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The Characterization of Floral Organs of Halfeti Black Rosa (*Rosa Odorata* L. Cv. Louis XIV) During the Flowering Stages

Sabri Erbaş[1,2,a], Murat Mutlucan[2,b,*]

[1]*Isparta University of Applied Sciences, Faculty of Agriculture, Department of Field Crops, Isparta, Türkiye*
[2]*Isparta University of Applied Sciences, Rose and Aromatic Plants Implementation and Research Center, Isparta, Türkiye*
[*]*Corresponding author*

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ABSTRACT

In recent years, research on the Halfeti black rose (*Rosa odorata* L. cv. Louis XIV), which has become a symbol of the Halfeti district in western Şanlıurfa, has begun to gain further depth. The present study investigates some physical and color changes in the Halfeti black rose during bud and flower development across five distinct. The result of study determined that the Halfeti black rose flower has an average of 28 petals, 5 sepals, 70 anthers, and 40 pistils. The petal content in the flower increased from 68.59% in the stage 1 to 83.04% in the stage 5, the sepal, hypanthium, anther+filament, style+stigma content decreased from 9.96, 14.18, 5.00 and 2.28% in the stage 1 to 5.79, 7.84, 1.51 and 1.82% in the stage 5, respectively. When the fresh and dry weights and diameters of the flower were examined, it was found that the flower diameter expanded (from 18.78 mm to 55.19 mm) and the fresh (from 2.55 g to 4.53 g) and dry weights (from 0.66 g to 0.90 g) increased throughout the development period. It was observed that the moisture content of the petals decreased from the 1. to the 5th stage (from 78.22% to 75.85%). Although the anther diameter showed a fluctuating development throughout the development period, it was observed to decrease towards the end of flowering. The filament elongated from the 1. period (6.60 mm) to the 5. period (10.64 mm). On the other hand, the style stigma lengths elongated from 10.54 mm to 12.02 mm until the 3. period and their legths were measured as 11.51 mm and 11.88 mm in the 4 and 5. periods, respectively. While the L* and a* values of the petals did not change significantly throughout the development period, the b value decreased. The hue angle declined throughout the development period. As a result, it was determined that the highest petal content, flower diameter, and fresh and dry flower weight were in the 5. period. However, the flowers in this period need to be analyzed for the secondary metabolite productivity mentioned above.

[a] *sabrierbas@isparta.edu.tr* *https://orcid.org/0000-0003-0691-6127* | [b] *muratmutlucan@isparta.edu.tr* *https://orcid.org/0000-0002-6990-277X*

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Introduction

Roses, which have a widespread distribution worldwide and are estimated to have been used medicinally by various civilizations for at least 5,000 years, hold a special significance in human history (Baydar et al., 2007). Roses belong to the genus *Rosa* in the *Rosaceae* family. The genus Rosa contains over 100 species widely distributed in Europe, Asia, the Middle East, and North America (Nilsson, 1997). In Türkiye, there are approximately 25 old garden roses and 45 wild and cultivated species (Ercişli, 2005; Özçelik, 2010). In addition to being used in the production of rose oil due to their distinctive fragrance, old garden roses have been used throughout history in the development of modern rose varieties. Rose species in the world are divided into classes such as "Old Garden Roses", "Shrubs", "Hybrid Teas", "Miniature Roses (*R. chinensis* var. *minima*)", "Climbers", "Grandifloras", "Floribundas", "Polyanthas". Today, landscape, potted, and cut roses are classified as "Noisette," "Bourbon" (*R. chinensis* × *R. damascena* hybrids), "Portland," "Pernetiana," "Polyantha" (*R. chinensis* × *R. multiflora* hybrids), "Hybrid China," "Hybrid Tea," and "Grandiflora hybrids" (Floribunda × Tea rose hybrids) (Tucker & Maciarello, 1988; Weiss, 1997; Baytop, 2001; Baydar & Kazaz, 2013; Erbaş & Baydar, 2016). Rose species used in global rose oil production include *R. damascena* Miller, *R. gallica* L., *R. centifolia* L., *R. alba* L., *R. bourboniana* Desp., *R. rugosa* L., and *R. moschata* Herrm. These rose species are used in cosmetics and perfumery, as well as in pharmaceuticals and food (Önder et al., 2023).

Halfeti black rose (genus Rosa; Indicae subsection (section 7), *R. odorata* L. cv. Louis XIV, 2n=4x=28),

which has become the symbol of the Halfeti district located west of Şanlıurfa in our country, has started to gain importance in the cosmetics industry in recent years, as well as being used in the production of foods such as jam and rose water in dry arrangements. The Halfeti rose has been cultivated in Halfeti for a long time. Although it is not known when and where it came to Halfeti, it is known that it was registered and commercialized in Europe by the French rose grower Guillot in 1859 under the name "Louis XIV" (Baytop, 2001). In addition, records indicate that it was brought to Halfeti by a person named "Lomo Sait" and spread widely (İkinci and Akmeşe, 2019). However, in 2021, it was patented with a geographical indication by the Halfeti Municipality under the name of origin 'Halfeti black rose/Halfeti black rose' (C2019/131). Halfeti rose, which is still widely cultivated in home gardens, has begun to be grown in large areas (1.5 hectares in greenhouses and 10 hectares in open fields) with the initiatives of public and private companies.

Halfeti black rose generally exhibits deciduous, shrubby and climbing landscape characteristics. Its leaves are solitary or in clusters with 5 leaflets. Hatipoğlu (2022) reported that the plant height of Halfeti black rose is 61.33 cm, crown width is 52.34 cm, crown/height ratio is 0.77 and it grows in an upright form, with leaf width being 5.69 cm, leaf length being 5.54 cm, leaf area being 18.61 cm^2 and pedicel length being 0.96 cm. The N content in the leaves was determined as 1.47%, P content as 0.09%, K content as 1.68%, Mg content as 0.28%, Ca content as 1.53% and Fe content as 78.7 ppm (Hatipoğlu, 2022). The flowers of Halfeti black rose are solitary and large on the shoots. The color is red, almost black, during the budding period and lightens to dark red during the flowering period (Baytop, 2001; Özden, 2013; Özçelik & Koca, 2021). It has been reported that under the ecological conditions where it is grown, the flower color, while red in summer, turns to dark red, almost black, in late autumn (Özden, 2013). The flowers have a light and pleasant scent, but the scent decreases as the flowers begin to age. The pollen viability rate is 50.56%, the morphologically normal pollen rate is 68.38%, and the pollen germination rate is 45.51% (Kılıç & Kazaz, 2023). The fruits are red and showy, with an average weight of 1 g, a length of 12.80 mm, a width of 11.65 mm, a seed count of 20-25, and a flesh weight of 0.85 g (Hatipoğlu & Ak, 2021; Kılıç, 2020). The average oil content of Halfeti black rose seeds is 5.48% and the oil contains 0.05% lauric acid, 0.05% myristic acid, 7.02% palmitic acid, and 4.13% stearic acid (Hatipoğlu, 2022). Since the petals of this rose are generally dried, the flowers are harvested before the fruit forms.

Seedlings of repeat-blooming roses, such as *R. odorata*, carry a mutant gene that shortens the juvenile phase after germination, causing them to flower in the same year. However, in these species, flowers appear only on shoots with six to seven leaves. Only in climbing repeat-blooming roses do flowers appear on shoots with 14 leaves. Shortly after bud burst, the shoot apical meristem undergoes a physiological change. It ceases to produce leaves and initiates an inflorescence consisting of one or more flowers. The transition from vegetative to reproductive growth is accompanied by flattening of the meristematic dome. A few weeks later, in late spring or early summer, flowers finally emerge from the terminal bud. Growth of the primary axis culminates in the flowering phase (Roberts & Bloke, 2003). During plant development, flowering is often associated with senescence and death. In most cases, the flower is the organ with the shortest lifespan (Halevy & Mayak, 1981). This developmental process is characterized by several stages, including differentiation, cell division, cell growth, and eventual death, i.e., senescence. Much of the petal growth is related to the outcome of cell growth (Eason & Webster, 1995). The process controlling flower development regulates the partitioning of assimilates between the flower and the rest of the shoot system. Developing flowers serve as sinks, and the rate of carbohydrate flux from the source to the sink is related to the activity rates of enzymes such as invertase (Barthe & Vaillant, 1993; Sood et al., 2006). This study was conducted to determine some physical and chemical changes in the flower during the floral development period of the Halfeti black rose.

Materials and Methods

The study used flowers and buds from five different stages of two-year-old Halfeti black roses (*Rosa odorata* L. cv. Louis XIV) growing in the Halfeti district of Şanlıurfa (Old Halfeti-Özmüş village) in 2025. The Halfeti rose garden where the study was conducted was established in October 2023 with 3 m row spacing and 50 cm within rows. The following standard maintenance, fertilization, and pesticide application program was implemented until the study was conducted in 2025. Rejuvenation pruning was performed on the plants each November (10 cm above the soil surface), and plants were irrigated regularly with drip irrigation throughout the growing season. In winter, the plants were treated with 40 kg/da of 10-18-12 + (20 SO_3) base fertilizer. When the shoots of the plants started to wake up, 1 lt fulvic acid, 2 kg 10-52-10 (N-P-K) and 1 kg micronutrient fertilizers [Boron 1.3 w/w, Copper 2.2 w/w, Iron 7 w/w, Manganese 8 w/w, Molybdenum 0.03 w/w, Zinc 4.3 w/w] were applied via drip irrigation at 15-day intervals (2 kg/da) starting 15 days before flowering (early March). On the other hand, when the leaf tips started to appear on the plants, 15 kg/da Bordeaux mixture was applied and pesticides recommended for combating aphids, powdery mildew, rust and black spot found on Halfeti black rose were applied. Buds and flowers were collected at five flowering stages between the period when the petal leaves began to appear and the period when the petal leaves were fully opened, according to the bud and flower size in mid-April. The characteristics of the buds and flowers in the collection stages are as follows (Figure 1);

- Stage 1: Petals are closed and tightly surrounded by sepals.
- Stage 2: Petals have not opened, but sepals are beginning to open.
- Stage 3: Petals have begun to open, but stamens are not visible.
- Stage 4: Petals have opened into a cupped shape, and stamens appear pale yellow.
- Stage 5: Petals are fully opened, and stamens appear dark yellow-brown.

Figure 1. Flowering stages of the Halfeti black rose

Morphological characteristics such as anther diameter (mm), filament length (mm), stigma diameter (mm), style length (mm), ovary length (mm), flower diameter (mm) anther and stigma number (number/flower) were examined in 50 randomly collected buds and flowers from each stage. Length and diameter measurements were made with the help of calipers and the number of pieces was counted by hand. The moisture contents of petals, sepals, ovary, anther+filament, and style+stigma were determined by drying them in an oven at 70°C for 48 hours throughout the developmental period. The proportional distribution (%) of these organs within the flower was calculated based on their fresh and dry weights. The color value in the flower was measured with a colorimeter (Minolta CR300, Ramsey, NJ, USA). Color measurements were performed at two points on both surfaces of petals selected randomly in each replication and given as the mean value of both surfaces. The colorimeter apparatus was calibrated according to standard white plate (Y=93.9, x=0.313 and y=0.321). The values were evaluated according to CIEL* (represents brightness–darkness changing from 0 to 100), a (+a*: red, −a*: green) b (+b*: yellow, −b*: blue), C* (represents vividity of color) and h◦ (represents perceived color) system. The chroma (C*) and hue angle (h◦) values were calculated with the following formulas: h◦= tan−1(b*/a*), C*= $[(a^*)^2+(b^*)^2]^{1/2}$. The mean values and standard errors of all obtained results are presented.

Results and Discussion

Halfeti black rose is an old rose species with intensely fragrant, double-layered, dark cherry-black flowers that generally bloom between April and September. In this study, color changes, moisture contents, and morphological changes in buds and flowers of Halfeti black rose were investigated throughout the flower development period. The results are presented in Table 1. In our study, it was determined that one Halfeti black rose flower has 28.00 petals, 5.00 sepals, 70.00 anthers, and 40.00 pistils (Table 1). In studies conducted on Halfeti rose, Kılıç (2023) reported an average of 25 petals and an average of 40 stigmas. On the other hand, Kılıç and Kazaz (2023) reported that the number of petals varied between 25 and 30, with an average of 27. Our results are consistent with those of Kılıç (2023) and Kılıç and Kazaz (2023). However, no data on anther number were found in these studies and literature searches. In the Rosa genus, the ABCE genes regulate the formation of petals, stamens, and carpels according to the flower developmental model (Smulders et al., 2019). Within this homeotic gene (MADS-box) group, the A gene is involved in the formation and differentiation of petals, the B gene in petals and stamens, and the C gene in the formation and differentiation of stamens and carpels. Here, the C function gene (RhAG) causes an increase in the number of petals and a decrease in the number of stamens in double roses (Bendahmane et al. 2013; Kılıç, 2020). Thus, in taxa with the trait of pluripotency, expression of this RhAG gene is downregulated (reduced number and activity of surface receptors), restricting its expression domain to the center of the floral meristem, resulting in fewer stamens and a greater number of petals (Tešanovic et al. 2018; Kılıç, 2020). Therefore, it is thought that the discrepancy between the results of our study and those of other researchers may be due to the homeotic transformation of stamens into petals.

According to the proportional distribution of floral organs throughout the flowering stage, the bud to petal ratio was 68.59% in the first stage, increasing throughout the development period and reaching 83.04% in the last stage. The sepal ratio varied between 5.79 and 9.96% throughout the flowering stage and decreased regularly. The hypanthium ratio, which is a structure composed of the calyx, petal, and bases of the stamens that form a cup-shaped tube called the floral receptacle or tube, decreased from 14.18% to 7.84% throughout the flowering stage. Similarly, the male and female organ ratios decreased from 5.00% to 1.51% and from 2.28% to 1.82%, respectively, throughout the flowering stage (Figure 1). It was found that the flower diameter expanded (from 18.78 mm to 55.19 mm), and the fresh weights (from 3.55 g to 4.53 g) and dry weights (from 0.66 g to 0.90 g) increased throughout the flowering stage. It was observed that the moisture content of the petals decreased from the first to the last stage (from 78.22% to 75.85%). While the moisture content of the sepals increased from the first to the 4. stage (from 68.00% to 70.69%), it decreased in the last stage (68.70%). The moisture content of the hypanthium decreased throughout the flowering stage (from 79.38% to 76.25%). The moisture content of the anther+filament increased until the 4. stage and decreased in the last stage. The moisture content of the style+stigma increased throughout the flowering stage (Table 1).

Shortly after bud burst in roses, the shoot apical meristem undergoes a physiological change. It ceases to produce leaves and initiates an inflorescence consisting of one or more flowers. The transition from vegetative to reproductive growth is accompanied by flattening of the meristematic dome. A few weeks later, in late spring or early summer, flowers finally emerge from the terminal bud (Roberts & Bloke, 2003). After this stage, the distribution of assimilates in plants is significantly influenced by the changes that occur during the transition from the vegetative to the reproductive phase (Halevy & Mayak, 1981).

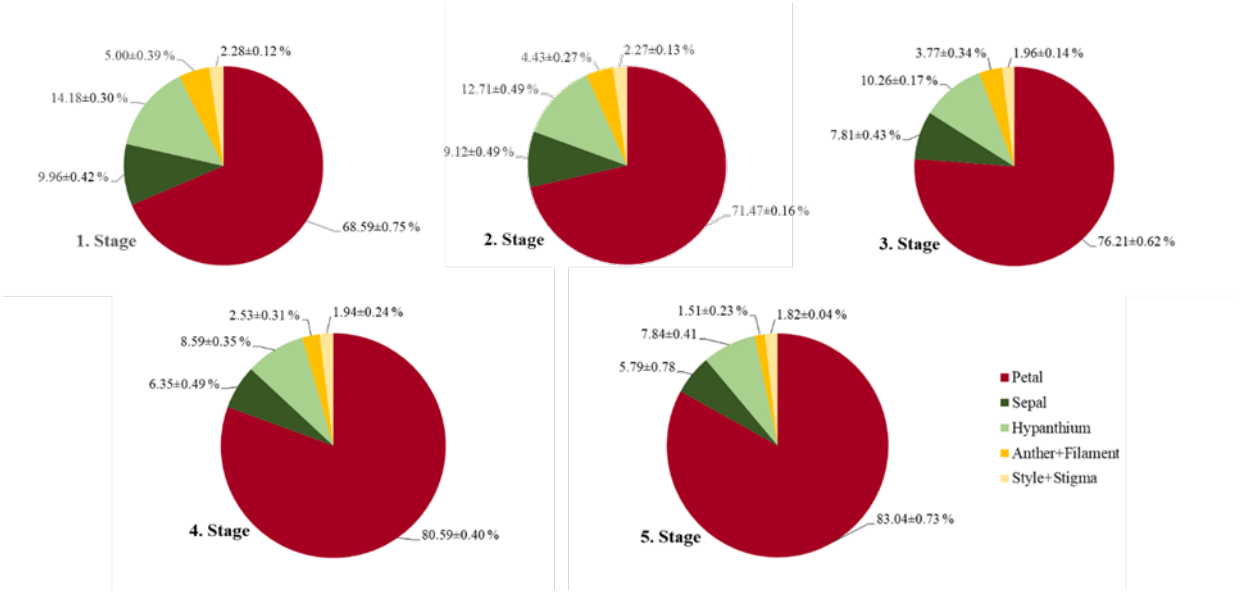

Figure 1. Percentages of organs in flowers (%)

Table 1. Changes in the flower organs of Halfeti black rose depending on the development stages

Characters		1. Stage	2. Stage	3. Stage	4. Stage	5. Stage
Fresh weight (g/flower)		3.55±0.26	3.71±0.15	3.90±0.14	4.21±0.30	4.53±0.25
Dry weight (g/flower)		0.66±0.04	0.75±0.03	0.79±0.02	0.87±0.05	0.90±0.12
Flower diameter (mm)		18.78±0.50	21.97±1.10	25.29±1.21	32.94±0.38	55.19±1.48
Anther diameter (mm)		3.03±0.17	1.82±0.07	2.00±0.04	1.46±0.07	1.35±0.08
Filament length (mm)		6.60±0.06	6.93±0.25	7.25±0.27	8.51±0.31	10.64±0.29
Style+ Stigma length (mm)		10.54±0.17	11.76±0.40	12.02±0.16	11.51±0.43	11.88±0.44
Moisture content (%)	Petal	78.22±0.12	77.50±0.10	77.48±0.18	77.29±0.24	75.85±0.12
	Sepal	68.00±1.92	69.12±1.98	69.23±3.02	70.69±1.58	69.70±0.31
	Hypanthium	79.38±4.82	76.47±0.42	76.60±2.93	76.34±2.03	76.25±1.53
	Anther+Filament	62.94±3.06	66.25±0.91	71.73±3.01	74.16±1.09	70.82±1.97
	Style+Stigma	71.83±2.69	74.64±4.51	74.94±4.51	77.65±1.01	78.31±0.61
Color of flower	*L**	24.45±0.86	22.44±0.55	24.61±0.30	28.63±0.99	25.82±0.81
	*a**	32.14±1.75	32.55±1.27	33.31±1.39	33.02±0.56	31.88±0.78
	*b**	14.14±0.57	4.97±0.92	3.69±0.96	-3.48±0.64	0.82±0.44
	*Hue**	23.05±0.41	8.37±1.53	5.97±1.65	-6.82±0.77	1.73±0.88
	Chrome	36.18±1.64	32.73±1.45	32.67±1.29	33.85±0.23	31.75±0.83
Number of petal (piece/flower)				28.00±2.00		
Number of sepal (piece/flower)				5.00±0.00		
Number of anthers (piece/flower)				70.00±9.30		
Number of pistils (piece/flower)				40.00±5.50		

The developing petal in roses continues to import dry matter throughout its development, with reducing sugars and starch accounting for 50% of its dry weight (Ho & Nichols, 1977). Therefore, hydrolysis of stored starch is likely related to flower opening because the decrease in water potential promotes water flow through the petal tissue and promotes cell growth. Our study also observed that moisture content in the petals decreases during flower development. In roses, the growth of the primary axis ends with the flowering period. In repeat-flowering species such as Halfeti rose, secondary shoots arise from buds in the leaf axils of the primary shoots (Roberts & Bloke, 2003). Petal development in roses can be divided into two main phases: an initial slow growth phase, primarily due to cell division, and a second rapid growth phase, primarily due to cell expansion (Martin & Gerats, 1993).

Normally, at the end of cell division, petals reach only 35-40% of their final size, and further growth and expansion are primarily a result of differential cell elongation. This suggests that the cell expansion phase plays an important role in flower development. Similar results have been observed in R. damascena and R. bourboniana (Sood et al., 2006). The role of sugars in flower development may be multifunctional: they act as energy sources (Moalem-Beno et al., 1997), osmotic regulators (Bieleski, 1993), and precursors of metabolic processes. In general, flower petal tissue contains high invertase activity, and most of the sugar pool of mature petals consists of invert sugars (Woodson & Wang, 1987; Önder et al., 2022). Soluble sugars in rose flowers have been suggested to be important as osmotically active substances in promoting petal growth (Ho & Nichols, 1977).

Sood et al. (2006) reported that starch content in rose flowers increases significantly throughout flower development until the 4. stage. Similar results were confirmed by Önder et al. (2022). Önder et al. (2022) also reported that in *R. damascena*, flower width did not differ between the first and second stage, increased rapidly after the 3. stage, and reached its highest level in the 5. stage. Fresh flower weight peaked in the last stage, and flower length increased until the 3. stage, decreasing as the petals opened. In our study, the fresh and dry weight of Halfeti black rose was faster especially after the 3. stage and reached the highest level in last stage.

In our study, although anther diameter showed a fluctuating development throughout the development period, it was observed to decrease towards the end of flowering. The anther diameter, which was 3.03 mm in the first stage, decreased to 1.35 mm in the last stage. It is thought that the anther diameter decreases due to the decrease in moisture content in the anthers. The filament to which the anthers are attached elongated from the first stage (6.60 mm) to the last stage (10.64 mm). On the other hand, the style+stigme lengths extended from 10.54 mm to 12.02 mm until the 3. stage and were measured as 11.51 mm and 11.88 mm in the 4. and last stages, respectively. No literature was found on anther diameter in Halfeti black rose (Table 1). Erbaş et al. (2015) reported that the diameter of *R. damascena* anthers was 2.62 mm and 2.69 mm in the first and second stages, while the diameter decreased in the 3. stage (2.16 mm). Similarly, in their study with lily anthers, Nelson et al. (2012) determined that the turgor in the outer epidermis of the anthers decreased periodically, causing the apex to open. On the other hand, it was also determined that filament length increased during the flower development stage in *R. damascena* (Erbaş et al., 2015).

In our study, when the color values of the Halfeti black rose were examined, the L* value (brightness) varied between 22.44 and 28.63 throughout the flowering stages, reaching its highest level in the 4. stage. The a* value varied between 31.88 and 33.31 throughout the flowering stage, giving similar values in all flowering stages. Significant differences were observed in the b* value throughout the flowering stage. The +b* value indicates an increase in yellow, and the -b* value indicates an increase in blue. In our study, the b* value was 14.14 in the first period, decreasing to -3.48 in the 4. stage and increasing to 0.82 in the 5. Stage (Table 1). The C* value, which does not correspond to any color, expresses the vividness or dullness of the color measured. It has been reported that as the C* value increases, colors appear more vivid, clear, and bright (Erbaş & Koyuncu, 2016). In our study, the C* value showed a decreasing fluctuating change throughout the flowering stage (from 36.18 to 31.75). The hue angle is a color circle and represents the colors that can be perceived by the eye. It takes values between 0° and 360° on the color circle, with 0°-90° indicating the transformation from red to yellow, 90°-180° from yellow to green, 180°-270° from green to blue, and 270°-360° from blue to red. In varieties with red or purple skin colors, as the value approaches 0°, the color darkens (Erbaş & Koyuncu, 2019). In our study, the hue angle decreased from the first period (23.05) to the 4. period (-6.82). In the last period, it increased to 1.73.

In the Halfeti Black Rose, the buds, which are red to almost black, are reported to turn dark red during the flowering period (Baytop, 2001; Özden, 2013; Özçelik & Koca, 2021). Furthermore, under the ecological conditions in which it is cultivated, it has been reported that the flower color, while red in summer, turns to dark red, almost black, in late autumn (Özden, 2013). In most plants, color development is associated with anthocyanin pigments in addition to primary pigments. Anthocyanin accumulation is an integral part of flower development in most plants and appears to be regulated by the same factor that controls petal growth (Weiss, 2000). In most plants, petal pigmentation occurs in the second phase of flowering, and its accumulation is tightly linked to cell expansion (Sood et al., 2006). However, there is no universal trend in the anthocyanin content of petals. While the level remains constant in some flowers and decreases greatly in others, a dramatic synthesis is evident in some flowers (Sakata & Uemoto, 1976; Sood et al., 2006). In Halfeti black rose, a decrease in b value throughout flower development indicates that the yellow value increases, but the red color increases due to a decrease in hue angle, and the petal color lightens from first stage to last stage. Particularly in red rose cultivars, a visible change in color is observed during flower development and senescence (Schmitzer et al., 2009). In roses, it is common to observe several flower developmental stages on a single plant. Often, the intense color of rose buds' changes to lighter shades of fully opened flowers. This phenomenon can be attributed to several factors: Firstly, changes in the content of various phenolic compounds and, secondly, an increase in the pH level of the cell sap. Sood and Nagar (2003) and Sood et al. (2006) investigated the levels of total anthocyanins as well as acidic and bound phenols in rose petals and measured an increase in the early stages of flower development followed by a decrease in the fully open stage.

Conclusion

The Halfeti Black Rose's repetitive blooming and intense fragrance suggest that it can be used not only as a landscape rose but also in the fragrance industry. However, research on the Halfeti Black Rose is still scarce. In our study, we examined some of its physical and chemical properties throughout the developmental period, a gap in the literature. Our results will guide future studies. However, in addition to physical properties, more detailed examination of chemical contents such as scent analysis, phenolic compounds, antioxidant activities, and color analysis, based on climatic changes during harvest time, will allow us to expand the potential uses of the Halfeti Black Rose. Our study found that the highest petal ratio, flower diameter, and fresh and dried flower weight were observed in the fifth stage of development. However, flowers from this stage should be analyzed for the secondary metabolite productivity. Our future studies will include changes in scent analysis, phenolic content, antioxidant activities, and color analysis during the developmental period.

Declarations

Ethical Approval Certificate

No ethical approval was required for the study.

Author Contribution Statement

Data collection, research, formal analysis, and writing the original draft were performed equally by both authors.

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Conflict of Interest

The authors declare no conflict of interest.

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