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Research Article

Energy dispersive X-ray Spectroscopy technique as a taxonomic indicator in the classification of *Klanchoe blossfeldiana* Poelln. cultivars

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Abstract
Determining elemental mapping in living plant samples using the energy–dispersive X-ray Spectroscopy technique (SEM-EDS) can be used as an indicator to solve classification problems among closely related taxonomic ranks, such as cultivars and other intraspecific categories. Four cultivars of *Klanchoe blossfeldiana* Poelln.: *K. blossfeldiana* Clandiva Monro (white petals), *K. blossfeldiana* Clandiva Hayworth (red petals), *K. blossfeldiana* Clandiva Fonda (yellow petals), and *K. blossfeldiana* Clandiva Bardot (orange petals) were investigated by (SEM – EDS) to explain element distribution in relation to petals colour to find out similarities and differences among these cultivars. All the investigated elements were common in all studied cultivars' petals except (Al^{3+}) ; (Si^{3+}) ; (Ni^{2+}) were absent in some studied cultivars and present in the others . Scanning electron microscope (SEM) Microphotograph of four *K. blossfeldiana* Cultivars petals showed different patterns from layer composition of zigzag form and very fine structures in *K. blossfeldiana* Clandiva Monro to regular composition of circular grooves and chains of luminous white structures of irregular thickness in *K. blossfeldiana* Clandiva Hayworth, and white Y-shaped structures appeared higher than grey ones in *K. blossfeldiana* Clandiva Fonda and *K. blossfeldiana* Clandiva Bardot, which had orange petals showing slightly zigzag structures with light gray to white in color, and irregular-shaped small structures. This research highlights the possibility of using elemental mapping and linking it with petal colour traits in plant classification.

Keywords: Crassulaceae , Elemental mapping , Energy dispersive x-ray , *Klanchoe blossfeldiana,* Spectroscopy technique, Taxonomy

INTRODUCTION

Klanchoe blossfeldiana is a species of the family (Crassulaceae sub.fam. Kalanchooideae), it is an infrageneric group restricted naturally in Madagascar (Ghobadifard *et al.,* 2019). It employs several reproductive strategies that enable it to survive and spread across various climates (Shitein *et al*., 2021), and has a wide range of cultivars that are challenging to categorize (Shaw, 2008). These cultivars result from hybridization among species of the same genus (interspecific), thereby controlling polygenic dependence on additive inheritance, which can lead to hybrid intermediacy (Al-Naib and Al-Wattar, 2021). Therefore, it is crucial to establish accurate classification criteria to distinguish these cultivars. Due to the presence of cardiac glycosides, Kalanchoe flowers are toxic to both humans and other animals (Siroka, 2023). This is why it became vital to investigate their exact chemical composition. The elemental mapping in living plant samples can be determined using the energy dispersive X-ray spectroscopy technique. This method depends on X-ray techniques, which are excited by electron scanning microscopy and induce protons by X-ray emission. A precise X-ray study of the metal distribution in tissue requires that the sample be kept in conditions that preclude elemental redistribution, ultrastructural changes, and chemical modification.

Techniques for elemental mapping using energy-dispersive X-ray spectroscopy play a significant role in plant metalloid homeostasis (Ent *et al*., 2018). EDS can be performed using Scanning electron microscopy (SEM), which is an important technique for analyzing elements. To investigate fresh biological samples, it is necessary to perform complex pre-treatment to stabilize the specimens under high vacuum and high resolution of a scanning electron microscope (Takaku *et al*.,2020) . The physiological importance of the major elements and others determines the accumulation of elements in plant organs (leaves, roots), tissues (phloem and epidermis), cells, cellular organelles, transporters, and chaperones. The distribution and concentrations of elements provide a framework for understanding gene expression and element transition across membranes, enabling comparisons across various plants and different dose treatment regimens, as well as between plants from different habitats (Ent *et al*., 2018).

Using the technique of Energy Dispersive X-ray Fluorescence (EDXRF), it was possible to explore the concentration of elements in Jasmine blossoms. It was found that the highest element in the sample was potassium, followed by phosphorus. Other elements that were discovered as trace elements include calcium, sulfur, iron, zinc, rubidium, manganese, copper, strontium, titanium, bromine, and nickel (Win *et al*., 2020). Another study employed energy-dispersive X-ray spectroscopy to analyze fresh leaves of Kalanchoe integra, which is used as an antihypertensive drug in Ghana. The results showed the presence of 12 macro and 26 micro elements in the extract, and the quantitative assessment for element amounts is pharmacologically significant, revealing that potassium, magnesium, and calcium, magnesium and calcium content in the extracts can be utilized to manage arrhythmias and hypertension conventionally (Frimpong–Manso *et al*., 2015). In a study that discussed element composition and the risk of heavy metals on the environment using some medicinal plants investigated with the EDS technique, the plants under study showed high levels of (Ni^{2+}) and (Cu^{2+}), while their extracts contained levels that ranged within the acceptable limits (Sánchez-Lara *et al.,* 2022). The color of five samples of *Bletilla striata* which had significant color differences (purple, pink, and yellow) for testing metal ion content of (Al^{3+}, Ca^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Na^{+}, P^{5+}, Zn^{2+}, Mo^{6+}, Cu^{2+}, and Mn^{2+}) and found that ($Al^{3+)}$ was in the highest concentration in yellow flowers, while the (Ca^{2+}) was the highest in purple flowers, as compared to the concentration of Na^{+} which was the highest in white flowers (Xie *et al*., 2023).

Therefore, this study aimed to identify the relationship between flower colour and element distribution in petal flower cultivars of *K. blossfeldiana*, and to recommend using it as a genetic character for taxonomic purposes. Additionally, the study aimed to identify intraspecific differences and similarities among the same species.

MATERIALS AND METHODS

Plant materials

Flowers of *K. blossifeldiana cultivars* were collected in full bloom stage from the University of Mosul gardens. Most of these cultivars were originally exported from the Netherlands, featuring various flower colours. They were kept as indoor potted plants in a cold, full-sun location, with temperatures ranging between 18-22 °C.

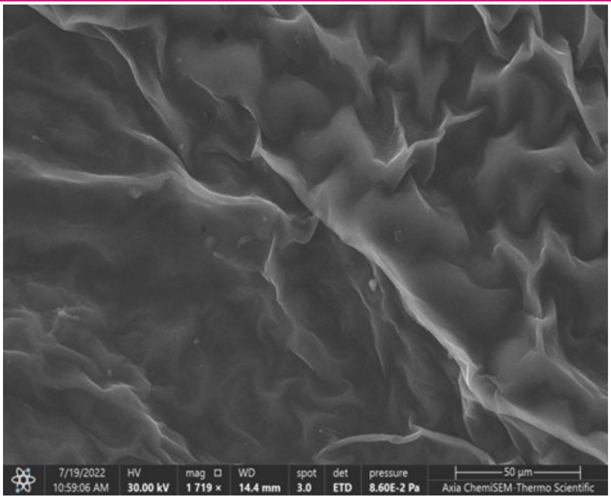
Distribution analysis of elements

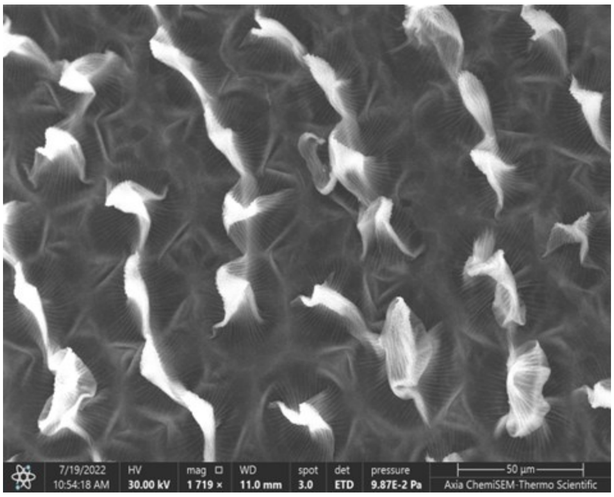
Before analysis, the fresh plants were preserved in 70% ethanol. Petal samples were carefully removed using needles and forceps and then rinsed twice with water. Petals were dried prior to examination with SEM and the recording of microphotographs. The identification of elements is carried out using an energy–dispersive X–ray spectrometer (EDS), as mentioned elsewhere (Takaku *et al*., 2020).

RESULTS AND DISCUSSION

Energy-dispersive X-ray spectroscopy (SEM-EDS) is a method that uses an electron beam to scan samples and is a common method used to determine the distribution of elements in plant tissues (Husted *et al*., 2011). The SEM–EDS analysis revealed that Kalanchoe blossfeldiana cultivars comprise a diverse range of elements (Table 1a, b, c, d) and (Fig. 1A, B, C, D), which illustrate the EDS spectra of the plant's fresh petals. The results showed that the elements belonged to two groups, essential elements composed of two groups (essential mineral elements (Si^{3+} ; P^{3+} ; Cl^{1-} ; K^{+}; Ca^{2+} ; Al^{3+} ; S^{2-} ; Ni^{2+} ; Mg^{2+}) and essential non-mineral elements (C^{2+} , O^{2-}). These elements were distributed among plant cultivars in different ways; some were common to all cultivars, while others were specific to a single cultivar.

Results demonstrate the Scanning electron microscopy (SEM) of the studied cultivars' petals (Fig. 1), which is considered one of the main techniques for characterizing materials, providing structural and morphological characteristics (Fig. 1B). This image shows the SEM microstructures of *K. blossfeldiana Clandiva* Monro petals, demonstrating the adsorbent layer's composition in a zigzag form and extremely small structures. The images explaining variance in petal microstructures among other cultivars displayed the layer composition in zigzag form and featured extremely small structures. The SEM microstructures of *K. blossfeldiana* Clandiva Hayworth petals are shown in Fig. 1B. These petals exhibit a regular composition of circular grooves and chains of luminous white structures of irregular thickness. This structure may be a result of the crystallization of certain elements (K+ Ca2+, Mg^{2+}). The SEM microstructures *K. Blossfeldiana* Clandiva Fonda are shown in Fig. 1C. White y-shaped structures are displayed higher than grey ones, reflecting *the elements'* adsorption characteristics. Fig. 1D displays the SEM microstructures of *K. Blossfeldiana* Clandiva Bar-



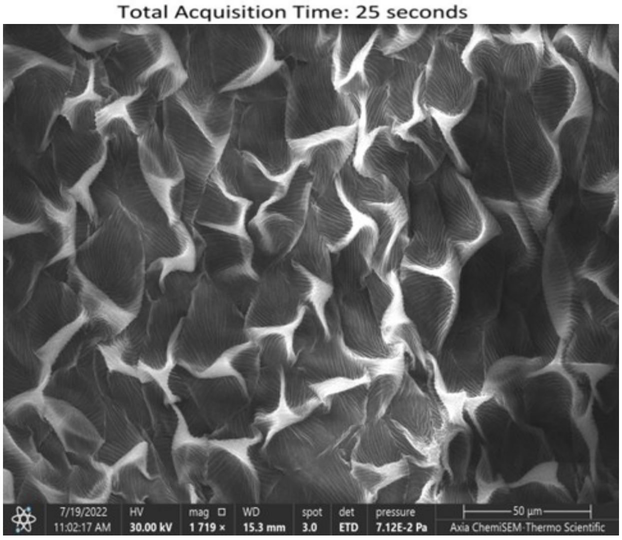


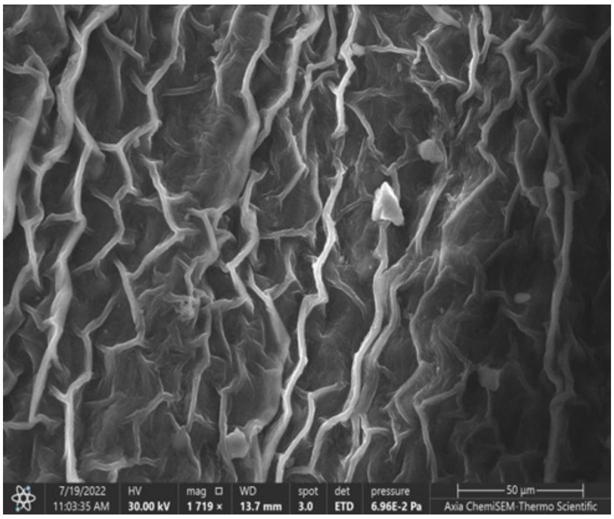
Klanchoe blossfeldiana Clandiva Monro *Klanchoe blossfeldiana* Clandiva Hayworth

(HV: High voltage; mag: Magnification (% image area; scanned area); WD: working distance between the objective lens and the sample; det ETD Everhart–Thornley Detector, which collects secondary electrons with low energy emitted from the specimen; spot: electron beam diameter)

Total Number of Counts: 160 297
Average Count Rate: 1 612 cps
Acceleration Voltage: 30 kV
Total Acquisition Time: 25 seconds

Total Number of Counts: 43 521
Average Count Rate: 1 530 cps
Acceleration Voltage: 30 kV
Total Acquisition Time: 30 seconds





Klanchoe blossfeldiana Clandiva Fonda *Klanchoe blossfeldiana* Clandiva Bardot

(HV: High voltage; mag: Magnification (% image area; scanned area); WD: working distance between the objective lens and the sample; det ETD Everhart –Thornly Detector, which collects secondary electrons with low energy emitted from the specimen; spot: electron beam diameter)

Total Number of Counts: 43 521
Average Count Rate: 1 530 cps
Acceleration Voltage: 30 kV
Total Acquisition Time: 30 seconds

Total Number of Counts: 47 011
Average Count Rate: 1 631 cps
Acceleration Voltage: 30 kV
Total Acquisition Time: 30 seconds

Fig. 1. *Scanning electron microscope microphtograph of four K. blossfeldiana Cultivars' petals; Maps resolution: 768 x 512*

dot, which shows orange petals. These petals exhibited slightly zigzag structures, with a light grey to white colour, and irregularly shaped small structures.

According to Table 1a and Fig. 2, the *K. blossfeldiana* Clandiva Monro 's white petals contain each of the elements (C^{2+}, O^{2-}, P^{3+}, Cl^{1-}, K^{+}, Ca^{2+}, Al^{3+} and Mg^{2+}). This cultivar showed the lowest atomic (23.6%) and weight (18.3%) of carbon (C^{2+}) in comparison with oxygen (O^{2-}), which had the highest atomic percentage (74.0%), while the highest weight was (77.0%). Other elements showed equal or lower values; however, this cultivar recorded the lowest number of elements among the studied cultivars.

The cultivar *K. blossfeldiana* Clandiva Hayworth with the red petals, showed the highest number of elements (C^{2+}; O^{2-}; P^{3+}; Cl^{1-}; K^{+}; Ca^{2+}; Al^{3+}; Mg^{2+}; Al^{3+}; S^{2-}; Ni^{2+}), and it had a high atomic carbon (C^{2+}), atomic percentage (50.1 %) as well as for the weight percentage (Table 1b; Fig. 3), which was equal to *K. blossfeldiana* Clandiva Fonda in its carbon percentage as in Table 1c and Fig. 4 showed lowest phosphorus (P^{3+}) (0.1%), with weight (0.3%) and higher chlorine (Cl^{1-}) atomic percentage (0.4 %) with weight (0.4 %). The highest aluminum (Al^{3+}) atomic percentage reached (1.1) and Weight (2.0%) in the cultivar *K. blossfeldiana* Clandiva Hayworth (Table 1b; Fig. 3), which showed aluminum weight percentage similar to the aluminium weight percentage found in *Bauhinia scandens* tissues (Lianah *et al.,* 2019), who found that this element had medical use.

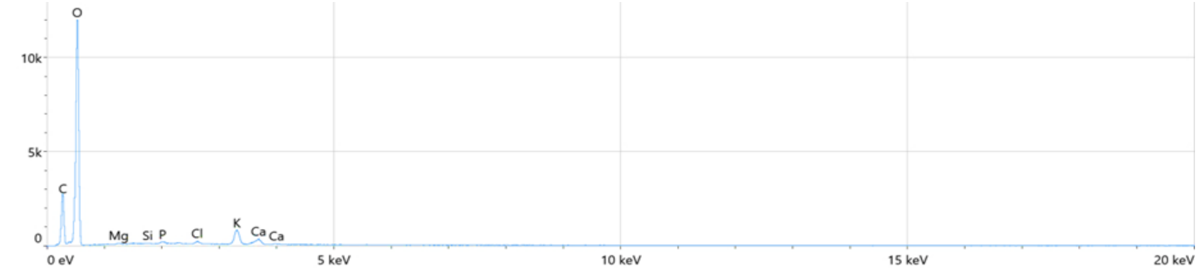
K. blossfeldiana Clandiva Hayworth contained Sulfur (S^{2-}**)** with an atomic percentage of 0.1%,Weight of 0.1%. In contrast, in *K. blossfeldiana* Clandiva Fonda, the sulfur atomic percentage was 0.1% and Weight of 0.3%, also, the red petals cultivar was similar to K. blossfeldiana Clandiva Monro and *K. blossfeldiana* Clandiva Fonda in magnesium (Mg2+) atomic percentage (0.2%). Still, the weight percentage varied in the studied cultivars. The cultivar *K. blossfeldiana* Clandiva Hayworth showed the only recorded Nickel (Ni^{2+}) atomic percentage (0.1%) with weight percentage (0.5%) as in Table 1b and Fig. 3. The yellow petals of the cultivar *K. blossfeldiana* Clandiva Fonda recorded an equal oxygen (O^{2-}) atomic percentage to the petals of the cultivar *K. blossfeldiana* Clandiva Hayworth (46.0%) and closely weight percentage, while it had a similar atomic percentage in Silica (Si^{3+}) (0.1 %) to the cultivar *K. blossfeldiana* Clandiva Bardot which had orange petals, but the weight percentage was different in the orange petals cultivar which recorded (0.2%) as in Table 1b and Fig. 5. Silica increases plant tolerance to biotic and abiotic stress (Manimaran *et al.,* 2025) The cultivar *K. blossfeldiana* Clandiva Fonda showed the highest atomic percentage in phosphorus (0.3%) with weight reached (0.6%) and also in potassium percentage which recorded (2.2%) atomic percentage with weight percentage reached (5.8%). This cultivar showed similarity in chlorine (Cl^{1-}) atomic percentage (0.2%) and the weight reached (0.4%) to the cultivars *K. blossfeldiana* Clandiva Monro and *K. blossfeldiana* Clandiva Bardot, as in Table 1a,c,d and Fig. 2,3,5. The cultivar *K. blossfeldiana* Clandiva Fonda was similar to the cultivar *K. blossfeldiana* Clandiva Bardot in each of calcium atomic percentage (0.1%), but they differed in weight percentage of (Ca^{2+}) slightly 2.0 – 1.9 % (Table 1c,d; Fig. 4,5). The cultivar *K. blossfeldiana* Clandiva Bardot differed from other studied cultivars in Carbon (C^{2+}) atomic percentage (45.5%) with weight (36.8%) and the micronutrient element Potassium (K^{+}) atomic percentage (1.6%), and weight (4.3%) as in Table 1d; Fig. 5.This element is an alkali element (Bhardwaj *et al.* 2025) , its functions in plants is to give tolerance to abiotic effects and stress caused by chilling, drought, high light and heat intensity, and nutrient deficiency (Lianah *et al*., 2019) and reached the highest atomic percentage magnesium (Mg^{2+}) (0.5%), while weight reached 0.8% as in Table 1,d; Fig. 5. As shown above, this element is important in several biochemical and physiological functions (Wang *et al*., 2023) .

The cultivar *K. blossfeldiana* Clandiva Bardot and the cultivar *K. blossfeldiana* Clandiva Fonda showed similarity in atomic percentage of four elements (Si^{3+}, Cl^{1-}, Ca^{2+}, Al^{3+}), while the weight percentage of these elements varied as shown in Table 1c,d. At the same time, *K. blossfeldiana* Clandiva Fonda showed similarity with the cultivar *K. blossfeldiana* Clandiva Hayworth in the atomic percentage of four elements (C^{2+}, O^{2-}, S^{2-}, Mg^{2+}), also the weight percentage was not identical as seen in Table 1c,b. The cultivar *K. blossfeldiana* Clandiva Monro recorded differences among other cultivars, in the absence of four elements (Al^{3+}, S^{2-}, Ni^{2+}, Si^{3+}) as shown in Table 1a; Fig. 2. Calcium (Ca^{2-}) had the highest atomic percentage in each of *K. blossfeldiana* Clandiva Bardot and *K. blossfeldiana* Clandiva Fonda (0.7%), while the lowest in atomic percentage was detected in the cultivar *K. blossfeldiana* Clandiva Monro as seen in Table 1,c,d; Fig. 2,4,5 as seen from (Table 1a; Fig. 2. The white petals of the cultivar *K. blossfeldiana* Clandiva Monro contained each of the element (C^{2+}, O^{2-}, P^{3+}, Cl^{1-}, K^{+}, Ca^{2-}, Al^{3+}, and Mg^{2+}), this cultivar showed the lowest atomic percentage of carbon (C^{2+}) (23.6 %) and the lowest weight (18.3%) in comparison with (74.0%) for oxygen (O^{2-}) which recorded the highest atomic percentage and the highest weight (74.0%).

Other elements were equal to or fewer than those of other studied cultivars; this cultivar recorded the lowest number of elements among the studied cultivars.

The cultivar *K. blossfeldiana* Clandiva Hayworth which had red petals showed the highest number of elements among cultivars (C^{2+}, O^{2-}, P^{3+}, Cl^{1-}, K^{+}, Ca^{2-}, Al^{3+}, Mg^{2+}, Al^{3+}, S^{2-}, Ni^{2+}), it had a high atomic carbon (C^{2+}) and an atomic percentage of (50.1) as well as for the weight percentage, this percentage was equal to *K. blossfeldiana* Clandiva Fonda carbon. This cultivar showed the lowest phosphorus (P^{3+}) atomic percentage (0.1%), with Weight (0.3%) and the highest chlorine (Cl^{1-}) atomic percentage (0.4%) with weight (0.4%), Aluminum (Al^{3+}) atomic percentage reached the highest (1.1%) and the highest with Weight (2.0%) as shown in (Table 1b) and (Fig. 4) . In the cultivar *K. blossfeldiana* Clandiva Hayworth, which showed aluminum weight percentage similar to the aluminum weight percentage found in *Bauhinia scandens* plant tissues (Lianah *et al*., 2019), who mentioned that this element had a great medical role, *K. blossfeldiana* Clandiva Hayworth showed Sulfur (S^{2-}) atomic percentage (0.1%)

Intensity of elements% %



X-ray Energy (keV)

Intensity of elements% % = Obtained integrated intensity of the element in the unknown specimen;
KeV = Ke: kinetic energy of the encharged particles; V: electric potential {voltage} measured in volts.

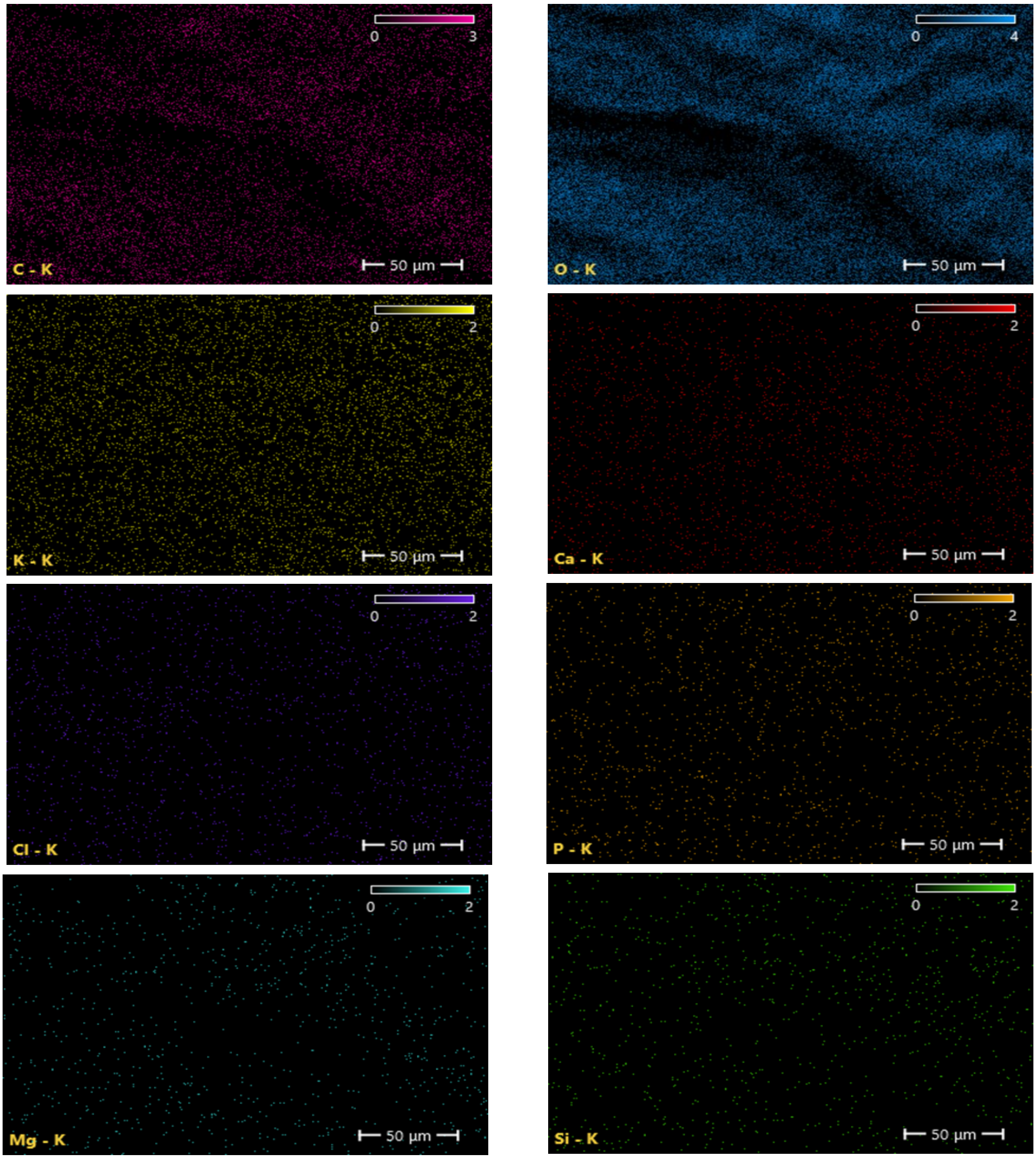


Fig. 2. *EDS spectrum under SEM. Elemental mapping diagrams of (C^{2+}, Ca^{2+}, Si^{3+}, O^{2-}, Mg^{2+}, P^{3+}, Cl^{1-}), showing a uniform distribution of these elements in petals of the cultivar K. blossfeldiana Clandiva Monro (K ratio of the characteristic X-ray = the correct ratio of intensity from standard specimen to that from unknown specimen)*

Table 1. Percentages of essential elements and trace elements present in the flowers parts (petals) of Kalanchoe *blossfeldiana cultivaes* petals

Element	Atomic percent-age	Atomic Error percent-age	Weight percent-age	Weight Error percent-age
C	23.6	0.3	18.3	0.2
O	74.5	0.4	77.0	0.4
Mg	0.2	0.0	0.2	0.0
Si	0.0	0.0	0.1	0.0
P	0.2	0.0	0.3	0.0
Cl	0.2	0.0	0.4	0.0
K	1.0	0.0	2.6	0.1
Ca	0.4	0.0	1.0	0.1

a. *Kalanchoe blossfeldiana* Clandiva Monro

Element	Atomic percent-age	Atomic Error percent-age	Weight percent-age	Weight Error per-centge
C	50.1	0.5	40.8	0.4
O	46.0	0.7	50.0	0.8
Mg	0.2	0.0	0.2	0.0
Al	1.1	0.0	2.0	0.1
P	0.1	0.0	0.3	0.0
S	0.1	0.0	0.1	0.0
Cl	0.4	0.0	1.0	0.0
K	1.4	0.0	3.7	0.1
Ca	0.5	0.0	1.4	0.0
Ni	0.1	0.0	0.5	0.1

b. *Kalanchoe blossfeldiana Clandiva* Hayworth

Element	Atomic percent-age	Atomic Error percent-age	Weight percent-age	Weight Error percent-age
C	50.1	0.3	40.6	0.3
O	46.0	0.5	49.6	0.5
Mg	0.2	0.0	0.4	0.1
Al	0.1	0.0	0.2	0.0
Si	0.1	0.0	0.1	0.0
P	0.3	0.0	0.6	0.0
S	0.1	0.0	0.3	0.0
Cl	0.2	0.0	0.4	0.0
K	2.2	0.0	5.8	0.1
Ca	0.7	0.0	2.0	0.1

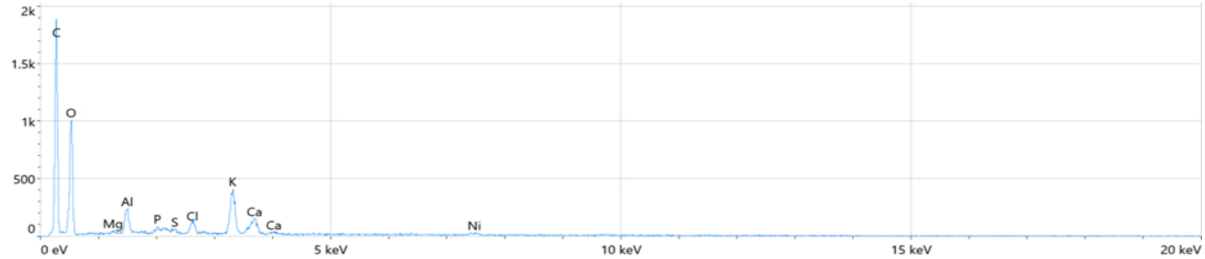
c. *Kalanchoe blossfeldiana* Clandiva Fonda

Element	Atomic percent-age	Atomic Error percent-age	Weight percent-age	Weight Error percent-age
C	45.5	0.5	36.8	0.4
O	51.2	0.7	55.1	0.7
Mg	0.5	0.1	0.8	0.1
Al	0.1	0.0	0.2	0.0
Si	0.1	0.0	0.2	0.0
P	0.2	0.0	0.4	0.0
Cl	0.2	0.0	0.4	0.0
K	1.6	0.0	4.3	0.1
Ca	0.7	0.0	1.9	0.1

d. *Kalanchoe blossfeldiana* Clandiva Bardot

and weight (0.1 %), while in *K. blossfeldiana* Clandiva Fonda, the sulfur atomic percentage (0.1%) and weight (0.3%). Also, the red petals cultivar was similar to *K. blossfeldiana* Clandiva Monro and *K. blossfeldiana* Clandiva Fonda in magnesium (Mg2+) atomic percentage (0.2%), but the weight varied in the studied cultivars. The cultivar *K. blossfeldiana* Clandiva Hayworth was the only recorded Nickel (Ni2+) atomic percentage (0.1%) with weight (0.5%) (Table 1a,b,c; Fig. 2, 3). The yellow petals of the cultivar *K. blossfeldiana* Clandiva Fonda recorded an equal oxygen (O_2-) atomic percentage to the petals of the cultivar *K. blossfeldiana* Clandiva Hayworth (46.0) and a closely weighted percentage. At the same time, it had a similar atomic percentage in Silica (Si3+) (0.1) to the cultivar *K. blossfeldiana* Clandiva Bardot, which had orange petals. Still, the weight percentage differed in the orange petals cultivar, which recorded 0.2. Silica raises plant tolerance to biotic and abiotic stress (Manimaran *et al*., 2025). Cultivar *K. blossfeldiana* Clandiva Fonda exhibited the highest atomic percentage in phosphorus (0.3) and the highest weight percentage (0.6). Additionally, it showed the highest atomic percentage of potassium (2.2%) and the highest weight percentage (5.8%). This cultivar showed similarity in chlorine (Cl^{1-}) atomic percentage (0.2) and weight percentage reached (0.4) to the cultivars *K. blossfeldiana* Clandiva Monro and *K. blossfeldiana* Clandiva Bardot. The cultivar *K. blossfeldiana* Clandiva Fonda was similar to the cultivar *K. blossfeldiana* Clandiva Bardot in each of calcium atomic percentage (0.7), but they differed in weight percentage of (Ca^{2+}) slightly (2.0 – 1.9) in Table 1a,b,c,d; Fig. 2,3,4,5. The cultivar *K. blossfeldiana* Clandiva Bardot in Table 1,d; Fig. 5 differ from other studied cultivars in Carbon (C^{2+}) atomic percentage (45.5) with weight percentage (36.8) and the micronutrient element Potassium (K^{+}) atomic percentage (1.6), and weight percentage (4.3) which is considered as alkaline element (Bhardwaj *et al*., 2025). .In plants, they have important functions to give tolerance to abiotic effects and stress caused by chilling, drought, , high light and heat intensity, and nutrient deficincy (Lianah, *et al*., 2019) and reached the highest atomic percentage in magnesium (Mg^{2+}) (0.5), while weight percentage reached (0.8), as shown above, this element is an essential component of chlorophyll and it is important for some enzymes involved in

Intensity of elements %



X-ray Energy (keV)

Intensity of elemnts % = The obtained integrated intensity of the element in the unknown specimen .

KeV = Ke: kinetic energy of the encharged particls ; V : electric potential {voltage} measured in volts

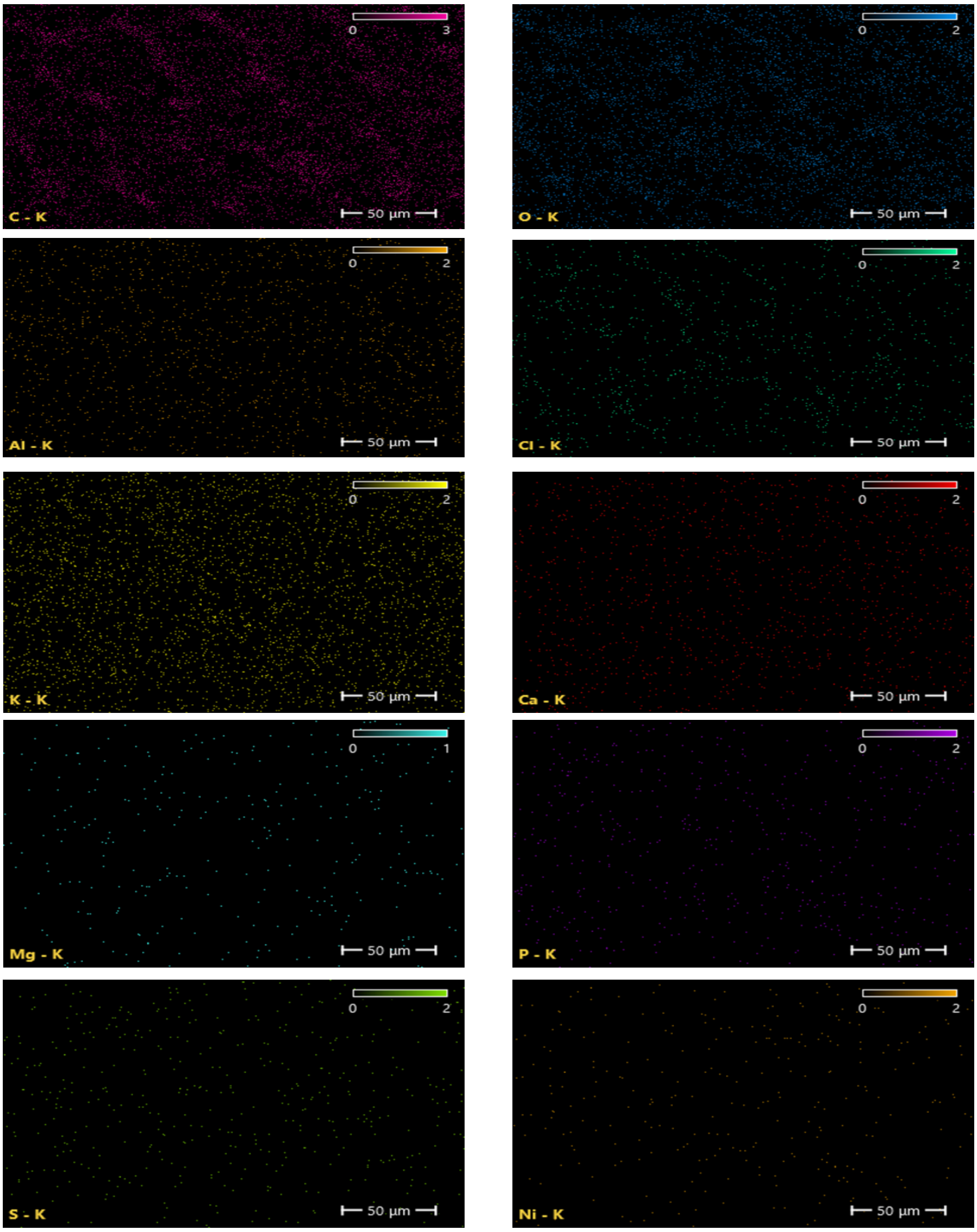
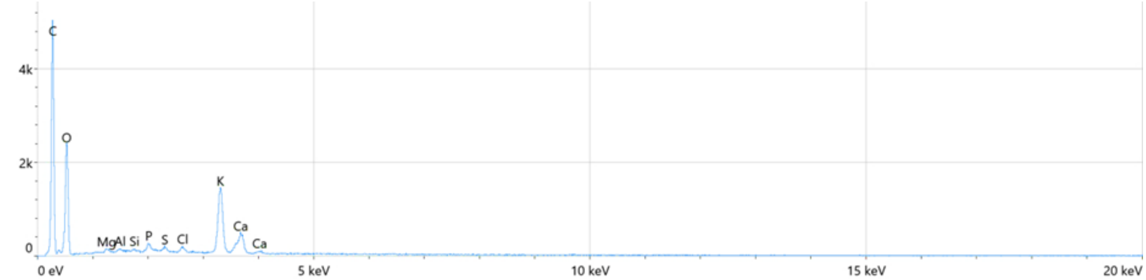


Fig. 3. *EDS spectrum under SEM The elemental mapping diagrams of (K^{+} ,C^{2+}, Ca^{2+} , O^{2-}, Mg^{2+} , P^{3+} , Cl^{1-} , S^{2-} ,Ni^{2+}) , showing a uniform distribution of these elements in petals of the cultivar K. blossfeldiana Clandiva HayWorth (K ratio of the characteristic X-ray = the correct ratio of intensity from standard specimen to that from unknown specimen)*

Intensity of elements%



X-ray Energy (keV)

Intensity of elements% % = Obtained integrated intensity of the element in the unknown specimen.

KeV = Ke: kinetic energy of the encharged particls ; V : electric potential {voltage} measured in volts.

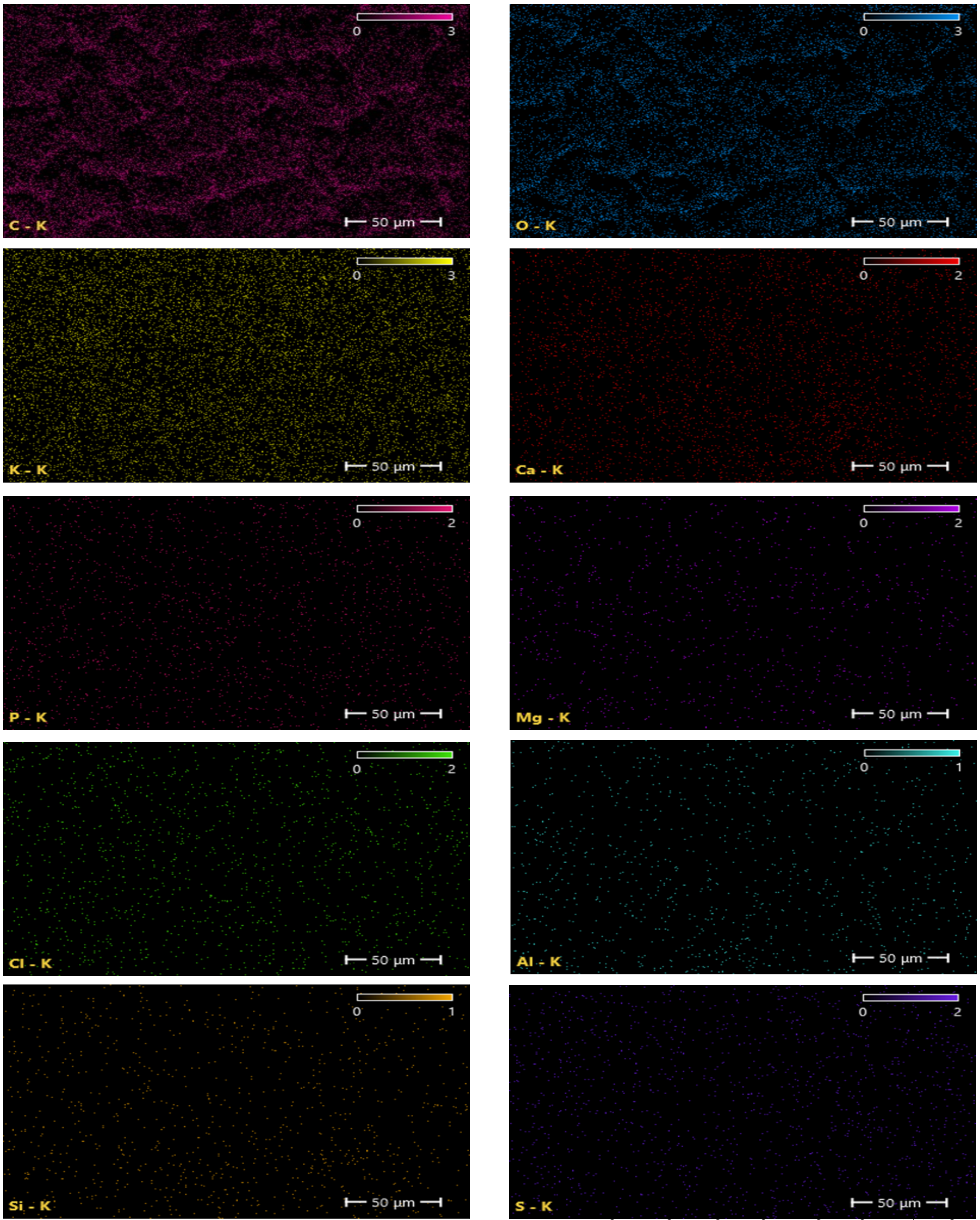
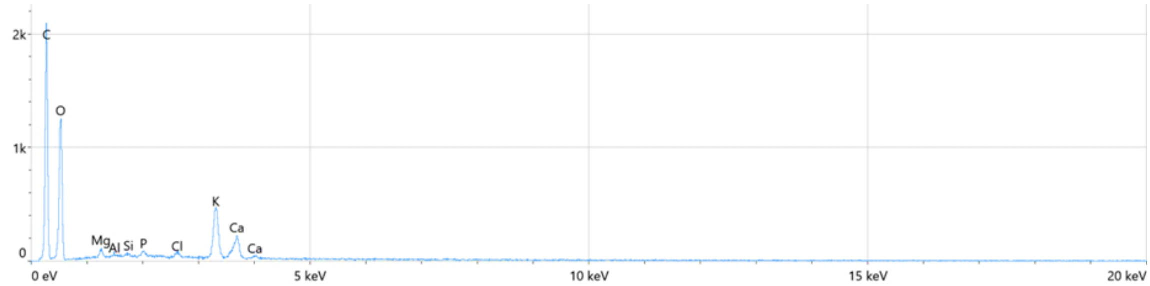


Fig. 4. *EDS spectrum under SEM The elemental mapping diagrams of (C^{2+}, Ca^{2+}, Si^{3+}, O^{2-}, Mg^{2+}, P^{3+}, Cl^{1-}, S^{2-}, Al^{3+}) showing a uniform distribution of these elements in petals of the cultivar K. blossfeldiana Clandiva Fonda (K ratio of the characteristic X-ray = the correct ratio of intensity from standard specimen to that from unknown specimen)*

Intensity of elements %



X-ray Energy (keV)

Intensity of elements % = Obtained integrated intensity of the element in the unknown specimen.

KeV = Ke: kinetic energy of the encharged particles; V : electric potential {voltage} measured in volts

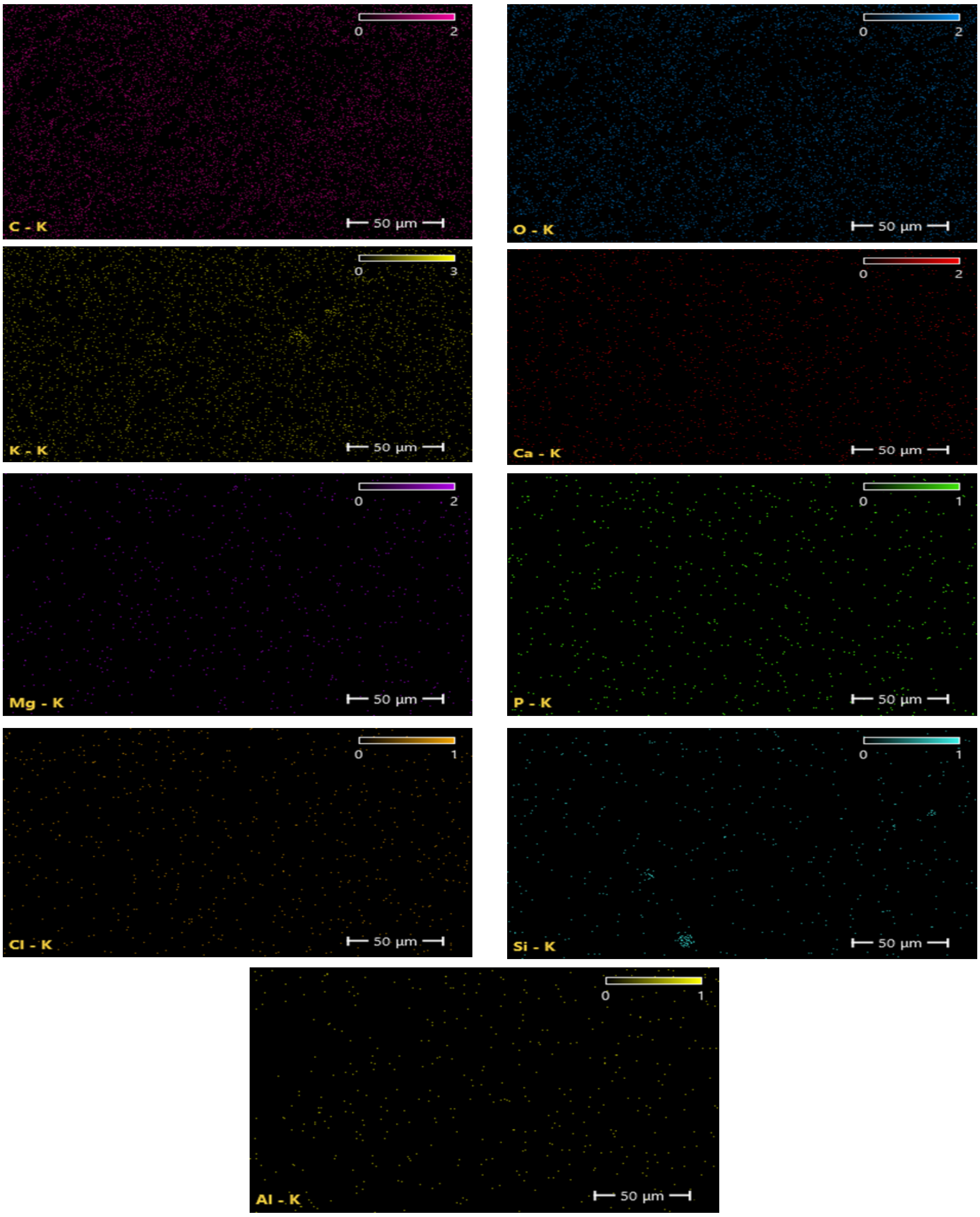


Fig. 5. *EDS spectrum under SEM The elemental mapping diagrams of (K^+, C^{2+}, Ca^{2+} Si^{3+}, O^{2-}, Mg^{2+}, P^{5+}, Cl^{1-}, Al^{3+}), showing a uniform distribution of these elements in petals of the cultivar Kalanchoe blossfeldiana Clandiva Bardot (K ratio of the characteristic X-ray = the correct ratio of intensity from standard specimen to that from unknown specimen)*

in metabolic prosses (Wang *et al*., 2023).

The cultivars *K. blossfeldiana* Clandiva Bardot and *K. blossfeldiana* Clandiva Fonda exhibited similarities in the atomic percentages of four elements (Si3+, Cl1-, Ca^{2+}, Al^{3+}), although the weight percentages of these elements varied. At the same time, *K. blossfeldiana* Clandiva Fonda showed similarity with the cultivar *K. blossfeldiana* Clandiva Hayworth in the percentage of four atomic elements (C^{2+}, O^{2-}, S^{2-}, Mg^{2+}). Also, the weight percentages were not identical. The cultivar *K. blossfeldiana* Clandiva Monro has record differences among other cultivars, in the absence of four elements (Al^{3+}; S^{2-}; Ni^{2+}; Si^{3+}), Calcium (Ca^{2+}) was the highest atomic percentage in each of *K. blossfeldiana* Clandiva Bardot and *K. blossfeldiana* Clandiva Fonda (0.7), while the lowest atomic percentage was in the cultivar *K. blossfeldiana* Clandiva Monro in Table 1a,b,c,d; Fig. 2,3,4,5).

(SEM - EDS) It depends on the reaction between fluorescent X-rays and the elements. When the metallic sample is introduced to a focused beam of fluorescent X-rays, electrons or protons excite the elements. The degree of element excitation depends on the energy of the incident X-rays. Protons or electrons will be detected and quantified by a detector. When an X-ray beam is incident on a sample, it does not destroy it. Nevertheless, free radicals may be generated, causing damage to the sample's tissues without causing sample heating (Acharya, 2023). The SEM-EDS technique measures elements that exceed 1000 ppm with high accuracy. So, this technique is not suitable for measuring heavy metals in plant tissues. This kind of technique is suitable for analyzing macro elements (Lu, 2024).

The colour of the flower is considered a key character in taxonomy and in determining the quality and economic importance of different ornamental plants. It also plays an ecological role in pollinator attraction (Trunschke *et al*., 2021). The flower colour results from the reflection of light in spongy tissue and its passage through a pigmentation layer to the eye. The flowers' reflectance is related to wavelength absorption by pigments like anthocyanins and carotinoids, as well as the scatter of light. The flower colour is influenced by various physicochemical and cellular factors, including pigment content, petal cell type, pigment shape, metal ions, and pH within the cell (Erickson and Pessoal, 2022).

The cell surface shape of petals affects light refraction and reflection in the cells, and affects petal colour brightness (Xie *et al*., 2023). The element profile differs in whole plant parts, while the element distribution tendency is still similar throughout growth stages (Nakanishi, 2021).

The weight percentage of the element can be defined as the element weight measured in the plant sample divided by the total weight of all measured elements multiplied by 100. While the atomic percentage is defined as the element atoms number at that weight percentage divided by the total atoms number in the plant sample multiplied by 100. Therefore, the atomic percentage is calculated by dividing the element weight percentage by the element atomic weight (Norton, 2021) .

Conclusion

In conclusion, the petal microstructure demonstrated different layer compositions amongst the studied cultivars of *K. blossfeldiana*, which showed zigzag form in *K. blossfeldiana* Clandiva Monro, circular grooves and a chain of luminous white structures of irregular thickness in *K. blossfeldiana* Clandiva Hayworth, white Y-shaped structures in *K. blossfeldiana* Clandiva Fonda, zigzag structures with light gray to white color, and irregular shape small structures in *K. blossfeldiana* Clandiva Bardot. The major components of the petals of Kalanchoe blossifeldiana cultivar flowers are carbon (C^{2+}) and oxygen (O_2-), followed by potassium (K^+) and calcium, with varying weight percentages. Notably, *Kalanchoe blossifeldiana* Calandiva Hayworth differs in its aluminium (Al^{3+}) weight percentage. The results suggest that petal microstructures can serve as a taxonomic indicator to differentiate cultivars within the same group. Specifically, elements such as Ni and S can be used to distinguish among *K. blossfeldiana* cultivars, whereas most elements are common across all cultivars.

Conflict of interest

The authors declare that they have no conflict of interest.

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