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Effect of Fusaric Acid (FA) Bio-stress on Certain Morphological Parameters for Potato (*Solanum tuberosum. L*) Cultivars *in Vitro*

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Abstract:
The experiment was carried out with the aim of studying the effect of biological stress on some morphological parameters of ten varieties of potatoes grown in vitro. Biological stress was applied by adding different concentrations of fusaric acid (0, 0.0125, 0.025, 0.05, 0.1, 0.2 mM), to the growth medium MS, and some growth parameters were measured, such as plant height (cm), number of leaves (leaf/plant¹), leaf area (mm²), number of roots (root.plant¹) and length it (cm), wet and dry weight of the plant (g). The results showed that the studied varieties were different in the response to biological stress according to the studied parameters. The addition of fusaric acid led to reduce all growth parameters compared with the control. The cluster analysis showed that based on the sum of the relative values of the studied growth parameters, the studied varieties were distributed in three different groups: The first group includes three tolerant varieties to biological stress, and these are Toronto, Barcelona, and Suria). The second group includes four Moderate varieties of bio-stress, and these are Fabulla, Nectare, Spunta, and Ardappel. The third group included the following sensitive varieties, 7-four-7, Farida, and Joly. The results indicate that the in vitro screening technology can be used as a fast and efficient method to investigate the genetic variation of biological stress tolerance in potatoes.

Keywords: potatoes, biological stress, fusaric acid, in vitro, cluster analysis.

1. Introduction

Potato (*Solanum tuberosum L.*) is one of the most important and widespread vegetable crops [1]. It is cultivated in about 140 countries [2] and belongs to the genus *Solanum* and family *Solanaceae*, which approximately includes about 83 genera and 2671 cultivars [3]. Potato production reached about 562342 tons, with a yield of 25298 kg/ha on a total area of 22229 ha [4]. The plant faces a large number of bio-stresses such as diseases and their consequences from toxic substances and abiotic stresses such as heat, salinity, and drought [5]. Vascular wilt and dry rot of potatoes are bio-stresses caused by Fusarium spp. [6] and their secretions, causing losses in global annual production ranging (10-53%) [7], [8], [9]. Thus, the toxic substances cause morphological and biochemical changes to plant tissues by 10-30% of crops [10], as well as losses in potato production up to 25% [4]. Fusaric acid (5-butylpyridine-2-carboxylic acid) is a non-specialized toxic fungal organic substance with the chemical formula $C_{10}H_{13}O_2N$ [12] and systemic toxicity that destroys the cytoplasmic membrane of cells [13]. It prevents water transfer through the ethmoid vessels to the leaves [54], affecting the overall physiological processes [10], [11], [14] especially ATP

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concentration in the cytoplasmic membrane and inhibition of the enzyme activity of the cytoplasmic membrane H+-ATPase [15]. It also increases Reactive Oxygen Species (ROS) and destroys the defense system in the plant cell [16], [17], [18]. Furthermore, wilting symptoms appear and inhibit plant growth and regeneration [10], [19], [20], [21], [22]. Some studies showed adverse effects of high concentrations of FA≥1.0 mmol, preventing plant growth, increasing the number of lateral roots, reducing their length at a rate of 2 mmol, decreasing the early differentiation process in the cells of the elongation zone, and destroying the apical meristem at a rate of 5 mmol. This explains morphologically and anatomically the short length of roots and increased number of lateral roots close to the root tip and accelerated root aging [23]. Ions of some elements such as copper and zinc mitigate the adverse effects inside the cytoplasm exposing to FA [24], [15] which captures and inhibits the oxidative enzymes containing such ions [34]. Mycotoxin screening and selection method using *in-vitro* plant tissue culture is a possible method to obtain disease-resistant plants by screening susceptible plants in a small area [17], [26], [27] by choosing a suitable screening agent such as adding fusaric acid to the culture medium [13] to produce plants tolerant of some Fusarium ssp. and their secretions that cause wilt disease of potatoes [33] and tomato [17], [26]. Thus, the objective of this study was to know the effect of different fusaric acid (FA) concentrations on the growth characteristics of ten potato cultivars, and identify the tolerated cultivars of vascular wilt disease and introduce them into the potato breeding program in Syria.

2. Materials and Methods

2.1 Duration and place of research implementation

The research was conducted at the laboratories of the Faculty of Agriculture, Damascus University, and Plant Biotechnology Laboratory at the General Commission for Biotechnology, Ministry of Higher Education during 2018-2020.

2.2 Plant material:

Ten cultivars of elected potatoes were used in the research implementation (Table 1). They were certified by the General Organization for Seed Multiplication (GOSM) in 2017, and are experimental cultivars at the Administration of Horticulture Research, General Commission for Scientific Agricultural Research (GCSAR) for screening: Joly, Farida, Ardappel, Suria, 7-Four-7, Nectar, Fabula, Barcelona, Spunta and Toronto.

Table 1: Morphological and productive traits of the studied potato varieties.

yield	Flesh color	Skin colour	Tuber shape	Dormancy Period	Maturity	Varieties
High	Light yellow	Yellow	Oval/Long oval	Medium	Late middle	**Joly**
High	Light yellow	Yellow	Oval/Long oval	Medium	Middle earl	**Barcelona**
Very high	Light yellow	Yellow	Very large, long	medium to long	Middle early	**Spunta**
High	Light yellow	Yellow	Oval	Long	Late middle	**Fabula**
Very high	Light yellow	Yellow	Oval/Long oval	Medium	Late middle	**Farida**
Very high	Light yellow	Light yellow	Oval -long	Medium	Early	**Nectar**
Very high	Light yellow	Dark yellow	Long oval, Oval	medium to long	Middle	**Suria**
High	Cream, White	Light yellow	Long oval, Oval	Medium, High	Middle early	**7-Four-7**
Very high	Cream	Light yellow	Long	Medium, High	Middle early	**Toronto**
High	Light yellow	Light yellow	Oval	Medium	Middle	**Ardappel**

The pink ends taken from the tuber top were planted in plastic pots containing sterilized peat. the growths were collected after 45 days of planting and cut into single cuttings of 1-1.5 cm each of which contains one lateral bud, washed with running water, immersed in 70% ethyl alcohol for 1 minute, and then soaked with sodium hypochlorite (NaOCl) at a concentration of (0.5)% for 10 minutes. Later on, the biopsies were washed 3 successive times with sterile distilled water, at a rate of 5 minutes each time. The cuttings were cultured in test tubes containing 12.5 ml sterile MS medium [28]. supplemented with 30 g/l^{-1} and 7 g/l^{-1} agar and pH=5.8. The cultured tubes were incubated in the growth chamber at 22±2 °C, with illumination of 16 hours/8 darkness, a light intensity of 3000 lux, and relative humidity of 70±10%.

2.2 Bio-stress treatments

Fusaric acid (Sigma-Aldrich) was dissolved in sterile cold distilled water and sterilized using a filter containing a nitrocellulose membrane (Millipore Filter 0.22 µm). Then the MS medium was sterilized at 121°C and at a pressure of 1.04 kg/cm^2 for 20 minutes. Different FA concentrations were added and distributed over five treatments (treatments of Fusaric Acid (TFA0….5) (mmol)) as follows: (TFA0=0, TFA1=0.2, TFA2=0.1, TFA3=0.05, TFA4=0.025 and TFA5=0.0125 mM) to the MS culture medium as a bio-stress factor for screening cultivars, and FA was not added to the control TFA0=0 according to [29]. Plants resulting from the propagation stage were divided into small cuttings (1.5-2 cm), containing a lateral bud with a leaf, and cultured into the stress medium for screening. The readings of growth parameters were taken 36 days after culture. The experiment was repeated twice, at a rate of 18 repetitions for each treatment.

2.3 Studied growth indicators

Growth indicators include measuring plant stem length (cm), number of leaves (leaf/plant), and length and number of roots (root/plant). Leaf area (mm^2) was measured using ImageJ software. The fresh and dry weights of plants were measured using a sensitive balance (accuracy±0.0000) after drying at 110 °C until the weight is stable [30].

2.4 Experimental design and statistical analysis

The experiment was done according to the Randomized Complete Block Design (RCBD) at a rate of 18 repetitions for each treatment. The results were analyzed using XLSTAT statistical software, and two-way analysis of variance was conducted using Fisher's test. The averages were compared by calculating the value of the Least Significant Difference (LSD) at the significance level (P≤0.01). The cluster analysis was conducted for the strains tolerance to FA bio-stress based on a set of relative values of the studied growth criteria between the control and stress factor according to [31].

$$RV_{SY-C.n} = \sum \left(\frac{S_{p1 \to p9} * 100}{C_{p1 \to p9}} \right) \qquad \text{[1, eq. (31)]}$$

Where $RV_{SY-C.n}$: the sum of the relative values of the cultivar, $S_{p1 \to p9}$ value of the studied parameters (seven parameters) in the stressed plant, $C_{p1 \to 9}$ value of the parameters in the control plant.

3. Results and Discussion

The sensitivity of different plant tissues treated with FA is partly due to cell viability, which depends on energy metabolism and water uptake, and varies by different concentrations of FA [32], [23].

3.1 Growth parameters under bio-stress with FA in vitro

3.1.1 Plant height

The data in Table 2 shows that there are significant differences in plant height (P≤0.01) between cultivars exposed to FA bio-stress added to MS. The average plant height decreased in each cultivar with increasing stress intensity. The lowest average values of height were recorded in TFA1 and TFA2 (2.06 and 2.73 cm), respectively, and the highest significant value of average height in the control TFA0 (without FA) was (14.8 cm), with significant differences for the other treatments. The results of the statistical analysis showed that there were significant differences between studied potato cultivars: Nectar recorded the highest value for the average plant height (8.72 cm), and the lowest value (3.73 cm) for Ardappel, with significant differences among other cultivars. As for the interaction between cultivars and stress treatments and their mutual interaction, the average plant height was significantly higher in Nectar for TFA0 (18.16 cm), while the lowest was significantly in Ardappel for TFA1 (1.16 cm). These results are consistent with those of other studies [25], [36]. Low plant height is due to preventing the uptake of water entering the cells as a result of FA destruction of the membrane of sensitive plants cells [35], causing the occurrence of osmotic stress that inhibits cells elongation [38] and reduces the overall plant growth [39] as a result of a disturbance in the shape and structure of cells and their physiological processes [40], [37]. FA reduces plant ability to withstand environmental and pathological stresses by capturing some of the micro-minerals involved in the synthesis of oxidative enzymes that prevent the increase of Reactive Oxygen Species (ROS) that destroy the cell and its defenses [41], [34] and then plant wilt and mortality [26], [19].

Table 2: Effect of fusaric acid TFA on the plant height in the studied varieties

Varieties	Plant height (cm)						Mean of varieties
	Treatments of fusaric acid (TFA) (mM)						
	TFA1	TFA2	TFA3	TFA4	TFA5	TFA0	
Joly	$2.00^{tuvstwxy}$	$3.94^{mnopqrs}$	4.69^{mn}	4.74^{mn}	10.63^{ghi}	12.89^{ef}	**6.48^{BCD}**
Farida	$2.28^{qrstuvwxy}$	$2.49^{qrstuvwx}$	$2.71^{opqrstuvwxy}$	$3.06^{nopqrstuv}$	13.81^{de}	16.22^{bc}	**6.76^{BCD}**
Ardappel	1.16^{xy}	1.26^{wxy}	1.62^{tuvwxy}	6.75^{kl}	7.78^{jk}	9.19^{ij}	**3.73^{F}**
Suria	$2.49^{qrstuvwx}$	$2.56^{pqrstuvwx}$	$2.64^{pqrstuvwxy}$	$3.08^{nopqrstu}$	9.01^{ghi}	17.16^{efg}	**6.47^{BCD}**
7-Four-7	1.28^{vwxy}	$2.71^{opqrstuvwx}$	$2.80^{opqrstuvwxy}$	$3.03^{nopqrstuvw}$	11.38^{fgh}	13.08^{hi}	**8.21^{A*}**
Nectar	$2.47^{qrstuvwxy}$	3.96^{mnopqr}	5.44^{lm}	4.78^{mn}	17.50^{ab}	18.16^{a}	**8.72^{A}**
Fabula	$2.21^{qrstuvwxy}$	$2.79^{opqrstuvwx}$	4.34^{mnop}	4.63^{mn}	10.44^{ghi}	16.69^{abc}	**6.85^{BCD}**
Barcelona	$2.14^{stuvwxy}$	$2.46^{qrstuvwxy}$	1.84^{tuvwxy}	3.99^{mnopq}	15.00^{ab}	16.12^{ab}	**7.58^{AB}**
Spunta	1.41^{uvwxy}	1.74^{tuvwxy}	$2.19^{rstuvwxy}$	$2.56^{pqrstuvwx}$	7.25^{k}	12.03^{efg}	**4.53^{EF}**
Toronto	$3.20^{nopqrstu}$	$3.40^{nopqrst}$	$3.93^{mnopqrs}$	4.44^{mno}	12.88^{ef}	14.23^{cd}	**7.18^{ABC}**

Mean of treatments		2.06^{DE}	2.73^{D}	3.22^{CD}	3.80^{C}	12.01^{B}	14.8^{A}	6.15
Treatments	**$LSD_{0.01}$**				**0.74**			
Varieties					**1.70**			
Interaction					**1.80**			
CV%					**38.2**			

*Values followed by the same letters in the same row (between treatments) or column (between types) are not significantly different at (Least significant differences of means LSD at P<0.01), cv is coefficients of variation, mM is mill moll.

3.1.2 Number of leaves

Our results, after statistical analysis, showed that there were significant differences in plants (P≤0.01) between FA different bio-stresses and cultivars and their mutual interaction. A significant decrease was found in the number of leaves on one plant among the considered cultivars and within the same cultivar with increasing FA concentration in the applied stress factors in the growth medium (Table 3). Significant differences were recorded in the number of plant leaves between cultivars. A reduction in the average number of plant leaves was observed in each cultivar in general with increasing intensity of FA bio-stress. The lowest value of the average number of leaves was recorded in TFA1 and TFA2 where it was (3.43 and 4.23 leaves.plant¹) respectively, and the highest value of the average number of leaves was in the control TFA0 (13.69 leaves.plant¹) with significant differences in other treatments. The results also demonstrated that there were significant differences between the considered potato cultivars, but the cultivars Farida, Nectar, and Barcelona did not have any significant difference, recording the highest value for the average number of plant leaves (8.48, 8.5, and 8.06 leaves.plant¹) respectively, while Ardappel recorded the lowest value for the average number of plant leaves (5.6 leaves.plant¹) with significant differences among other cultivars. As for the interaction between cultivars and different stress factors and their mutual interaction, the number of plant leaves was the highest in 7-Four-7 for the control TFA0 (17.13 leaves.plant^{1}), while the lowest was in Ardappel for TFA1 (2.5 leaves.plant¹). The results in Table 3 shows that treating plants with FA significantly reduced the number and growth of leaves, and these results are in agreement with those of several studies [42], [55]. Reduced number of plant leaves exposed to FA bio-stress explains the low uptake of water and minerals, resulting in water stress and adverse impact on organogenesis and cellular differentiation processes [43], [44]. This leads to a reduction in plant height and the number of stem nodes and leaves, causing a decrease in the leaf surface exposed to light to reduce the transpiration rate [45]. Demonstrated [13] that increased FA concentrations led to cytoplasmic damage of FA-treated cucumber leaf cells and lysis of mesophyll cells, as well as adverse effects of FA on the cytoplasmic membrane on maize [32], tobacco [37], and tomato [46].

Table 3: Effect of fusaric acid treatments (FA) on the number of leaves of plants of the studied varieties.

Varieties	Number of leave						Mean of varieties
	Treatments of fusaric acid (TFA) (mM)						
	TFA1	TFA2	TFA3	TFA4	TFA5	TFA0	
Joly	3.13^{qrstu}	$3.75^{nopqrstu}$	$5.00^{jklmnop}$	5.38^{ijklmn}	9.38^{h}	12.83^{ef}	**6.54^{BCD}**
Farida	$4.75^{klmnopq}$	5.50^{ijklm}	5.88^{ijkl}	6.00^{ijk}	13.63^{cde}	15.13^{bc}	**8.48^{A*}**
Ardappel	2.50^{u}	2.63^{tu}	2.88^{stu}	3.13^{qrstu}	9.13^{h}	13.28^{def}	**5.6^{D}**
Suria	1.03^{qrstu}	$3.63^{opqrstu}$	$4.13^{mnopqrstu}$	5.13^{jklmno}	10.88^{bcd}	13.13^{ef}	**7.33^{ABC}**
7-Four-7	$3.63^{opqrstu}$	$3.75^{nopqrstu}$	$4.25^{lmnopqrst}$	$4.75^{klmnopq}$	9.63^{h}	17.13^{a}	**7.19^{ABC}**
Nectar	3.38^{pqrstu}	5.63^{ijklm}	6.88^{i}	5.13^{jklmno}	15.13^{bc}	14.88^{bcd}	**8.5^{A}**
Fabula	3.00^{rstu}	$4.63^{klmnopqr}$	5.50^{ijklm}	6.00^{ijk}	11.75^{fg}	12.63^{ef}	**7.25^{ABC}**
Barcelona	$3.75^{nopqrstu}$	$4.75^{klmnopq}$	$5.00^{jklmnop}$	5.75^{ijklm}	15.63^{ab}	14.50^{cde}	**8.06^{A}**
Spunta	2.63^{tu}	$4.75^{klmnopq}$	$5.25^{ijklmno}$	5.50^{ijklm}	9.00^{h}	10.38^{gh}	**6.25^{CD}**
Toronto	$4.50^{klmnopqrs}$	3.25^{qrstu}	6.00^{ijk}	6.50^{ij}	13.63^{cde}	12.00^{bcde}	**7.98^{AB}**
Mean of treatments	3.43^{E}	4.23^{D}	5.08^{C}	5.33^{C}	12.18^{B}	13.59^{A}	**7.32**
Treatments	**$LSD_{0.01}$**			**0.62**			
Varieties				**1.44**			
Interaction				**1.70**			
CV%				**30**			

*Values followed by the same letters in the same row (between treatments) or column (between types) are not significantly different at (Least significant differences of means LSD at P<0.01), cv is coefficients of variation, mM is mill moll.

3.1.3 Leaves area

The results of the statistical analysis showed that there were significant differences (P≤0.01) between different FA bio-stresses and cultivars and their mutual interaction in each considered plant. The results in Table 4 shows the effect of stress factors on the total area of leaves with increasing intensity of applied stress by cultivar. It was also observed that the average leaf area was significantly higher in the control TFA0 (3886.8 mm^2), and the leaf area decreased at increasing FA concentration in the growth medium, where the lowest leaf area significantly was in TFA1 (318.7 mm^2) with significant differences for other treatments. The results also showed that there were no significant differences in the value of the leaf area between Farida and Joly (1853.83 mm^2 and 1735.4 mm^2), respectively, but they were more significant than other cultivars, whereas 7-Four-7 recorded the lowest value (713.6 mm^2). As for the interaction between cultivars and FA stress factors and their interaction, the leaf area was significantly higher in Farida for the control TFA0 (5831.6 mm^2), while the lowest significant was 7-Four-7 for TFA1 (105.9 mm^2). These results are consistent with those of several studies showing that FA stress adversely affects leaf number and area by causing osmotic stress [43] and water stress [45]. Low leaf area is directly related to reduced plant height and number of leaves, as its reduction is the first morphological indicator affected by

water stress [35], and low number of leaves on the plant is only a result of stress effect on organogenesis and cellular differentiation [44], leading to low number of leaves and leaf surface exposed to sunlight to reduce transpiration rate and photosynthesis, and thus to save plant water for stress tolerance [35].

Table 4: Effect of fusaric acid treatments (TFA) on Leave area the of plants in the studied varieties:

Varieties	Leave area (mm^2)						Mean of varieties
	Treatments of fusaric acid (TFA) (mM)						
	TFA1	TFA2	TFA3	TFA4	TFA5	TFA0	
Joly	$454.5^{lmnopq\ rstu}$	$517.0^{klmnop\ qrstu}$	873.5^{hijkl}	930.9^{ghijk}	2836.6^{ef}	4933.3^{b}	**1757.6^{A}***
Farida	$519.8^{klmnop\ qrstu}$	$557.3^{klmnop\ qrstu}$	$638.6^{klmnopq\ rstu}$	1108.9^{ghij}	2466.6^{f}	5831.6^{a}	**1853.8^{A}**
Ardappel	171^{rstu}	190.9^{qrstu}	$423.3^{lmnopqr\ stu}$	$698.5^{klmnop\ qrstu}$	$579.1^{klmnop\ qrs}$	4361.7^{cd}	**1074.2^{CD}**
Suria	312.8^{pqrstu}	$362.8^{nopqrst\ u}$	$404.8^{mnopqrs\ tu}$	$451.0^{lmnopq\ rstu}$	4859.4^{b}	3702.7^{c}	**1682.3^{AB}**
7-Four-7	105.9^{u}	133.4^{tu}	$398.5^{mnopqrs\ tu}$	$418.3^{lmnopq\ rstu}$	$792.6^{hijklm\ no}$	2432.8^{f}	**713.6^{D}**
Nectar	$454.6^{lmnopq\ rstu}$	$517.1^{klmnop\ qrstu}$	$583.6^{klmnopq\ rst}$	1243.9^{gh}	1378.2^{g}	3423.3^{cd}	**1266.8^{B}**
Fabula	$372.5^{nopqrst\ u}$	$539.8^{klmnop\ qrstu}$	$582.6^{klmnopq\ rst}$	$877.4^{jklmnop\ q}$	842.2^{hijklm}	3281.7^{cde}	**1082.7^{CD}**
Barcelona	106.5^{u}	$342.5^{nopqrst\ u}$	$767.1^{ijklmnop}$	948.9^{ghijk}	1149.4^{ghi}	3211.4^{de}	**1087.6^{CD}**
Spunta	$547.4^{klmnop\ qrstu}$	$608.1^{klmnop\ qrs}$	$713.6^{ijklmnop}$	$810.5^{hijklmn}$	1149.4^{ghi}	3425.8^{cd}	**1209.1^{BC}**
Toronto	142.6^{stu}	212.4^{qrstu}	$329.6^{opqrstu}$	$404.0^{mnopqr\ stu}$	$420.4^{lmnopq\ rstu}$	5285.0^{b}	**1132.3^{C}**
Mean of treatments	318.76^{E}	398.13^{DE}	571.52^{D}	789.23^{C}	1649.46^{B}	3988.93^{A}	**832.24**
Treatments	**$LSD_{0.01}$**			**0.22**			
Varieties				**0.46**			
Interaction				**0.47**			
CV%				**49.2**			

*Values followed by the same letters in the same row (between treatments) or column (between types) are not significantly different at (Least significant differences of means LSD at P<0.01), cv is coefficients of variation, mM is mill moll.

3.1.4 Number of roots

The results showed that there were significant differences in the plant (P≤0.01) between FA different bio-stresses and between cultivars and their mutual interaction. A considerable adverse effect of bio-stress factors on root number and growth with increasing applied stress intensity by cultivar was observed (Table 5). The average number of roots decreased significantly with increasing FA concentrations, and the number of roots was significantly lower in TFA1 (0.08 root.plant^{-1}) with significant differences with other treatments, while it was significantly higher in the control TFA0 (8.96 root.plant^{-1}). The results revealed that there were no significant differences between studied potato cultivars, where Nectar recorded the highest value for the number of roots (3.81 root.plant^{-1}), followed by the three cultivars Surya, Toronto, and Joly (3.67, 3.6, and 3.47 root.plant^{-1}), respectively with no significant

differences. However, Ardappel recorded the lowest value for the number of roots (1.99 root.plant^{-1}). As for the interaction between cultivars and different stress factors and their interaction, the number of roots was significantly higher in 7-Four-7 for the control TFA0 (11.25 root.plant^{-1}), whereas the lowest significant number was for TFA1 and TFA2 (0.13 root.plant^{-1}). These results are consistent with those of several studies [43], [47]. Other studies showed that the plant response to FA osmotic stress begins with morphological and physiological changes in the roots, resulting in adverse changes in the uptake of water and minerals, and production of hormones responsible for sending signals to the vegetative system, thus affecting the entire biological, physiological and metabolic processes in the plant [48], [49]. FA stress causes a reduction in the growth of the root system, resulting in a decrease in the osmotic content of tissues, and thus a decrease in the turbulent pressure of the cell, which inhibits growth and elongation [38]. Therefore, the plant is considered stress-tolerant if it develops a strong root system under the effect of this stress [50].

Table 5: Effect of fusaric acid treatments (FA) on the number of roots of plants in the studied varieties

Varieties	Number of roots						Mean of varieties
	Treatments of fusaric acid (TFA) (mM)						
	TFA1	**TFA2**	**TFA3**	**TFA4**	**TFA5**	**TFA0**	
Joly	0.00^{o}	0.00^{o}	0.25^{o}	0.38^{o}	5.38^{jk}	9.50^{def}	**3.47 A***
Farida	0.00^{o}	0.00^{o}	0.00^{o}	0.50^{o}	6.63^{ij}	8.63^{efg}	**2.63 AB**
Ardappel	0.00^{o}	0.00^{o}	0.00^{o}	6.17^{ij}	7.20^{ghi}	7.65^{ghi}	**1.99 B**
Suria	0.00^{o}	0.00^{o}	0.88^{no}	1.50^{mno}	9.75^{cdef}	10.25^{cde}	**3.67 A**
7-Four-7	0.13^{o}	0.13^{o}	0.88^{no}	1.50^{mno}	6.50^{ij}	11.25^{bc}	**2.79 AB**
Nectar	0.63^{no}	0.75^{no}	1.13^{mno}	1.63^{mno}	6.88^{hij}	10.13^{cde}	**3.81 A**
Fabula	0.00^{o}	0.00^{o}	1.38^{mno}	1.50^{mno}	12.88^{b}	7.38^{ghi}	**3.11AB**
Barcelona	0.00^{o}	1.00^{mno}	1.25^{mno}	1.33^{mno}	3.63^{l}	6.63^{ij}	**2.55 AB**
Spunta	0.00^{o}	0.00^{o}	1.00^{mno}	1.33^{mno}	15.63^{a}	10.75^{cd}	**2.82 AB**
Toronto	0.00^{o}	2.25^{lmn}	2.63^{lm}	3.83^{kl}	8.33^{fgh}	5.38^{ghi}	**3.6 A**
Mean of treatments	0.08^{E}	0.40^{DE}	0.94^{D}	1.98^{C}	8.28^{B}	8.96^{A}	**3.05**
Treatments	**0.67**						
Varieties	**1.42**						
Interaction	**1.7**						
CV%	**65.4**						

LSD$_{0.01}$

*Values followed by the same letters in the same row (between treatments) or column (between types) are not significantly different at (Least significant differences of means LSD at P<0.01), cv is coefficients of variation, mM is mill moll.

3.1.5 Length of roots

The results showed that there were significant differences in the plant (P≤0.01) between different FA stresses and cultivars and their mutual interaction. The results into Table 6 shows the effect of bio-stress factors on the length of roots with increasing intensity of applied stress by cultivar, noting that the average root length was significantly higher in the control TFA0 (10.88 cm). The root length decreased with increasing FA concentration in the growth medium, where the root length was lower significantly in FTA1 (0.05 cm), with no significant differences in TFA2 (0.14 cm). The results also showed that there were significant differences between considered potato cultivars: Barcelona was more significant than other cultivars. As for the considered cultivars Joly and Farida, no significant differences were observed, and the same was for Nectar and Fabula, whereas Spunta recorded the lowest value (2.31 cm). As for the interaction between cultivars and applied bio-stress factors and their mutual interaction, the root length was significantly higher in Joly for the control TFA0 (16.94 cm), and the lowest was significantly for TFA1 and TFA2 with a value of (0 cm). These results are consistent with those of several studies [15], [36] Several studies showed that the addition of high FA concentrations to the growth medium destroyed the structure of the cell walls of sieve vessels carrying the plant, morphological and physiological changes in the roots and a reduction in osteoporosis pressure due to lack of cellular water content and reduced elongation and plant growth [38], [49]. Other studies showed that plant exposure to high FA doses leads to low water uptake and production of growth regulators responsible for sending signals to the vegetative system that affects other physiological processes inside the cells [47]. This study is also consistent with other studies showing that high concentrations of FA ≥ 1.0 mmol led to short roots, increased number and accelerated aging [23], as FA affects the processes of phosphorous oxidation and energy synthesis ATP needed for their growth and development [32], [43].

Table 6: Effect of fusaric acid treatments (TFA) on the root length of plants in the studied varieties

Varieties	Roots length (cm)						Mean of varieties
	Treatments of fusaric acid (TFA) (mM)						
	TFA1	**TFA2**	**TFA3**	**TFA4**	**TFA5**	**TFA0**	
Joly	0^{l}	0^{l}	0.04^{l}	0.25^{l}	4.15^{hi}	16.94^{a}	**2.54^{CDE}***
Farida	0^{l}	0^{l}	0^{l}	0.28^{l}	5.33^{h}	10.20^{cd}	**2.62^{CDE}**
Ardappel	0^{l}	0^{l}	0^{l}	2.48^{jk}	2.98^{ij}	9.16^{de}	**2.46^{DE}**
Suria	0^{l}	0^{l}	0.68^{l}	1.26^{kl}	9.19^{de}	10.89^{bc}	**3.73^{ABCD}**
7-Four-7	0.23^{l}	0.24^{l}	0.93^{l}	1.04^{kl}	5.5^{h}	8.83^{def}	**3.40^{BCD}**
Nectar	0.3^{l}	0.35^{l}	0.7^{l}	0.63^{l}	8.69^{ef}	12.19^{b}	**3.52^{BC}**
Fabula	0^{l}	0^{l}	0.79^{l}	0.88^{l}	7.88^{efg}	9.14^{de}	**3.85^{BC}**
Barcelona	0^{l}	0^{l}	0.5^{l}	0.43^{l}	7.56^{fg}	10.02^{def}	**4.79^{A}**
Spunta	0^{l}	0.2^{l}	0.36^{l}	0.77^{l}	3.06^{ij}	10.91^{bc}	**2.31^{E}**
Toronto	0^{l}	0.61^{l}	1.09^{kl}	1.12^{kl}	7^{g}	10.73^{b}	**4.08^{AB}**
Mean of treatments	0.05^{D}	0.14^{D}	0.51^{CD}	0.91^{C}	6.12^{B}	10.88^{A}	**3.33**

Treatments	**$LSD_{0.01}$**	**0.67**
Varieties		**1.42**
Interaction		**1.7**
CV%		**62.7**

*Values followed by the same letters in the same row (between treatments) or column (between types) are not significantly different at (Least significant differences of means LSD at P<0.01), cv is coefficients of variation, mM is mill moll.

3.1.6 Plant fresh weight

The results showed that there were significant differences in the plant (P≤0.01) between different FA stresses and cultivars and their mutual interaction. The results into Table 7 shows the effect of FA stress factors on the plant fresh weight with increasing applied stress intensity by cultivar. It is noted that the average fresh weight was significantly higher in the control TFA0 (0.9083 g), and the fresh weight decreased at increasing FA concentration in the growth medium. The lowest fresh weight was significant in TFA1 (0.0804 g) with no significant difference for TFA2 and TFA3 (0.1002 and 0.1147 g), respectively. The results also showed that there were significant differences between studied potato cultivars: Barcelona was more significant than Ardappel with a value of (0.3861 g), while Ardappel recorded the lowest value (0.2113 g). As for other studied cultivars, significant differences were observed between them. As regards the interaction between cultivars and FA stress factors and their mutual interaction, the fresh weight was significantly higher in the two cultivars, Nectar and Farida for the control TFA0 (1.2057 and 1.1197 g), respectively, while the lowest was significantly in TFA1 (0.0804 g), noting that there was no significant difference between this treatment and the two treatments TFA2 and TFA3 and the value of each (0.1002 and 0.1147), respectively. The results are in agreement with those of other studies [42], [55]. The results showed that all FA factors led to a reduction in the fresh weight value due to a decrease in most growth indicators such as plant length, the number of leaves, length and number of roots [39], stem and root diameter [51], and plant leaf surface and physiological processes [43].

Table 7: Effect of fusaric acid treatments (TFA) on the wet weight of plants in the studied varieties

Varieties	Wet weight (g)						Mean of varieties
	Treatments of fusaric acid (TFA) (mM)						
	TFA1	**TFA2**	**TFA3**	**TFA4**	**TFA5**	**TFA0**	
Joly	0.0826^{mno}	0.0881^{mno}	0.0983^{mno}	0.1487^{klmno}	0.3545^{hi}	0.9912^{b}	**0.2890^{ABC}**
Farida	0.1021^{mno}	0.1116^{mno}	0.1454^{klmno}	0.1521^{klmno}	0.3402^{hi}	1.1197^{a}	**0.3285^{AB}**
Ardappel	0.0619^{no}	0.0788^{mno}	0.0836^{mno}	0.3237^{hij}	0.3721^{h}	0.6015^{g}	**0.2113^{C}**
Suria	0.0860^{mno}	0.0992^{mno}	0.1055^{mno}	0.1124^{mno}	0.8127^{def}	0.7401^{f}	**0.3260^{AB}**
7-Four-7	0.0662^{no}	0.0547^{o}	0.0673^{no}	0.1162^{mno}	0.3414^{hi}	0.8732^{cde}	**0.2532^{BC}**
Nectar	0.1242^{lmno}	0.1268^{lmno}	0.1758^{klm}	0.3078^{hij}	0.2931^{hij}	1.2057^{a}	**0.3722^{A*}**

Fabula	0.0524^{o}	0.0858^{mno}	0.0958^{mno}	0.1000^{mno}	0.3000^{hij}	0.9099^{bcd}	**0.2573^{BC}**
Barcelona	0.0597^{no}	0.1065^{mno}	0.1121^{mno}	0.1649^{klmn}	0.8950^{bcde}	0.9787^{bc}	**0.3861^{A}**
Spunta	0.1001^{mno}	0.1104^{mno}	0.1201^{lmno}	0.1583^{klmno}	0.2499^{ijk}	0.7905^{ef}	**0.2549^{BC}**
Toronto	0.0691^{mno}	0.1406^{lmno}	0.1431^{klmno}	0.2253^{jkl}	0.3630^{h}	0.8630^{de}	**0.3007^{ABC}**
Mean of treatments	0.0804^{D}	0.1002^{D}	0.1147^{D}	0.1809^{C}	0.4319^{B}	0.9083^{A}	**0.30**
Treatments	**$LSD_{0.01}$**			**0.04**			
Varieties				**0.10**			
Interaction				**0.11**			
CV%				**47**			

*Values followed by the same letters in the same row (between treatments) or column (between types) are not significantly different at (Least significant differences of means LSD at P<0.01), cv is coefficients of variation, mM is mill moll.

3.1.7 Dry weight

The results showed that there were significant differences in the plant (P≤0.01) between different FA bio-stresses and cultivars and their mutual interaction. The results into Table 8 shows the effect of stress factors on plant dry weight with increasing intensity of applied bio-stress by cultivar. It is also observed that the average dry weight was significantly higher for the control TFA0 (0.1435 g), and the dry weight decreased with increasing FA concentration in the culture medium. The significantly lowest dry weight was for TFA1 (0.0503 g) with significant differences for TFA2 (0.0273 g). The results also revealed that there were significant differences between the considered cultivars. Ardappel was more significant than Spunta with a value of (0.0678), while 7-Four-7 recorded the lowest value (0.0344 g) with no significant difference between it and both Fabula and Spunta (0.0352 and 0.0362). As for other studied cultivars, no significant differences were observed. As for the interaction between cultivars and different stress factors and their interaction, the dry weight was significantly higher in Ardappel for the control TFA0 (0.3165 g), while the lowest was significantly in 7-Four-7 for TFA2 (0.0067 g). High dry weight is an advantageous trait for plants as it is related to plant tolerance to any type of stress. One study demonstrated a durable relationship between the accumulation of dry matter in the plant and its productive ability under stress conditions [52]. Other studies showed that all FA factors led to a reduction in the value of fresh and dry weights due to a decrease in most growth indicators [43], [51]. Reduced size of leaf area, which is active in the photosynthesis process, reduces the concentration and fixation of CO_2 in the triple carbon return ring available within the chloroplasts, decreasing dry weight in general [53].

Table 8: Effect of fusaric acid treatments (TFA) on the dray weight of plants in the studied varieties

Varieties	Dray weight (g)						Mean of varieties
	Treatments of fusaric acid (TFA) (mM)						
	TFA1	TFA2	TFA3	TFA4	TFA5	TFA0	
Joly	0.0146^{jklmno}	0.0151^{jklmno}	0.0161^{ijklmno}	0.0251^{hijklmno}	0.0373^{ghijkl}	0.1353^{cd}	**0.0399^{BC}***
Farida	0.0111^{lmno}	0.0124^{klmno}	0.0231^{hijklmno}	0.0260^{hijklmno}	0.0303^{hijklmno}	0.1575^{bc}	**0.0434^{BC}**
Ardappel	0.0289^{hijklmno}	0.0117^{klmno}	0.0107^{lmno}	0.0356^{ghijklm}	0.0414^{ghij}	0.3165^{a}	**0.0678^{A}**
Suria	0.0155^{jklmno}	0.0154^{jklmno}	0.0165^{ijklmno}	0.0175^{ijklmno}	0.1045^{ef}	0.095^{f}	**0.0441^{BC}**
7-Four-7	0.0125^{klmno}	0.0067^{o}	0.0084^{o}	0.0158^{jklmno}	0.0384^{ghijk}	0.1249^{de}	**0.0344^{C}**
Nectar	0.0234^{hijklmno}	0.0241^{hijklmno}	0.0355^{ghijklmn}	0.0415^{ghij}	0.0459^{gh}	0.1681^{b}	**0.0564^{AB}**
Fabula	0.0092^{mno}	0.0106^{lmno}	0.0183^{ijklmno}	0.0209^{hijklmno}	0.0429^{ghi}	0.1093^{def}	**0.0352^{C}**
Barcelona	0.0076 ^{o}	0.0178^{ijklmno}	0.0165^{ijklmno}	0.0615^{g}	0.0947^{f}	0.1197^{def}	**0.0530^{ABC}**
Spunta	0.0217^{hijklmno}	0.0221^{hijklmno}	0.0236^{hijklmno}	0.0242^{hijklmno}	0.0310^{hijklmno}	0.0945^{f}	**0.0362^{C}**
Toronto	0.0086^{no}	0.0195^{hijklmno}	0.0208^{hijklmno}	0.0288^{hijklmno}	0.0383^{ghijk}	0.1249^{dc}	**0.0401^{BC}**
Mean of treatments	0.0153^{E}	0.0155^{E}	0.0188^{D}	0.0273^{C}	0.0505^{B}	0.1435A	0.0450
$LSD_{0.01}$ Treatments				**0.74**			
$LSD_{0.01}$ Varieties				**1.70**			
$LSD_{0.01}$ Interaction				**1.80**			
CV%				**78.5**			

*Values followed by the same letters in the same row (between treatments) or column (between types) are not significantly different at (Least significant differences of means LSD at P<0.01), cv is coefficients of variation, mM is mill moll.

3.2 Cluster analysis

based on the sum of relative values of studied growth parameters, led to the division of studied potato cultivars according to their tolerance to FA bio-stress, so the studied potato cultivars were distributed to three different groups (Figure 1). The first group included three cultivars tolerant to FA stress: Toronto, Spunta, and Surya; the second group included four cultivars that are moderately tolerant to FA stress: Ardappel, Nectar, Fabula, and Spunta; and the third group included three cultivars sensitive to FA: Joly, Farida, and 7-Four-7, as the latter cultivar is the most sensitive.

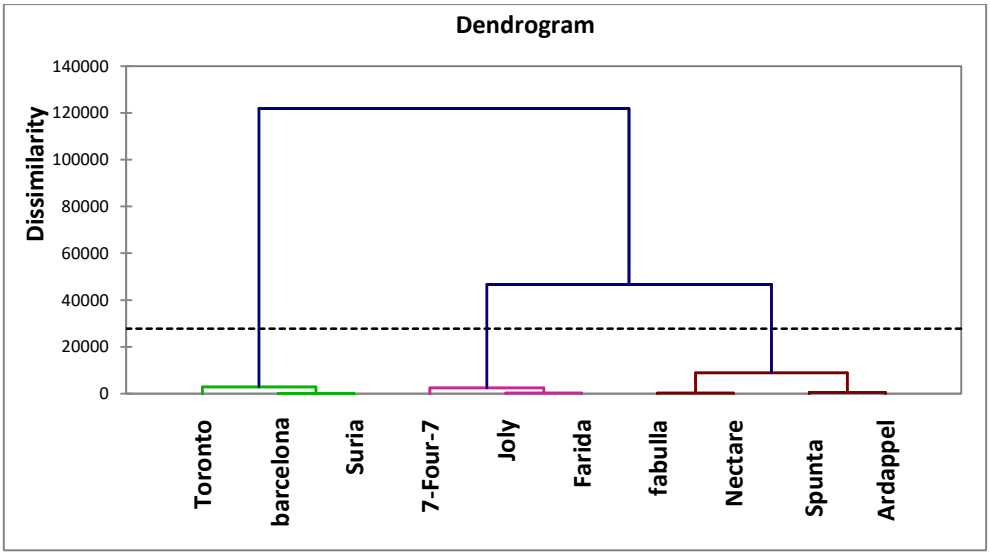

Figure 1: Dendrogram based on relative values of growth parameters of the ten potato varieties under the effect of different fusaric acid (FA) treatments.

Conclusions

The growth parameters of potato cultivars significantly decreased by increasing the intensity of FA bio-stress for all traits compared with the control. Potato cultivars differed in their response to stress by cultivar and stress intensity, so Toronto was the most stress-tolerant, while 7-Four-7 was the most sensitive.

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