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Effects of methyl jasmonate, salicylic acid and phenylalanine on aloe emodin and aloin in diploid and tetraploid *Aloe barbadensis*

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Summary: *Aloe vera* is one of the most famous medicinal plants. Aloin and aloe emodin are the most important active compounds in this plant. The purpose of this research was the comparison of aloin and aloe emodin production after the elicitation by methyl jasmonate, salicylic acid, and phenylalanine in diploid and tetraploid *Aloe vera* plants in greenhouse conditions. The plants were treated with the concentrations of 25, 50, and 100 µM. The amounts of aloin and aloe emodin were determined 24 and 48 hours after application of the treatment. HPLC analysis showed that the leaves of the control diploid plants (without applying elicitors) had more aloin (1.20 fold) and aloe emodin (1.14 fold) than the control tetraploid plants. The maximum concentration of aloin (1.15 ± 0.07 µg mg^{-1} dry weight) was obtained after the elicitation by 25 µM methyl jasmonate, 24 hours after treatment, in diploid plants) 6.36 fold compared to the control (0.18 µg mg^{-1} dry weight). In addition, the maximum concentration of aloe emodin (0.28 µg mg^{-1} dry weight) was obtained after the elicitation by 25 µM salicylic acid, 24 hours after treatment, in diploid plants) 6.18 fold compared to the control (0.04 µg mg^{-1} dry weight)). The long-term effect of three studied elicitors (after 240 days) on plant health and survival was also studied. This investigation showed that only methyl jasmonate at a concentration of 100 µM was resulted in the death of *Aloe vera* plants.

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Key words: elicitation, polyploidy, secondary metabolites, HPLC, flow cytometry

Introduction

Aloe vera (2x=2n=14), is one of the richest medicinal plants (Mahor et al., 2016). About 500 species of *Aloe vera* have been identified (Cousins et al., 2012). There are more than one hundred reports on the properties of active compounds found in *Aloe vera* (Pal et al., 2013; Sánchez et al., 2020). Aloin and aloe emodin are the most important active compounds in this plant (Shi et al., 2021). The presence of aloin and aloe emodin has been proved in *Aloe vera* plant latex (Elsohly et al., 2007; Wang et al., 2012; Karnama et al., 2015). These metabolites have important properties including strong antioxidant, antiviral, anti-leukemia, the inhibitory effect on breast cancer and reducing blood vessel obstruction (Ido izhakj et al., 2002; Tabolacci et al., 2010; Chen et al., 2014; Birari et al., 2020; Dong et al., 2020; Sharanya et al., 2020; Wang et al., 2020; Kaparakou et al., 2021; Xu et al., 2021). In order to increase the amount of important plant metabolites, different methods are used such as elicitation and polyploidy induction (Thakur et al., 2019; Makowski et al., 2020). Most *Aloe vera* plants are diploid (Cavallini et al., 2012); As a result, the study of secondary metabolites was conducted only in diploid plants (Gantait et al., 2014) and there is no record in tetraploid plants (Pan et al., 2007; Zhang et al., 2007; Bano & Sharma 2020; Yesmin et al., 2021). Ploidy level has an effect on quantity of secondary metabolites. Many studies have reported the positive effect of increasing ploidy level on the amount of secondary metabolites (Lavania et al., 2012; Bagheri et al., 2015; Salma et al., 2017; Hamrashid et al., 2021; Kumar, 2021). There are also reports of negative effects in this regard (Hayat et al., 2010; Abdoli et al., 2013). Therefore, the comparison of secondary metabolites in diploid and tetraploid *Aloe vera* plants and the amount of response to elicitation in them can answer many questions in this regard.

Elicitation is a common method for improving the production of plant secondary metabolites and several factors such as the selection of the appropriate elicitor, effective concentrations of elicitor and the duration of its application on the plant must be carefully determined. Various elicitors were applied in the *Aloe vera* plant (Ardebili et al., 2012; Martinez-Romeo et al., 2013; Raei et al., 2014; Kavianifar et al., 2018; Anjum et al., 2019; Hatami et al., 2019). Methyl jasmonate and salicylic acid are two of the most commonly used elicitors in plant secondary metabolites researches. Methyl jasmonate, as a plant hormone can increases the amount of secondary metabolites in plants (Gadzovska et al., 2007; Hao et al., 2015). It has also increased the amount of aloin and aloe emodin in *Aloe vera* in *in vitro* conditions (Choudhri et al., 2018; Yin et al., 2020). Salicylic acid is another important elicitor (Ejtahed et al., 2015; Lanka, 2018).

Salicylic acid (SA) is one of the most important plant phenolics that affects seed germination, stomatal movements, pigment accumulation, photosynthesis, ethylene biosynthesis, enzyme activities, abscission reversal, nutrient uptake, flower induction, membrane functions, legume nodulation, metabolic activities, overall development of the plants and postharvest disease reductions (e.g. Ezzat et al., 2017ab, 2020, 2021). Due to its hormone-like activity, SA has also been employed to different plant species, both *in vivo* and *in vitro*, to explore its role in the secondary

metabolite synthesis and accumulation. Salicylic acid can proficiently recover the biosynthesis of secondary metabolites in plants (Ali et al., 2020). The effect of salicylic acid on the growth of *Aloe vera* plants (Abdolahi et al., 2011) and the increase of primary and secondary metabolites in it has been proven (Lee et al., 2013).

The use of precursors is another way to improve the production of plant secondary metabolites. Phenylalanine is an aromatic amino acid and a precursor to the enzyme phenylalanine ammonia-lyase (PAL). This enzyme is present at the beginning of the biosynthesis pathway of many secondary metabolites in plants (Sa & Elsayed, 2021). The role of phenylalanine has been proven as an essential amino acid and an important precursor in the biosynthesis pathway of many secondary plant compounds.

The most effective concentrations used for the elicitors mentioned above are 25, 50 and 100 µmol (Raghavendra et al., 2012; Cai et al., 2013; Barrientos et al., 2014; Lee et al., 2015; Li et al., 2015; Qaderi et al., 2016; Hassini et al., 2017; Wang et al., 2017; Andi et al., 2019; Dantas et al., 2020; Li et al., 2021; Mehravaran et al., 2021). Also, according to the latest research, the highest amount of secondary metabolites was observed 24 and 48 hours after the application of elicitor (Alavi Mehryan et al., 2020; Behzadirad et al., 2020; Hoseinpanahi et al., 2020; Martin et al., 2020; Madani et al., 2021; Nisha et al., 2021; Pesaraklu et al., 2021; Sangpueak et al., 2021).

It should be noted that the use of elicitors that have less toxic effects on the plant is an advantage. In this study, to find out this feature in selected elicitors, the viability of plants treated with elicitor was investigated after 240 days.

On the other hand, elicitation has been performed with the aim of increasing secondary metabolites only applied in *in vitro* conditions. While the application of elicitors in greenhouse conditions is easier and more practical and reduces costs. In this research, we tried to study many of the factors related to the increase of metabolites as carefully as possible, so to better understand the nature of *Aloe vera* plants, we also compared tetraploid and diploid *Aloe vera* plants morphologically. Of course, there are reports of comparative morphology of diploid and tetraploid *Aloe vera* plants (Parai & Mukherjee, 2014; Ramirez et al., 2015), but there are some contradictions in them, and this research will help clarify the issue.

Given the importance of *Aloe vera* as a valuable medicinal plant and the properties of the aloin and aloe emodin, and taking into account all of the above, it seems that the effects of methyl jasmonate, salicylic acid and phenylalanine at concentrations of 25, 50 and 100 µM, 24 and 48 hours after treatment and comparison of results in diploid and tetraploid plants, can provide researchers with useful information about the increase of important metabolites. Also, the study of long-term effects of treatment can show the sensitivity of *Aloe vera* plant to the toxicity of each of these elicitors. This is very important in maintaining the health and survival of the plant during the experiment.

Materials and methods

In this study, diploid and tetraploid *Aloe vera* plants were used. Tetraploid plants were previously obtained by colchicine treatment and multiplied via micro propagation through the cultivation of terminal buds in MS medium containing l mg L^{-1} of BAP and 1 mg L^{-1} of IAA (Molsaghi et al., 2014). The ploidy levels of plants were confirmed by flow cytometry analysis (Partech GmbH, Munster, Germany) before starting the experiment. Plants were grown in a plastic greenhouse located at 51° and 43 min north latitude, 35° and 8 min east longitude, and 1215m above sea level. Elicitation and sampling of treated plants was also performed in a greenhouse. Irrigation of *Aloe vera* plants was done by a drip irrigation system.

To compare the morphology of diploid and tetraploid *Aloe vera* plants, mature plants of similar age were selected (5-year-old plants) and measurements were performed on 12 plants including 6 diploids and 6 tetraploid plants.

All treatments were applied to plants of the same age (about one-year-old). Methyl jasmonate, salicylic acid and phenylalanine were in powder form, which after converting the µmol to mg L^{-1}, all of them were dissolved in distilled water and brought to a volume of 1 liter. Tween 20 at a concentration of 10 ml was used for better absorption of elicitors by plants (Hazrati et al., 2012). All elicitors were prepared in the same way, with three concentrations of 25, 50, and 100 µmol, and sprayed on all aerial parts of diploid and tetraploid plants with three replications for each treatment. Then, sampling was performed 24 and 48 hours later. Control plants were sprayed with distilled water.

The plant latex was obtained from the leaves of the middle part of the plant. To achieve latex, the base of the leaves was cut transversely, and then the leaves were placed one hour vertically. Finally, the latex secreted from the base of the leaves was collected ***(Figure 1)***.

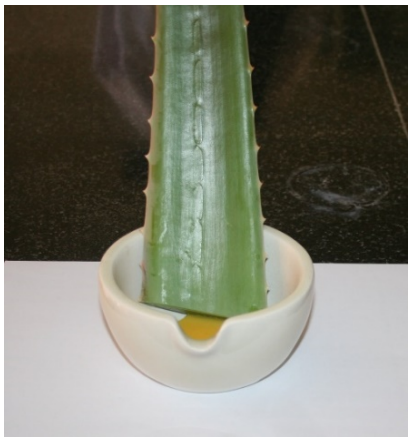

Figure 1. Obtaining latex from the leaves of the *Aloe vera* plant.

To prevent physical stress in the plants, for each concentration of treatments, the elicitor was sprayed on four plants. The reason for this was that when the plants are sampled after 24 hours, the plants will suffer from new stress by cutting the leaves, and after 48 hours, the results are trustworthy for applying the elicitor. Therefore, 48 hours after elicitation, we sampled plants that had not already been sampled.

Extraction of aloin

First, the collected latex was freeze-dried (SCANVAC Model, Cool safe 55 9, Serial no: 0609038) for 48 hours. Then 0.5 g of weighing powder added to 10 ml of ethanol. The resulting suspensions were placed in an ultrasonic water bath (LC 140h, Elma, Germany) at 50 °C for one hour. Then the suspensions were centrifuged at 3000 rpm for 10 min. The supernatants were carefully filtered through 0.45 µm pore size nylon membrane filters (Park et al., 1998).

Extraction of aloe emodin

Extracted plant latex was freeze-dried for 24 hours. Then 10 mg of dried powder was accurately weighed and transferred into a polypropylene vial; 500 µL of methanol was added, and the sample was vortex-mixed for 5 minutes and also centrifuged (4000 rpm) for 5 min. Finally, the supernatants filtered through 0.45 µm pore size nylon membrane filters (Mandrioli et al., 2011).

HPLC analysis

Aloe emodin and aloin contents of each sample were determined through high performance liquid chromatography (HPLC). Equivalent volume (20 µL) of each replication was manually injected into the HPLC system equipped with a C18 column (250 × 4.6 mm, pore size 5 µm; Nucleodur). The mobile phase was an Isocratic Methanol: Ultra-pure water (80: 20) at flow rate of 1 mL min $^{-1}$ run for 20 min. The UV detector (K-2501) was adjusted at 254 and 365 nm with a band width of 10 nm. In fact, the best wavelengths for detection of aloe emodin and aloin, in our study, were respectively 254 and 365 nm which was already reported by some researchers (Mandrioli et al., 2011; Machado et al., 2016).

Aloin and aloe emodin standard references (CAS Number: 481-72-1) and HPLC grade methanol were purchased from Sigma-Aldrich (Germany). Standard solutions in eight different concentrations (5, 10, 25, 50, 100, 250, 500 and 1000 ppm) were prepared from the stock solution (1mg mL^{-1}) and diluting with mobile phase, and directly injected into the HPLC system. All the prepared sample solutions were centrifuged, and the supernatant was injected into the HPLC.

To understand the peak location of aloin and aloe emodin, on the output diagram of the HPLC detector, the standard diagram was compared with the sample diagram ***(Figure 2)***. Also, with the aim of quantifying the amount of metabolites, software Empower 1 was used to draw the standard curves of aloin and aloe emodin, and to obtain the curve equation. The R2 value was 0/9998 and the standard curve linear formula was Y = 24689X–185381.

Aiming to assess aloin and aloe emodin at the same time, the Machado method was successful (Machado et al. 2016). However, methanol as a solvent, and isocratic program instead of the gradient was used in the current study.

Statistical analysis

Effects of elicitors and precursor (methyl jasmonate, salicylic acid and phenylalanine) were investigated in three experiments, separately. All experiments were conducted as factorial based on a completely randomized design. The factorial arrangement of the treatments in each experiment consisted of three factors containing ploidy level with two levels (diploid and tetraploid), duration of elicitation with three levels (0, 24 and 48 hours) and concentration of elicitors and precursor (methyl jasmonate, salicylic acid and phenylalanine) with four levels (0, 25, 50 and 100 µM). The normality and equal variance hypotheses were met and conventional parametric statistics were applied for the analysis. The data were analyzed using analysis of variance (ANOVA) and mean comparisons were performed by least significant difference (LSD) using SPSS (ver.16).

Results

A comparative study of some morphological characteristics of diploid and tetraploid plants was performed due to better understand the nature of *Aloe vera* plants. The results showed that, in tetraploid plants, plant height and leaf length were shorter, while the number of leaves per plant was higher. Also, leaf width in tetraploid plants was lower than that of diploid plants. Similar results were observed in other plants such as *Hyoscyamus* (Lavania et al., 1991), *Dracocephalum moldavica* (Yavari et al., 2000), *Ocimum basilicum* (Mirzaei et al., 2001), *Pomegranate* (Shao et al., 2003), *Serenoa repens* (Madon et al., 2005), *Echinacea purpurea* L. (Abdoli et al., 2013).

Effects of methyl jasmonate on aloe emodin and aloin in diploid and tetraploid Aloe vera

The maximum concentration of aloin (1.15 µg mg^{-1} dry weight) was obtained after the elicitation by 25 µM methyl jasmonate, 24 hours after treatment, in diploid plants. This amount has increased 6.36 fold compared to the control (0.18 µg mg^{-1}).

The maximum concentration of aloe emodin (0.21 µg mg^{-1}) was obtained after the elicitation by 25 µM methyl jasmonate, 24 hours after treatment which followed by the elicitation by 100 µM methyl jasmonate, 48 hours after treatment in diploid plant. In tetraploids, the maximum concentration of aloe emodin (0.15 µg mg^{-1}) was obtained after the elicitation by 100 µM methyl jasmonate, 48 hours after treatment ***(Figure 3)***.

Effect of salicylic acid on aloe emodin and aloin in diploid and tetraploid Aloe vera

The highest amount of aloe emodin (0.28 µg mg^{-1}) was produced by diploid plants treated with 25 µM of salicylic acid and 24 hours after treatment ***(Figure 4)***. This amount has increased 6.18 fold compared to the control (0.04 µg mg^{-1}). On the other side, the highest amount of aloin produced by tetraploid plants treated with a 100 µM concentration of salicylic acid, 24 hours after treatment (0.52 µg mg^{-1}).

Effect of phenylalanine on aloin and aloe emodin in diploid and tetraploid Aloe vera

The greatest amounts of aloin (0.68 ± 0.06, 0.78 ± 0.03 and 0.69 ± 0.09 µg mg^{-1}) were observed in tetraploid plants using the three concentrations of 25, 50 and 100 µM of phenylalanine, respectively, 24 hours after treatment. The maximum amount of aloe emodin (0.16 ± 0.01 µg mg^{-1}) was also obtained in tetraploid plants and using 50 µM of phenylalanine, 24 hours after treatment ***(Figure 5)***.

Also, the study of the long-term effects (240 days after treatment) of the use of elicitors on the treated plants showed that methyl jasmonate at a concentration of 100 µM would cause plant death ***(Figure 6)***. While about fifty percent of the leaves of plants treated with salicylic acid were completely healthy. And the leaves of the plants treated with phenylalanine were completely healthy and juicy and even produced suckers.

Some morphological characteristics were also compared in diploid and tetraploid plants. Results showed that in tetraploid plants, plant height and leaf length are shorter and the number of leaves per plant is higher. Also, leaf width in tetraploid was less than in diploid plants ***(Table 1)***.

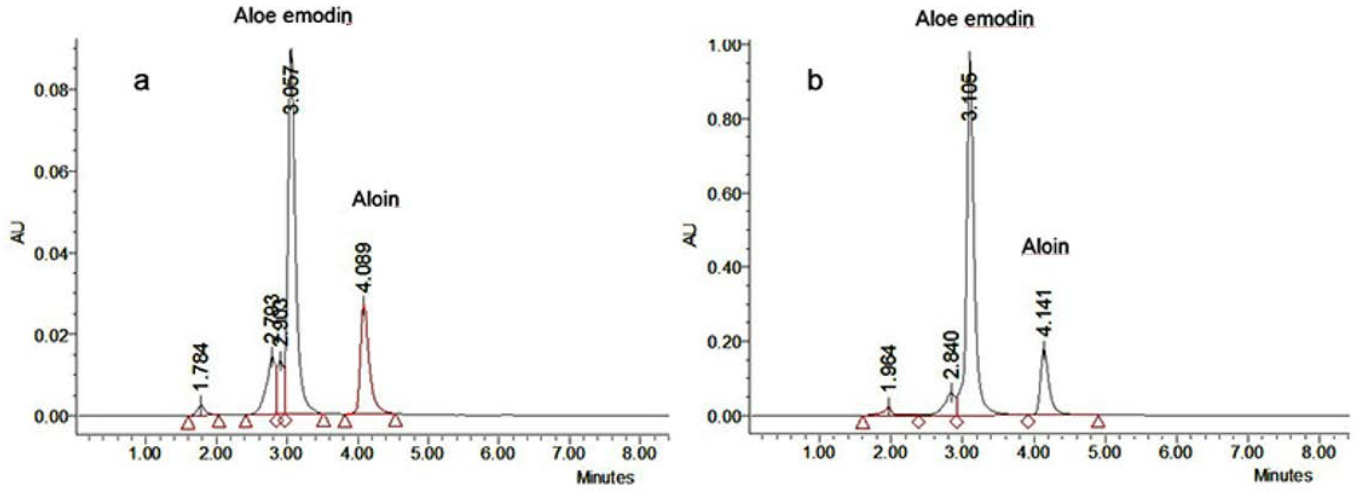

Figure 2. Chromatograms for aloe emodin and aloin. a: Standard, b: A sample treated with 25 µM salicylic acid after 24 hours in diploid *Aloe vera* plant.

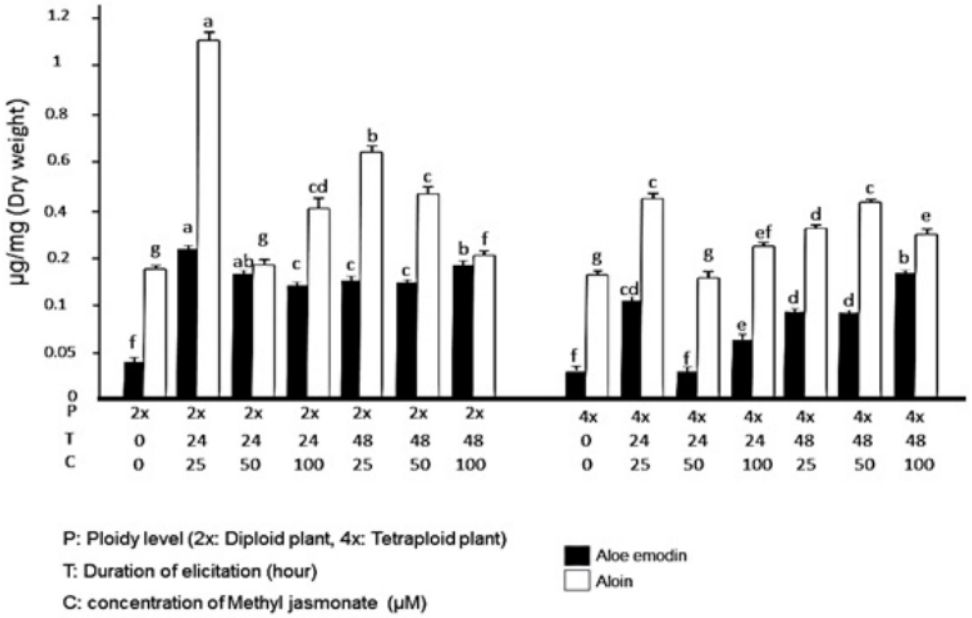

Figure 3. Effects of methyl jasmonate on aloe emodin and aloin in diploid and tetraploid *Aloe vera.*

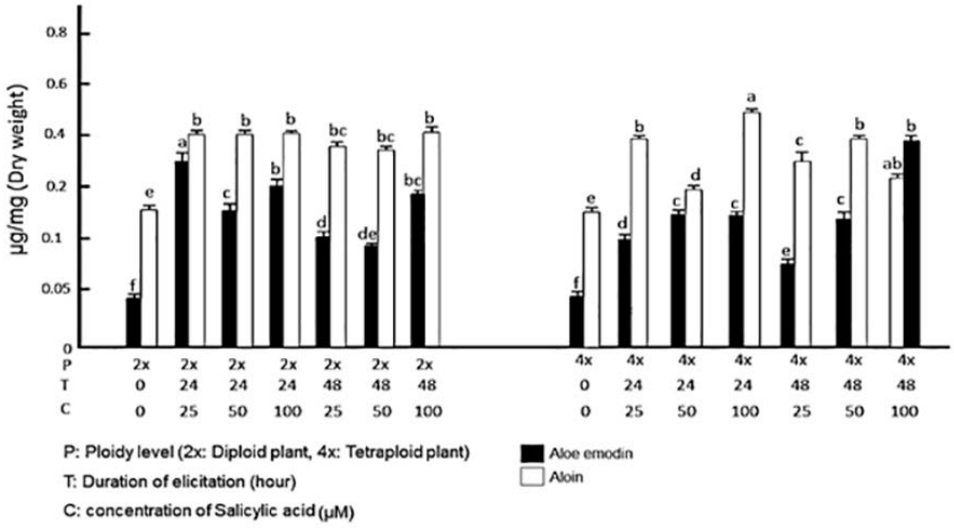

Figure 4. Effects of salicylic acid on aloe emodin and aloin in diploid and tetraploid *Aloe vera.*

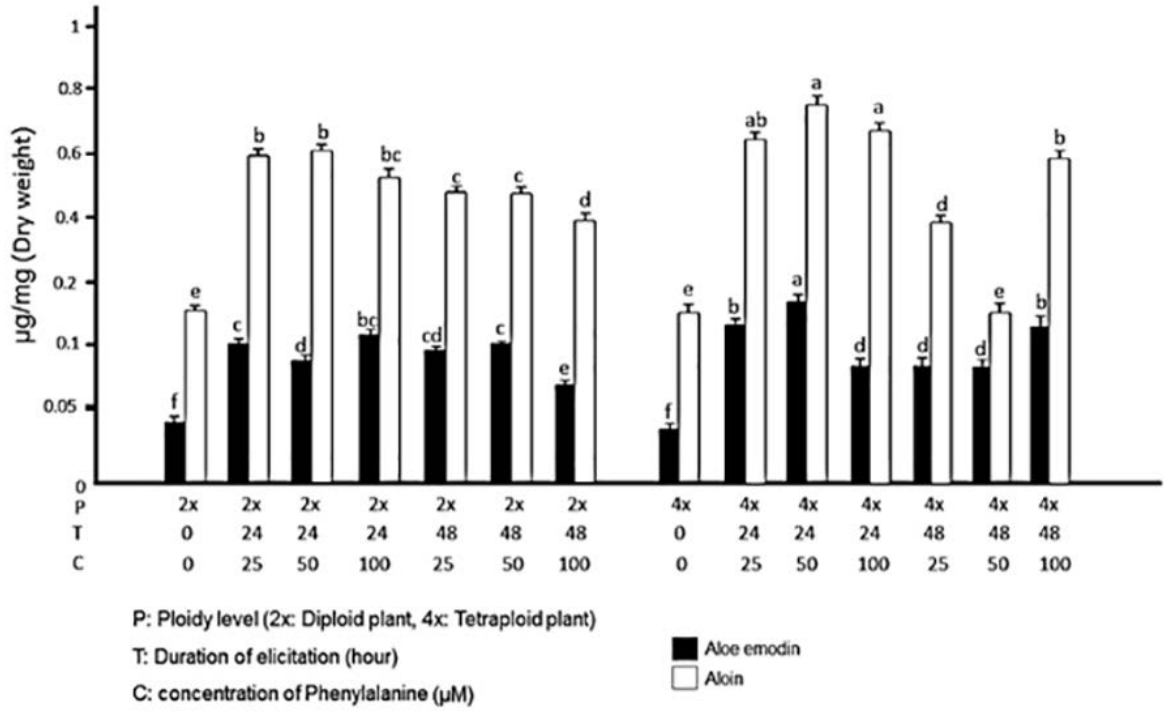

Figure 5. Effects of phenylalanine on aloe emodin and aloin in diploid and tetraploid *Aloe vera*.

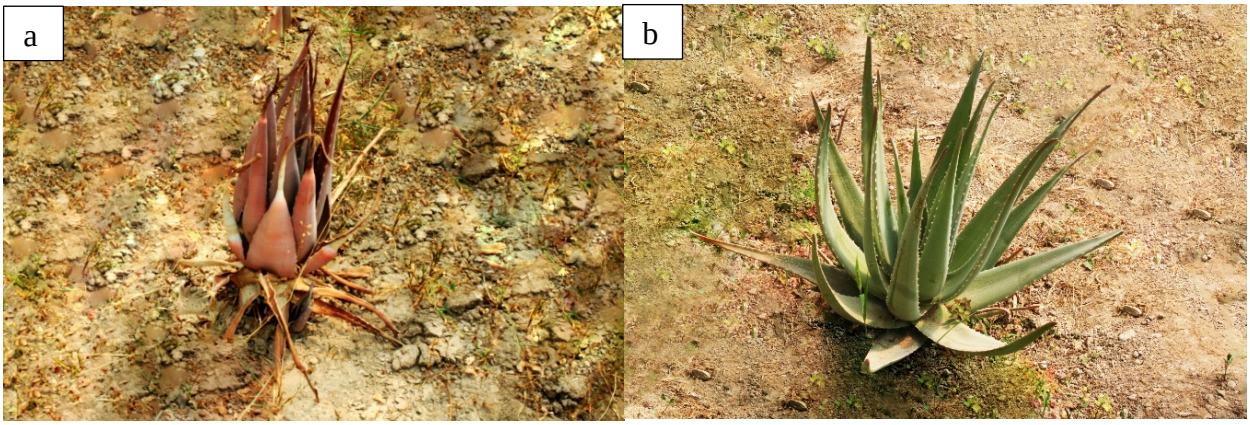

Figure 6. Long term effect of methyl jasmonate on *Aloe vera.* a: the treated plant (100 µM); b: the control.

Table 1. Comparison of morphological characteristics in diploid and tetraploid *Aloe vera* plants.

	Average leaf width	Average leaf length	Average number of leaves per plant	Average plant height
Diploid	100 mm	2 cm	16 ± 2	78 cm
Tetraploid	80 mm	43 cm	20 ± 3	65 cm

Discussion

Aloe vera plant has amazing medicinal and therapeutic metabolites (Danish et al., 2020). The quantity of these metabolites is normally quite low. It has been known for years that the production of secondary metabolites can be increased by using elicitors (Ramachandra & Ravishankar 2002; Radman et al., 2003; Namdeo, 2007). In this research, an attempt has been made to correctly examine some aspects of using the elicitors including elicitor type, elicitor concentration, duration of elicitation treatment and toxic effects of elicitors on plant survival. This study was performed simultaneously on diploid and tetraploid plants to determine the effect of elicitors on different ploidy levels.

Effect of methyl jasmonate on aloe emodin and aloin in diploid and tetraploid Aloe vera

Normally, without the application of elicitor, the amount of aloin is more than the amount of aloe emodin in *Aloe vera* plants (Kumar et al., 2017). In our study, we can generally conclude that the treatment of *Aloe vera* diploid plants with methyl jasmonate (25 µM) produced the highest amountbof aloe emodin (1.15 ± 0.07 µg mg^{-1}) in comparison to other elicitors. This maximum production was observed 24 hours after plant treatment, after which the amount of this metabolite was significantly reduced. Sometimes polyploidization leads to a decrease in the production of active ingredients. This phenomenon can have many reasons at the molecular level, such as gene silencing (Segraves & Thompson 1999; Hansen et al.,

2007; Halverson et al., 2008b; Kubátová et al., 2008; Schlaepfer et al., 2008), unusual frequent copies of the gene, a form of gene silencing (Matzke et al., 1995; Smyth, 1997; Depicker; Van Montagu 1997) and suppression of some genes that involve in the biosynthesis pathway of secondary metabolites (Dhawan & Lavania 1996; Hullsanders et al., 2009; Abdoli et al., 2013). Also, the results showed that, unlike tetraploid plants, the diploid plants have produced more production of aloin. Some other studies have reported similar results (Kumar et al., 2017). Each of the reasons stated above can lead to a decrease in the amount of aloin in tetraploid *Aloe vera* plants. Maybe some of these findings have implicated RNA as an agent in co-suppression (Metzlaff et al., 1997). By applying the elicitor and inducing the biosynthetic pathway, the difference between diploids and tetraploids was clearly observed, so that in most treatments, the amount of metabolites in tetraploids was less than that of diploids (Smyth, 1997). The results of this study also showed that in diploid plants, the amount of aloe emodin after 24 hours decreased with increasing the concentration of methyl jasmonate. This decrease can be due to an ultra-sensitivity response to an increase in the concentration of elicitor (Roewer et al., 1992). So in some treatments, methyl jasmonate appears to be toxic at high concentrations and it acts as an inhibitor and prevents the biosynthesis of aloe emodin.

Positive effects of methyl jasmonate on the improvement of the production of some secondary metabolites in various diploid plants have already been reported, such as increasing beta cyanine in *Purtulaca* (Nazmul et al., 2003). Jasmonic Acid (JA) and its methyl ester, methyl jasmonate (MJ), are derived from linoleic acid and are compounds with a circular pentagon structure. Jasmonate is probably involved as a signaling compound in stimulating the stages leading to transcription and translation in the biosynthesis of secondary metabolites in plants and induced transcription activities of the genes involved in the formation of secondary metabolites (Yukimun et al., 1996). Many reports emphasize the importance of methyl jasmonate to stimulate the production of secondary metabolites in plants (Kim et al., 2009; Qu et al., 2011; Veerashree et al., 2012). There are several hypotheses in relation to the mechanism of the defense response regulating by the methyl jasmonate, such as connecting elicitor to a receptor in the plasma membrane, the flow of calcium from extracellular sources and intracellular such as vacuoles into the cytoplasm, rapid change of protein phosphorylation pattern and protein kinase activity (Vasconsuelo & Boland, 2007). Connecting the elicitors to the receptors, the plant receptors are activated, leading to the activation of ionic channels, proteins bound to GTP (G-protein), and kinase protein. The second messengers are intracellular signaling molecules released by the cell in response to exposure to the elicitors, and they are also the triggers of intracellular signal transduction cascades (Blume et al., 2000). Methyl jasmonate belongs to the family of cyclopentanone compounds and regulates a wide range of defense responses to the plant (Sembdner & Parthier 1993; Creelman & Mullet 1997). Jasmonic acid and its active derivatives are an important signal in plants that induce the biosynthesis of a wide range of secondary metabolites (Pauwels et al., 2009).

Effect of salicylic acid on aloe emodin and aloin in diploid and tetraploid Aloe vera

The maximum amount of aloe emodin (6.18 fold compared to the control) was produced by diploid plants treated with 25 µM of salicylic acid, 24 hours after treatment. The maximum amount of aloin produced by tetraploid plants treated with 100 µM of salicylic acid, 24 hours after treatment. One of the reasons for increasing the production of aloe emodin and aloin under the influence of salicylic acid is that salicylic acid is one of the intrinsic and key signals of the cell that interfere with the activation of plant defense responses (Ajungla et al., 2009). Salicylic acid rapidly accumulates in response to stress and causes a reaction to high plant sensitivity and it spreads in other parts of the plant and creates a wide range of defense responses. Salicylic acid also causes the expression of genes associated with biosynthesis and the production of many secondary metabolites in the plant (Draper, 1997). The results of this study showed that salicylic acid can be used to improve the biosynthesis of two important metabolites of aloin and aloe emodin in *Aloe vera*. However, the positive effect of this elicitor on increasing aloin production was much greater than that of aloe emodin.

Effect of phenylalanine on aloin and aloe emodin in diploid and tetraploid Aloe vera

The results of this study are consistent with the results of studies that have reported a positive correlation between the increase in genomic DNA and the increase in secondary metabolites in some medicinal plants (Adaniya & Shira, 2001; Berkov & Philipov, 2002; Gonzalez & Weathers, 2003; Bertea et al., 2005; Koul et al., 2010; Majidi et al., 2010; Omid Beigi et al., 2010). On the other hand, the role of phenylalanine has been proven as an essential amino acid and an important precursor in the biosynthesis pathway of many secondary plant compounds. Phenylalanine has been successfully applied to enhance the metabolite production in numerous plants (Biswas et al., 2020). Among macronutrients, nitrogen is the most essential nutrient for plant growth. Plants can uptake different forms of nitrogen containing amino acid, nitrate and ammonium. Phenylalanine as an organic source of nitrogen can also be effective in increasing the plant growth and the production of plant metabolites (Andi et al., 2019). In this study, we used phenylalanine alone, as an important precursor, to investigate its effect on the studied secondary metabolites. In many studies, feeding with phenylalanine precursor along with other elicitors as an elicitation support had significant effects of the increase of secondary metabolites (Swieca et al., 2014; Swieca et al., 2016) such as successful use of phenylalanine with salicylic acid (Govindaraju et al., 2018; Li et al., 2021) and methyl jasmonate (Portu et al., 2017; Arano-Varela et al., 2020).

Plant health and survival should also be taken into account in research projects for secondary metabolite production. Accordingly, the production of secondary metabolites using the elicitor should be continued until it does not harm the plant. In this study, due to the drip irrigation system in the greenhouse and not washing the aerial parts of the plant, it was possible to investigate the response of plants to the elicitors in the long-term. Continual exposure of elicitation can lead to increasing cell death. Although cellular sensitivity of treatment duration depends on many factors such as plant type (Mangas et al., 2006) and production of ethylene in elicited plant. Any of these reasons can be cause to reducing cell growth and cell death rates (Qin & Lan, 2004)**.** The investigation of the survival rate of the treated plants after 240 days showed a high mortality rate of plants treated with methyl jasmonate (100 µM). *Aloe vera* plants may have a low level of resistance to this elicitor toxicity. While salicylic acid elicitor showed much less toxic effects than methyl

jasmonate on *Aloe vera* plants. Finally, phenylalanine with minimal effect on the health of *Aloe vera* plants did not harm them, and it can be said that *Aloe vera* plants are compatible with phenylalanine.

Aloe vera is one of the most important medicinal plants in the world and many researchers call it a pharmacy in a plant (Boundreau et al., 2006). This plant contains highly valuable and effective metabolites in the treatment of various diseases. In this study, the elicitation was applied to *Aloe vera* plants in greenhouse conditions. We examined some factors influencing this process and the results can be used for further research on increasing the production of *Aloe vera's* important metabolites.

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