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

Shafighi, S.1, Moieni, A.2 & Monfared, S. R.1

1Department of Agricultural Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, P.O. Box 14115-336, Iran
2Department of Plant Genetics and Breeding, Faculty of Agriculture, Tarbiat Modares University, Tehran, P.O. Box 14115-336, Iran

Author for correspondence: moieni_a@modares.ac.ir

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.

Shafighi, S., Moieni, A., Monfared, S. R. (2022): Effects of methyl jasmonate, salicylic acid and phenylalanine on aloe emodin and aloin in diploid and tetraploid Aloe barbadensis. International Journal of Horticultural Science 28: 86-96. https://doi.org/10.31421/ijhs/28/2022/9304

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; Sharyna 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; Yessim et al., 2021). Ploidy level has an effect on the amount 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; Martínez-Romeo et al., 2013; Rai 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 (Ejtehadi 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; Ramírez 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 1 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 1215 m 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 tetraploids 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 (Hazarati 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.](image)

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).
Effects of methyl jasmonate, salicylic acid and phenylalanine on aloe emodin and aloin...

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 = 0.4698X – 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), Draccephalum 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 amount of 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; Kabátová 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 & Lavanja 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 aloe 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 Portulaca (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 aloe 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 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 aloe and aloe emodin in Aloe vera. However, the positive effect of this elicitor on increasing aloe production was much greater than that of aloe emodin.

Effect of phenylalanine on aloe emodin and aloin 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 alone or 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. Continued 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...
Effects of methyl jasmonate, salicylic acid and phenylalanine on aloe emodin and aloin…  92

jasmone 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 the world and many researchers call it a pharmacy in the world. 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.

References

Abdoli, M., Moieni, A., Badi, H. N. (2013): Morphological, physiological, cytological and phytochemical studies in diploid and colchicine-induced tetraploid plants of Echinacea purpurea (L.). Acta Physiologiae Plantarum, 35(7), 2075-2083.

Abdollahi, M., Jafarpour, M., Zeinali, H. (2011): Effect of various salicylic acid concentrations on growth of Aloe vera L. International Journal of AgriScience, 1(5), 311-313.

Adaniya, S., Shira, D. (2001): In vitro induction of tetraploid Ginger (Zinger officinalis Roscoe) and its pollen fertility and germinability. Sci Hortic., 88: 277–287.

Ajungla, L., Patil, P. P, Barmukh, R. B. & Nikam, T. D. (2009): Influence of biotic and abiotic elicitors on accumulation of hyoscyamine and scopoline in root cultures of Datura metel L. Indian J Biotech., 8: 317-322.

Ali, B. (2020). Salicylic acid: An efficient elicitor of secondary metabolite production in plants. Biocatalysis and Agricultural Biotechnology, 10(1), 884-893.

Andi, S. A., Gholami, M., Ford, C. M., Maskani, F. (2019): The effect of light, phenylalanine and methyl jasmonate, alone or in combination, on growth and secondary metabolism in cell suspension cultures of Viitis vinifera. Journal of Photochemistry and Photobiology B: Biology, 199(9), 111-125.

Anjum, S., Anjum, I., Hano, C., Kousar, S. (2019): Advances in nanomaterials as novel elicitors of pharmacologically active plant specialized metabolites: Current status and future outlooks. RSC Advances, 9(69), 40404-40423.

Arano-Varela, H., Cruz-Sosa, F., Estrada-Zúñiga, M. E., Fernández, F. J. (2020): Effects of phenylalanine and methyl jasmonate on verbascoide production in Buddleja cordata Kunth cell suspension cultures. South African Journal of Botany, 135, 41-49.

Ardebili, Z. O., Moghadam, A. R. L., Ardebili, N. O., Pashaie, A. R. (2012): The induced physiological changes by foliar application of amino acids in Aloe vera L. plants. Plant Omics, 5(3), 279-294.

Bagheri, M., Mansouri, H. (2015): Effect of induced polyploidy on some biochemical parameters in Cannabis sativa L. Applied Biochemistry and Biotechnology, 175(5): 2366-2375.

Bano, M., Sharma, G. (2020): Meiotic irregularities in tetraploid Aloe arborescens Miller and their consequences. National Academy Science Letters, 30(9), 1-4.

Barrientos Carvacho, H., Pérez, C., Zúñiga, G., Mahn, A. (2014): Effect of methyl jasmonate, sodium selenate and chitosan as exogenous elicitors on the phenolic compounds profile of broccoli sprouts. Journal of the Science of Food and Agriculture, 94(12), 2555-2561.

Behzadrad, M., Naghavi, M. R., Shahnejat Bushehri, A. A. (2020): Importance of hormonal elicitors in inducing morphine biosynthesis in the cell culture of (Papaver bracteatum Lindl.). Journal of Agricultural Science and Technology, 22(1), 261-270.

Berkov, S., Philpov, S. (2002): Alkaloid production in diploid and autotetraploid plants of Datura stramonium. Pharma Biol., 40: 617–621.

Berta, C. M., Azzolin, C. M. M., Bossi, S., Doglia, G. and Maffei, M. E. (2005): Identification of an EcoRI restriction site for a rapid and precise determination of β-asarone-free Acorus calamus cytotypes. Phytochemistry, 66: 507-514.

Birari, L. A., Mahajan, U. B., Patil, K. R., Patil, D. D., Bagul, N. A., Belemkar, S., Patil, C. R. (2020): Aloe protects against arsenic trioxide-induced myocardial membrane damage and release of inflammatory cytokines. Naunyn-Schmiedeberg's Archives of Pharmacology, 393(8), 1365-1372.

Biswas, T., Mathur, A., Gupta, V., Luqman, S., Mathur, A. K. (2020): Elicitation and phenylalanine precursor feeding based modulation of in vitro anthocyanin production, enzyme activity and gene expression in an Indian ginseng congener-Panax sikkimensis Ban. Industrial Crops and Products, 145, 111986.

Blume, B., Nünberner, T., Nass, N., Scheel, D. (2000): Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. The Plant Cell, 12(8), 1425-1440.

Boudreau, M. D., Beland, F. A. (2006): An evaluation of the biological and toxicological properties of Aloe barbadensis (Miller), Aloe vera. Journal of Environmental Science and Health Part C, 24(1), 103-154.

Cai, Z., Kastell, A., Speiser, C., Smetanska, I. (2013): Enhanced resveratrol production in Viitis vinifera cell suspension cultures by heavy metals without loss of cell viability. Applied Biochemistry and Biotechnology, 171(2), 330-340.

Cavallini, A., Natali, L., Sanchez, I. C. (2012): VI Aloe barbadensis Mill. (= A. vera L.). Medicinal and Aromatic Plants III, 15(1), 95-108.

Chen, R., Zhang, J., Hu, Y., Wang, S., Chen, M., Wang, Y. (2014): Potential antineoplastic effects of Aloe-emodin: a comprehensive review. The American Journal of Chinese Medicine, 42(02), 275-288.

Choudhri, P., Rani, M., Sangwan, R. S., Kumar, R., Kumar, A., Chhokar, V. (2018). De novo sequencing, assembly and characterisation of Aloe vera transcriptome and analysis of expression profiles of genes related to saponin and anthraquinone metabolism. BMC Genomics, 19(1), 1-21.
Cousins, S. R., Witkowski, E. T. F. (2012): African aloe ecology: A review. J. Arid Environ. 85: 1–17.

Creelman, R. A., Mullet, J. E. (1997): Biosynthesis and action of jasmonates in plants. Annual Review of Plant Biology, 48 (1): 355-381.

Danish, P., Ali, Q., Hafeez, M. M., Malik, A. (2020): Antifungal and antibacterial activity of Aloe vera plant extract Biological and Clinical Sciences Research Journal, 2020(1), 418-432.

Dantas, L. A., Rosa, M., Resende, E. C., Silva, F. G., Pereira, P. S., Souza, A. C. L., Neto, A. R. (2020): Spectral quality as an elicitor of bioactive compound production in Solanum aculeatissimum JACC cell suspension. Journal of Photochemistry and Photobiology B: Biology, 204, 111-119.

Depicker, A., Van Montagu, M. (1997): Post-transcriptional gene silencing in plants. Curr Opin Cell Biol. 9:373-382.

Dhawan, O. P., Lavanía, U. C. (1996): Enhancing the productivity of secondary metabolites via induced polyplody: a review. Euphytica, 87: 81-89.

Dong, X., Zeng, Y., Liu, Y., You, L., Yin, X., Fu, J., Ni, J. (2020): Aloe-emodin: a review of its pharmacology, toxicity, and pharmacokinetics. Phytotherapy Research, 34(2), 270-281.

Draper, J. (1997): Salicylate × superoxide synthesis and cell suicide in plant defense. Trends Plant Sci., 2: 162-165.

Ejtehad, R. S., Radjabian, T., Tafreshi, S. A. H. (2015): Suicide in plant defense. Trends Plant Sci., 2: 162-165.

Ejtahed, R. S., Radjabian, T., Tafreshi, S. A. H. (2015): Suicide in plant defense. Trends Plant Sci., 2: 162-165.

Ezat, A., Ammar, A., Szabó, Z., & Holb, I. (2017): Salicylic acid treatment saves quality and enhances antioxidant properties of apricot fruit. Horticultural Science, 44(2): 73-81.

Ezat, A., Ammar, A., Szabó, Z., Nyéki, J., Holb, I. J. (2017): Postharvest treatments with methyl jasmonate and salicylic acid for maintaining physico-chemical characteristics and sensory quality properties of apricot fruit during cold storage and shelf-life. Polish Journal of Food and Nutrition Sciences, 67(2):159-166.

Ezat, A., Hegedűs, A., Szabó, S., Ammar, A., Szabó, Z., Nyéki, J., Molnár, B., Holb, I. J. (2020): Temporal changes and correlations between quality loss parameters, antioxidant properties and enzyme activities in apricot fruit treated with methyl jasmonate and salicylic acid during cold storage and shelf-life. Applied Sciences, 10(22): 8071.

Ezat, A., Szabó, S., Szabó, Z., Hegedűs, A., Berényi, D., Holb, I. J. (2021): Temporal patterns and inter-correlations among physical and antioxidant attributes and enzyme activities of apricot fruit inoculated with Monilinia laxa under salicylic acid and methyl jasmonate treatments under shelf-life conditions. Journal of Fungi, 7(5): 341.

Gadzovska, S., Maury, S., Delaunay, A., Spasenoski, M., Joseph, C., Hagege, D. (2007): Jasmonic acid elicitation of Hypericum perforatum L. cell suspensions and effects on the production of phenylpropanoids and naphthodianthrones. Plant Cell, Tissue and Organ Culture, 89(1), 1-13.

Gantait, S., Sinniah, U. R., Das, P. K. (2014): Aloe vera: a review update on advancement of in vitro culture. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 64(1), 1-12.

Gonzalez, L. D. J., Weathers, P. J. (2003): Tetraploid Artemisia annua hairy roots produce more artemisinin than diploids. Plant Cell Rep., 21: 809-811.

Govindaraju, S., Arulselvi, P. I. (2018): Effect of cytokinin combined elicitors (l-phenylalanine, salicylic acid and chitosan) on in vitro propagation, secondary metabolites and molecular characterization of medicinal herb—Cowles aromaticus Benth (L.). Journal of the Saudi Society of Agricultural Sciences, 17(4), 435-444.

Halverson, K., S. B. Heard, J. D. Nason, J. O. Stireman III. (2008b): Origins, distribution, and local co-occurrence of polyplody cytotypes in Solidago altissima (Asteraceae). American Journal of Botany 95: 50 – 58.

Hamarakshid, S. H., Khaledyan, Y., Soleimani, F. (2021): In vitro polyplody-mediated enhancement of secondary metabolites content in Stachys byzantina L. Genetic Resources and Crop Evolution, 72(1), 1-10.

Hansen, D. L., Lambertini, C., Jampeetong, A., Brix, H. (2007): Clone-specific differences in Phragmites australis: Effects of ploidy level and geographic origin. Aquatic Botany 86: 269 – 279.

Hao, X., Shi, M., Cui, L., Xu, C., Zhang, Y., Kai, G. (2015): Effects of methyl jasmonate and salicylic acid on tanninshone production and biosynthetic gene expression in transgenic Salvia miltiorrhiza hairy roots. Biotechnology and Applied Biochemistry, 62 (1): 24-31.

Hassini, I., Baenas, N., Moreno, D. A., Carvajal, M., Bougannini, N., Martínez Ballesta, M. D. C. (2017): Effects of seed priming, salinity and methyl jasmonate treatment on bioactive composition of Brassica oleracea var. capitata (white and red varieties) sprouts. Journal of the Science of Food and Agriculture, 97(8), 2291-2299.

Hatami, M., Naghdi Badi, H., Ghorbanpour, M. (2019): Nano-elicitic of secondary pharmaceutical metabolites in plant cells: A review. Journal of Medicinal Plants, 18(71), 6-36.

Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I. (2010): Soil beneficial bacteria and their role in plant growth promotion: a review. Annals of Microbiology, 60(4), 579-598.

Hazarati, S., Sarvestani, Z. T., Babaei, A. (2012): Enhancing yield and aloin concentration of Aloe vera plants by simultaneous application of N and benzyl adenine. Journal of Medicinal Plants Research, 6(10), 1834-1841.

Hoseinpanah, B., Bahranejad, B., Majdi, M., Dastan, D., Ashengroh, M. (2020): The effect of different elicitors on hairy root biomass and resveratrol production in wild Vitis vinifera. Journal of Applied Biotechnology Reports, 7(1), 25-31.

Hull-Sanders, H. M., Johnson, R. H., Owen, H. A., Meyer, G. A. (2009): Effects of polyplody on secondary chemistry, physiology, and performance of native and invasive genotypes of Solidago gigantea (Asteraceae). American Journal of Botany, 96(4), 762-770.
Ido Izhakj, I. (2002): Emodin—a secondary metabolite with multiple ecological functions in higher plants. New Phytologist, 155(2): 205-217.

Kanaana, S.K., Viljoen, A.M., Kamaatu, G.P., Chen, W., Sandasi, M., Adhami, H.R. (2015): Simultaneous quantification of anthrones and chromones in Aloe ferox (“Cape aloe”) using UHPLC-MS. Phytochemistry Letters, 13: 85–90.

Kaparakou, E. H., Kanakis, C. D., Gerogianni, M., Maniati, K., (2010): Development, micropropagation and characterization of colchiploid of Echinacea purpurea. Journal of Medicinal Plants Research, 4(18), 1912-1921.

Kim, K. S., Zhao, Y., Jang, H., Lee, S. Y., Kim, J. M., Kim, K. S., Hong, B. H. (2009): Large-scale pattern growth of graphene films for stretchable transparent electrodes. Nature, 457(7230), 706.

Koul, S., Sambyal, M., Kitchlu, S. K., Bakshi, S. K., Kaul, M. K. (2010): Development, micropropagation and characterization of colchloid of Echinacea purpurea (L.) Moench. Indian J Biotech., 9: 221-224.

Kubátová, B., Trávnícek, P., Bastlová, D., Vladislav, C. (2011): The effect of nano elicitors on callus induction and mucilage production in tissue culture of Linum usitatissimum L. Journal of Medicinal Plants, 17(67), 45-54.

Kim, K. S. Chung, Y. D., Lee, Y. K., Cho, Y. S. (2012): The effect of 5-aminolevulinic acid elicitation on adventitious root formation in alevia leaves. Korean J. HortSci., 45 (1): 16–21.

Machado, D. I., López-Cervantes, J., Mariscal-Dominguez, M. F., Cruz-Flores, P., Campos-Bayopoli, O. N., Cantú-Soto, E. U., Sanches-Silva, A. (2016): An HPLC procedure for the quantification of aloin in latex and gel from Aloe barbadensis leaves. Journal of Chromatographic Science, 55(3), 251-257.

Madani, V., Zare, N., Asghahri Zakaria, R. (2021): The effect of elicitors on the biochemical properties and expression of the genes involved in sesquiterpenes biosynthesis pathway in the hairy root cultures of medicinal plant Valeriana officinalis L. Iranian Journal of Plant Biology, 12(4), 19-42.

Madon, M., Clyde, M. M., Hashim, H. (2005): Polyploidy induction of oil palm through Colchicine and oryzalin treatments. J OI Palmer Res., 17: 110-123.

Mahor, G., Ali, S. A. (2016): Recent update on the medicinal properties and use of Aloe vera in the treatment of various ailments. Biosci Biotechnol Res Commun, 9:273-288.

Majdi, M., Karimzadeh, G., Malboobi, M. A., Omidbaigi, R., Mirzaghaderi, G. (2010): Induction of tetraploidy to feverfew (Tanacetum parthenium Schulz-Bip.): morphological, physiological, cytological, and phytochemical changes. HortSci., 45 (1): 16–21.

Makowski, W., Tokarz, K. M., Tokarz, B., Basanski, R., Wittek, K., Królacka, A. (2020): Elicitation-based method for increasing the production of antioxidant and bactericidal phenolic compounds in Dionaea muscipula J. Ellis tissue. Molecules, 25(8), 179-194.

Mandrioli, R., Mercolini, L., Ferranti, A., Fanali, S., Raggi, M. A. (2011): Determination of aloe emodin in Aloe vera extracts and commercial formulations by HPLC with tandem UV absorption and fluorescence detection. Food Chemistry, 126(1), 387-393.

Mangas, S., Bonfill, M., Osuna, L., Moyano, E., Tortoriello, J., Cusido, R. M.,..., Palazón, J. (2006): The effect of methyl jasmonate on triterpene and sterol metabolisms of Centella asiatica, Ruscus aculeatus and Galphimia glauca cultured plants. Phytochemistry, 67(18), 2041-2049.

Martin, R. L., Le Bouch, P., Clin, P., Schwarzemberg, A., Yvin, J. C., Andrillon, D.,..., Val, F. (2020): A comparison of PTI defense profiles induced in Solanum tuberosum by PAMP and non-PAMP elicitors shows distinct, elicitor-specific responses. Plos One, 15(8), 236-253.

Martinez-Romero, D., Guillén, F., Pérez-Aguilar, H., Castillo, S., Serrano, M., Zapata, P. J., Valero, D. (2013): Is it possible to increase the aloin content of Aloe vera by the use of ultraviolet light? Journal of Agricultural and Food Chemistry, 61(9), 2165-2170.
Matzke, M. A., Matzke, A. J. (1995): How and why do plants inactivate homologous (trans) genes. Plant Physiology, 107(3), 679.

Mehravaran, L., Omidi, M., Naghavi, M. R., Fakheri, B. A. (2021): Effect of some elicitors on morphophysiological, biochemical and molecular traits of Stevia. Russian Journal of Plant Physiology, 68(2), 347-355.

Metzlaff, M., O’Dell, M., Cluster, P.D., Flavell, R. B.(1997): RNA-mediated RNA degradation and chalcone synthase A silencing in petunia. Cell, 88:845-854.

Mirzaei, M., Shafiee, A., Foroumadi, A. (2001): Antituberculosis agents II. Evaluation of in vitro antituberculosis activity and cytotoxicity of some 2-(1-methyl-5-nitro-2-imidazolyl)-1, 3, 4-thiadiazole derivatives. Il Farmaco, 56(8), 621-623.

Molsaghi, M., Moieni, A., Kahrizi, D. (2014): Efficient protocol for rapid Aloe vera micropropagation. Pharmaceutical Biology, 52(6), 735-739.

Namdeo, A. G. (2007): Plant cell elicitation for production of secondary metabolites: a review. Pharmacogn Rev, 1(1), 69-79.

Nazmul, A. M., Sugahara, S., Tanaka, M. (2003): Ferromagnetism and high Curie temperature in semiconductor Nazmul, A. M., Sugahara, S., Tanaka, M. (2003): Ferromagnetism and high Curie temperature in semiconductor Nazmul, A. M., Sugahara, S., Tanaka, M. (2003): Ferromagnetism and high Curie temperature in semiconductor Nazmul, A. M., Sugahara, S., Tanaka, M. (2003): Ferromagnetism and high Curie temperature in semiconductor Nazmul, A. M., Sugahara, S., Tanaka, M. (2003): Ferromagnetism and high Curie temperature in semiconductor Nazmul, A. M., Sugahara, S., Tanaka, M. (2003): Ferromagnetism and high Curie temperature in semiconductor

Nisha, S., Bora, A., Gogoi, H. K., Dwivedi, S. K., Handique, P. J. (2021): A Study on the effect of different elicitors on capsaisin accumulation in cell suspension cultures of Capsicum annuum (Bhut Jolokia). Research Square, 10(20), 12-23.

Omid, B. R., Mirzaei, M., Hassani, M. E., Sedghi Moghadam, M. (2010): Induction and identification of polyploidy in basil (Ocimum basilicum L.) medicinal plant by colchicine treatment. Int J Plant Prod., 4 (2): 87–98.

Pal, S., Sahrawat, A., Prakash, D. (2013): Aloe vera: composition, processing and medicinal properties; International Journal of Current Discoveries and Innovations, 2: 106–122.

Pan, Y., Hu, Z. L., Chen, G. P., Liu, Y., Wang, X. Y., Chen, X. Q. (2007): Callus induction of Aloe vera L. var. chinensis (Haw) Berg [J]. Journal of Chongqing University (Natural Science Edition), 5(1), 834-850.

Parai, P., Mukherjee, A. (2014): Cytomorphological studies of Aloe variegata L. and Aloe zebrina Baker (Xanthorrhoeaceae) by an image analyzing system. Cytologia, 79(2), 281-286.

Park, M. K., Park, J. H., Kim, N. Y., Shin, Y. G., Choi, Y. S., Lee, J. G., ..., Lee, S. K. (1998): Analysis of 13 phenolic compounds in Aloe species by high performance liquid chromatography. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques, 9(4), 186-191.

Pauwels, L., Inzé, D., Goossens, A. (2009): Jasmonate-inducible gene: what does it mean? Trends in Plant Science, 14(2), 87-91.

Pesaraku, A., Radjabian, T., Salami, S. A. (2021): Methyl jasmonate and Ag+ as effective elicitors for enhancement of phenolic acids contents in Salvia officinalis and Salvia verticillata, as two traditional medicinal plants. South African Journal of Botany, 141, 105-115.

Portu, J., López, R., Santamaria, P., Garde-Cerdán, T. (2017): Elicitation with methyl jasmonate supported by precursor feeding with phenylalanine: Effect on Garnacha grape phenolic content. Food Chemistry, 237, 416-422.

Qaderi, A., Akbari, Z., Kalateh-Jari, S., Fatehi, F., Tolyat, M., Jalali Moghadam, M., Naghdi Badi, H. (2016): Improving trigonelline production in hairy root culture of fenugreek (Trigonella foenum-graecum). Journal of Medicinal Plants, 15(59), 73-80.

Qin, W. M., Lan, W. Z. (2004): Fungal elicitor-induced cell death in Taxus chinensis suspension cells is mediated by ethylene and polyamines. Plant Science, 166(4), 989-995.

Qu, S. Q., Dumay, J. (2011): The qualitative research interview. Qualitative Research in Accounting & Management, 8(3), 238-264.

Radman, R., Saez, T., Bucke, C., Keshavarz, T. (2003): Elicitation of plants and microbial cell systems. Biotechnology and Applied Biochemistry, 37 (1): 91-102.

Raei, M., Angaji, S. A., Omidi, M., Khodayari, M. (2014): Effect of abiotic elicitors on tissue culture of Aloe vera. Int. J Biosci, 5(1), 74-81.

Raghavendra, S., Kumar, V., Ramesh, C. K., Khan, M. M. (2012): Enhanced production of L-DOPA in cell cultures of Mucuna pruriens L. and Mucuna prurita H. Natural Product Research, 26(9), 792-801.

Ramachandra, R. S., Ravishankar, G. A. (2002): Biotransformation of isoeugenol to vanilla flavor metabolites and capsaicin in freely suspended and immobilized cell cultures of Capsicum frutescens: study of the influence of β-cyclodextrin and fungal elicitor. Process Biochemistry, 35, 341-348.

Ramirez Godina, F., Robledo Torres, V., Reyes Valdés, M. H., Escobedo Bocardo, L., Torres Tapia, M. A., García Osuna, H. T. (2015): Histological and morphological study of autotetraploid and diploid plants of tomatillo. Revista Mexicana de Ciencias Agrícolas, 6(SPE12), 2291-2299.

Roever, I. A., Cloutier, N., Nessler, C. L., De Luca, V. (1992): Transient induction of tryptophan decarboxylase (TDC) and strictosidine synthase (SS) genes in cell suspension cultures of Catharanthus roseus. Plant Cell Reports, 11 (2): 86-89.

Sa, T. E., El Sayed, S. A. (2021): Evaluating biotic elicitation with phenylalanine and/or yeast for rosemary (Rosmarinus officinalis L.) Sustainable improvement under traditional and organic agriculture. Agricultural Sciences, 12(3), 273-292.

Salma, U., Kundu, S., Mandal, N. (2017): Artificial polyploidy in medicinal plants: advancement in the last two decades and impending prospects. Journal of Crop Science and Biotechnology, 20(1), 9-19.

Sánchez, M., González-Burgos, E., Iglesias, I., Gómez-Serranillos, M. P. (2020): Pharmacological update properties of Aloe vera and its major active constituents. Molecules, 25(6), 13-24.

Sanguepak, R., Phansak, P., Thuman, K., Siriwong, S., Wongkaew, S., Buensantei, N. (2021): Effect of salicylic acid formulations on induced plant defense against cassava anthracnose disease. The Plant Pathology Journal, 37(4), 35-56.

Schlaepfer, D. R., Edwards, P. J., Semple, J. C., Billetter, R. (2008): Cytogeography of Solidago gigantea (Asteraceae) and its invasive ploidy level. Journal of Biogeography 35: 2119 – 2127.
Seagraves, K. A., J. N. Thompson. (1999): Plant polyploidy and pollination: Floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. Evolution 53: 1114–1127.

Sembdner, G. A. P. B., Parthier, B. (1993): The biochemistry and the physiological and molecular actions of jasmonates. Annual Review of Plant Biology, 44(1), 569-589.

Shao, J., Chen, C., Deng, X. (2003): *In vitro* induction of tetraploid in pomegranate (*Punica granatum*). Plant Cell, Tiss Organ Cult., 75: 241-246.

Sharanya, C. S., Arun, K. G., Sabu, A., Haridas, M. (2020): Aloe emodin shows high affinity to active site and low affinity to two other sites to result consummately reduced inhibition of lipoxygenase. Prostaglandins & Other Lipid Mediators, 150, 106-123.

Shi, G., Jiang, H., Feng, J., Zheng, X., Zhang, D., Jiang, C., Zhang, J. (2021): *Aloe vera* mitigates dextran sulfate sodium-induced rat ulcerative colitis by potentiating colon mucus barrier. Journal of Ethnopharmacology, 279, 114-128.

Smyth, D. R. (1997): Gene silencing: suppression at a distance. Current Biology, 7(12), R793-R796.

Świeca, M. (2016): Potentially bioaccessible phenolics, antioxidant activity and nutritional quality of young buckwheat sprouts affected by elicitation and elicitation supported by phenylpropanoid pathway precursor feeding. Food Chemistry, 192, 625-632.

Świeca, M., Sęczyk, Ł., Gawlik-Dziki, U. (2014): Elicitation and precursor feeding as tools for the improvement of the phenolic content and antioxidant activity of lentil sprouts. Food Chemistry, 161, 288-295.

Tabolacci, C., Lentini, A., Mattioli, P., Provenzano, B., Oliverio, S., Carlomostì, F., Beninati, S. (2010): Antitumor properties of aloe-emodin and induction of transglutaminase 2 activity in B16–F10 melanoma cells. Life Sciences, 87(9-10), 316-324.

Thakur, M., Bhattacharya, S., Khosla, P. K., Puri, S. (2019): Improving production of plant secondary metabolites through biotic and abiotic elicitation. Journal of Applied Research on Medicinal and Aromatic Plants, 12, 1-12.

Vasconsuelo, A., Boland, R. (2007): Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Science, 172(5), 861-875.

Veerashree, V., Anuradha, C. M., Kumar, V. (2012): Elicitor-enhanced production of gymnemic acid in cell suspension cultures of *Gymnema sylvestre* R. Br. Plant Cell, Tissue and Organ Culture (PCTOC), 108(1), 27-35.

Wang, F., Zhi, J., Zhang, Z., Wang, L., Suo, Y., Xie, C., ..., Sun, H. (2017): Transcriptome analysis of salicylic acid treatment in *Rehmannia glutinosa* hairy roots using RNA-seq technique for identification of genes involved in acteoside biosynthesis. Frontiers in Plant Science, 8, 787-803.

Wang, P., Zhou, W., Wamer, W., Kryniitsky, A.J., Rader, J.I. (2012): Simultaneous determination of aloin A and aloe-emodin in products containing *Aloe vera* by ultra-performance liquid chromatography with tandem mass spectrometry: Analytical Methods, 4: 3612–3619.

Wang, Z., Tang, T., Wang, S., Cai, T., Tao, H., Zhang, Q., ..., Qi, Z. (2020): Aloin inhibits the proliferation and migration of gastric cancer cells by regulating NOX2–ROS-mediated pro-survival signal pathways. Drug Design, Development and Therapy, 14, 145-162.

Xu, Q., Fan, Y., Loor, J. J., Liang, Y., Lv, H., Sun, X., ..., Xu, C. (2021): Aloin protects mice from diet-induced non-alcoholic steatohepatitis via activation of Nrf2/HO-1 signaling. Food & Function, 12(2), 696-705.

Yavari, A., Sarkani, S., Moyer Jr, E. T. (2000): On applications of generalized functions to beam bending problems. International Journal of Solids and Structures, 37(40), 5675-5705.

Yesmin, R., Islam, M. D., Deepo, D. M., Kim, H. Y., Kim, C. K., Lim, K. B. (2021): Cytogenetic assessment of Haworthia using flow cytometry and fluorescence in situ hybridization. Horticulture, Environment, and Biotechnology, 121, 1-9.

Yin, Q., Han, X., Han, Z., Chen, Q., Shi, Y., Gao, H., ..., Chen, S. (2020): Genome-wide analyses reveal a glucosyltransferase involved in rutin and emodin glucoside biosynthesis in tartary buckwheat. Food Chemistry, 318, 126-147.

Yukimune, Y., Tabata, H., Higashi, Y., Hara, Y. (1996): Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in *Taxus* cell suspension cultures. Nature Biotechnology, 14(9), 11-29.

Zhang, Z. C., Zhang, S. N., Zhang, W., Zhang, H. L. (2007): Induction of tetraploidy of non-heading Chinese cabbage with late-bolting and identification of chromosome configuration. Acta Botanica Boreali-Occidentalia Sinica, 27(1), 28-44.