved in the secondary tissue production process and forms secondary phloem facing out and secondary xylem facing in towards the root's center. These tissues constitute the major parts of the conducting tissues of the vascular cylinder.

In all varieties investigated, schizogenous secretion channels are found in the bark parenchyma and in the central cylinder. Secretion channels of the bark parenchyma are more abundant and larger in size compared to those of the central cylinder, which are less abundant and smaller in size. The secretory structures seen in roots were rounded to

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oval, with an epithele consisting of 7–8 cells around each. The secretory structures seen in the roots were schizogenous channels with rounded to oval outlines and each surrounded by an epithele composed of 7–8 cells. Green Gold cultivar exhibited the least channel diameter (74.18 ± 0.83 µm) among the three cultivars, followed by Imperial Star cultivar and Violetto cultivar (Fig. 2, Table 1). In anatomical observations, secretory structures were also observed related to the occurrence of biologically active compounds (Fig. 2, Table 1). It was found that the central cylinder was the largest anatomical region of the root, comprising about 70% of the total diameter. Most of this area had been filled by secondary xylem and related woody tissues. The xylem had a diffuse pattern with the conducting elements occurring irregularly in chains and a loosely circular array in the stele. The diameters of the secondary conducting elements differed between cultivars. Green Gold (64.35 ± 0.71 µm) and Violetto (59.38 ± 0.71 µm) had the largest values, whereas Imperial Star had a smaller mean diameter of 42.59 ± 0.48 µm. Primary conducting tissues were present and still identifiable in the innermost part of the central cylinder in all three cultivars and persisted until the end of the growing season (Fig. 2, Table 3).

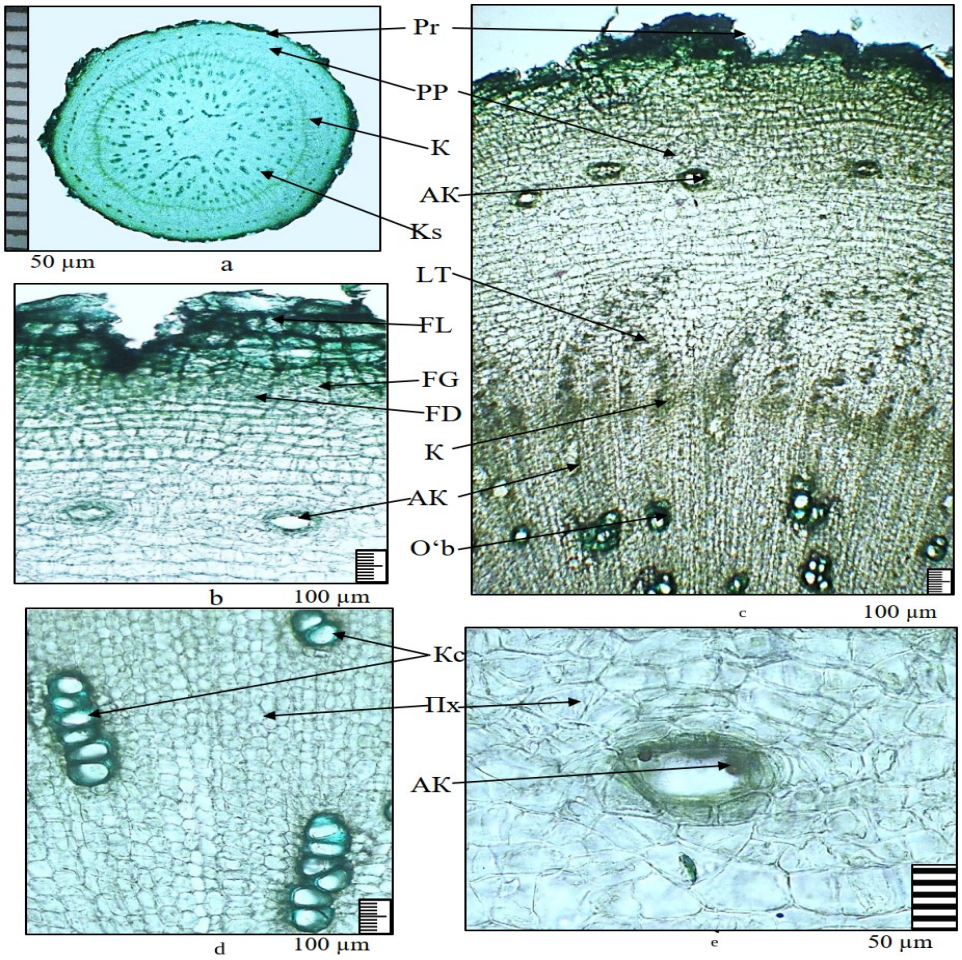


Fig. 2: Anatomical structure of the root of the *Green Gold* variety: a – General appearance of the root; b – periderm and bark parenchyma; c – detail; d – parenchyma and secondary conducting bundle; e – schizogenous excretory duct. **Legend:** AK – secretory canal (schizogenous duct), K – cambium, Ks – xylem, LT – bast fibers, PP – bark parenchyma, Pr – periderm, Px – parenchyma cells, FG – phellogen, FD – phelloderm, FL – phloem, O'B – conducting bundle.

Transverse cross sections of the root of the Imperial Star cultivar showed a circular outline and no apparent lignification and a non-fascicular organization. The outer surface had a periderm developed into three layers, phellem, phellogen and phelloderm. The phellogen consisted of radially elongated rectangular cells, from which elongated, non-living suber cells formed towards the exterior and larger, living cells towards the interior. The latter were clearly different from the adjacent cortical parenchyma.

The cells of the cortical parenchyma were thin walled and rounded, oval or isodiametric. The mean diameter of these cells in Imperial Star was 60.93 ± 0.68 µm which was greater than that recorded for Green Gold and Violetto. The vascular cambium was made up of brick shaped cells in a few layers. It was responsible for secondary tissue formation, producing secondary phloem towards the outside and secondary xylem towards the central cylinder (Fig. 3, Table 1).

Secretory channels were found to be schizogenous in the central cylinder as well as the cortical parenchyma. The

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number and size of the channels were different in the two regions with more and larger channels in the cortical parenchyma and smaller and fewer channels in the central cylinder. In the channels, a rounded to oval cross-sectional shape and lining by 7-8 epithelial cells were noted. In the plant, their mean diameter in the plant has reached 149.95 ± 1.51 µm in Imperial Star, which is larger than in Green Gold and Violetto.

The central cylinder made up about 71% of the total root diameter and was mainly composed of secondary xylem and associated woody tissues. No distinct pattern of xylem elements was observed; they were irregularly arranged in chains and radiated out in a roughly circular outline in the stele. The mean diameter of the secondary conducting elements measured was 42.59 ± 0.48 µm; this value was lower than in the other cultivars, Green Gold and Violetto. Primary conducting tissues were found in the centre part of the stele and were easily recognized until the end of the growing season (Fig. 3, Table 1).

Table 1: Quantitative indicators of root anatomical features in promising artichoke varieties

Indicators	Green Gold	Imperial	Violetto
Root diameter, mm	12.00 ± 0.20 c	15.63 ± 0.21 a	13.88 ± 0.18 b
Thickness of bark parenchyma, µm	1840.63 ± 24.65 b	1950.57 ± 27.65 a	1450.80 ± 19.00 c
Proportion of bark parenchyma in root diameter, %	30.00 ± 0.5 a	29.00 ± 0.5 ab	28.60 ± 0.5 b
Diameter of bark parenchyma cells, µm	33.33 ± 0.50 c	60.93 ± 0.83 a	39.46 ± 0.55 b
Diameter of schizogenous secretion channels, µm	74.18 ± 1.02 c	149.95 ± 1.85 a	92.10 ± 1.15 b
Thickness of woody parenchyma (libriform), µm	4293.33 ± 50.85 b	4775.50 ± 61.90 a	3621.87 ± 45.65 c
Proportion of woody parenchyma (libriform) in root diameter, %	70.00 ± 0.5 b	71.00 ± 0.5 ab	71.40 ± 0.5 a
Diameter of parenchyma cells, µm	78.56 ± 1.09 a	47.51 ± 0.62 c	62.53 ± 0.81 b
Diameter of xylem vessels in conducting bundles, µm	64.35 ± 0.87 a	42.59 ± 0.59 c	59.38 ± 0.87 b

Values (mean ± SD, n = 3 biological replicates per variety) within each row bearing different letters differ significantly (one-way ANOVA with Tukey's HSD post-hoc test, P<0.05).

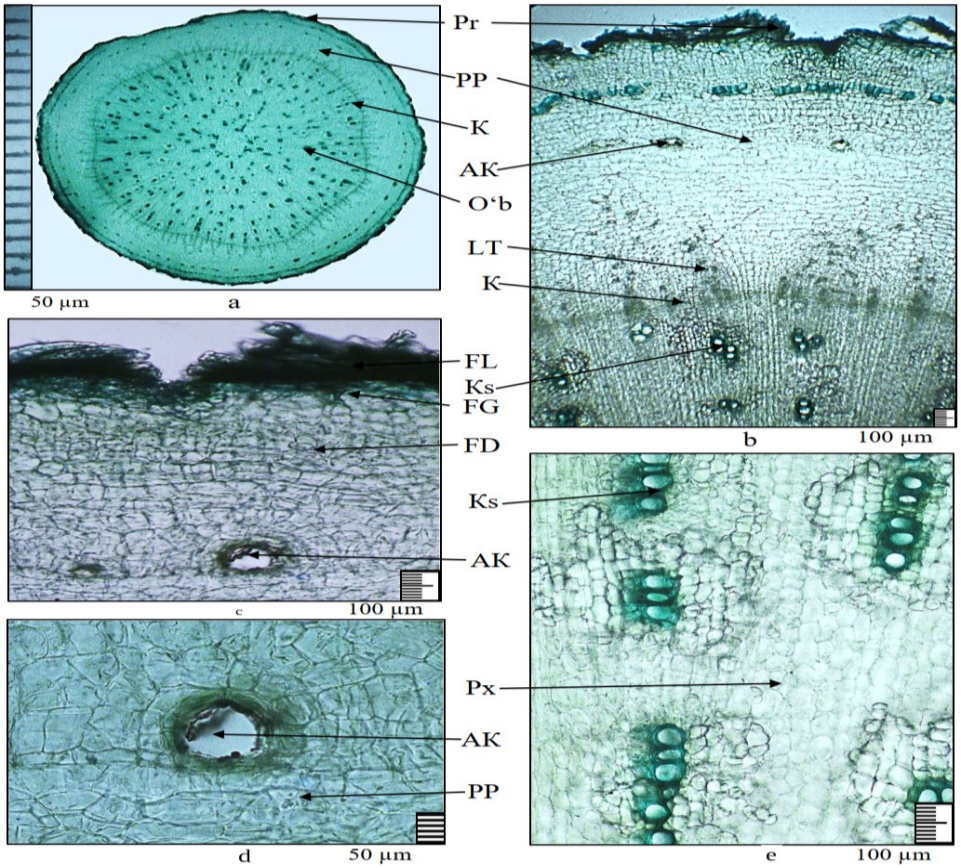


Fig. 3: Anatomical structure of the *Imperial variety* root: a – General appearance of the root; b – detail; c – periderm and bark parenchyma; d – schizogenous excretory duct; e – parenchyma and secondary conducting bundle. **Legend:** AK – secretory canal (schizogenous duct), K – cambium, Ks – xylem, PP – bark parenchyma, Pr – periderm, Px parenchyma cells, FG – phellogen, FD – phelloderm, FL – phloem, O'B – conducting bundle.

Violetto roots were cross-sectionally examined and found to be circular in shape, non-fascicular and lacking in lignification. Root surface was peridermed and the phellogen produced suberin rich non-living phellem cells toward the exterior and larger living rectangular phelloderm cells towards the interior. The phelloderm was easily identified from the surrounding cortical parenchyma (Fig. 4, Table 1). The cells of the cortical parenchyma were rounded, oval

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or isodiametric with relatively thick walls. The mean diameter of their was 39.46 ± 0.45 µm, which was found to be smaller than that of Imperial Star. The thickness of the cortical area was 1,450.77 ± 15.53 µm, which represent 28.6% of the total diameter of the root. The vascular cambium consisted of brick-shaped cells in several layers. The cells formed secondary phloem towards the outer tissues and secondary xylem towards the central cylinder.

The secretory channels were schizogenous and found in the cortical parenchyma and central cylinder. They were more numerous and larger in the cortex, while in the central cylinder they are comparatively smaller and less in number (Fig. 2, Table 1). The channels had rounded or oval profiles. There were 5–6 epithelial cells lining small channels and 9–10 lining larger ones. The mean channel diameter for the sample was 92.1 ± 0.94 µm which is lower than what was recorded for Imperial Star. In the cortical parenchyma, the channels were packed in rows and correlated with the accumulation of secretory substance. The central cylinder was the main part of the root. The thickness of woody tissue measured 3,621.85 ± 37.23 µm which accounted for 71.4% of the total root diameter. In the secondary xylem, the conducting elements were irregularly arranged in chains with a diffuse distribution of the xylem. The mean diameter of the measured secondary xylem elements was 59.38 ± 0.71 µm, which is higher than that of Green Gold and Imperial Star (Fig. 4, Table 1).

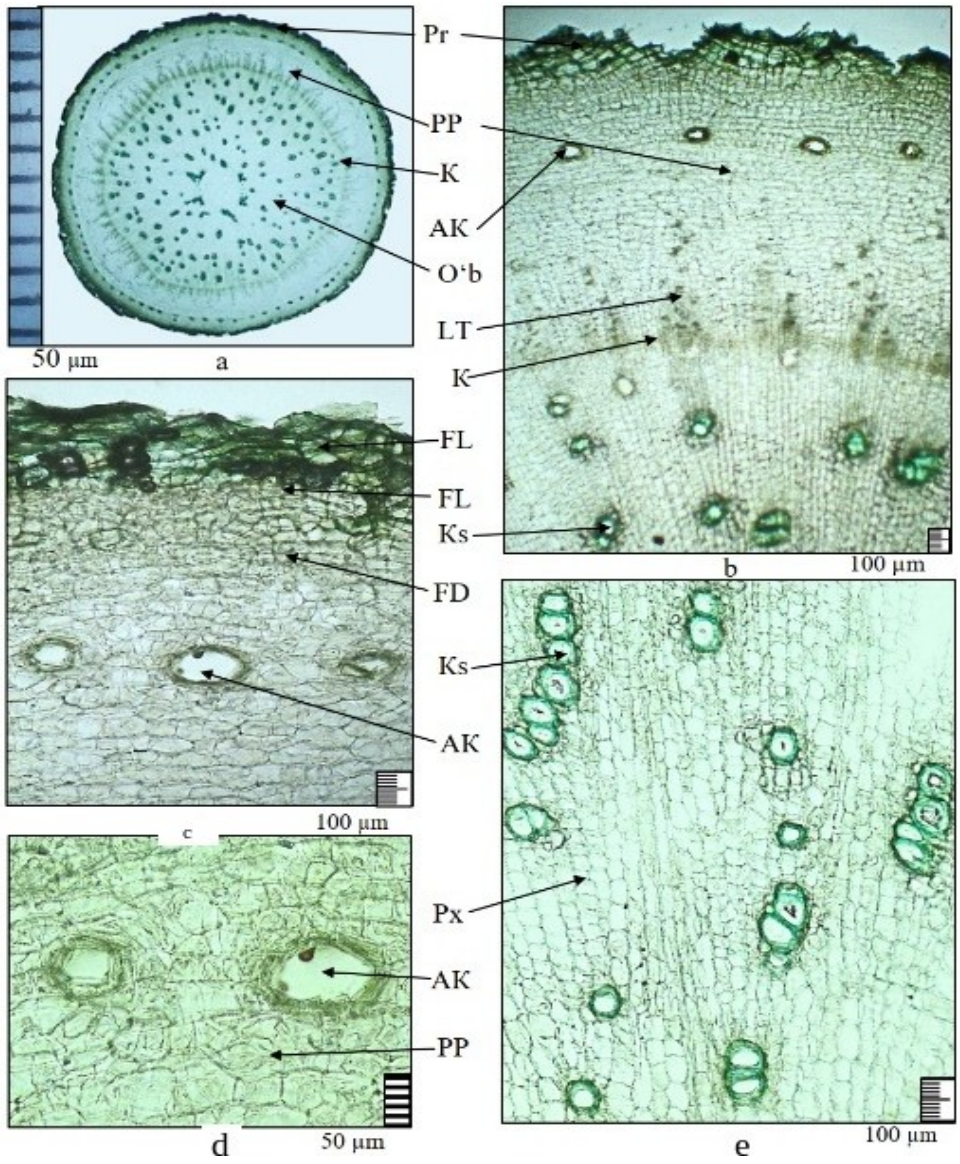


Fig. 4: Anatomical structure of the root of the *Violetto* variety: A–General appearance of the root; B–detail; C–periderm and bark parenchyma; D–schizogenous excretory duct; E–parenchyma and secondary conducting bundle. **Legends:** AK–secretory canal (schizogenous duct), K–cambium, Ks–xylem, PP–bark parenchyma, Pr–periderm, Px parenchyma cells, FG – phellogen, FD – phelloderm, FL – phloem, O'B – conducting bundle.

Comparative analysis of root anatomical and biometric properties of Green Gold, Imperial Star and Violetto showed that both shared and cultivar-specific properties exist. All three cultivars had a circular root shape and non-fascicular root pattern in transverse cross sections. Root diameter was found to differ among the cultivars, with the

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highest value being Imperial Star (15.63 mm) and the lowest being Green Gold (12.0 mm) (Fig. 2–4). In all cultivars studied the outside protective layer was composed of periderm. The phellogen formed suberin-containing phellem cells on the outside and phelloderm cells on the inside, as is typical in the protective tissues of the root. The cultivars also differed in cortical cell dimensions, the distribution and diameter of secretory channels, and the dimensions of secondary xylem elements.

The inner side of the phellogen develops living large and rectangular phelloderm cells which appear distinctly different from the inner bark parenchyma cells. Imperial variety had the largest diameter of bark parenchyma cells (60.93 ± 0.68 µm) which accounted for 29% of root diameter; Green Gold variety had the least diameter of bark parenchyma cells (33.33 ± 0.41 µm) which accounted for 30% of root diameter and Violetto variety had intermediate diameter (39.46 ± 0.45 µm) which accounted for 28.6% of root diameter. Schizogenous secretion channels were not found in the vegetal organs of any variety studied, except in the roots, in both the bark parenchyma and the central cylinder. The secretion channels of the bark parenchyma are bigger and more numerous than those of the central cylinder. For the Imperial variety, the maximum diameter of the schizogenous secretion channels was 149.95 ± 1.51 µm; for Violetto, 92.1 µm ± 0.94; and for Green Gold, the least, 74.18 µm ± 0.83. The Green Gold variety had the maximum diameter of parenchyma cells in the central cylinder (78.56 ± 0.89 µm), while the Violetto variety had the minimum diameter of parenchyma cells in the central cylinder (62.53 ± 0.66 µm), and the Imperial variety had the minimum diameter of parenchyma cells in the central cylinder (47.51 ± 0.51 µm).

3.1. Phytochemical Analysis of the Root

One of the factors influencing the possible value of medicinal plant material is its chemical constituents. We therefore evaluated the root nitrogen level, calculated protein content and selected flavonoids and mineral elements in the three varieties of artichoke.

Literature data on the investigation of the aboveground parts of artichoke show that they contain protein (3%), carbohydrates (7-15%), carotene (0.4 mg%) and vitamin C (3-11 mg%), as well as fats, nucleic acids, vitamins B, B, P, K, E (total 1.27%), dietary fiber, tannins and mineral elements. Among the carbohydrates, there is inulin (Mirrakhimova et al., 2014).

Mean root nitrogen was numerically greatest in Imperial Star (1.31 ± 0.03%), followed by Violetto (1.29 ± 0.03%) and Green Gold (1.22 ± 0.03%). The corresponding total protein values were 8.02 ± 0.16%, 7.89 ± 0.16% and 7.65 ± 0.16%. The apparent differences were not, however, statistically significant for either nitrogen (P = 0.147) or protein (P = 0.314; Table 2).

Table 2: Phytochemical composition of the roots in artichoke varieties

Indicator	Green Gold	Imperial Star	Violetto
Nitrogen content (%)	1.22 ± 0.03 a	1.31 ± 0.03 a	1.29 ± 0.03 a
Total protein content (%)	7.65 ± 0.16 a	8.02 ± 0.16 a	7.89 ± 0.16 a

Values (mean ± SD, n = 3 biological replicates per variety) within each row bearing different letters differ significantly (one-way ANOVA with Tukey's HSD post-hoc test, P<0.05).

Flavonoids are polyphenolic secondary metabolites of plant metabolism in plants. Although they are a secondary function, they play a significant role in plant growth and development for their protection from parasites, insect damage, bacterial, viral and fungal infections. Their main roles are oxidative stress protection via antioxidant activity (Mukumov & Rasulova, 2019). We then analyzed the flavonoid content (rutin, quercetin, dihydroquercetin, gallic acid) in the roots of the artichoke varieties. The content of rutin in the roots showed significant differences among Green Gold (0.75 ± 0.03 mg/g), Imperial Star (0.32 ± 0.02 mg/g) and Violetto (0.31 ± 0.02 mg/g) (one-way ANOVA with Tukey's HSD, P<0.05). In contrast, Imperial Star (0.83 ± 0.04 mg/g) and Violetto (0.76 ± 0.03 mg/g) did not differ significantly and had significantly higher quercetin content in the roots than Green Gold (0.36 ± 0.02 mg/g). In the root of Green Gold, 1.2 mg/g of dihydroquercetin was found, while in Imperial Star and Violetto, it was not found (Table 3). Likewise, 0.50-0.51 mg/g of gallic acid was detected in Imperial Star and Violetto, but not in Green Gold (Table 3).

The elemental composition of the root samples was also analysed by mass spectrometry for each variety. Table 4 shows the concentrations of the measured trace elements.

The differences were considerable in the microelement content of the root of the three varieties of artichoke as reported in Table 4. The highest amounts of copper (4.98 ± 0.20 mg/kg), iron (1080.5 ± 38.5 mg/kg), manganese (4.07 ± 0.20 mg/kg), and zinc (20.5 ± 0.9 mg/kg) were found in the Violetto variety, which were significantly higher than Green Gold and Imperial Star, Tukey's HSD (P<0.05). The iron content of Green Gold (186.2 ± 6.4 mg/kg) and of Imperial Star (202.5 ± 7.1 mg/kg) were not significantly different, and were both considerably lower than that of Violetto. Imperial Star (19.9 ± 0.9 mg/kg) and Violetto (20.5 ± 0.9 mg/kg) did not differ significantly, but both were

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significantly higher than Green Gold (4.62 ± 0.21 mg/kg). All three varieties of copper differed significantly (Violetto exceeded Green Gold and Imperial Star). In contrast, for manganese, Violetto differed significantly from both Green Gold and Imperial Star, which did not differ from each other (Table 4). Table 5 reports the concentrations of the measured macroelements separately.

Table 3: Flavonoid content in the root of artichoke varieties (mg/g dry weight; rutin and quercetin

Samples	Concentration (mg/g dry weight)	Concentration (mg/g dry weight)	Concentration (mg/g dry weight)	Concentration (mg/g dry weight)
Samples	Rutin	Quercetin	Dihydro quercetin	Gallic acid
Green Gold	*Green Gold*	*Green Gold*	*Green Gold*	*Green Gold*
Root	0.75 ± 0.03 [a]	0.36 ± 0.02 [b]	1.2	0
Imperial Star	*Imperial Star*	*Imperial Star*	*Imperial Star*	*Imperial Star*
Root	0.32 ± 0.02 [b]	0.83 ± 0.04 [a]	0	0.50
Violetto	*Violetto*	*Violetto*	*Violetto*	*Violetto*
Root	0.31 ± 0.02 [b]	0.76 ± 0.03 [a]	0	0.51

Values (mean ± SD, n = 3 biological replicates per variety) within each row bearing different letters differ significantly (one-way ANOVA with Tukey's HSD post-hoc test, P<0.05). Dihydroquercetin and gallic acid based on a single determination per variety).

Table 4: Microelement content in the roots of artichoke varieties

Samples (vegetative organs)	Cu	Fe	Mn	Zn
Green Gold	*Green Gold*	*Green Gold*	*Green Gold*	*Green Gold*
Root	3.96 ± 0.14 [b]	186.2 ± 6.4 [b]	1.63 ± 0.06 [b]	4.62 ± 0.21 [b]
Imperial Star	*Imperial Star*	*Imperial Star*	*Imperial Star*	*Imperial Star*
Root	3.40 ± 0.12 [c]	202.5 ± 7.1 [b]	1.79 ± 0.07 [b]	19.9 ± 0.9 [a]
Violetto	*Violetto*	*Violetto*	*Violetto*	*Violetto*
Root	4.98 ± 0.20 [a]	1080.5 ± 38.5 [a]	4.07 ± 0.20 [a]	20.5 ± 0.9 [a]

Values (mean ± SD, n = 3 biological replicates per variety) within each column bearing different letters differ significantly (one-way ANOVA with Tukey's HSD post-hoc test, P<0.05).

Table 5: Macroelement content in artichoke roots

Samles	Ca	P	S	Mg	K	Na
Green Gold	*Green Gold*	*Green Gold*	*Green Gold*	*Green Gold*	*Green Gold*	*Green Gold*
Root	4711.6 ± 71.6 [b]	462.1 ± 6.9 [c]	240.2 ± 7.2 [ab]	293.4 ± 8.8 [b]	2372.3 ± 71.3 [b]	445.7 ± 13.4 [c]
Imperial Star	*Imperial Star*	*Imperial Star*	*Imperial Star*	*Imperial Star*	*Imperial Star*	*Imperial Star*
Root	4441.1 ± 67.5 [c]	528.5 ± 7.9 [b]	222.8 ± 6.7 [b]	259.5 ± 7.8 [c]	2791.6 ± 84.3 [a]	679.3 ± 20.3 [a]
Violetto	*Violetto*	*Violetto*	*Violetto*	*Violetto*	*Violetto*	*Violetto*
Root	5812.1 ± 88.1 [a]	709.1 ± 11.3 [a]	273.0 ± 8.2 [a]	405.4 ± 12.1 [a]	2485.4 ± 74.9 [b]	495.4 ± 14.9 [b]

Values (mg/kg dry weight: mean ± SD, n = 3 biological replicates per variety) within each column bearing different letters differ significantly (one-way ANOVA with Tukey's HSD post-hoc test, P<0.05).

There were significant differences among the three varieties for all six elements measured by one-way ANOVA (P<0.05) in the macroelement content of the root (Table 5). Violetto had the highest calcium (5812.1 ± 88.1 mg/kg); phosphorus (709.1 ± 11.3 mg/kg); and magnesium (405.4 ± 12.1 mg/kg) levels, which were all significantly higher than in Green Gold and Imperial Star. Violetto (273.0 ± 8.2 mg/kg) was significantly higher than Imperial Star (222.8 ± 6.7 mg/kg), and Green Gold (240.2 ± 7.2 mg/kg) was not statistically different from either of these. Potassium and sodium differed: Imperial Star contained the highest amounts of both (2791.6 ± 84.3 and 679.3 ± 20.3 mg kg-1, respectively), both of which were significantly higher than the amounts in Green Gold and Violetto. Green Gold and Violetto did not differ significantly from each other regarding potassium. Green Gold was intermediate for calcium and phosphorus and differed significantly from Imperial Star and Violetto (Table 5).

4. DISCUSSION

Previous investigations on halophytes and salt-tolerant plant species have provided substantial insight into the physiological, biochemical, and nutritional responses of plants growing under saline conditions (Li et al., 2020; Agudelo et al., 2021; Corrado et al., 2021; Alexopoulos et al., 2023; Atzori et al., 2025). Studies of Mediterranean halophytes such as Atriplex halimus, Salicornia fruticosa, and Cakile maritima, together with work on seawater vegetables, baby lettuce cultivars, and species such as Hedypnois cretica and Urospermum picroides, have primarily focused on salt-tolerance mechanisms and their potential nutritional or functional implications. Researchers have also documented non-stomatal limitations of photosynthesis under salinity and salinity-related growth impairment in milk thistle (Pan et al., 2021; Zahra et al., 2021). Despite this substantial body of work, comparative anatomical and phytochemical characterization of Cynara root tissue across varieties grown under the same saline-soil conditions has remained limited, a gap the present study addresses.

The three artichoke varieties examined here shared the same fundamental root organization - periderm, bark

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parenchyma, and central cylinder consistent with previous anatomical descriptions of Cynara root structure (Isomov et al., 2024, 2025; 2026). Within this shared plan, however, the varieties differed considerably in tissue dimensions. Imperial Star combined the largest bark parenchyma cells and the largest schizogenous secretion channels with the smallest xylem vessel diameter. In contrast, Green Gold showed the reverse pattern: comparatively small bark parenchyma and secretion channels but the largest xylem vessels. Larger xylem vessel diameter is generally associated with greater hydraulic capacity but potentially higher vulnerability to cavitation, while more extensive parenchyma and secretion-channel tissue may favor storage of osmotically active solutes and secondary metabolites (Hanin et al., 2016; Wiszniewska et al., 2021). Interpreted cautiously, these anatomical contrasts suggest that the three varieties may rely on different combinations of water-transport efficiency and storage capacity within the root under the same moderately saline conditions, rather than converging on a single common structural strategy. Because this study imposed no salinity gradient, these anatomical differences cannot be attributed to differential salinity tolerance; they are best interpreted as genotype-specific structural traits whose functional significance across a range of salinity levels would require a dedicated gradient experiment to resolve.

The phytochemical results point to a similar pattern of genotype-specific divergence layered on top of a largely conserved primary metabolism. Root nitrogen and total protein content did not differ significantly among the three varieties, suggesting that basic nitrogen assimilation and protein accumulation in the root were not strongly differentiated by variety under the growing conditions of this study. In contrast, the flavonoid profile diverged sharply: Green Gold accumulated significantly more rutin, while Imperial Star and Violetto accumulated significantly more quercetin, and only Green Gold contained detectable dihydroquercetin. Because rutin, quercetin, and dihydroquercetin lie along the same flavonol biosynthetic branch, with quercetin as a common precursor that can be glycosylated to rutin or reduced to dihydroquercetin, these differences may reflect variety-specific regulation of downstream flavonol-modifying enzymes rather than a difference in overall flavonoid biosynthetic capacity (Petropoulos et al., 2018; Zayed et al., 2020). Flavonoids of this class are widely reported to contribute to antioxidant and anti-inflammatory defense in Cynara and related Asteraceae (Gould & Lister, 2006; Cerulli et al., 2024; Nechchadi et al., 2024), and the pronounced varietal differences observed here suggest that Green Gold and the Imperial Star/Violetto group may differ in the specific antioxidant compounds available in the root. However, this study did not measure antioxidant activity directly, so this functional link remains to be tested. This pattern of variety-dependent flavonoid partitioning is consistent with an earlier comparative study of Imperial Star and other *C. scolymus* varieties, which likewise reported significant differences in phenolic and flavonoid content by variety and by plant organ (Wang et al., 2003), as well as with more recent phytochemical comparisons of distinct C. scolymus varieties emphasizing genotype-specific metabolite profiles (Luca et al., 2022).

Mineral accumulation likewise showed a genotype-dependent pattern rather than a uniform response, consistent with earlier reports that saline stress differentially alters mineral composition, phenolic acids, and flavonoids across artichoke and cardoon genotypes (Colla et al., 2013). Violetto accumulated the highest concentrations of calcium, phosphorus, magnesium, copper, iron, and manganese, while Imperial Star accumulated the highest potassium and sodium. This divergence for the monovalent cations potassium and sodium is consistent with the general observation that ion uptake and partitioning under saline conditions can vary substantially among genotypes of the same species, with some varieties favoring greater potassium retention relative to sodium and others showing the opposite pattern (Hanin et al., 2016; Pan et al., 2021). Because soil sodium content was not directly measured in this study and no salinity gradient was applied, these mineral differences should be viewed as descriptive evidence of genotype-specific isonomic profiles under one saline-soil condition rather than as direct evidence of differential salt-exclusion or salt-inclusion strategies; testing that hypothesis would require paired measurements of soil and plant ion content across a range of salinity levels. The pronounced divergence in potassium accumulation also parallels findings that potassium fertilization strongly modulates growth and chemical constituents of globe artichoke, further supporting genotype-specific sensitivity to potassium availability and uptake (Petropoulos et al., 2022).

This study has several limitations that should guide the interpretation of its findings and the design of future work. First, the three varieties were compared under a single, moderately saline soil condition at one site (Vobkent District, Bukhara Region) without a controlled salinity gradient; consequently, the anatomical and phytochemical differences reported here reflect varietal divergence under this particular saline environment rather than dose-dependent responses to salinity itself. Second, we examined only root tissue phytochemically, and destructive anatomical sampling did not allow repeated measurements on the same individual plants over time. Future studies exposing these three varieties to a controlled range of soil salinity levels, with parallel anatomical and phytochemical sampling of root, stem, and leaf tissue, are needed to determine whether the genotype-specific patterns identified here also confer differential tolerance to increasing salinity stress.

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5. CONCLUSION

We compared the anatomy and chemical constituents of roots from Green Gold, Imperial Star and Violetto artichokes grown in moderately saline soil in Bukhara Region. By examining anatomical measurements together with nitrogen, protein, flavonoids, and mineral elements, the study extends the available descriptive evidence for these varieties under local field conditions.

The main anatomical organization was consistent across varieties, but several dimensions varied by cultivar. Imperial Star had the largest overall root diameter, cortical cells, and schizogenous secretory channels; Green Gold had the widest xylem vessels. Nitrogen and calculated protein did not vary significantly. In contrast, Green Gold had the highest rutin, while Imperial Star and Violetto had more quercetin. Dihydroquercetin was detected only in Green Gold. Violetto generally showed higher concentrations of many of the analyzed mineral elements.

These results identify distinct root traits that could inform subsequent studies of artichoke germplasm and root composition. They should not, however, be interpreted as proof of superior salt tolerance: the comparison involved one site and one soil-salinity category, without a controlled salinity gradient. Replicated trials across several salinity levels and environments are needed before making recommendations about relative tolerance.

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Author's Contributions: This study was a collaborative effort among all authors. Eldor Isomov, Yigitali Tashpulatov, Nodirjon Bobokandov, Gulchekhra Tastanova, Bakhrom Tukhtarov, Mekhriniso Bobokandova, Gulnoza Odilova, Ozoda Alimova, Vakhob Rakhmonov, Gulnoza Djumayeva, and Iroda Saydullayeva contributed to the study design and data analysis. Eldor Isomov, Yigitali Tashpulatov, Vakhob Rakhmonov, and Nodirjon Bobokandov conducted the laboratory experiments, analyzed the data, interpreted the results, and drafted the manuscript. All authors reviewed and approved the final manuscript.

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