Effect of soaking with colchicine on some anatomical traits, growth and yield of wheat *Triticum aestivum* L.

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Abstract

During the winter agricultural season of 2022-2023, a field experiment was carried out in Al-Basateen Al-Sharqiah region, which is located approximately 4 km away from the center of Al-Muthanna governorate. The objective of the experiment was to investigate various anatomical and phenotypic parameters of wheat cultivars under the influence of the mutagenic alkaloid colchicine, at concentrations of 0, 250, 500, and 750 mg L-1. The study employed the RCBD design was used with the arrangement of the split plots with three replications. The main plots were assigned to different concentrations of colchicine, while the sub-plots were assigned to different cultivars (Ibaa 99, Mawadah, Bohuth22, Babel).

The statistical analysis yielded considerable variations among the cultivars in terms of both phenotypical features and histological sections of the leaves. The cultivar Bohuth22(V3) shown exceptional performance in terms of spike density, with an mean of 242.3 spikes per square meter, as well as grain yield, with an mean of 65.90 grains per spike. On the other hand, the cultivar Babel(V4)exhibited superiority in terms of the weight of 1000 grains, averaging at 54.27 grams. Regarding the cultivar Ibaa 99(V1), it had a notable advantage in relation to the thickness of the vascular bundles found in the leaf tissue, measuring 115.33 µm.

The results clearly indicated the positive impact of mutagenic colchicine on various traits. Specifically, at a concentration of 250 mg L^{-1}, and the weight of 1000 grains was 51.04 g. However, when the concentration exceeded 750 mg L^{-1}, the effects were not further enhanced. The flag leaf area is measured to be approximately 36.66 cm^2, the biological yield is recorded as 14.17 tons ha^{-1}, and the grain yield is recorded as 4.574 tons ha^{-1}. Additionally, the concentration surpasses 500 mg per liter as the number of grains per spike reaches 64.38.

Regarding the interaction between the mutagenic compound colchicine and the cultivars, was significant for most studied traits.

Keywords: Wheat cultivars, colchicine alkaloid, Vascular bundles.

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Introduction:

The wheat crop, scientifically known as *Triticum aestivum* L., holds a prominent position among strategic crops due to its significant nutritional value. It serves as a crucial staple for global food security, catering to over 35% of the world's population. Wheat also takes the lead in terms of cultivated area and productivity. This is primarily attributed to its high carbohydrate content, constituting approximately 50% of its composition, which serves as a fundamental source of energy. Additionally, wheat contributes around 20% of the protein and calorie intake for human consumption (1). The significance of cultivars in determining yield is of significant importance, as evidenced by several studies that have examined the performance of different cultivars and their influence on the productivity of economic production(2). The presence of a substantial quantity of genes poses challenges in terms of their improvement by direct selection, mostly due to their weak inheritance and susceptibility to environmental influences(3). Nevertheless, numerous studies suggest that productivity remains low, despite its prominent position on the list of national goals. To enhance both the quantity and quality of crop production and achieve self-sufficiency, it is imperative to augment the productivity of Wheat by utilizing existing resources effectively. This is crucial to meet the growing food demand(4). Consequently, plant breeders focus on enhancing grain yield by indirectly improving the traits associated with it. Enhancing crop quality can be achieved through the implementation of indirect selection methods that prioritize traits exhibiting high heritability(5). The genetic factors governing the growth and productivity characteristics of crop cultivars play a crucial role in this process. Additionally, the interplay between these genetic factors, environmental conditions, and the integration of contemporary agricultural technologies in field practices significantly influences the enhancement of phenotypic and productive traits in cultivars(6). Plant breeders employ various means and tools to enhance production levels and enhance the quality of crops(3). One such means is the utilization of mutagenesis technology, which induces mutations and harnesses them in a beneficial manner to attain these objectives. This technology serves as a significant source of variation in diverse plant characteristics, rendering it one of the foremost methods employed by plant breeders. The chemical mutagenesis process involves the utilization of colchicine alkaloid. This compound functions by inhibiting the formation of spindle threads during the regular replication of somatic cells. Consequently, it prevents the proper segregation of chromosomes to the poles of the cell. As a result, the chromosomal number in the cell is doubled, leading to an increase in cell size. Subsequently, there is an augmentation in metabolic activities, which consequently leads to an elevation in the

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synthesis of primary and secondary chemicals, so leading in enhanced production(7).

Materials and methods

In the winter agricultural season of 2022-2023, a field experiment was conducted on November 15, 2022 in Al-Basateen Al-Sharqiah region. The study focused on various factors and employed employed RCBD design was used with the arrangement of the split plots with three replications to divide and distribute treatments. The main plots were assigned to different concentrations of the colchicine compound, denoted as control 0 (C0), 250 mg L^{-1} (C1), 500 mg L^{-1} (C2), and 750 mg L^{-1} (C3). The sub-plots, which were of greater significance, represented different cultivars and were symbolized as Ibaa99 (V1), Mawadah (V2), Bohuth22 (V3), and Babel (V4).

In the study, the seeds were subjected to a solution of colchicine following its dissolution in water, adhering to the prescribed concentrations and a set duration of 24 hours. The seeds were thoroughly rinsed with water and thereafter sown directly (3). The board in question consisted of five rows, with wheat seeds being sowed at a rate of 2.4 grams each row. In order to attain a plant density of 120 kg ha^{-1}(8), appropriate measures were taken, including fertilization operations carried out in accordance with recommended guidelines (9), as well as timely irrigation and weeding when required.

The traits that were examined in this study include many phenotypic indicators such as the number of days from culture till physiological maturity, flag leaf area, number of spikes per square meter, number of grains per spike, weight of 1000 grains, biological yield, and grain yield.

The leaf blade was subjected to vertical histological sectioning, which involved obtaining cross-sectional tomography of the leaf tissue. Random leaves were selected from plants for each experimental unit, and samples were prepared and analyzed following the prescribed steps (10). Subsequently, the models were examined, and measurements of the sections and their dimensions were conducted using a microscope equipped with a lens. The ocular structures were captured using a CH3 Olympus compound microscope equipped with a camera.

Results and Discussion

1 -Appearance indicators

Days from cultivation to physiological maturity

The analysis of the data presented in Table 1 reveals a statistically significant disparity in colchicine concentrations. The concentration of 750 exhibited superior responsiveness as it accelerated the plants' attainment of physiological maturity by 132 days, whereas the plants treated with the concentration of 250 experienced a delay of 147 days in achieving physiological maturity.

The observed extension in the duration between cultivation and physiological maturity can potentially be attributed to the impact of a

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heightened concentration of colchicine. This elevated concentration is known to induce cellular damage and mutations within plant cells, consequently impeding plant growth and elongating the transition period from vegetative growth to fruiting growth.

With respect to the phenomena of overlap, the data reported in the table demonstrate a statistically significant influence of the colchicine concentrations and cultivars on the observed attribute .

The most superior combination seen in this study is V4 C3, with a mean value of 118.00 days for early maturation. On the other hand, V1C1 exhibited the most delayed maturation, with a mean value of 154.33 days .

The findings presented align with the observations made in reference (11) regarding the impact of colchicine on the duration and timing of growth stages, as well as the influence of genetic variations among different cultivars and the diverse flowering durations. Additionally, the environmental conditions experienced by each cultivar during cultivation and maturation play a crucial role in determining the duration of each growth stage. The maturation of the crop is determined by the cumulative caloric units necessary for each specific cultivar, as a result of the equilibrium between two distinct stages.

Table 1 the impact of cultivars and the use of colchicine, and their interaction, on the duration in days from planting to physiological maturity.

Cultivars	colchicine mg L^{-1}				**Mean**
	C_0(0)	**C_1 (250)**	**C_2(500)**	**C_3(750)**	
Ibaa99(V1)	147.00	154.33	149.33	138.00	147.17
Mawadah(V2)	144.67	142.00	140.00	135.67	140.58
Bohuth22(V3)	144..33	142.33	134.00	137.00	139.42
Babe(V4)	144.00	149.00	148.33	118.00	139.83
Mean	145.00	146.92	142.92	132.17	
L.S.D	cultivars =**N.S**		colchicine = **5.025**		**C× V = 10.643**

Flag leaf area (cm^2)

The findings presented in Table 2 indicate that the application of colchicine alkaloid had a substantial impact on the flag leaf area. Specifically, the treatment with a concentration of 750 mg L^{-1} resulted in the highest mean of 36.66 cm^2, whereas the comparison treatment yielded the lowest mean of 26.29 cm^2.

The observed superiority in this particular capacity can be attributed to the treatment of

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the seeds with mutagenic colchicine. This treatment resulted in an increase in cell size through chromosomal duplication, leading to an increase in leaf area. This increase in leaf area is necessary to maintain a consistent cytoplasm ratio with the size of the nucleus. Furthermore, the increase in cell components has a positive impact on the flag leaf area (12).

The findings presented in Table 2 indicate that there were statistically significant variations in the bilateral overlap observed in the flag leaf area characteristic among different colchicine treatments and cultivars. Specifically, the combination (C3V3) exhibited the highest mean of 43.63 cm², while the combination (C0V4) displayed the lowest mean of 22.74 cm² for this particular characteristic. This finding aligns with the conclusions made by (13) in their study on wheat crop treated with varying doses of colchicine. Specifically, the study found that the highest concentration of colchicine resulted in the highest mean interaction in this particular attribute.

Table 2: the impact of cultivars and the use of colchicine, and their interaction, on leaf area (cm^2)

Cultivars	colchicine mg L^{-1}				**Mean**
	C_0(0)	**C_1 (250)**	**C_2(500)**	**C_3(750)**	
Ibaa99(V1)	31.15	31.47	31.95	34.44	32.25
Mawadah(V2)	25.96	34.07	36.01	35.74	32.95
Bohuth22(V3)	25.32	33.05	36.49	43.63	34.62
Babe(V4)	22.74	31.27	34.33	32.84	30.30
Mean	26.29	32.47	34.69	36.66	
L.S.D	cultivars = **n.s**		colchicine = **1.647**		**C× V = 4.026**

Number of spikes(m^{-2})

The findings shown in Table 3 indicate that there were statistically significant variations observed among the different cultivars in terms of the number of spikes per unit area. Specifically, the cultivar Bhooth 22(V3) demonstrated superior performance with an mean of 242.3 spikes per square meter, whilst the cultivar Ibaa 99 (V1)exhibited the lowest mean of 178.1 spikes per square meter.

The enhanced performance of the Bohuth22 cultivar can be linked to its genetic capacity for tiller production and its ability to capitalize on favorable growth conditions to generate supportive materials that facilitate the development of productive tillers. The distinguishing factor among them is the density of spikes per unit area.

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Table 3: the impact of cultivars and the use of colchicine, and their interaction, on Number of spikes(m^{-2})

cultivars	colchicine mg L^{-1}				**Mean**
	C_0(0)	**C_1 (250)**	**C_2(500)**	**C_3(750)**	
Ibaa99(V1)	196.0	176.0	180.4	160.0	178.1
Mawadah(V2)	234.2	242.2	203.6	223.1	225.8
Bohuth22(V3)	253.8	229.3	220.0	266.2	242.3
Babe(V4)	218.7	199.3	180.0	172.9	192.7
Mean	225.7	211.7	196.0	205.6	
L.S.D	cultivars = **48.62**		colchicine = **n.s**		**C × V =n.s**

The number of grains (spike^{-1})

A statistically significant difference was seen among the colchicine treatments in relation to this particular feature, as indicated in the results presented in Table 4. The concentration of 500 mg L^{-1} exhibited the highest mean, with a recorded value of 64.38 grains spike-1. In contrast, the comparison concentration (0) displayed the lowest mean for this characteristic, measuring 59.13 grains spike^{-1}. This represents a percentage increase of 8.88%.

The potential explanation for the observed increase in grain quantity within the spike may be attributed to the enhanced efficiency of vegetative cover resulting from the influence of colchicine. Consequently, this improved efficiency leads to an increase in the production of dry matter, which is subsequently transported from the source to the sinkand ultimately transformed into grains.

The findings presented in Table 4 demonstrate a statistically significant variation among the cultivars in terms of this particular trait. Specifically, the Bohuth 22 (V3) cultivar exhibited superior performance, with an mean of 65.90 grains per spike. In contrast, the Babel (V4) cultivar displayed the lowest mean of 54.93 grains per spike, representing a notable increase of 19.97%.

The variation in the number of grains in the spike can be attributed to the genetic composition of the wheat cultivars, as it is the primary determinant in this regard. This finding aligns with the conclusions drawn by (14), which demonstrated significant disparities in the number of grains in the spike among different wheat cultivars.

The table presented in the study indicates a statistically significant difference in the two-way interaction between the cultivars and the concentrations of colchicine in relation to the

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trait of number of grains per spike. Specifically, the combination of cultivar V1 and concentration C2 exhibited the highest mean of 70.27 grains per spike, while the lowest mean of 48.20 grains per spike was observed in the combination of cultivar V4 and concentration C0. The findings of this study align with the observations made in reference (15) about the mutagenic impact of colchicine, namely its ability to enhance grain production in rice crops.

Table 4: the impact of cultivars and the use of colchicine, and their interaction, on The number of grains (spike^{-1})

cultivars	colchicine mg L^{-1}				**Mean**
	C_0(0)	**C_1 (250)**	**C_2(500)**	**C_3(750)**	
Ibaa99(V1)	54.20	63.73	70.27	67.87	64.02
Mawadah(V2)	66.73	60.73	65.13	66.53	64.78
Bohuth22(V3)	67.40	70.20	69.47	56.53	65.90
Babe(V4)	48.20	54.73	52.67	64.13	54.93
Mean	59.13	62.35	64.38	63.77	
L.S.D	cultivars = 4.103		colchicine= **3.321**		**C× V =6.650**

Weight of 1000 grains

Significant differences were seen among the colchicine treatments in terms of this capacity, as indicated in Table 5. The concentration of 250 mg L^{-1} yielded the greatest mean of 51.04 g, while the concentration of 500 mg L^{-1} resulted in the lowest mean of 43.47 g, representing a percentage increase of 17.41%.

The findings presented in Table 5 demonstrate notable variations among the cultivars in terms of the weight of 1000 grains. Specifically, the Babylon(V4) cultivar exhibited the highest mean weight of 54.27 g, while the Bohuth 22(V3) cultivar displayed the lowest grain weight, mean at 44.16 g. This discrepancy reflects a growth rate of 22.89%. The potential dominance of a certain cultivar over another can be attributed to the phenomena of compensation in the yield components of grain crops, as well as the influence of the genetic composition of the cultivar in relation to this trait. The processing of processed foodstuffs during the flowering period until physiological maturity is influenced by various factors, including the grain and the rate, duration, and efficiency of processing. Additionally, the number of grains per spike plays a role in this process as there is competition for foodstuffs within a single spike. This study aligns with previous research (16) which suggests that

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there is a notable variation in this characteristic among different cultivars.

In relation to the impact of the binary interaction between cultivars and colchicine concentrations, it can be observed from Table (5) that there exists a statistically significant influence on the weight of 1000 grains. The combination (C1 V4) exhibited the highest mean weight of 58.67 g, whereas the lowest grain weight was observed in the combinations (C2V1) and (C2V2), with an mean of 40.00 g. This finding aligns with previous research (17) that suggested an increase in the weight of 1000 grains when treating plants with colchicine alkaloid. The superior performance of most combinations in terms of grain weight can be attributed to the mutagenic effect of colchicine, which leads to an increase in dry matter production and a doubling of plant cell volume. This, in turn, has a positive impact on the transport of nutrients during grain filling.

Table 5 the impact of cultivars and the use of colchicine, and their interaction, on Weight of 1000 grains (g)

cultivars	colchicine mg L^{-1}				**Mean**
	C_0(0)	**C_1 (250)**	**C_2(500)**	**C_3(750)**	
Ibaa99(V1)	46.77	46.83	40.00	48.97	45.64
Mawadah(V2)	48.67	52.03	40.00	53.33	48.51
Bohuth22(V3)	45.43	46.63	39.83	44.73	44.16
Babe(V4)	53.33	58.67	54.03	51.03	54.27
Mean	48.55	51.04	43.47	49.52	
L.S.D	cultivars = **4.159**		colchicine= **2.893**		**C× V = 6.079**

Grain yield(ton ha^{-1})

The findings shown in Table 6 indicate that the application of a treatment with a concentration of 750 mg L^{-1} resulted in a considerably higher grain yield characteristic. This treatment yielded an mean of 4.574 tons ha-^{1}, which was the greatest among all treatments. In contrast, the comparison treatment (0) yielded the lowest mean of 3.736 tons ha-^{1}, representing a percentage increase of 22.43% compared to the treatment with 0 mg L^{-1}. The potential cause for this observed advantage may be attributed to its higher number of grains per spike, as seen in Table 4, which therefore had a beneficial impact on grain yield.

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Table 6 the impact of cultivars and the use of colchicine, and their interaction, on Grain yield(ton ha-1)

cultivars	colchicine mg L^{-1}				**Mean**
	C_0(0)	**C_1 (250)**	**C_2(500)**	**C_3(750)**	
Ibaa99(V1)	3.468	3.752	4.155	4.181	3.889
Mawadah(V2)	4.865	5.093	4.919	4.994	4.968
Bohuth22(V3)	3.437	4.670	4.216	4.823	4.287
Babe(V4)	3.173	3.863	3.687	4.298	3.755
Mean	3.736	4.345	4.244	4.574	
L.S.D	cultivars = **n.s**		colchicine = **0.4210**		**C× V =n.s**

Biological yield(ton ha^{-1})

According to the findings, Table 7 demonstrates that the treatment with a concentration of 750 mg L^{-1} exhibited a significantly greater capacity, yielding an mean of 14.17 tons ha^{-1}. Conversely, the treatment with a concentration of 0 mg L^{-1} yielded the lowest mean for this particular characteristic, amounting to 12.56 tons ha^{-1}.

The observed superiority can perhaps be attributed to the augmentation of all vegetative growth indicators resulting from the emergence of novel mutations and the enlargement of plant cells when subjected to mutagenic colchicine treatment. This augmentation is manifested by a rise in the biological yield, encompassing both grain and straw.

Regarding the issue of overlap, the findings revealed a noteworthy impact of treatment overlap on the biological yield. Specifically, the combination (C3V3) exhibited the highest mean value for this attribute, reaching 16.09 tons ha^{-1}. Conversely, the combination (C0 V1) demonstrated the lowest mean value for this particular characteristic, amounting to 10.40 tons ha^{-1}.

The potential explanation for this phenomenon may be attributed to the influence of colchicine on promoting overall vegetative growth and enhancing the spike's grain count. The four components, as depicted in Table 4, collaborated synergistically to enhance the overall grain yield. The table shown in the study illustrates the relationship between two components, namely grain and straw, and their impact on the biological yield. It is observed that an increase in these components leads to a corresponding rise in the biological yield, which aligns with the findings reported by the author (reference 18). The use of colchicine alkaloid resulted in a significant enhancement of the biological yield.

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Table 7 the impact of cultivars and the use of colchicine, and their interaction, on biological yield (ton ha^{-1})

cultivars	colchicine mg L^{-1}				**Mean**
	C_0(0)	**C_1 (250)**	**C_2(500)**	**C_3(750)**	
Ibaa99(V1)	10.40	11.07	11.89	14.19	11.89
Mawadah(V2)	14.01	14.47	12.77	13.53	13.70
Bohuth22(V3)	14.90	12.38	14.34	16.09	14.43
Babe(V4)	10.93	13.76	14.56	12.89	13.03
Mean	12.56	12.92	13.39	14.17	
L.S.D	cultivars = **n.s**		colchicine = **1.102**		**C× V = 2.792**

2-Anatomical indicators

Leaf tissue anatomy vertical

The leaf tissue vascular bundle thickness

The findings presented in Table 8 indicate that there was a significant difference in the thickness of the leaf tissue's vascular bundles among the different cultivars. Specifically, the cultivar Ibaa 99(V1) exhibited the highest mean thickness of 115.33 µm, while the Mawadah (V2) had the lowest mean thickness of 79.93µm. This represents a notable increase of 44.32% between the two cultivars.

The observed variation in this feature among cultivars can likely be attributed to the distinct genetic composition of each cultivar.

Regarding the intersection of the treatments, notable disparities were observed. Specifically, combination (C2 V1) exhibited the highest mean value of 122.53 µm, whereas the combination (C2 V2) displayed the lowest mean value of 66.33 µm.

The potential explanation for this perceived superiority may be attributed to the impact of colchicine, which has been observed to increase cell size and induce alterations in the cytoskeleton and cellular components. These outcomes align with the findings reported in reference 19.

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Table 8 the impact of cultivars and the use of colchicine, and their interaction, on the leaf tissue vascular bundle thickness

Cultivars	colchicine mg L^{-1}				**Mean**
	C_0(0)	**C_1 (250)**	**C_2(500)**	**C_3(750)**	
Ibaa99(V1)	103.43	113.57	122.53	121.77	115.33
Mawadah(V2)	89.07	70.07	66.33	94.27	79.93
Bohuth22(V3)	107.20	105.97	87.73	71.73	92.91
Babe(V4)	103.87	84.20	105.89	83.13	94.27
Mean	100.89	93.20	95.62	92.73	
L.S.D	cultivars **=1.68**		colchicine **=N.S**		**C× V =3.19**

Transverse leaf tissue cross-sectional tomography

It is evident from Figure 1 that the histological microtome technique was employed to examine the vertical sections of the wheat crop leaf. The figure illustrates the various components of the leaf, including the upper epidermis layer covered with cuticles. The epidermis is comprised of a single layer of oval-shaped cells. Adjacent to the epidermis is the mesophyll layer, which consists of normal parenchyma cells. Following the mesophyll layer is the lower epidermis, composed of small oval-shaped cells. The vascular bundles are distributed throughout the mesophyll, with each bundle being protected by a layer of sclerenchyma tissue above and below. This layer is connected to both the upper and lower leaf epidermis. Additionally, each vascular bundle possesses its own vascular system. The structure consists of an outside layer known as the bark, followed by a layer of wood above it, and beneath the wood lays another layer of wood. Within this lower layer of wood is a hollow area referred to as the cavity of the first wood, which is a dissociative fissile intercellular space. Below this cavity, a Schizogenous-lysigenous intercellular space is produced. The bundle is enveloped with Bundle sheath fibers externally.

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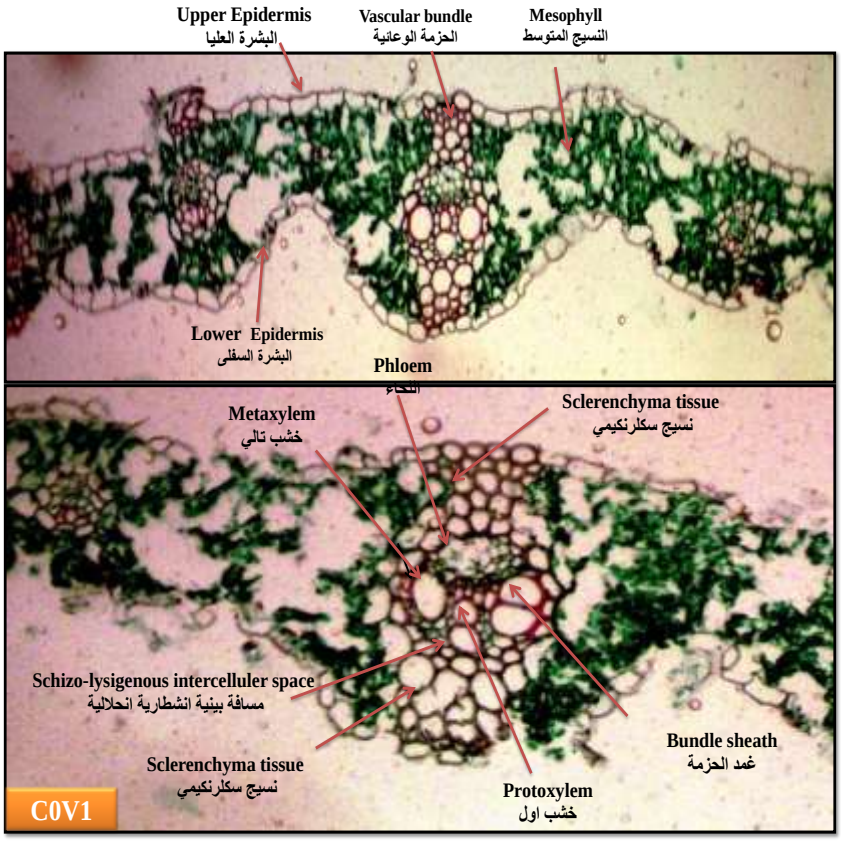

Figure 1 : Transverse leaf tissue cross-sectional tomography

The findings depicted in Figure 2 indicate that the treatment labeled as C2V1 resulted in the occurrence of rupture and damage in both the upper and lower epidermal cells of the leaves. Additionally, damage and fracture were observed in the mesophyll cells. Conversely, treatment C1V2 exhibited a deformation in the shape of the vascular bundle, along with the emergence of filaments in the upper epidermis. The observed outcome of the treatment (C2V2) can be attributed to a defensive response, whereas the treatment (C1V4) resulted in the rupture of both upper and lower epidermal cells and mesophylls. No discernible impact was detected in the remaining treatments.

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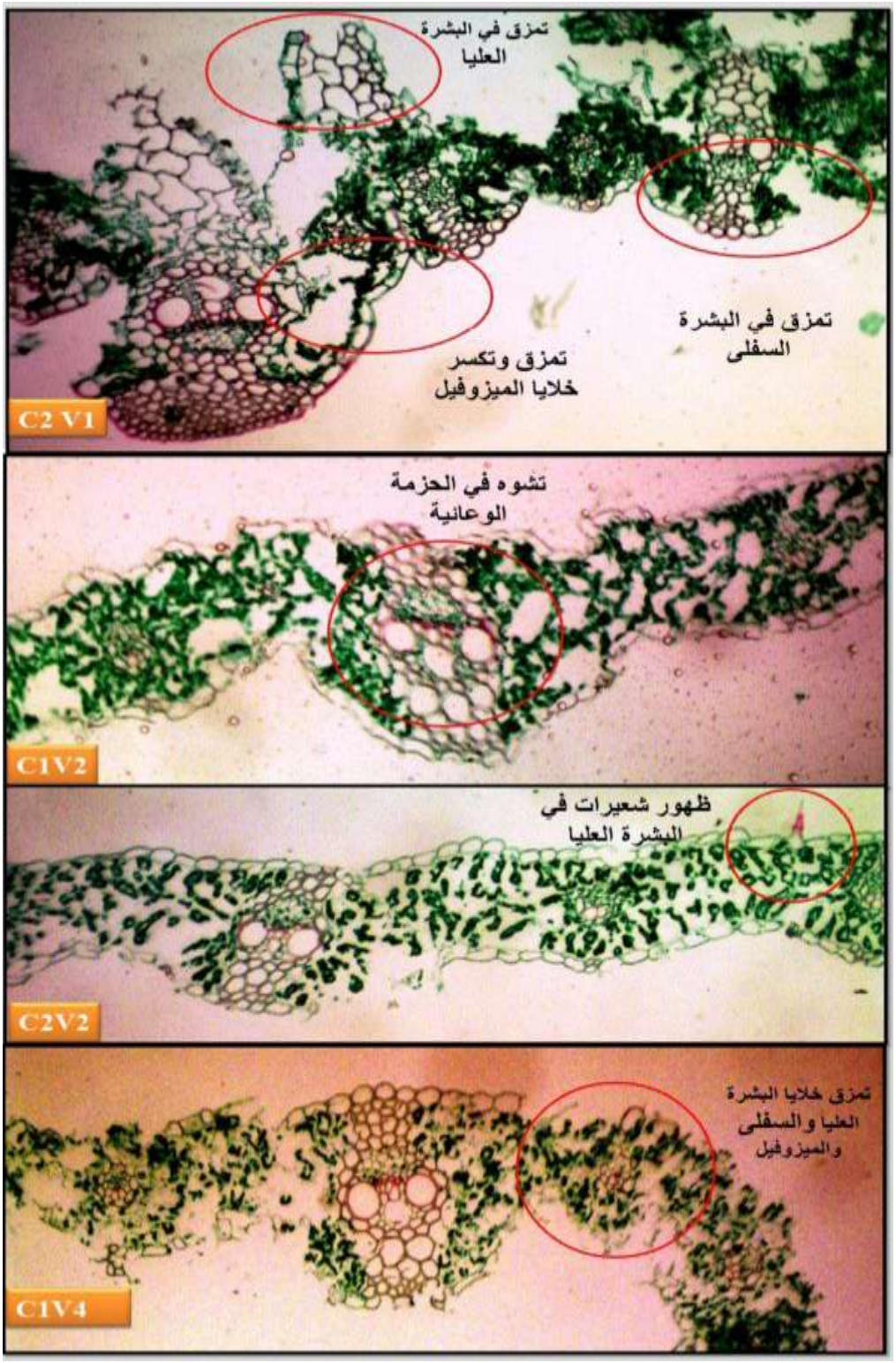

Figure 2 : the impact of interaction cultivars and the use of colchicine, on Transverse leaf tissue

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