



ACCUMULATION OF FERULIC ACID UNDER DROUGHT STRESS IN *IN VITRO* CULTURE OF *GARDENIA JASMONIDE* VARIEGATA

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ABSTRACT

Gardenia is regarded as an ornamental and medicinal plant that includes important phenolic compounds, as well as some of anti-inflammatory flavonoids. The most prevalent hydroxyl cinnamic acid in plants is ferulic acid, which is 4-hydroxy-3-methoxy cinnamic acid. It demonstrates a wide range of biological activity that can be utilized as a substrate for the synthesis of chlorogenic acid. The current study aimed to evaluate the accumulation of ferulic acid and antioxidant capacity in response to ABA and PEG and mimic the draught stress, in the *in vitro* culture of *Gardenia jasmonides* Variegata. Different concentrations of abscisic acid (ABA) and polyethylene glycol (PEG) (MW: 4000–6000) were applied individually in the culture medium of *Gardenia jasmonide* Variegata callus cultures to evaluate their effects on the growth as well as the ferulic acid content and antioxidant capacity. The results showed that maximum values for growth indicators (fresh and dry weight) at the fourth week of culturing (9.524 and 0.446 g, respectively) with the addition of 5 g/l PEG (M.W. 6000). As for ferulic acid, PEG (M.W. 6000) at 7.5 g/l gave 2.708 µg/g dry weight ferulic acid in the second week, which is remarked as the highest ferulic acid content. The highest antioxidant activity was recorded using 7.5 g/l PEG (M.W. 4000) in the fourth week, which was recorded at 2491.6–812.61 µM Trolox equivalent / 100mg sample when 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) were subjected, respectively. Finally, it can be concluded that using PEG in various treatments had a positive impact on the growth and accumulation of ferulic acid in *Gardenia jasmonide* Variegata calli cultures and subsequently increased the anti-oxidant activity as opposed to using ABA.

INTRODUCTION

Plants produce a variety of defence secondary metabolites, mainly phenolic and nitrogen-containing compounds, to reduce the effects of various climate change-related stresses (such as warming due to increased greenhouse gas emissions, drought, cold, ozone layer depletion, and harmful UV radiation) (Julkunen *et al.*, 2014). Plants affected by climate change may be a significant source for drug development because the manufacturing of defence compounds in plants (including medicinal plants) is frequently elevated and these compounds have varied pharmacological qualities. Researchers who are looking for alternative options for the drug discovery process may use other wild plants that have been subjected to different climatic or abiotic stresses. When plants are under stress, bioactive compounds are elevated. The output of bioactive compounds from plants may be increased by cultivating target plant tissues on a large scale in a controlled environment using different abiotic stresses. As a result, plant tissue culture would provide academics and the pharmaceutical industry with an additional platform to scale up the production of valuable phytochemicals under climate change factors (Yeshe *et al.*, 2022). Recently, plant cell and tissue culture techniques have been regarded as a valuable platform to mimic environmental conditions and/or environmental stress on plants (i.e., drought, salinity, etc.) (Gabr *et al.*, 2020). Currently, research on the principal effects of climate change on plants (salinity and drought) is considered to be a key foundation for

understanding how plants respond by creating secondary metabolites (Gabr *et al.*, 2020). Secondary metabolites are generated as a result of defence reactions that elicitors can activate and elevate (Radman *et al.*, 2003). Since then, plants have been regarded as a major source of the biosynthesis of bioactive compounds, which are used as pharmaceuticals. About 25% of all human-made medications are thought to be derived from medicinal plants (De Luca *et al.*, 2012-Wurtzel and Kutchan, 2016). The growth phase and physiology of the plant, as well as the environmental growth conditions, affect the production of bioactive compounds by different plant species. A useful tool for simulating environmental stress on plants is *in vitro* elicitation (Gabr *et al.*, 2020). One of the most practical and successful methods for increasing the synthesis of secondary metabolites in plants is the elicitation process. Elicitation in biotechnology is frequently described as enhancing and activating the formation of metabolites through the addition of low amounts of elicitors (Isah *et al.*, 2018). Elicitors may be used to increase the synthesis of secondary metabolites in plants. They may also play a significant role in several biosynthetic pathways to increase the production of commercially important bioactive compounds. The two main groups, abiotic and biotic, are the most practical and widely accepted classifications for elicitors [Namdeo, 2007-Patel and Krishnamurthy, 2013].

The non-toxic polymer polyethylene glycol (PEG), which has varying molecular weights, is thought to reduce water uptake by tissues by lowering the osmotic potential in the culture medium

when added to *in vitro* plant cultures (Lawlor, 1970- Aazami *et al.*, 2010). Its effect resembles, to a great extent, drought stress. The impact of PEG treatment *in vitro* on plant development and biochemical changes has been studied in a variety of species, including *Agave salmiana* (Puente-Garza *et al.*, 2017)] and *Allium hirtifolium* [Ghassemi-Golezani *et al.*, 2018). On the other hand, the ability of plants to adapt to drought circumstances is related to abscisic acid's involvement in several plant growth processes and its stress-related responses. Stomatal closure caused by ABA under drought stress lowers water loss by lowering the transpiration rate. Additionally, ABA promotes root cell elongation and gradually raises hydraulic conductivity, allowing plants to recover from water shortages (Daszkowska-Golec, 2016).

Gardenia is a member of the Rubiaceae plant family, which includes attractive and therapeutic plants. The Gardenia species jasmonide with its subspecies Variegata is utilized in this investigation. Gardenia has demonstrated that it can be used to treat inflammatory diseases and relieve pain. Since it includes numerous beneficial phenolic components, such as cinnamic acid and chlorogenic acid, as well as numerous anti-inflammatory flavonoids, such as rutin (Raj and Singh, 2022). 4-hydroxy-3-methoxycinnamic acid, often known as ferulic acid, was first extracted from the ferule foetida. It is a naturally occurring phenolic phytochemical found in various plants, seldom in its free form but typically in conjugated form with the plant cell wall. It gets its name from the scientific name of the plant and is thought to be the most prevalent

hydroxyl cinnamic acid in the plant world (Mathew and Abraham, 2004). The synthesis of numerous other significant organic molecules, including vanillin, depends on ferulic acid. It serves as a potential precursor for the production of natural vanillin. Ferulic acid, which possesses anti-oxidant, anti-inflammatory, and anti-thrombotic effects, is effective in treating several respiratory disorders (Xie *et al.*, 2021). Recently, the severe acute respiratory illness corona virus 2 (SARS-CoV-2) is thought to be the source of the 2019 corona virus disease (COVID-19), which has turned into a global pandemic disaster and claimed an incalculable number of lives [18]. It has been discovered that ferulic acid, a therapeutic molecule with anti-tumor and antiviral properties, may have pharmaceutical applications. Therefore, the purpose of this investigation was to identify the pharmacological mechanisms of ferulic acid as well as the prospective therapeutic targets of ferulic acid in the treatment of patients with COVID-19 (Merad *et al.*, 2022). It demonstrates a wide range of biological activities, including antiviral, anti-inflammatory, antimicrobial, anti-allergic, hepatoprotective, and anti-carcinogenic (Pang *et al.*, 2022). Ferulic acid has a lipid-lowering effect by lowering triglyceride and cholesterol levels, which lowers the risk of heart disease and acts as an anti-atherogenic (BOZ, 2015). Curcumin and ferulic acid's combined action mechanisms may be effective in preventing and reversing the development of Alzheimer's (Ohashi *et al.*, 2022). It may be used to reduce diabetes and high blood pressure (Wang *et al.*, 2021). It could have anti-ageing,

neuroprotective, and anti-apoptotic properties. It is regarded as one of the most photo-protective ingredients included in sunscreens and skin creams. (Bumrungpert *et al.*, 2018). Because ferulic acid is a 4-hydroxy-3-methoxy cinnamic acid that can be used as a substrate for the manufacture of chlorogenic acid, (Kumar and Pruthi, 2014] and Gardenia is abundant in these phenolic compounds (such as cinnamic acid and chlorogenic acid) (Uddin *et al.*, 2015).

The current study aimed to evaluate the accumulation of ferulic acid and antioxidant capacity in response to ABA and PEG, to mimic the draught stress, in the *in vitro* culture of *Gardenia jasmonides* Variegata.

MATERIALS AND METHODS

Plant material

Gardenia jasmonides Variegata growing in El Zouhria botanical garden, Cairo, Egypt, was used as the starting material. Shoot segments of about 5 cm were cut and put under tap water for one hour. Then sterilized using 70 % ethanol for one min. Then 30 % clorox for five min. And rinsed three times with distilled water. The plantlets were subcultured three times on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 2 mg/l BA for shoots multiplication needed for callus induction for this study (El-Ashry *et al.*, 2022).

Calli cultures

Following El Ashry *et al.*, 2022 callus induction was carried out by cutting the leaves of *in vitro*-growing *Gardenia jasmonide* Variegata plantlets into segments that were approximately

0.5cm and placing them on MS medium supplemented with 0.5 mg/l BA and 0.5 mg/l picloram for one subculture (four weeks) in the dark. For the callus formation required for this study, the inducted callus was subcultured on Murashige and Skoog (MS) medium enriched with 4 mg/l TDZ (control treatment).

Effect of abscisic acid (ABA) on fresh and dry weights of calli

Approximately 0.5 g of friable fresh calli were transferred to MS medium (Murashige and Skoog, 1962) that was supplemented with 4 mg/l TDZ (control treatment) with different concentrations of ABA (0.25, 0.5, and 1 mg/l) as follows:

T1: MS medium + 4 mg/l TDZ (control)

T2: Control + 0.25 mg/l ABA

T3: Control + 0.5 mg/l ABA

T4: Control + 1 mg/l ABA

Samples from three replicates were harvested at the end of the first, second, third, and fourth weeks. Fresh weights were recorded, and the samples were dried using a freeze dryer, and the dry weights were recorded.

Effect of polyethylene glycol (PEG) on fresh and dry weights of calli

Approximately 0.5 g of friable fresh calli were transferred to MS medium supplemented with 4 mg/l TDZ (control treatment) with different concentrations (2.5, 5, 7.5 g/l) of PEG (M.W. either 4000 or 6000) as follows:

T1: MS medium + 4 mg/l TDZ (control)

T5: Control + 2.5 g/l PEG M.W. 4000

T6: Control + 5 g/l PEG M.W. 4000

T7: Control + 7.5 g/l PEG M.W. 4000

T8: Control + 2.5 g/l PEG M.W. 6000

T9: Control + 5 g/l PEG M.W. 6000

T10: Control + 7.5 g/l PEG M.W. 6000

Samples from three replicates were harvested at the end of the first, second, third, and fourth weeks. Fresh weights were recorded, and the samples were dried using a freeze dryer, and the dry weights were recorded.

Sample extraction

Following Gabr *et al.*, 2017, the extraction was carried out in complete darkness. For each treatment, 100 mg of grounded dried samples of calli were extracted in 1.5 ml of 80% methanol for 24 hours. After that, the extracts were sonicated for 20 minutes in an ultrasonic water bath (Grant, United Kingdom). Samples were centrifuged for 5 min. at 6000 rpm (Sigma, 2–16 PK, and Germany). The extracts were collected and the pellets were extracted twice with 500 µl of the same solvent. The extracts were stored at - 20 °C until further use.

Effect of ABA and PEG on ferulic acid content using high performance liquid chromatography (HPLC)

Ferulic acid content was assessed weekly by HPLC on a Unicam Crystal 200 Liquid Chromatograph (Column: Kromasil C18 5µm250*4.66 mm). The mobile phase consisted of methanol and water (both acidified with 0.3% orthophosphoric acid w/v). The flow rate was 1.4 ml/min. Substances were detected by absorption at $\lambda= 320$ nm, and their identifications were carried out by the comparison of retention times and absorption spectra with the ferulic standard. The ferulic standard used was manufactured by Sigma Aldrich. Sample content was expressed as µg/g dry weight and derived using a known concentration of standard and sample peak areas.

Antioxidant analysis

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) analysis

Using the ABTS radical, total antioxidant capacity was calculated. At 734 nm, absorbance was measured every minute for six minutes. After six minutes, the radical scavenging activity was expressed as a percentage of ABTS discoloration. Additionally, the ABTS Radical Scavenging Activity was ascertained through comparison with Trolox's standard calibration curve. Trolox equivalents (µM of TE) per 100 mg dry weight (100 mg DW) were the unit of presentation for the results (Robereta *et al.*, 1999).

Ferric reducing antioxidant power (FRAP) analysis

The method described by (Benzie *et al.*, 1996) was applied with minor adjustments to determine the ability to reduce ferric ions. For the standard curve, fresh Trolox working solutions were employed. The linear calibration curve was used to compute the antioxidant capacity, which was expressed as µM Trolox equivalents per 100 mg dry weight of sample. The antioxidant capacity relied on the sample's ability to reduce ferric ions.

Statistical analysis

All analysis was in triplicate and data reported as Mean \pm Standard Deviation (SD). Data were subjected to analysis of variance (One-way ANOVA) ($p<0.05$). Results were processed by Excel (Microsoft office 2010).

RESULTS AND DISCUSSIONS

Effect of ABA on fresh and dry weights of calli

The effects of three ABA concentrations (0.25, 0.5, and 1 mg/l) on

calli fresh and dry weights during the four weeks of cultivation. The values for fresh and dry weight were varied based on the different treatments, as shown in Figs. 1 and 2. The fresh and dry weights generally follow the same trend since they both gradually increased until the third week, when they started to decline. At the end of the third week, the fresh and dry weights recorded their maximum values. The third week showed the highest fresh weight of fresh calli with the control (without ABA). Increasing ABA concentration typically causes a reduction in fresh weight (Fig. 3). The fresh weight was observed to decrease by using 0.5 mg/l ABA, reaching its lowest value (2.725 g of fresh calli) in the first week. Additionally, the first week of treatment with 0.5 mg/l of ABA resulted in the lowest dry weight (0.075 g of dried calli). While, the highest dry weight (0.265 g of dried calli) was obtained in the third week with the control (without ABA) treatment. During the plant's life cycle, abscisic acid is known to be produced as an endogenous hormone to regulate several physiological processes (Tripathi and Tuteja, 2007). Our findings on ABA were supported by those from others (Nasab *et al.*, 2012], who stated that the increase in ABA reduced the proliferation of calluses in wheat. And with (Rumińska *et al.*, 2013) findings, who reported that applying a high concentration of ABA reduced calli proliferation, the lower ABA concentration improved the increase in calli fresh weight in their investigation on the indirect somatic embryogenesis of cacti.

Effect of PEG on fresh and dry weights of calli

The effects of PEG with two molecular weights (M. W. 4000 and 6000) at different concentrations (2.5, 5, and 7.5 g/l) on calli fresh and dry weights during the four weeks of cultivation are presented in Figs. 4 and 5. The values for fresh and dry weight were varied based on the different treatments. With adding 5 g/l PEG (M.W. 6000), the fresh and dry weights reached their peak values at the end of the fourth week, which were (9.524 g of fresh calli and 0.446 g of dried calli, respectively). While adding 7.5 g/l PEG (M.W. 4000) in the third week resulted in the lowest fresh weight (2.745 g of fresh calli), while adding 2.5 g/l PEG (M.W. 4000) in the first week resulted in the lowest dry weight (0.085 g of dried calli) (Fig. 6). The non-toxic polymer polyethylene glycol (PEG), which has varying molecular weights, is thought to reduce water uptake by tissues by lowering the osmotic potential in the culture medium when added to plant cultures of tomato (Aazami *et al.*, 2010). According to the data, our findings match those of other researchers (Sarmadi *et al.*, 2019), who observed that, as PEG concentration increased, the fresh weight (FW) and dry weight (DW) in the calli decreased by lowering the calli's water content. *Taxus baccata* L. callus culture. The largest fresh callus culture weight was produced with 10% PEG in the absence of ABA, according to Jameel and Abdulaziz, 2012 in their study on the somatic embryo development in date palm (*Phoenix dactylifera* L.), and even 1 M of ABA added to the suspensions stopped growth.

Biochemical analysis

Effect of ABA on ferulic content using HPLC

Data were recorded weekly for four weeks using HPLC to assess the impact of abscisic acid on the amount of ferulic acid (Fig. 7). It was discovered that ferulic acid could be found in varying quantities in samples from calli cultures that had undergone various treatments. According to the findings in Figs. 7 and 8, the amount of ferulic acid was changed greatly depending on the various treatments. In terms of the variation in ferulic acid content, the control treatment showed the maximum ferulic acid content in the third week (2.789 µg/g dry weight). It is obvious that applying ABA at various concentrations generally has a negative effect or even reduces the ferulic acid content during the four weeks of culturing, except when it is used at a low concentration (0.25 mg/l) in the second week, it recorded 2.152 µg/g dry weight. As ABA concentration rises, ferulic acid gradually declines over weeks, since when the calli are cultivated on MS medium supplemented with 1 mg/l ABA for three weeks, the ferulic acid reaches its lowest level (0.05 µg/g dry weight). In accordance with our findings, ferulic acid steadily declines as ABA concentration and time increase. According to several reports, ABA is an endogenous hormone that regulates a variety of physiological functions throughout a plant's life cycle (Rumińska *et al.*, 2013). Our findings can be explained by what was reported by (Wakabayashi *et al.*, 1997) who reported that, while coleoptiles of dark-grown wheat (*Triticum aestivum* L.) grew, so did the levels of wall-bound diferulic

acid (DFA) and ferulic acid (FA); however, the increases were significantly inhibited by ABA. On the other hand, their contents increased and approached control coleoptile levels when ABA was eliminated. The mechanical properties of cell walls and the amounts of DFA and FA were shown to be closely correlated. In the control coleoptiles, the activity of phenylalanine (PAL) and tyrosine-ammonia-iyase (TAL) increased rapidly. The increases in this enzyme activity were significantly inhibited by ABA. According to these results, ABA inhibits the elevation of PAL and TAL activities in wheat coleoptiles, which lowers the amount of wall-bound FA. This, in turn, could decrease the level of DFA and preserve the flexibility of the cell wall. Our findings regarding ABA are consistent with those made by others (Li *et al.*, 1993), who claimed that ABA and phenolic acids, particularly p-coumaric acids and ferulic acid, had significant interactions on lettuce seed germination. Together with ABA, these phenolic substances demonstrated additive inhibitory effects on seed germination.

Antioxidant activity using ABTS assay

The data in Fig. 9 show that, applying 0.25 mg/l ABA recorded the maximum antioxidant activity, particularly during the second week of culturing (2646.5 µM Trolox equivalent per 100 mg sample). As the ABA concentration increased, the antioxidant activity steadily declined. This pattern overlaps with the ferulic acid pattern, indicating a relationship between antioxidant activity and ferulic acid concentration.

Antioxidant activity using FRAP assay

The data in Fig. 10 show the FRAP method's measurements of antioxidant

activity during the four weeks of cultivation. The data in Fig. 10 show that 0.25 mg/l ABA showed the highest antioxidant activity, particularly in the second week of cultivation (869.27 μ M Trolox equivalent/100 mg sample), which is supported by the results of the ABTS method in Fig. 9. It can be said that the antioxidant activity is related to the ferulic acid content since this pattern is often associated with the antioxidant activity using ABTS and the ferulic acid pattern. Since it decreases as ABA concentration rises. It was found that when ABA concentration increased, antioxidant activity steadily decreased. Our findings may conflict somewhat with those of other researchers (Gagne *et al.*, 2011– Sandhu *et al.*, 2011), who showed that grapes can accumulate anthocyanins when exogenous ABA is applied. It has been demonstrated that ABA treatment can raise the anthocyanin content of strawberries, which increases the fruit's anti-oxidant activity (Jiang and Joyce, 2003).

Effect of PEG on ferulic acid content using HPLC

HPLC was performed to assess the impact of polyethylene glycol on the amount of ferulic acid, and data were collected weekly (Fig. 11). Ferulic acid could be detected in samples extracted from calli cultures that had undergone different treatments with varied concentrations. According to Figs. 11 and 12, the amount of ferulic acid changed greatly depending on the various treatments. It was determined by displaying the different treatments of polyethylene glycol that it has a positive effect on the ferulic acid content. It was found that using MS medium

supplemented with 7.5 g/l PEG (M.W. 6000) in the second week recorded the highest ferulic acid content (2.708 μ g / g dry weight). While the MS medium supplemented with 2.5 g/l PEG (M.W.4000) in the second week showed the lowest ferulic acid level (0.048 μ g/g dry weight), however. In general, it can be recognized that ferulic acid increases gradually with further weeks in different polyethylene glycol treatments. Except when 7.5 g/l PEG (M.W. 6000) was added, which resulted in 2.708 μ g/g dry weight of ferulic acid at the end of the second week and a fast decline to 0.513 μ g/g dry weight at the end of the third week (Fig. 11).

By reducing leaf growth, ferulic acid, which is covalently bonded to the cell wall's carbohydrates, supports drought adaptation. Drought may have produced defensive mechanisms, such as the reported increase in cell-wall-bound ferulic acid concentration in response to a water deficit in the leaf. Another valid biochemical indicator of a plant's resistance to drought stress may be its ability to accumulate phenolic compounds in dehydrated leaves (Hura *et al.*, 2009). It has become clear that exposure of plants to water shortage leads to an increase in the intensity of blue fluorescence emitted by plants and results from phenolic compounds, especially ferulic acid (Hura *et al.*, 2006). By comparing the various treatments with polyethylene glycol, it became apparent that applying PEG had a positive impact on the ferulic acid level. Since when PEG is added to plant cultures, the osmotic potential in the culture medium decreases, reducing the amount of water that tissues can absorb

and simulate the effects of drought stress on plants then adding PEG to the culture medium resembles the effect of drought on plants in which increasing the ferulic acid content. (Li *et al.*, 1993- Gagne *et al.*, 2011). Our findings correspond with other researcher's findings (Chakhchar *et al.*, 2016) who reported that, ferulic acid was the most common phenolic component found in wheat under water stress caused by the application of PEG 6000 and seemed to be mostly related to the inhibition of germination.

Antioxidant activity using ABTS assay

To study how PEG affected antioxidant activity using ABTS, samples were taken every week through four weeks of cultivation. By presenting Fig. 13, it can be observed that, the maximum antioxidant activity was observed when 7.5 g/l PEG (M.W. 4000) was used, especially during the fourth week of culturing (2491.6 μ M Trolox equivalent/100 mg sample).

Antioxidant activity using FRAP assay

The data in Fig. 14 show the FRAP assay assessments of antioxidant activity during the four weeks of cultivation. It was found that, by using 7.5 g/l PEG (M.W. 4000), the maximum antioxidant activity was recorded, particularly in the fourth week of culturing (812.61 μ M Trolox equivalent/100 mg sample), as shown by the results in Fig. 14. This finding is supported by the ABTS assay in Fig. 13. Typically, ABTS is used to confirm this pattern of antioxidant activity.

It has become clear that exposure of plants to water shortage leads to an increase in the intensity of blue fluorescence emitted by plants resulting from phenolic compounds, especially ferulic acid and increasing the phenolic

compounds increased the antioxidant activity (Hura *et al.*, 2006). Our findings agree with other authors findings (Hajihashemi *et al.*, 2018), who showed that all PEG treatments enhanced the total antioxidant capacity. Additionally, they discovered that the greatest *Stevia rebaudiana* Bertoni antioxidant activity was obtained on MS medium supplemented with 4% of PEG (M.W. 6000) and this proved the validity of the hypothesis that PEG encourages multiple phenolic compounds to deal with the drought stress generated by PEG. Our findings conflict with those of other researchers (Khan *et al.*, 2019), who indicated that antioxidant activity decreased as polyethylene glycol concentration increased in tomato.

CONCLUSION

In contrast to the application of ABA, which has a negative effect on the fresh, dry weights, and ferulic acid of *Gardenia jasmonide* Variegata calli cultures. The application of PEG, might be determined to have a beneficial influence on these factors. Additionally, PEG treatments have a beneficial impact on ferulic acid concentration and antioxidant activity, since the application of PEG in the culture medium resembles to a great extent, drought stress. Also, drought may have produced defensive mechanisms in plants, such as the reported increase in cell-wall-bound ferulic acid concentration in response to water deficit in the leaf. Then the PEG various treatments have positive impact on ferulic acid content.

Ethics approval and consent to participate

Not applicable

Availability of data and material

All data supporting the conclusions of this article are provided with the article

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Conflicts of interest

The authors declare there are no conflicts of interest.

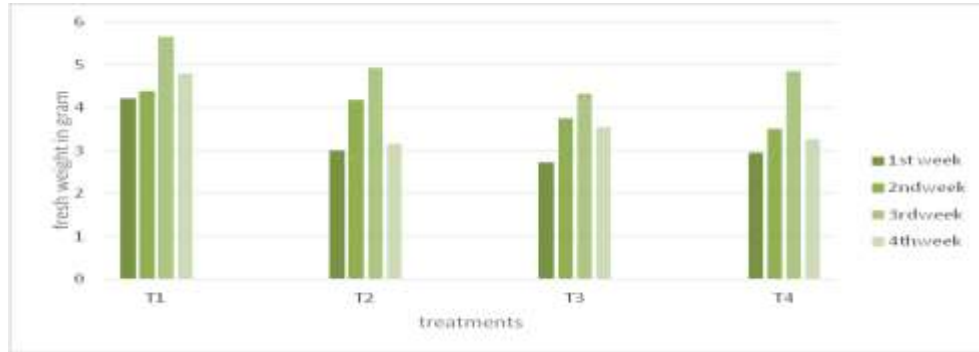


Fig.1 Effect of abscisic acid on fresh weight (g) of Gardenia jasmonide Variegata calli cultures during four weeks of culturing on T1: MS medium + 4 mg/l TDZ (control)

- 128 T2: Control + 0.25 mg/l ABA
- 129 T3: Control + 0.5 mg/l ABA
- 130 T4: Control + 1 mg/l ABA

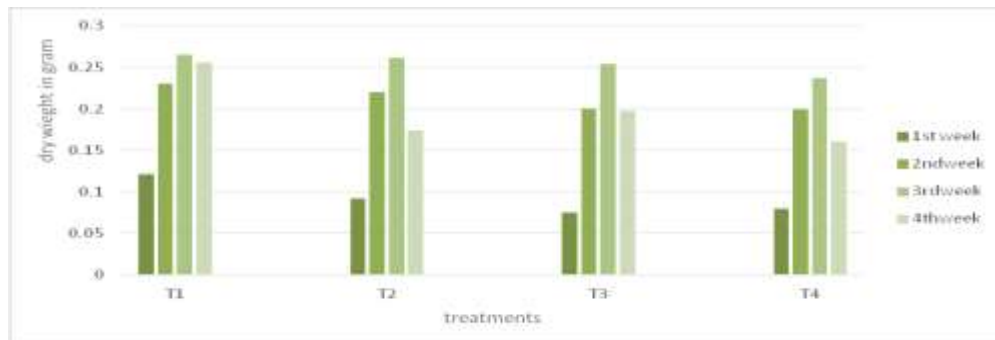


Fig. 2 Effect of abscisic acid on dry weight (g)of Gardenia jasmonide Variegata calli cultures dry during four weeks of culturing on T1: MS medium + 4 mg/l TDZ (control)

- 128 T2: Control + 0.25 mg/l ABA
- 129 T3: Control + 0.5 mg/l ABA
- 130 T4: Control + 1 mg/l ABA

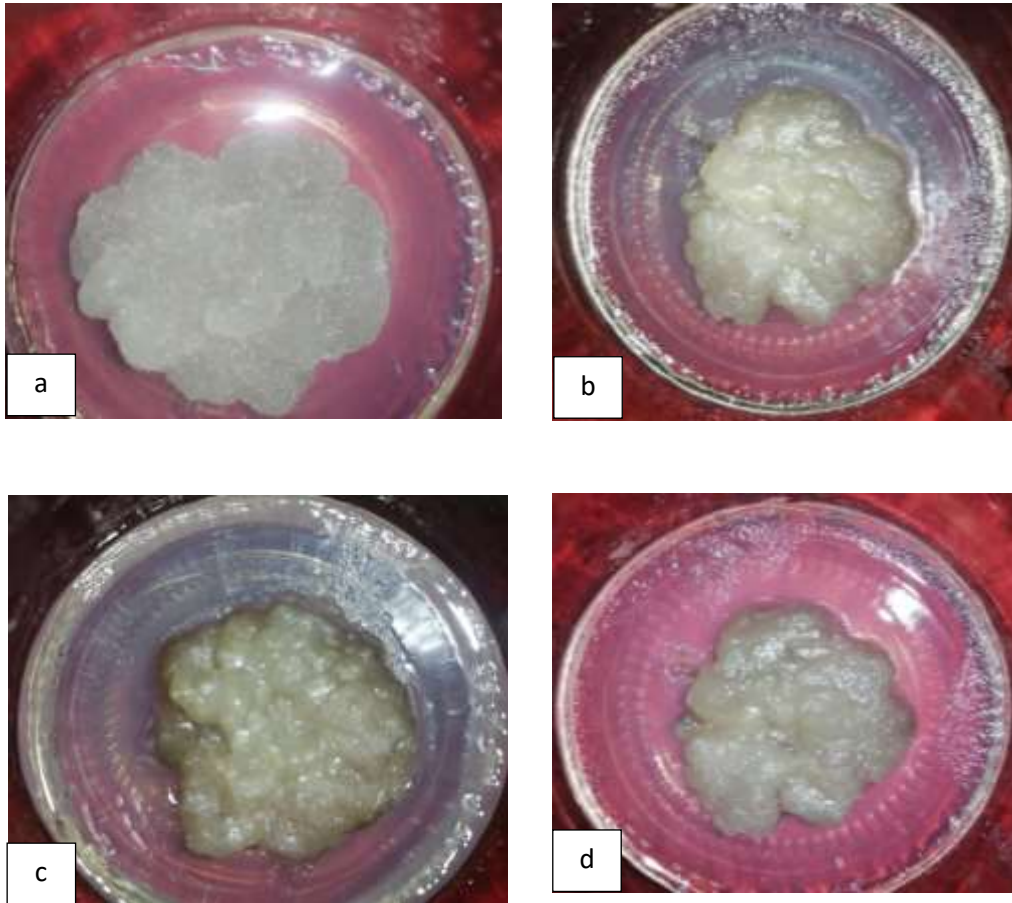


Fig.3 *Gardenia jasmonide* Variegata calli cultures after four weeks of culturing on

- a- T1: MS medium + 4 mg/l TDZ (control)
- b-. T2: Control + 0.25 mg/l ABA
- c- T3: Control + 0.5 mg/l ABA
- d- T4: Control + 1 mg/l ABA

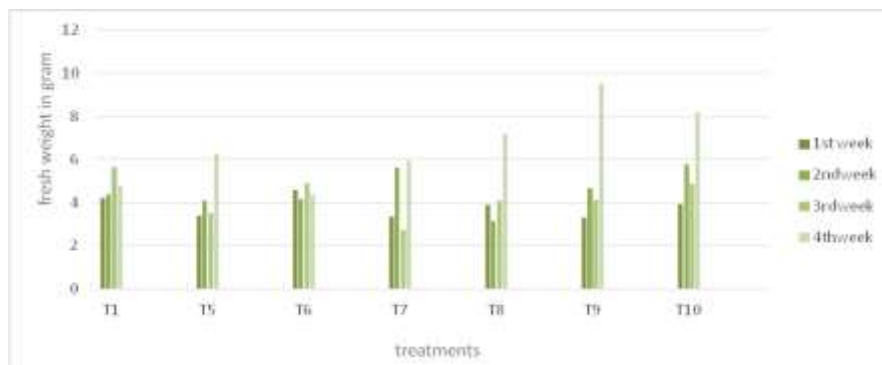


Fig.4 Effect of polyethylene glycol on fresh weight (g) of Gardenia jasmonide Variegata calli cultures during four weeks of culturing on

- T1: MS medium + 4 mg/l TDZ (control)
- T5: Control + 2.5 g/l PEG M.W. 4000
- T6: Control + 5 g/l PEG M.W. 4000
- T7: Control + 7.5 g/l PEG M.W. 4000
- T8: Control + 2.5 g/l PEG M.W. 6000
- T9: Control + 5 g/l PEG M.W. 6000
- T10: Control + 7.5 g/l PEG M.W. 6000

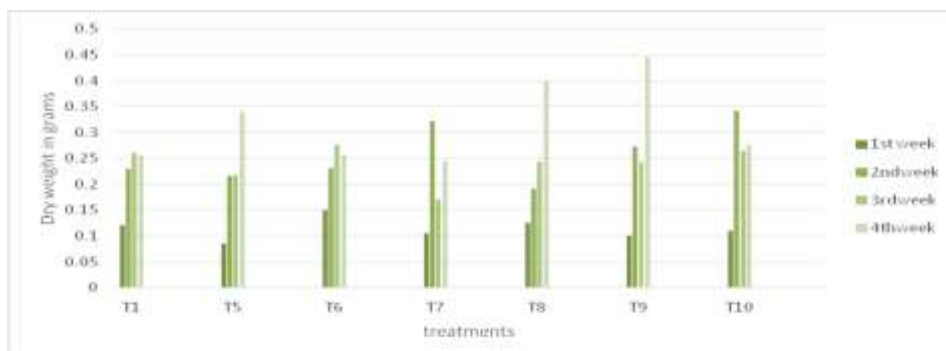


Fig.5 Effect of polyethylene glycol on dry weight(g) of Gardenia jasmonide Variegata calli cultures during four weeks of culturing on

- T1: MS medium + 4 mg/l TDZ (control)
- T5: Control + 2.5 g/l PEG M.W. 4000
- T6: Control + 5 g/l PEG M.W. 4000
- T7: Control + 7.5 g/l PEG M.W. 4000
- T8: Control + 2.5 g/l PEG M.W. 6000
- T9: Control + 5 g/l PEG M.W. 6000
- T10: Control + 7.5 g/l PEG M.W. 6000

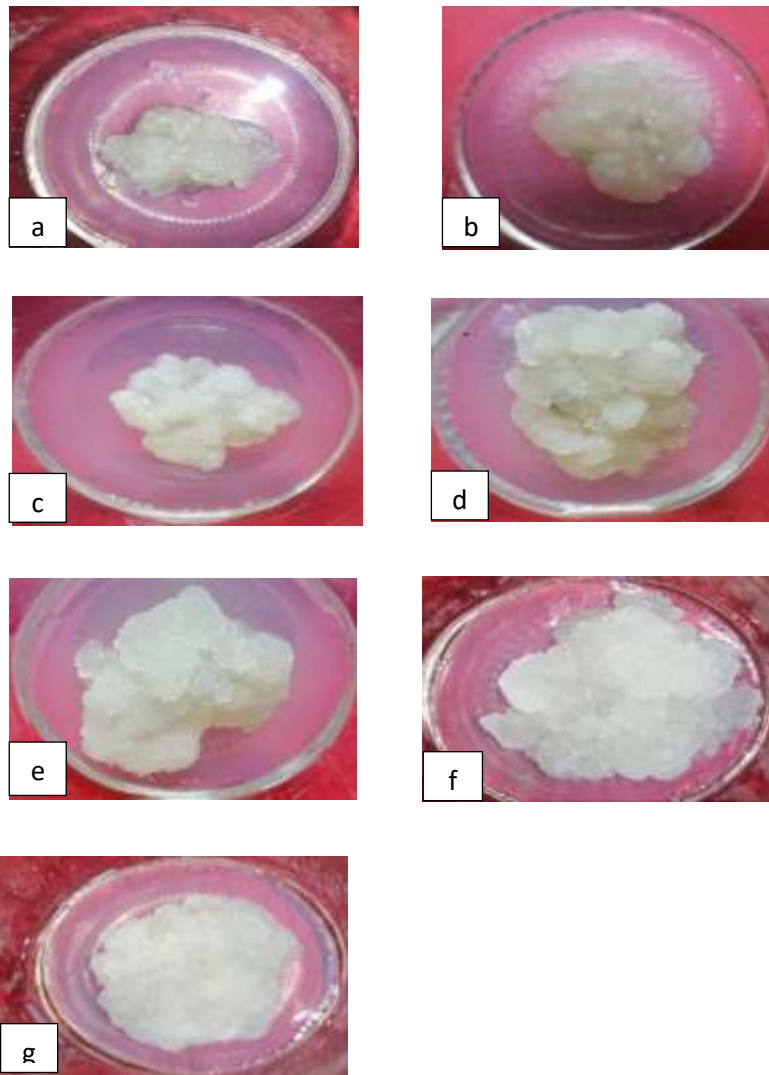


Fig.6 *Gardenia jasmonide* Variegata calli cultures after four weeks of culturing on

- T1: MS medium + 4 mg/l TDZ (control)
- T5: Control + 2.5 g/l PEG M.W. 4000
- T6: Control +5 g/l PEG M.W. 4000
- T7: Control +7.5 g/l PEG M.W. 4000
- T8: Control + 2.5 g/l PEG M.W. 6000
- T9: Control +5 g/l PEG M.W. 6000
- T10: Control + 7.5 g/l PEG M.W. 6000

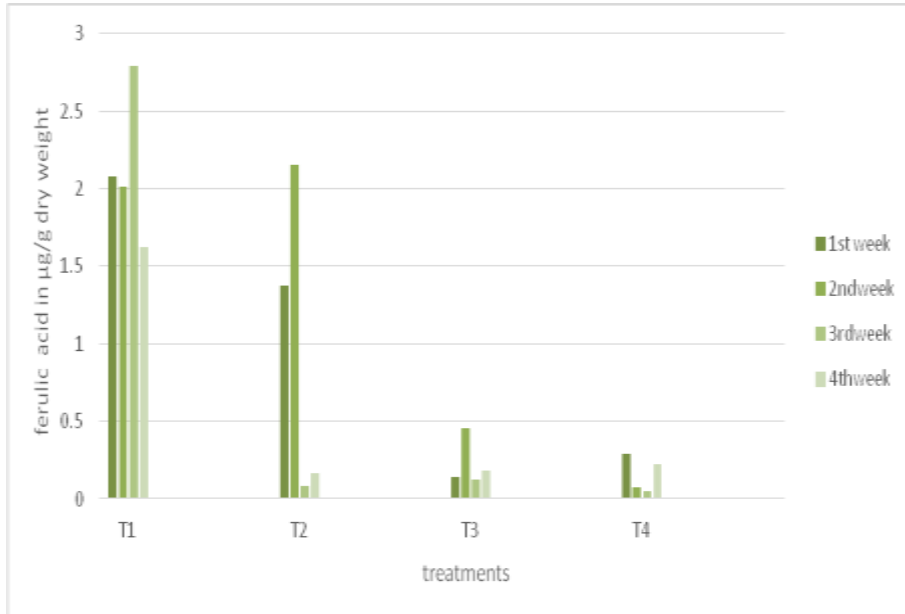


Fig.7 Effect of abscisic acid on ferulic acid content ($\mu\text{g} / \text{g}$ dry weight) of *Gardenia jasmonide* Variegata calli cultures during four weeks of culturing on T1: MS medium + 4 mg/l TDZ (control)

- 128 T2: Control + 0.25 mg/l ABA
- 129 T3: Control + 0.5 mg/l ABA
- 130 T4: Control + 1 mg/l

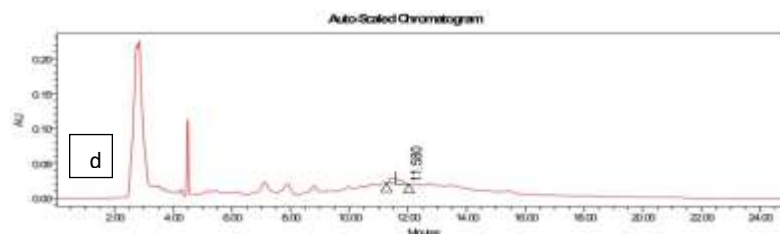
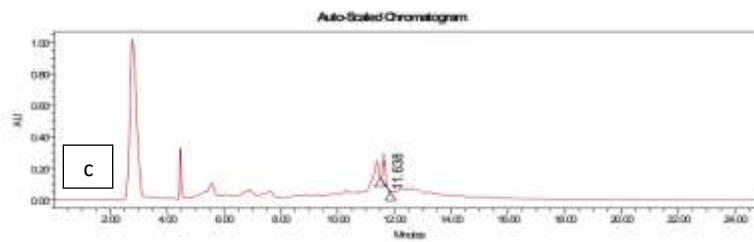
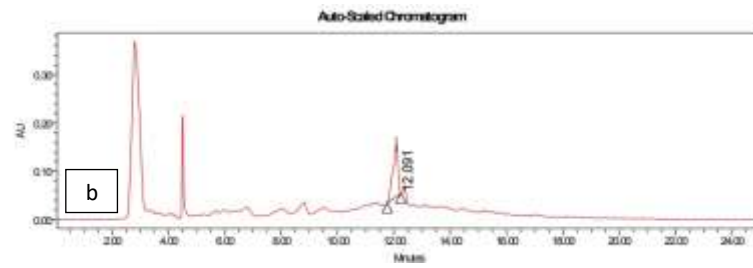
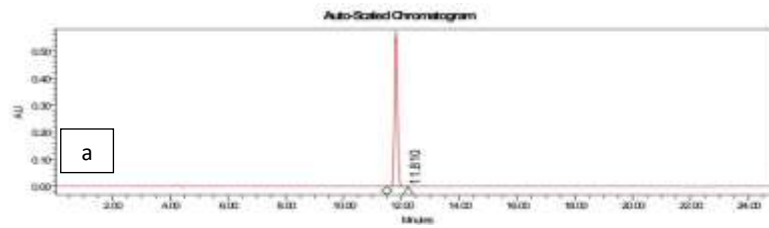


Fig.8 Detection of ferulic acid by HPLC

- a: Standard of ferulic acid
- b: ferulic acid in *Gardenia jasmonide* Variegata calli cultures on the control treatment in the third week (highest ferulic content)
- c: ferulic acid in *Gardenia jasmonide* Variegata calli cultures on the control treatment supplemented with 0.25 mg/l ABA in the second week
- d: ferulic acid in *Gardenia jasmonide* Variegata calli cultures on the control treatment supplemented with 1mg/l ABA in the third week (lowest ferulic content)

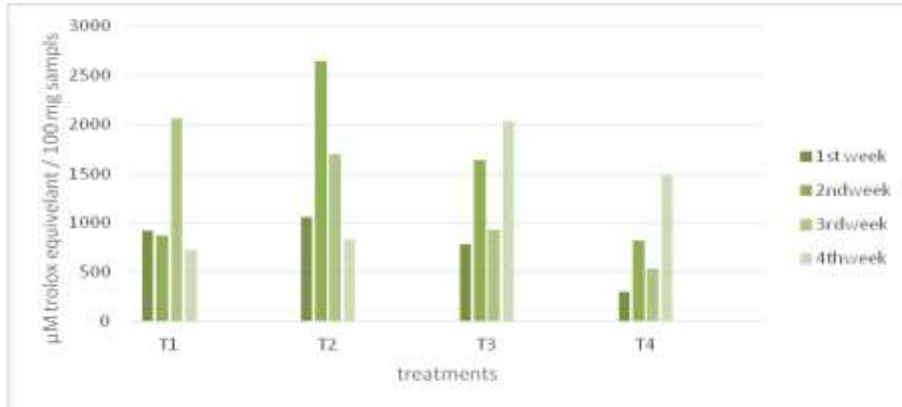


Fig.9 Effect of abscisic acid on antioxidant activity (µM Trolox equivalent \ 100 mg sample) using ABTS assay of *Gardenia jasmonide* Variegata calli cultures during four weeks of culturing on T1: MS medium + 4 mg/l TDZ (control)

- T2: Control + 0.25 mg/l ABA
- T3: Control + 0.5 mg/l ABA
- T4: Control + 1 mg/l

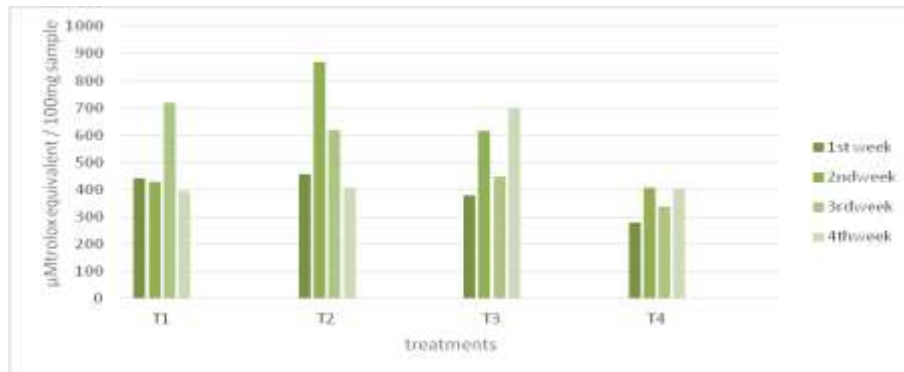


Fig.10 Effect of abscisic acid on antioxidant activity (μM Trolox equivalent \ 100 mg sample) using FRAP assay of *Gardenia jasmonide* Variegata calli cultures during four weeks of culturing on

- T1: MS medium + 4 mg/l TDZ (control)
- T2: Control + 0.25 mg/l ABA
- T3: Control + 0.5 mg/l ABA
- T4: Control + 1 mg/l

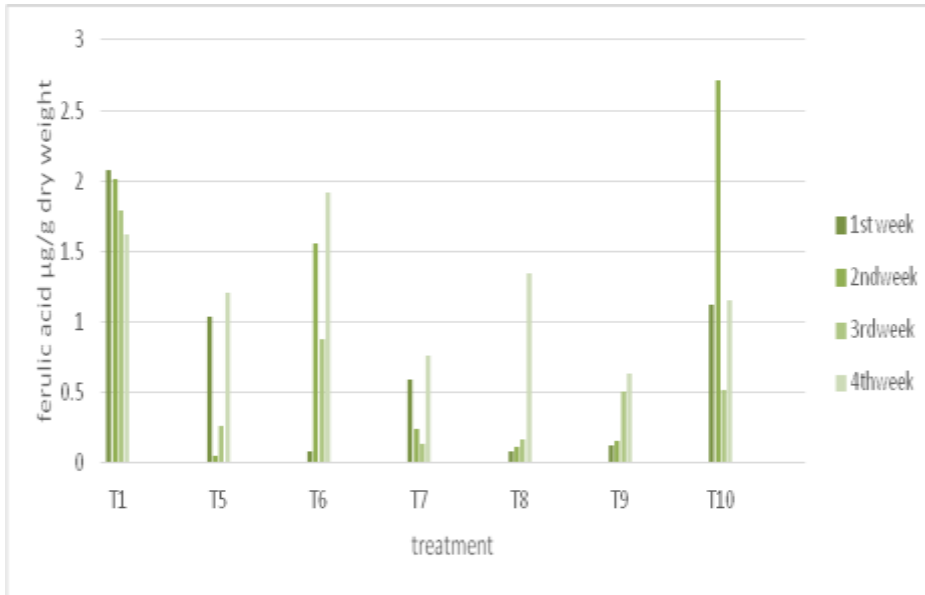


Fig.11 Effect of polyetheylene glycol on ferulic acid content (μg / g dry weight) of *Gardenia jasmonide* Variegata calli cultures in grams during four weeks of culturing on

- T1: MS medium + 4 mg/l TDZ (control)
- T5: Control + 2.5 g/l PEG M.W. 4000
- T6: Control +5 g/l PEG M.W. 4000
- T7: Control +7.5 g/l PEG M.W. 4000
- T8: Control + 2.5 g/l PEG M.W. 6000
- T9: Control +5 g/l PEG M.W. 6000
- T10: Control + 7.5 g/l PEG M.W. 6000

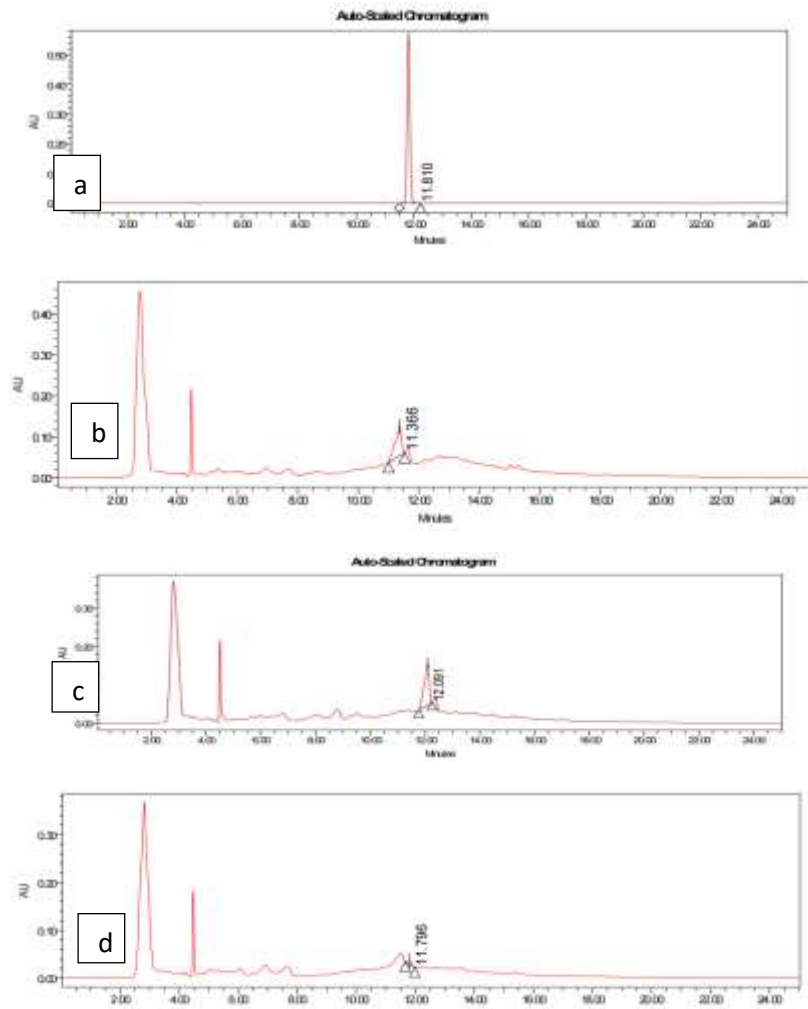


Fig.12 Detection of ferulic acid by HPLC

- a: Standard of ferulic acid
- b: Ferulic acid in *Gardenia jasmonide* Variegata calli cultures on the control treatment in the first week
- c: Ferulic acid in *Gardenia jasmonide* Variegata calli cultures on the control treatment supplemented with 7.5 g/l PEG in the second week (highest ferulic content)
- d: Ferulic acid in *Gardenia jasmonide* Variegata calli cultures on the control treatment supplemented with 2.5 g/l PEG in the second week (lowest ferulic content)

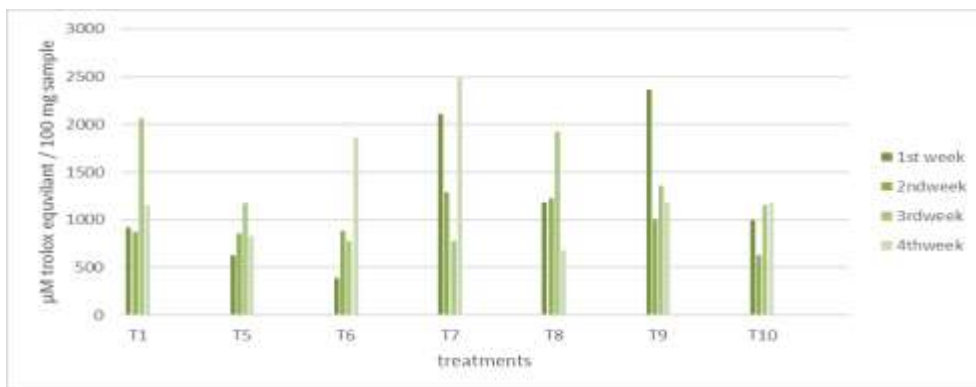


Fig.13: Effect of poly ethylene glycol on antioxidant activity(µM Trolox equivalent \ 100 mg sample) using ABTS assay of *Gardenia jasmonide Variegata* calli cultures during four weeks of culturing on

- T1: MS medium + 4 mg/l TDZ (control)
- T5: Control + 2.5 g/l PEG M.W. 4000
- T6: Control + 5 g/l PEG M.W. 4000
- T7: Control + 7.5 g/l PEG M.W. 4000
- T8: Control + 2.5 g/l PEG M.W. 6000
- T9: Control + 5 g/l PEG M.W. 6000
- T10: Control + 7.5 g/l PEG M.W. 6000

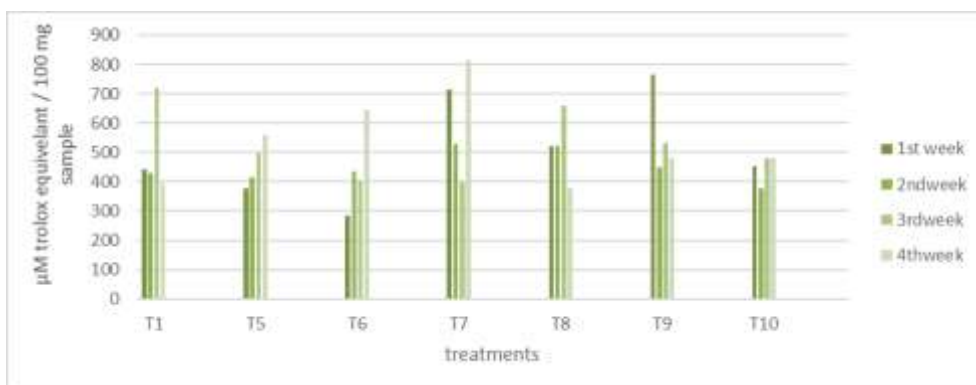


Fig.14 Effect of poly ethylene glycol on antioxidant activity(µM Trolox equivalent \ 100 mg sample) using FRAP assay of *Gardenia jasmonide Variegata* calli cultures during four weeks of culturing on

- T1: MS medium + 4 mg/l TDZ (control)
- T5: Control + 2.5 g/l PEG M.W. 4000
- T6: Control + 5 g/l PEG M.W. 4000
- T7: Control + 7.5 g/l PEG M.W. 4000
- T8: Control + 2.5 g/l PEG M.W. 6000
- T9: Control + 5 g/l PEG M.W. 6000
- T10: Control + 7.5 g/l PEG M.W. 6000

REFERENCES.

- Aazami, M.A., Torabi, M., Jalili, E. (2010).** In vitro response of promising tomato genotypes for tolerance to osmotic stress. *Afr. J. Biotechnol.*; 9:4014–4017.
- Aazami, M., Torabi, M., Jalili, E. (2010).** In vitro response of promising tomato genotypes for tolerance to osmotic stress. *Afr. J. Biotechnol.*; 9:4014–4017.
- Benzie, I.F. , Strain, J.J. (1996).** The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay *Analytical Biochemistry*; 239 : 70-76.
- BOZ, H. (2015).** Ferulic Acid in Cereals – a Review *Czech J. Food Sci.*; 33 (1): 1–7.
- Bumrungpert, A., Lilitchan, S., Tuntipopipat, S., Tirawanchai, N., Komindr, S. (2018)** Ferulic acid supplementation improves lipid profiles, oxidative stress, and inflammatory status in hyperlipidemic Subjects: A randomized, double-blind, placebo-controlled clinical trial. *Nutrients*.; 10(6):713
- Chakhchar , A. , Aissam, S., El Modafar, C. (2016).** Quantitative and qualitative study of phenolic compounds involved in germination inhibition of wheat under water deficit. *Technology and Investment.* ; 7: 86-95.
- Daszkowska-Golec, A. (2016).** Drought Stress Tolerance in Plants. Volume 2. Springer; Berlin/Heidelberg, Germany. The role of abscisic acid in drought stress: How ABA helps plants to cope with drought stress; pp. 123–151.
- De Luca, V., Salim, V., Atsumi, S.M., Yu, F. (2012).** Mining the Biodiversity of Plants: A Revolution in the Making. *Science*.;336: 1658-1661.
- El-Ashry, A. A. , Ebrahim, H. S. , Rabie, S. A. A. , El-Bahr, M.K., Gabr, A.M. M. (2022).** Influence of light quality on growth and secondary metabolite in tissue cultures of *Gardenia jasmonides*, *Variegata Egypt. J. Chem.* ; 65(2):695 - 702 .
- Gabr ,A. M., Arafa, N.M., El-Ashry, A.A., El-Bahr, M.K. (2017).** Impact of zeatin and thidiazuron on phenols and flavonoids accumulation in callus cultures of gardenia (*Gardenia jasminoides*). *Pakistan Journal of Biological Sciences*.; 20(7): 328-335
- Gabr, A. M.M. , Ebrahim, H. S., El-Ashry, A. A., El-Bahr , M. K. (2020)** Importance of Artificial Environment Conditions on Plant Biotechnology, Plant Growth, and Secondary Metabolites In:Abd El-Kader S M, El-Basioni B M M. (Eds.) *Precision Agriculture Technologies for Food Security and Sustainability.* IGI Global, 292 - 319.
- Gagne, S , Cluzet, S. , Merillon, J.M., Geny. L. (2011).** ABA initiates anthocyanin production in grape

- cell cultures. - J. Plant Growth Regul. ; 30: 1-10,.
- Ghassemi-Golezani, K., Farhadi, N., Nikpour-Rashidabad, N. (2018).** Responses of in vitro-cultured *Allium hirtifolium* to exogenous sodium nitroprusside under PEG-imposed drought stress. *Plant Cell Tissue Organ Cult.*; 133:237–248
- Hajihashemi, S., Rajabpoor, S., Djalovic, I. (2018).** Antioxidant potential in *Stevia rebaudiana* callus in response to polyethylene glycol, paclobutrazol and gibberellin treatments *Physiol. Mol. Biol. Plants.* ; 24(2) :334–335.
- Hura, T. , Hura, K. , Grzesiak, S.(2009).**Possible contribution of cell-wall-bound ferulic acid in drought resistance and recovery in triticale seedlings. *Journal of plant physiology.*:1720-1733
- Hura, T., Grzesiak, S., Hura, K., Grzesiak, M.T., Rzepka, A. (2006).** Differences in the physiological state between triticale and maize plants during drought stress and followed rehydration expressed by the leaf gas exchange and spectrofluorimetric methods. *Acta Physiologiae Plantarum.*,. 28 (pg. 433-443)
- Isah, T., Umar, S., Mujib, A. , Sharma , M.P. , Rajasekharan, P.E., Zafar , N., Fruk, A. (2018).**Secondary metabolism of pharmaceuticals in the plant in vitro cultures: Strategies, approaches, and limitations to achieving higher yield. *Plant Cell Tissue Organ Cult.*; 132: 239–265. [CrossRef]
- Jameel,,M. A., Abdulaziz, M. A. (2012).** Effect of Abscisic Acid and Polyethylene Glycol on the Synchronization of Somatic Embryo Development in Date Palm (*Phoenix dactylifera* L.) *Biotechnology*;11:318-325.
- Jiang, Y.M. , Joyce, D. C. (2003).** ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit .*J. Plant Growth Regul.*; 39: 171-174.
- Julkunen-Tiitto, R., Nenadis, N., Neugart, S., Robson, M., Agati, G., Vepsäläinen, J., Zipoli, G., Nybakken, L., Winkler, B., Jansen, M.A.K. (2014).** Assessing the response of plant flavonoids to UV radiation: An overview of appropriate techniques. *Phytochem. Rev.* 14: 273–297.
- Khan, R. T. ,Gerdezi, S. D. A., Habibi, T., Abbas, S. R. (2019).** DPPH (2, 2-Diphenyl -1-Picrylhydrazyl) free radical scavenging activity of tomato genotypes against PEG simulated drought stress.*F Pak. J. Bot.* ; 51(4) :1249-1253,
- Kumar ,N., Pruthi, V. (2014).**Potential applications of ferulic acid from natural sources. *Biotechnol. Rep. (Amst).*; 16(4):86-93.
- Lawlor, D.W. (1970)** Absorption of polyethylene glycol by plants and their effects on plant growth. *New Phytol.*; 69: 501–513
- Li, H. H. , Inoue, M. , Nishimura, H., Hasegawa, K., Mizutani ,J., Tsuzuki, E.(1993).** Interactions of trans-cinnamic acid, Its related

- phenolic allelochemicals, and abscisic acid in seedling growth and seed germination of lettuce. *Journal of Chemical Ecology*; 19: 1775-1787.
- Mathew, S., Abraham, T.E. (2004).** Ferulic Acid: An Antioxidant Found Naturally in Plant Cell Walls and Feruloyl Esterases Involved in Its Release and Their Applications. *Critical Reviews in Biotechnology*;24:59-83.
- Merad, M., Blish, C.A., Sallusto, F., Iwasaki, A. (2022).** The immunology and immunopathology of COVID-19. *Science*; 375(6585):1122–7.
- Murashige, T., Skoog, F. (1962).** A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Plant Physiology*; 15: 473-497.
- <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Namdeo, A.G. (2007).** Plant cell elicitation for production of secondary metabolites: A review. *Pharmacogn. Rev.* ;1: 69–79.
- Nasab, B. F., Omid, M., Amirtokaldan, M. (2012).** Callus induction and plant regeneration of wheat mature embryos under ABA treatment *Inter. J. of Agric. And Crop Sci.*; 4 (1): 17 -23.
- Ohashi, H., Tsuji, M., Oguchi, T., Momma, Y., Nohara, T., Ito, N., Yamamoto, K., Nagata, M., Kimura, A. M., Kiuchi, Y. (2022).** Combined treatment with curcumin and ferulic acid suppressed the A β -induced neurotoxicity more than curcumin and ferulic Acid Alone. *Int. J. Mol. Sci.*; 23: 9685.
- Pang, G., Yi, T., Luo, H., Jiang, L. (2022).** Preclinical findings: The pharmacological targets and molecular mechanisms of ferulic acid treatment for COVID-19 and osteosarcoma via targeting autophagy. *Front. Endocrinol.*;13:971687.
- Patel, H., Krishnamurthy, R. (2013).** Elicitors in plant tissue culture. *J. Pharmacogn. Phytochem.*; 2: 60–65.
- Puente-Garza, C.A., Meza-Miranda, C., Ochoa-Martínez, D., García-Lara, S. (2017).** Effect of in vitro drought stress on phenolic acids, flavonols, saponins, and antioxidant activity in *Agave salmiana*. *Plant Physiol. Biochem.*;115:400-407.
- Radman, R., Sacz, T., Bucke, C. and Keshvartz, T. (2003).** Elicitation of plants and microbial cell systems *Biotechnol. Appl. Biochem.*, 37:91-100.
- Raj, N., Singh, D. A (2022)** critical appraisal on ferulic acid: Biological profile, biopharmaceutical challenges and nano formulations *Health Sciences Review* 5; 100063
- Robereta, R., Nicoletta, P., Anna, P., Ananth, P., Min, Y., Catherine, R. (1999).** Antioxidant activity applying an improved ABTS radical cation

- decolouration assay. *J. Free Radic. Biol. Med.*; 26:1231 - 1237.
- Rumińska, J., Lema Goncerzewicz, K., Gabriel, M. (2013).** Influence of Abscisic Acid and Sucrose on Somatic Embryogenesis in *Cactus Copiapoa tenuissima* Ritt. forma *mostruosa* *The Scientific World Journal*; ArticleID 513985, 7 pages.
- Sandhu, A.K., Gray, D.J., Lu, J.A., Gu, L.W. (2011).** Effects of exogenous abscisic acid on antioxidant capacities, anthocyanins, and flavonol contents of muscadine grape (*Vitis rotundifolia*) skins *Food Chem.*; 126: 982-988
- Sarmadi, M., Karimi, N., Palazon, J., Ghassempour, A., Mirjalili, M. H. (2019).** Improved effects of polyethylene glycol on the growth, antioxidative enzymes activity and taxanes production in a *Taxus baccata* L. callus culture. *Plant Cell Tissue and Organ Culture.* ;137 (2)
- Tripathi, S.K., Tuteja, N. (2007).** Integrated signaling in flower senescence: An overview. *Plant Signal. Behav.*; 2:437-445
- Uddin, R., Saha, M.R., Subhan, N., et al. (2014)** HPLC-analysis of polyphenolic compounds in *Gardenia jasminoides* and determination of antioxidant activity by using free radical scavenging assays. *Advanced Pharmaceutical Bulletin.*;4(3):273-281.
- Wakabayashi, K., Hoson, T., Kamisaka, S. (1997).** Abscisic acid suppresses the increases in cell wall-bound ferulic and D I ferulic acid levels in Dark-Grown Wheat (*Triticum aestivum* L.)coleoptiles *Plant Cell Physiol.*; 38(7): 811-817
- Wang, E. J., Wu, M. Y., Lu, J. H. (2021).** Ferulic acid in animal models of Alzheimer's disease: a systematic review of Preclinical Studies. *Cells* ;10: 2653.
- Wurtzel, E.T., Kutchan, T.M. (2016)** Plant metabolism, the diverse chemistry set of the future. *Science* ; 353(6305):1232-1236.
- Xie, X., Yu, T., Hou, Y., Han, A., Ding, Y., Nie, H., Cui, Y. (2021).** Ferulic acid ameliorates lipo polysaccharide-induced tracheal injury via cGMP/PKGII signaling pathway. *Respiratory Research*; 22:308
- Yeshi, K., Crayn, D., Ritmejerýté, E., Wangchuk, P. (2022).** Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules*; 27 (1):313 - 344.

تراكم حمض الفيروليك تحت ظروف اجهاد الجفاف في مزارع الكالس الخاصة بالجاردينيا المبرقشة

Gardenia jasmonide Variegata

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تعتبر الجاردينيا من نباتات الزينة و النباتات الطبية و اللتي تحتوي علي العديد من المركبات الفينولية و ايضا الفلافونيدات المضادة للالتهابات. هيدروكسيل حمض السيناميك الاكثر انتشارا في النباتات هو حمض الفيروليك ، وهو 4 هيدروكسي 3 ميزوكسي حمض السيناميك و له نطاق واسع من النشاط البيولوجي و يمكن استخدامه كركيزة لتخليق حمض الكلوروجينيك.

تهدف الدراسة الي تقييم تراكم حمض الفيروليك في مزارع الانسجة الخاصة بالجاردينيا المبرقشة كاستجابة لتطبيق حمض الابسيسك و البولي ايثيلين جليكول و هما يحاكيان اجهاد الجفاف . تم تطبيق تركيزات مختلفة من حمض الابسيسك او البولي ايثيلين جليكول ذو الوزن الجزيئي المختلف (4000، 6000) كل علي حدا في مزارع الأنسجة الخاصة بالجاردينيا المبرقشة ومعرفة تأثيرهم علي النمو و محتوى حمض الفيروليك و ايضا نشاط مضادات الأكسدة. اظهرت النتائج ان اعلي معدل للنمو (الوزن الطازج و الجاف) تم الحصول عليها مع الاسبوع الرابع من الزراعة علي 5 جم / لتر من البولي ايثيلين جليكول (وزن جزيئي6000) (9.524 و 0.446 جم على التوالي). اما بالنسبة لتراكم حمض الفيروليك فانه عند استخدام 7.5 جم / لتر من البولي ايثيلين جليكول (وزن جزيئي 6000) اعطي 2.708 مكجم / جم وزن جاف من حمض الفيروليك في الاسبوع الثاني و هو يعد اعلي معدل لتراكم حمض الفيروليك. اعلي نشاط لمضادات الاكسدة تم تسجيله مع استخدام 7.5 جم / لتر من البولي ايثيلين جليكول في الاسبوع الرابع و هو ما يعادل 2491.6 – 812.6 ميكرومولر مكافئ ترولوكس / 100 مجم من علي التوالي. واخيرا فانه يمكن الاستنتاج ان استخدام المعاملات (FRAP) و (ABTS) العينة و ذلك باستخدام طريقة المختلفة من البولي ايثيلين جليكول لها تأثير ايجابي علي نمو وتراكم حمض الفيروليك في مزارع الأنسجة الخاصة بالجاردينيا المبرقشة و بالتالي زيادة نشاط مضادات الاكسدة.