www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2022.1109159
15/2 (2022) 218-224

A study on determining the morphological, physiological and chemical properties of Green rose (*Rosa chinensis* Jacq. 'Viridiflora') grown in greenhouse conditions

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Abstract

In this study, some morphological, physiological and chemical properties of the *Rosa chinensis* Jacq. 'Viridiflora' taxa, which does not form petals due to a flower anomaly called phlloidi and is called 'Greenrose' only because of the long and green sepals it forms, were discussed. In this context, plant growth characteristics, leaf characteristics, stomatal morphology, proportional water content of leaves, chlorophyll contents of leaves and micro and macro nutrient content of leaves were determined. As a result of the study, it was determined that the 'Green rose' plant, which was compared with different *Rosa* taxa, had a broad/upright structure, the average leaf area was 17.17 cm^2, the average pedicel length was 1.45 cm, the proportional water content of the leaf was 77.42%, and the chlorophyll amount was 40.87. It was concluded that the leaves of *R. chinensis* 'Viridiflora' plant had average values in stomatal morphology parameters. In line with reference values, nitrogen, phosphorus and manganese values were found to be low in Yeşilgül leaves, while boron and calcium values were found to be high. Potassium, magnesium, copper and iron contents have been determined within the reference range.

Key words: Green rose, *Rosa chinensis* Jacq. 'Viridiflora', morphology, plant physiology, nutrient element

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Sera koşullarında yetiştirilen Yeşilgüllerin (*Rosa chinensis* Jacq. 'Viridiflora') morfolojik, fizyolojik ve kimyasal özeliklerini belirlemek üzerine bir çalışma

Özet

Bu çalışmada Phlloidi adı verilen bir çiçek anomalisi nedeniyle taç yaprağı oluşturmayan ve sadece oluşturduğu uzun ve yeşil çanak yaprakları nedeniyle 'Yeşilgül' olarak adlandırılan *Rosa chinensis* Jacq. 'Viridiflora' bitkisinin morfolojik, fizyolojik ve kimyasal özellikleri incelenmiştir. Bu kapsamda bitki büyüme özellikleri, yaprak özellikleri, stoma morfolojisi, yaprak oransal su içerikleri, yaprakların klorofil içerikleri ve yaprakların mikro ve makro besin içerikleri belirlenmiştir. Çalışma sonucunda, farklı *Rosa* taksonları ile karşılaştırılan 'Yeşil gül' bitkisinin geniş/dik bir yapıya sahip olduğu, ortalama yaprak alanının 17.17 cm^2, ortalama sap uzunluğunun 1.45 cm olduğu, yaprağın orantılı su içeriğinin %77.42, klorofil miktarının ise 40.87 olduğu değerlerini aldığı belirlenmiştir. *R. chinensis* 'Viridiflora' bitkisinin yapraklarının stoma morfolojisi parametrelerinde ortalama değerlere sahip olduğu sonucuna varılmıştır. Yeşilgül yapraklarında azot, fosfor ve manganez değerleri düşük, bor ve kalsiyum değerleri ise yüksek bulunmuştur. Potasyum, magnezyum, bakır ve demir içerikleri referans aralığında belirlenmiştir.

Anahtar kelimeler: Yeşilgül, *Rosa chinensis* Jacq. 'Viridiflora', morfoloji, bitki fizyolojisi, besin elementi

1. Introduction

Evidence showing that roses were a part of social life in all civilizations established in different periods has reached the present day. In museums located in different cities of our country, located in the Anatolian geography,

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 Received: 26.04.2022; Published: 15.08.2022 BioDiCon. 1037-260422

where civilization dates back to ancient times, objects such as vases, goblets and bowls, coins with rose patterns, and jewelry, which are based on the 'rose' phenomenon in design, are exhibited. Roses depicted as wreaths on the floor mosaic found in Pergamon (Bergama) Antique City in İzmir can be shown as an example [1].It is known that there is a rose variety called sempervirens [2,3]. In the Ancient Greek Period, the red rose was known as the symbol of Aphrodite [4], and the white rose was known as the symbol of Selene [5]. During this period, rose festivals called 'Rosalia' were held for the dead. This custom continued in the Roman period and cemeteries were decorated with roses to commemorate the dead [6].

The *Rosales* order includes nine families of economic and ecological importance. The *Rosaceae* family, which generally consists of trees and shrubs important for landscaping and fruit growing, includes 90 genera and 3000 species. Members of the family have mostly worldwide distribution but are more concentrated in the northern temperate regions. The family is economically extremely important as a source of many fruits, essential oils, and countless varieties of landscape and ornamental plants [7]. *Rosa* L. taxa belonging to the *Rosaceae* family, which are generally distributed in all regions of Turkey, are found naturally in Central/Western Asia, Europe, the Caucasus, North Africa, Iran and the North and West parts of Iraq and Northern Afghanistan [8, 9, 10]. Roses have generally been identified with people throughout the historical process with their aesthetic flowers. The rosehip (*Rosa canina*) species, which is in the same genus taxonomically, is also important with its fruits. Not only *R. canina*, but also *R. rugosa* and *R. montana* are important rosehip species. In this review, the literature on the use of *Rosa* L. taxa in different areas in the historical period has been reviewed. For this, in addition to natural rose species, some cultural taxa and findings related to rose hips are also included.

1. Material and methods

The plant materials were collected from Halfeti Municipality Karagül Display Greenhouse, Ministry of Agriculture and Forestry Halfeti District Directorate 'Hidden Smell of a Calm City Karagül Project' Greenhouse in Şanlıurfa Province. The literature of Brummitt and Powell (1992) was taken as a reference for the standard writing of scientific names, including the authors, and was examined considering the UPOV (2010) criteria [11, 12]. Plants grown in pots are in Harran University R&D Greenhouse.

The findings obtained in the analyzes made; It has been revealed that the pH of the growing medium in which the study was carried out was 7.14, clayey / loamy structure and low in organic matter. Lime content was determined as 6.14% and salt content as 0.04%. When the chemical properties of the soil used in the greenhouse where the experiment was carried out were examined, the P value was high, the K value was critical, and the Fe, Cu, Zn, Mn and B values were found sufficient. It was determined that the growing environment was generally at sufficient levels in terms of the content of plant nutrients required for rose cultivation, no fertilization was made and the experiment was carried out in this way.

2.1. Methods

2.1.1. Determination of plant growth trait

Plants of different taxa propagated by cuttings were transplanted into 5 L pots, plant length (cm), plant crown width (cm), plant crown/height ratio measurements were made. Growth patterns of 2-year-old plants were determined according to height and crown width.

2.1.2. Determination of morphological characteristics

Leaves were determined by calculating the leaf area in cm² with the help of ImageJ program [13, 14].

The leaves and leaflets of the plants were counted one by one and the average number of leaves and leaflets per plant was determined [12, 14].

In the study, one leaf was taken from ten different plants and one summer shoot from each plant in each phenological development period. Considering that the distribution of stomata may vary in plants with compound leaves, stomata patterns were extracted from three different points (tip, middle, bottom) on each leaf. In *Rosa* L. species, the leaves are in the form of leaflets; Differences in stomatal characteristics between the tip (A), middle (B), and lower (C) leaflets of the leaves were also investigated. The “Nail Polish Method” was used to take the stoma molds [15, 16, 17].The lower surfaces of the leaves were painted with completely transparent nail polish and left to dry. Tape was attached to the dried nail polish leaves and then removed and attached to the slides. Thus, the stoma was examined under the microscope. Images taken with SOIF microscope with 10x objective, photographed with MShot camera, MShot-1.3.10. Counts were made in a computer program [16, 17, 18, 19].

The number of stomata detected in the 0.776 mm^2 field of view; It was determined by adapting to 1 mm^2 area. For stoma/pore length and width (µm) values, the length and width of 10 stomata in the stoma/pore pattern photographs were measured in µm by using the MShot-1.3.10 computer program [16, 17, 18, 19].

The wet weights of the leaf samples taken from the pots in the experiment were determined, and the turgor weight was determined as a result of keeping them in petri dishes containing 100 ml of water for 24 hours. The samples were dried in an oven at 65-70ºC and their dry weights were determined by weighing them on a precision balance [14, 20,21].

It was determined with the SPAD-502 instrument by 4 separate measurements made in 2020 and 2021 from the bottom, middle and tip parts of the compound leaflets [14, 20].

2.1.4. Determination of plant nutrients in leaves

For the analysis of macro and micronutrients, leaves that collected together with the petiole during the flower formation period, which is the period taken as an example in the study of Jones et al. (1991), and preserved in separate paper bags. Leaf samples were taken from the middle parts of annual shoots together with their stems, representing the bottom, middle and end leaflets of each plant. This process was performed in 3 replications for the samples taken. The samples brought to the laboratory were first thoroughly washed with running water and then washed with distilled water. The samples were left to dry for 7 days at room temperature, and were mixed 3 times a day to prevent moisture. After these processes, the samples that were dried in the oven were ground in a crucible mortar and put in plastic bags, giving their codes for analysis.

Dried leaf samples were burned with the wet burning method in 3 replications, 0.5 g sample in each replication. Nitrogen amount was calculated as % by Khejdal method [23, 24].Phosphorus in the leaves; It was determined by the colorimetric determination of the color in the spectrophotometer as a result of the dry burning process [25]. Determination of calcium and potassium in leaves; It was determined by reading the samples in a flamephotometer [23]. Determination of magnesium in leaves; After dilution of plant samples prepared by dry burning method, it was determined by reading with an atomic absorption instrument [23].

The specified method was applied for the nutrient analysis in the leaves. 1 ml of sulfuric acid and then 19 ml of ethyl alcohol were added to the leaf samples weighing 1 g. The ash was first burned in the furnace at 250˚C for 2 hours, then at 650˚C for 4 hours and left to cool. Then 5 ml to burnt leaves. HCL (Hydrochloric Acid) has been added. Leaf samples were filtered with the help of sterile filter paper, crucibles were filled with distilled water, and the process was repeated 2-3 times. Mn, Cu, Zn and Fe contents were calculated in ppm units in atomic absorption spectrophotometer [23].

2. Results

The morphological data obtained in the study were found to be compatible with the relevant references. In addition to these, some physiological and chemical parameters that were not studied before were also determined [26,27]. Morphological parameters according to the research results are given in Table 1.

Table 1. Morphological features of *R. chinensis* 'Viridiflora'

Parameters	**Value**
Plant height (cm)	48.03
Crown width (cm)	50.60
Crown/Height ratio	1.05
Leaf width (cm)	7.28
Leaf length (cm)	6.40
Leaf area (cm^2)	17.17
Pedicel length (cm)	1.45
Tip leaflet area (cm^2)	5.38
Middle leaflet area (cm^2)	3.42
Bottom leaflet area (cm^2)	1.11
Stoma size(µm)	23.75
Stoma width (µm)	20.43
Pore size (µm)	21.01
Pore width (µm)	14.81
Stoma density (pcs/mm^2)	210.90

The parameters determined in determining the leaf proportional water content of *R. chinensis* 'Viridiflora' are presented in Table 2.

Table 2. Dry, fresh and turgor weights and proportional water content of the leaves of *R. chinensis* 'Viridiflora'

Parameters	**Value**
Dry weight (g)	0.0161
Fresh weight (g)	0.0751
Turgor weight (g)	0.1104
Relative Water Content of Leaves (%)	**77.42**

The SPAD measurements and the average chlorophyll content value at different periods are presented in Table 3.

Table 3. Leaf chlorophyll content values of R. chinensis 'Viridiflora' taxa

SPAD Value	**Value**
1st	35.46
2nd	41.40
3rd	45.13
4st	41.50
Average	**40.87**

The macro and micro nutrients detected in the leaves are presented in Table 4.

Table 4. Macro and micro nutrient content of leaves of *R. chinensis* 'Viridiflora' taxa

Elements	**Value**
N (%)	1.33
P (%)	0.08
K (%)	1.50
Mg (%)	0.23
Ca (%)	2.77
Fe (ppm)	110.87
Cu (ppm)	11.35
Zn (ppm)	27.84
Mn (ppm)	22.42
B (ppm)	151.04

3. Conclusions and discussion

Apart from the leaves, flowers, thorns and colors of roses, plant development characteristics also vary greatly. For example, climbing growth type and stunting characteristics have been defined as dominant features in roses [22, 28]. In the studies carried out on *Rosa* L. taxa, the characteristics of the species or types such as plant height and plant crown were determined, in terms of plant development characteristics; It is classified as broad (diffuse), upright and splayed/upright. When the relevant studies[8, 29, 30] are examined, the *R. chinensis* 'Viridiflora' plant has been defined as 'flat/erect'.

Leaf width, length and area measurements can be considered as one of the distinguishing morphological features of rose species. In a study conducted in this context [31], leaf observations were made during the vegetation period of Damask Rose (*R.* x *damascena*). As a result of the study, the leaf development phase in roses was determined as 6 different periods, the average leaf width changed from 1.3 cm to 6 cm in this process; The average leaf length has reached from 0.8 cm to 5.6 cm.In the *R. chinensis* 'Viridiflora', the tip leaflet length value (4.27 cm) was found to be higher than that taxon.

Alp et al. (2016), stomatal characteristics of 5 *Rosa* taxa (*R. pulverulanta*, *R. canina*, *R. foetida*, *R.* x *damascena* and *R.* x *damascena* var. *semperflorens*) grown in Van ecology were determined. As a result of the research, it was revealed that stomata were mostly hypostomatic and the average number of stomata per mm^2 ranged between 290.21pcs mm^{-2}(*R.* x *damascena*) and 130.61pcs mm^{-2} (*R.* x *damascena* var. *semperflorens*), and stoma density tended to increase as stoma lengths decreased. As a result of the study, the highest stoma length was found in *R. pulverulanta* with 13.70 µm, and the highest value in the average stoma number was determined in *R.* x *damascena* with 290.21pcs mm^{-2}. In a similar study [32] stomatal characteristics of 3 different taxa (*R. canina*, *R. pimpinellifolia*, *R. iberica*) were examined, the stomatal length was highest in *R. pimpinellifolia* (31.8 µm), the densest stoma number was *R. canina*. It was determined that there were also (150.74pcs mm^{-2}). According to the common opinion of both studies, it was concluded that as stoma width and length decreased, stoma density per unit area increased in these rose taxa. Hatipoğlu

and Ak (2021) found that the highest stoma density was in *R.* x *damascena* (335.06pcsmm^{-2}), while the lowest stoma density was in *R. pisiformis* (169.50 pcs mm^{-2}).In this context, the green rose has taken average values in stomatal parameters according to the relevant literature.

Fanourakis et al. (2019), at 100, 200 and 400 µmol/(m^2•s) photosynthetic photon intensity of hybrid roses; stomatal response, stomatal anatomical features and cuticular transpiration, photosynthesis response of plants to light and CO2. It has been shown that light intensity leads to a significant increase in plant biomass, often with larger porous stomata [34]. In a similar study [33], the highest chlorophyll content was observed in *R. chinensis* 'Old Blush' (44.46), while the lowest chlorophyll content was observed in *R. foetida* (28.32). In this context, the Green rose received an above-average value. Chlorophyll, which is of vital importance for plants, is the main active ingredient of photosynthesis and the amount of chlorophyll in the plant varies according to ecological conditions and different taxa.

Yamane (1990) reported that the pH of the topsoil in the natural stands of *R. rugosa* varied between 5.1 and 7.6. In the regions where *R. rugosa* grows naturally in Denmark, pH values were determined as 4.66-7.74, lime rate 6.26% and organic matter content 1.20%. The results of the soil analysis carried out within the scope of the thesis and the aforementioned literature were found to be close to each other [35]. As a result of a research on the macro and micro nutrient contents of the leaves of a culton belonging to the *R. odorata* taxa [22], the optimum value ranges during the beginning of flower formation; 3.00-5.00% for nitrogen, 0.25-0.50% for phosphorus, 1.50-3.00% for potassium, 1.00-2.00% for calcium, 0.25-0.50% for magnesium, 30-60 ppm for boron, 7-25 ppm for copper, iron 60-200 ppm for manganese, 30-200 ppm for manganese, 18-100 ppm for zinc. In line with these reference values, nitrogen, phosphorus, and manganese values were found to be low in Green rose leaves, while boron and calcium values were found to be high. Potassium, magnesium, copper, and iron contents were within the reference range.

R. odorata and *R. chinensis* subspecies are rose taxa that have adapted to the local ecology and are used extensively in gardens and landscaping in Şanlıurfa. Some varieties belonging to these species are of local importance for Sanliurfa; they are known as 'Halfeti Rose' (*R. odorata* 'Louis XIV') and 'Green rose' (*R. chinensis* 'Viridiflora'). In addition, as a result of its adaptation to the region, 'Black Rose' received geographical indication as of 2021 in order to contribute to rural/ecological tourism and people living in rural areas to focus on different employment areas. In this context, it is thought that it is important to determine these parameters of the Green Rose, which is at least as important as the Halfeti Rose. This plant, which has a spicy and peppery odor, can also be evaluated in different areas of use [36]. It will make a significant contribution to the economy of both the country and Halfeti by protecting, cultivating, and bringing to tourism the green rose, which is a very important potential for Turkey. It is of great importance to give more weight to scientific studies to be carried out on green roses soon and to prevent the destruction of this natural and biological richness with the projects to be carried out.

Acknowledgements

The study was supported within the scope of HUBAP 19248 project and Council of Higher Education 100/2000 project. We would like to thank the Republic of Turkey Halfeti District Directorate of Agriculture and Forestry for helping with the supply of plant material.

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