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Determination of sugar contents in *Hyacinthella* Schur bulbs and identification of sugars by cluster analysis method

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Hyacinthella Schur soğanlarında şeker içeriğinin belirlenmesi ve şekerlerin kümeleme analizi yöntemiyle tanımlanması

Abstract: Türkiye has a rich floristic diversity and is accepted as the gene center of the *Hyacinthella* genus. Nineteen species of the *Hyacinthella* genus have been described in the World, and eleven of these species are distributed in Türkiye. Additionally, ten of the eleven *Hyacinthella* species are endemic to our country. Therefore, any study on these species will contribute to the protection, promotion and sustainability of our country's genetic resources. Plants may have different metabolite contents depending on their genetic structure and environmental conditions. Sugar content in bulbous plants depends on sucrose metabolism and varies with species and environmental effects. In this study, the sugar contents of 11 different *Hyacinthella* species distributed in our country were analyzed. The presence of 9 different sugar types in the species was analyzed qualitatively and quantitatively. In addition, depending on the sugar content, identification and grouping techniques were used with the Cluster Analysis Method. As a result of sugar analysis, while glucose, sucrose and fructose are found in all species, differences were detected between species in other sugar contents In cluster analysis, *Hyacinthella* species were divided into 3 different groups in terms of sugar content. The study both identifies the sugar contents found in the bulbs of *Hyacinthella* species and suggests a different identification method By combining morphological, molecular and metabolic data, a complete and accurate identification of species will be achieved.

Key words: Classification, hyacint, endemic

Özet: Türkiye floristik açıdan zengin bir çeşitliliğe sahiptir ve *Hyacinthella* cinsinin gen merkezi olarak Türkiye kabul edilmektedir. Dünya'da *Hyacinthella* cinsine ait on dokuz tür tanımlanmakla, bu türlerin on bir'i Türkiye'de yayılış göstermektedir. Ayrıca *Hyacinthella* cinsinin on bir türü'nün on'u ülkemiz için endemiktir. Bu nedenle, bu türler ile ilgili yapılacak her türlü çalışma, ülkemiz gen kaynaklarının korunması, tanıtılması ve sürdürülebilirliğine katkı sağlayacaktır. Bitkiler genetik yapıları ve çevresel şartlara göre farklı metabolit içeriklerine sahip olabilirler. Soğanlı bitkilerde şeker içeriği sukroz metabolizmasına bağlı olup, tür ve çevresel etkiler ile değişkenlik göstermektedir. Bu çalışmada ülkemizde yayılış gösteren 11 farklı *Hyacinthella* türüne ait şeker içerikleri analiz edilmiştir. Dokuz farklı şeker grubunun tür içinde varlığı kalitatif ve kantitatif olarak analiz edilmiştir. Ayrıca şeker içeriklerine bağlı olarak, küme analizi yöntemiyle tanımlama ve gruplama teknikleri kullanılmıştır. Sonuç olarak yapılan şeker analizlerinde glikoz, sukroz ve fruktoz tüm türlerde rastlanır iken, diğer şeker içeriklerinde türler arasında farklılıklar tespit edilmiştir. Kümeleme analizinde *Hyacinthella* türleri şeker içerikleri bakımından 3 farklı gruba ayrılmıştır. Yapılan çalışma hem *Hyacinthella* türlerinin soğanlarında bulunan şeker içeriklerini tanımlamakta hem de farklı bir tanımlama yöntemi önermektedir. Morfolojik, moleküler ve metabolik verilerin birleştirilmesi ile türlerin tam ve doğru bir şekilde teşhis edilmesi sağlanacaktır.

Anahtar Kelimeler: Sınıflandırma, sümbül, endemik

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1. Introduction

Türkiye is one of the most important centers of the world in terms of plant genetic resources also a gene center for many genera and species. *Hyacinthella* Schur is a genus of bulbous ornamental plants belonging to the Asparagaceae family. *Hyacinthella* species emerge from bulbs whose tunics are often covered in powdery white crystals. Nineteen taxa belonging to the *Hyacinthella* species have been identified in the world and 11 of them are distributed in Türkiye. The genus *Hyacinthella* consists of 11 taxa [10 species and 1 hybrid species [*H. micrantha* (Boiss.) Chouard × *H. heldreichii* (Boiss.) Chouard] (Davis, 1965; Persson and Wendelbo, 1982; Persson, 2000).

Leaves of plants are the primary photosynthetic organs that fix atmospheric carbon to make sucrose, which is then transported great distances to non-photosynthetic tissues via phloem. Sugars and sugar derivatives were originated from fixed carbon during the photosynthesis. Triose phosphates is the first sugar derivates that released from chloroplasts by carbon fixation and triose transformed to sugars in the cytosol of photosynthetic (source) cells (Malkin and Niyogi, 2000). This sugar reserve is ready for export to heterotrophic (sink) organs like bulb, corm, tuber, etc., The variety of different sugar concentrations throughout leaf development is related with the significant requirement of decreasing sugars for energy and carbon skeletons (Buchanan et al., 2000). Researchers reported a relationship between sucrose metabolizing enzymes and transcript levels

in four different developmental stage in *Hevea* Aubl. leaves.

Sugar contents interact with other signaling molecules, including phytohormones therefore effect plant growth and development because of their position as energy and carbon sources, as well as their regulatory activities (Rolland et al., 2006; Smeekens et al., 2010). Environmental conditions (crop management) and genetic variety of speices can have an impact effect on the sugar content of tubers and bulbs (Thompson et al., 2008). Many environmental factors and climate change can influence a variety of biochemical processes; balance of partitioning of sugars within plant cells and their transportation from source to sink organs.

Sugar levels, transportation, consumption, and storage are regulated and dependent on physiological activity, plant organs, extreme conditions, circadian rhythms, and physilogic age. (Lemoine et al., 2013). Sucrose can be degraded by a variety of enzymes (invertases and sucrose synthase) or regenerated from degradation products. Furthermore, sucrose, glucose, fructose, and trehalose operate as metabolic signaling molecules in host plant cells and stimulating the activation of gene sets, including defense genes.

Bulb growth requires starch accumulation and storage of carbohydrates (Xu et al., 2019; Wu et al., 2020; Li et al., 2021). In this context, growth of the bulb are tightly linked to starch and sucrose metabolism. Starch and sucrose metabolism is a complex biochemical process involving multiple enzymes (Liu et al., 2022). Soluble sugars are degradation product of starch and they can provide energy for morphogenesis, carbon fixation, emergence of leaves and development of flower buds (Liu et al., 2022).

The objective of this study was to determine the sugar content of bulbs of all *Hyacinthella* species grown in Türkiye. According to our research, no previous study has been found on this subject. Additionally, the data obtained was evaluated with the Cluster analysis method, which is a useful statistical technique used in the fields of recognition, classification and machine learning.

2. Materials and Method

2.1. Material

The materials of this study consists of all *Hyacinthella* taxa distributed in Türkiye. Between March and May of 2019 - 2021, samples of *Hyacinthella* genus taxa were collected both in flowering and fruiting form from their localities specified in the records in the Flora of Turkey. The collected samples were dried in accordance with herbarium methods, identified and preserved in the Research Laboratory of Yüzüncü Yıl University, Faculty of Science.

Eleven different *Hyacinthella* species distributed in Turkey were collected and bulbs of them were dried in shadow. Species names are given in Table 1.

2.2. Methods

2.2.1. Analysis of Sugars

Five grams of bulb samples of 11 different *Hyacinthella* species crushed with liquid nitrogen thoroughly by mortar and pestle. Fourty ml of methanol was added and the final mixture was homogenized with a magnetic stirrer for 20 minutes at 50 °C. After centrifugation at 3000 rpm for 10 minutes at the appropriate temperature, the volume of the supernatants was made up to 50 ml with methanol and mixed thoroughly. The methanol phase was then evaporated

Table 1. *Hyacinthella* species used in the study

No	Species name
1	*H. campanulata* K. Perss. & Wendelbo (End.)
2	*H. acutiloba* K. Perss. & Wendelbo (End.)
3	*H. glabrescens* (Boiss.) K.Perss. & Wendelbo (End.)
4	*H. heldreichii* (Boiss.) Chouard (End.)
5	*H. hispida* (J.Gay) Chouard (End.)
6	*H. lazulina* K.Perss.& Jim.Perss. (End.)
7	*H. lineata* (Steud. ex Schult. & Schult.f.) Chouard (End)
8	*H. micrantha* (Boiss.) Chouard (End.)
9	*H. nervosa* (Bertol.) Chouard
10	*H. siirtensis* B.Mathew (End.)
11	*H. venusta* K.Perss (End.)

evaporated in a rotary evaporator. The remaining solution was made up to 5 ml with water.

The extracts were load to Sep-Pak C18 cartridges and 7.5 ml of acetonitrile was added to 2.5 ml of the filtrate. Extractions were loaded to 0.45 µm membrane filter. Samples were injected into the HPLC device. Analyzes of free sugars were performed by Torija et al. (1998), and Karkacier et al. (2003).

2.2.1.2. HPLC conditions and calibration

Regression curves for linearity of the HPLC method were tested by injecting 9 different sugar standarts at 0.2, 0.5, 1.0, 2.5, and 5 mg/mL concentrations. Acetonitrile/water (80:20) was used as the mobile phase. Analyze sugars at 30 °C with an HPLC refractive index detector at a flow rate of 2 mL/min. NH_2 (Amino) column was used as the column. Sugar content and amounts were expressed as mg/ml. Sugar analyzes were completed in three replicates. HPLC was calibrated using standard sugars such as glucose, fructose, sucrose, maltose, galactose, ribose, xylose, triose, mannose, arabinose.

Nineteen different sugars standarts were tested to detect the sugars content in the bulbs of *Hyacinthella*, however 9 of them could be detected.

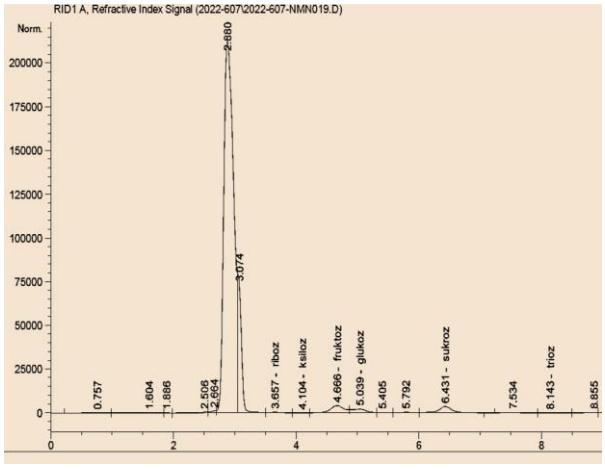

Figure 1. Chromatogram of sugar standards

2.2.2. Cluster Analysis

K-means is a clustering algorithm used to cluster a dataset into a certain number (K) of clusters or groups. This algorithm clusters data points according to a specific centroid, determining a set of centroids that represent the center of each cluster. Data points are assigned to the center closest to them, and this process is repeated until the clustering result of data points becomes stable.

The step-by-step working principle of the algorithm is as follows:

1. Initially, K random centers are selected. These centers may be randomly selected from the data set or determined according to a specific rule.
2. Each data point is assigned to the closest center. In this step, it is determined which cluster each data point is in.
3. A new center is calculated for each cluster. This is done by averaging all the data points of the cluster.
4. Steps 2 and 3 are repeated until a convergence criterion is reached where the centers change positions. The convergence criterion may be that the locations of the centers no longer change or that the amount of change does not exceed a certain threshold.

These steps are repeated until the dataset finds the optimal centers for clustering. The algorithm works by updating the position of the centroids and reclustering the data points at each iteration. As a result, each data point is associated with a cluster and the center of each cluster is determined.

3. Results

3.1. Sugar analysis

Although 19 different sugar standards were used for 11 different *Hyacinthella* bulbs in the study, 9 of them could be detected by HPLC analysis. Different sugar groups were obtained in different *Hyacinthella* species (Table 2.).

When the sugars in bulbs were evaluated qualitatively, glucose, fructose and sucrose sugars were found in all species naturally however the presence of other sugar types varied according to species (Table 2). It was determined that the differences between the species were mostly arabinose, triose and xylose sugars.

In the sugar analysis, the values differed in quantity levels. Ribose is the highest amount in *H. acutiloba*. Xylose, Glucose and Arabinose were found at higher level in *H. Campanulata*, Fructose at the highest amount founded in *H. Heldreichii*, Galactose at the highest levels in *H. Nervosa*. Sucrose and triose at the highest levels in *H. Venusta*.

Significant differences were detected between species, especially in terms of xylose and triose sugar amounts (Table 3).

Table 2. Qualitative analysis of sugars in *Hyacinthella* bulbs

Species	Ribose	Xylose	Arabinose	Fructose	Glucose	Galactose	Sucrose	Maltose	Triose
H. campanulata	-	-	+	+	+	+	+	+	+
H. acutiloba	+	+	+	+	+	+	+	+	+
H. glabrescens	-	+	-	+	+	+	+	+	+
H. heldreichii	-	-	-	+	+	+	+	-	+
H. hispida	-	+	-	+	+	+	+	-	+
H. lazulina	+	+	+	+	+	-	+	-	-
H. lineata	+	+	-	+	+	-	+	-	-
H. micrantha	-	+	-	+	+	-	+	-	+
H. nervosa	-	+	+	+	+	+	+	-	+
H. siirtensis	+	+	+	+	+	-	+	+	-
H. venusta	-	-	+	+	+	+	+	+	+

3.2. Cluster analysis

In this study, sugar data sets of 11 different *Hyacinthella* bulbs were used for cluster analysis. Clustering was done according to whether they were present or not. Clustering was performed by applying the K-means method to the data set. Plant species were clustered using the attributes of 9 different sugar types. In the K-means method, K=3 was chosen.

When the uure of the cluster analysis is examined, 3 different clusters are obtained. In the 1st group, *H. siirtensis*, *H. acutiluba*, *H. lazuilana* and *H. lineta* species (green), in the 2nd group, *H. campanulata* and *H. venusta* species (red), in the 3rd group, *H. glabrescens*, *H. nervosa*, *H. micrantha*, *H. hispida* and *H. helderichii* (blue) were defined. Same groups appear to be similar to each other.

4. Discussions

It was determined that there were significant differences between the species and their sugar contents. Naturally, glucose, fructose and sucrose sugars are found in all bulbs. These sugars are natural forms found in all plants. Differences were observed between species in sugar contents such as arabinose, triose and xylose. These sugar

Table 3. Quantitative analysis of sugars in *Hyacinthella* bulbs

EO	Ribose	Xylose	Arabinose	Fructose	Glucose	Galactose	Sucrose	Maltose	Triose
H. campanulata	1.09	9,.7	8.72	4.68	5.39	4.30	2.55	1.74	4.61
H. acutiloba	4.93	6.80	1.90	4.11	2.28	3.34	3.84	2.58	2.44
H. glabrescens	-	1.18	-	2.55	4.84	1.76	2.69	1.00	5.76
H. heldreichii	1.32	-	1.51	8.65	5.63	-	2.57	-	1.32
H. hispida	-	4.04	-	4.7	2.03	3.76	2.20	-	4.23
H. lazulina	-	3.85	6.16	1.60	1.24	-	3.08	-	-
H. lineata	-	3.85	6.16	1.60	1.24	-	3.08	-	-
H. micrantha	-	1.72	5.25	2.74	4.08	-	1.30	-	7.31
H. nervosa	2.55	4.86	-	3.01	5.24	-	3.16	3.29	5.00

H. siirtensis	-	3.98	1.98	3.02	3.20	5.64	2.58	-	4.60
H. venusta	2.33	2.38	2.38	6.25	3.69	-	2.84	1.89	7.67

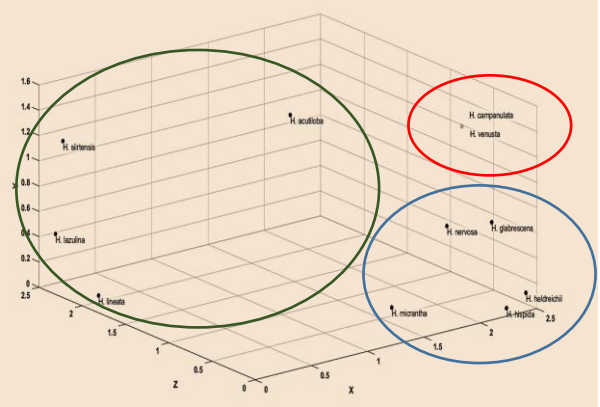

Figure 2. Cluster analysis of *Hyacinthella* species

were defined as specific sugars. Different sugar types play an active role in many metabolic events in plants; cell wall content to signaling, stress response to root tip elongation.

Responses to environmental changes; enzymatic and sugar content changes occurring in plant tissues are act as important indicators of the adaptive capacity of a species. This metbaolic process was defined as a necessity for the survival of the plant (López-Millán et al., 2000). In fact, the ability to maintain carbohydrate production and consumption, the stabilization of its levels, are the other responses, have been identified as a crucial adaptation for cold tolerance (Allen and Ort, 2001).

In the sugar analysis, it was seen that not only the sugar contents were different, but also their concentrations were variable. This may be due to the location and physiological age of the species, or due to their different adaptations. Locations of the *Hyacinthella* species are quite variable and their habitats also vary. Moreover, the environmental effects they exposed to are different from each other. In this context, sugar contents in *Hyacinthella* bulb may provide information about their habitat and adaptations.

There are quite different literature regarding sugar contents and diversity. These studies stated that sugar contents and concentrations may vary depending on metabolic and genetic differences, as well as ecological and stress situations. High sugar levels in plant tissues boost the plant's immunological response to fungal infections. Sugars most likely serve as priming chemicals that cause immunity in plants activated by pathogen-associated compounds (Morkunas and Ratajczak, 2014). Sugar content, qualitative composition, and transport in plant tissues change continually at day and night period also during all developmental phases. Plants have evolved an effective system by decreasing or increasing of sugar content related with metabolic process. Cell division, germination, vegetative development, flowering, and senescence are all impacted by changes in their concentrations. (Patric et al., 2013).

As a result, the reasons why sugar content varies in different *Hyacinthella* species studied are; It is thought to arise from the environment they are in and the conditions they are exposed to (biotic and abiotic), physiological conditions such as growth, development and flowering, and ultimately genetic differences.

In recent studies, anatomical and palynological features on *Hyacinthella* species were published (Eroğlu et. al, 2022; Sahin et al, 2022) However, the sugar content of bulbs belonging to *Hyacinthella* species were analyzed for the first time in current study. Moreover, the similarity and closeness between the species were tried to be evaluated by cluster analysis. More comprehensive and effective studies on *Hyacinthella* will contribute to the evaluation of this genus as a pharmaceutical or ornamental plant.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

Mehmet Acar: Analysis- Investigation-writing. Mehmet Emre Erez: Project administration – Investigation- review and editing. Hüseyin EROĞLU: Field investigation, anaylsis- writing.

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References

Allen DJ, Ort DR (2001). Impacts of chilling temperatures on photosynthesis in warm-climate plants. Trends in Plant Science 6: 36-42.

Buchanan BB, Gruissem W, Jones RL (2000). Biochemistry and Molecular Biology of Plants. Rockville: American Society of Plant Physiologists.

Davis PH (1965). Flora of Turkey and the East Aegean Islands, Vol. 1. Edinburgh: Edinburgh University Press.

Eroğlu H, Karaismailoğlu MC, Fidan M, Pınar SM (2022). Seed morphological and anatomical structures as taxonomy tool for Turkish *Hyacinthella* Schur (*Asparagaceae*) Taxa. Phytotaxa 564: 191-207.

Karkacier M, Erbas M, Uslu MK, Aksu M (2003). Comparison of different extraction and detection methods for sugars using amino-bonded phase HPLC. Journal of ChromatoGraphic Science 41(6): 331-333.

Lemoine R, La Camera S, Atanassova R, Dédaldéchamp F, Allario T, Pourtau N, Bonnemain JL, Laloi M, Coutos- Thévenot Prousset L et al., (2013). Source-to-sink transport of sugar and regulation by environmental factors. Frontiers Plant Science 4: 272-289.

Li J, Seng S, Li D, Zhang F, Liu Y, Yao T, Wu J (2021). Antagonism between abscisic acid and gibberellin regulates starch synthesis and corm development in *Gladiolus hybridus*. Horticulture Research. 8(1): 155.

Liu Y, Zhenyu Qi, Jinsen Wei, Jingquan Yu, Xiaojian Xia (2022). Brassinosteroids promote starch synthesis and the implication in low-light stress tolerance in *Solanum lycopersicum*. Environmental and Experimental Botany 201: 104990.

López-Millán AF, Morales F, Abadía A, Abadía J (2000). Effects of iron deficiency on the composition of the leaf apoplastic fluid and xylem sap in sugar beet: implications for iron and carbon transport. Plant Physiology 124: 885-897.

Malkin R, Niyogi K (2000). Photosynthesis. In: Buchanan BB, Gruissem W, Jones RL (eds). Biochemistry and Molecular Biology of Plants. Rockville: ASPP.

Morkunas I, Ratajczak L (2014). The role of sugar signaling in plant defense responses against fungal pathogens. Acta Physiologiae Plantarum 36: 1607-1619.

Patrick JW, Botha FC, Birch RG (2013). Metabolic engineering of sugars and simple sugar derivatives in plants. Plant Biotechnology Journal 11: 142-156.

Persson K (2000). Two new bulbous species from the central Taurus mountains of Turkey. New Plantsman 7(4): 200-208.

Persson K, Wendelbo P (1982). Taxonomy and cytology of the genus *Hyacinthella, Liliaceae, Scilloideae* with special reference to the species in Southwestern Asia. Scilloideae 513-541.

Rolland F, Baena-Gonzalez E, Sheen J (2006). Sugar sensing and signaling in plants: Conserved and novel mechanisms. Annual Review of Plant Biology 57: 675-709.

Smeekens S, Ma J, Hanson J, Rolland F (2010). Sugar signals and molecular networks controlling plant growth. Current Opinion Plant Biology 13: 273-278.

Sahin AA, Eroğlu H (2022). Pollen morphology of the genus *Hyacinthella* (*Asparagaceae*) and *Muscari azureum* in Turkiye. Phytotaxa 564: 39-58.

Thompson AL, Love SL, Sowokinos JR, Thornton MK, Shock CC (2008). Review of the sugar end disorder in potato (*Solanum tuberosum* L.). American Journal of Potato Research 85(5): 375-386.

Torija E, Díez C, Matallana C, Camara M, Camacho E, Mazarío P (1998). Influence of freezing process on free sugars content of papaya and banana fruits. Journal of the Science of Food and Agriculture 76(3): 315-319.

Wu Y, Ren Z, Gao C, Sun M, Li S, Min R, Xia Y (2020). Change in sucrose cleavage pattern and rapid starch accumulation govern lily shoot-to-bulblet transition in vitro. Frontiers Plant Science 11: 564713.

Xu JX, Li QZ, Yang LY, Li X, Wang Z, Yang Z, Zhang YC (2019). Changes in starch synthesis and metabolism within developing bulbs of *Lycoris radiata* during the vegetative growth stage. Journal of Plant Growth Regulation 39 (2): 785-794.