

WITHDRAWN: Optimization of Seed Germination, Callus Induction, and Secondary Metabolites Production in *Allium Jesdianum*: Elicitation by Methyl Jasmonate and Putrescine

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Abstract

Allium jesdianum is considered as the most important genera of the Liliaceae family with various pharmacological properties. The present study was carried out to determine the best pre-germination treatment in *A. jesdianum*. The highest seed germination (58.33%) was observed at chemical treatment including the combination of α -naphthaleneacetic acid (1 mgL^{-1}) and benzyl aminopurine (3 mgL^{-1}) on Murashige and Skoog (MS) media. The highest callus induction (86.7%) was observed at MS/2 media, which was supplemented by NAA (1 mgL^{-1}) and BAP (3 mgL^{-1}) from hypocotyl explants. Two elicitors including methyl jasmonate (MeJ) (0, 25, 50, and $100 \text{ }\mu\text{M}$) and putrescine (Pu) (0, 0.5, and 1 mM) were used to investigate their effects on lipid peroxidation, callus growth traits, total phenolics content (TPC), the contents of total flavonoids (TFD), total flavonols (TFL), anthocyanin, total chlorophyll, total carotenoids and on antioxidant activity through DPPH (1,1-diphenyl-2-picrylhydrazyl) method under callus culture. The results showed the superiority of MeJ over Pu for increasing the secondary metabolites and antioxidant activity in callused of *A. Jesdianum*, compared to the control. The highest TPC ($6.02 \text{ mg GAEg}^{-1} \text{ FW}$), TFD ($0.52 \text{ mg QEG}^{-1} \text{ FW}$), and TFL ($0.39 \text{ mg QEG}^{-1} \text{ FW}$) were observed under $50 \text{ }\mu\text{M}$ of MeJ. Meanwhile, the highest value for anthocyanin ($8.99 \text{ }\mu\text{mol g}^{-1} \text{ FW}$) was achieved at $25 \text{ }\mu\text{M}$ of MeJ. The highest DPPH activities were observed at 50 and $100 \text{ }\mu\text{M}$ of MeJ. Putrescine (0.5 mM) elicitation showed only superiority for callus growth rate (0.53 mm day^{-1}) and carotenoids ($0.045 \text{ mg g}^{-1} \text{ DW}$). In this study, effective seed germination and elicitation of *A. Jesdianum* callus cultures were developed for the first time. Thus, new protocols could be suitable for future studies in biotechnological aspects of this medicinal plant.

1. Introduction

Medicinal plants are traditionally considered among the main sources of secondary metabolites (SMs) and natural drugs with antioxidant properties (Yue et al. 2016). The discovery of new natural products with medicinal characteristics has been recently the subject of intense research (Yue et al. 2016). These SMs are important sources for producing different cosmetics, fragrances, flavors, food additives, and pharmaceutical and industrial products (Namdeo 2007; Erb and Kliebenstein, 2020). The technique of plant cell culture is based on a set of biotechnological methods for producing valuable plant-specific SMs on large scale, independent from environmental conditions (Vanisree et al. 2004; Smetanska 2008). *In vitro* culture techniques have been used for enhancing different phytochemical compounds as a promising bio-production way for desired and/or rare natural products (Dias et al. 2016; Yue et al. 2016). In this regard, *in vitro* elicitation is an efficient strategy to promote plants' defense mechanism for scavenging the reactive oxygen species (ROS) (Matkowski 2008). The subsequent modulating and production of plant SMs occurs by regulating the expression of genes that are involved in producing key enzymes implicated in the biosynthesis of different defense-related compounds as phytoalexins, flavones, flavonoids phenolics, and other bioactive compounds (Namdeo 2007) under the elicitation process. To enhance the production of these components, chemical elicitors have been widely applied in recent years as environmentally benign techniques (Narayani and Srivastava 2017). *In vitro* production of

worthy compounds using callus culture has been very well recognized and is being used widely for different industrial applications (Narayani and Srivastava 2017).

Jasmonates (JA) is considered an important chemical messenger for different signal transduction pathways in different ways such as elicitation for the biosynthesis of SMs, and participating in enzyme activation under environmental stresses (Thiruvengadam et al. 2015; Nabi et al. 2021). Methyl jasmonate (MeJ) is an essential signaling compound in the intracellular signal cascades that ultimately leads to the hyper production of various SMs (Narayani and Srivastava 2017; Singh and Dwivedi 2018). According to previous studies in the stimulation of flavonoids and phenolic compounds production, jasmonates and their derivatives such as MeJ have been used in many plant families as elicitors under cell cultures (Gabr et al. 2016; Ho et al. 2016; Mendoza et al. 2018).

Polyamines (PAs), as a group of phytohormone-like aliphatic amine natural compounds, could play critical roles in the defense mechanism of plants against environmental stresses (Chen et al. 2019). PAs are involved in many important cellular processes such as cell viability, cell division, and its prolongation, protein synthesis, replication of DNA, response to different abiotic stresses by acting as defense signaling molecules and scavengers of free radicals, regulation of different physiological processes as embryogenesis, floral development, and fruit ripening and senescence (Jiménez Bremont et al. 2014; Rangan et al. 2014; Chen et al. 2019).

The putrescine (Pu) with its plant formula as $C_4H_{12}N_2 \cdot 2H_2O$ is categorized as one of the plant hormones in the polyamines group (Jiménez Bremont, et al. 2014). This organic compound is considered as the primary source of other PA_s species such as spermidine and spermine (Jiménez Bremont et al. 2014; Rangan et al. 2014). Some studies have shown progressing effects of PA_s on increasing the plant tolerance to environmental stresses, but physiological responses to Pu as an elicitor has not been clear. Very few studies have been reported that the effect of exogenous PA_s is enhanced for somatic embryogenesis and shoot regeneration in some plants such as *Cucumis anguria* L. (Thiruvengadam and Chung 2015), *Dactylis glomerata* L. (Li and Burritt 2003), and *Nigella damascena* L. (Klimek-Chodacka et al. 2020).

The genus *Allium* in the Liliaceae family secures its place among the top-ranked multipurpose medicinal plants with huge commercial values and food industries (Bhat 2020). There are some reports on the use of this genus in traditional medicine as an antioxidant, antibacterial (Kyung 2012; Pirbalouti 2019), antiviral (Bhat 2020), antiparasitic, antifungal (Bhat 2020), anti-protozoa, anti-inflammatory (Pirbalouti 2019), anticancer (Bhat 2020), anti-diabetic, and beneficial effects on curing the cardiovascular diseases (Lorigooini et al. 2015) and common cold treatments (Pirbalouti 2019).

Allium jesdianum Boiss (*A. jesdianum*) is an important endemic, threatened, and underutilized plant species of Iran (known locally as “Bonsorkh” and “Yazdi onion”) that grows in high altitudes (1800–2600 m) of Zagros mountains in west and northwest regions of Iran (Kalantari et al. 2018; Pirbalouti 2019). This species is eaten raw or as a cooked vegetable or is used as a flavor additive to fresh or cooked foods

in Iran. In the field cultivation, its propagation rate is very slow and it takes years to produce a new variety. In folk medicine of Iran, bulbs and leaves of this plant are used for the treatment of cold, kidney problems, and rheumatic pains (Pirbalouti 2019). Also, other medical properties as antibacterial (Dorosti et al. 2017) and anticancer (Dorosti et al. 2017) are reported for this valuable species. In this regard, many attempts have been made to isolate and exploit the active medicinal compounds from it. Considering the potential value of *A. jesdianum* cell or tissue cultures for biotechnological application, there is an urgent need to develop strategies for the protection and conservation of this genus by the development of its fast seed germination and *in vitro* conservation protocols.

The present work was aimed to study (a) the effects of physical and chemical pre-treatments on seed germination of *A. jesdianum* under *in vitro* culture, (b) the optimization of an efficient procedure for callus induction of *A. jesdianum*, and (c) establish an efficient strategy for enhancement in commercially vital SMs of *A. jesdianum* under elicitation by Pu and MeJ through callus cultures.

2. Materials And Methods

2.1. Seed collection and seed germination experiments

A. jesdianum seeds were gathered from the highland of Sabzkooh province (31° 27' N 52° 40' E mountain about 2,140 m height), in Chaharmahal-o-Bakhtiari region of Iran. *A. jesdianum* seeds were identified by the experts of Botanical Researcher of Department of Natural Resources, Isfahan University of Technology (IUT), Isfahan, Iran. The seeds were surface disinfected with 70% (v/v) ethanol for 90 sec and surface sterilized thoroughly with 2% (v/v) sodium hypochlorite for 15 min. The seeds were then rinsed three times with sterile water to remove the remaining amount of disinfection liquid. The sterilized seeds were then placed on to MS medium (Murashige and Skoog, 1962) (Duchefa, Netherland) containing 0.8% (w/v) agar (Sigma–Aldrich, USA) and 3% (w/v) sucrose and 0.1 mg L⁻¹ myoinositol, with different chemical treatments. The seeds were cultured on MS medium with several chemical treatments, including: plant growth regulators as (1-naphthaleneacetic acid [NAA: Duchefa], 6-Benzylaminopurine [BAP: Duchefa], 2,4-dichlorophenoxyacetic[2,4-D: Duchefa] and gibberellic acid [GA₃: Duchefa]). Other seeds were treated with physical pre-treatments such as NaCl (1 M), cold (4°C for four weeks) and mechanical scarification, then were cultured on MS medium supplemented with different PGRs combinations (Table 1). The seeds were cultured on MS medium as a control group. In order to determine the effect of physical and chemical pretreatments on germination experiments were conducted in the photoperiod of 16/ 8 (light/dark) at 23 ± 2°C for seed germination.

Table 1

Effect of different pre-treatments on germination traits of *A. jesdianum* under *in vitro* culture conditions.

No.	Treatments	GP (%)	GR (mm day ⁻¹)
1	^I MS (Control)	¥52.17 ^b ± 2.3	0.26 ^d ± 0.02
2	MS + GA ₃ (10 mg L ⁻¹)	38.23 ^c ± 1.15	0.1 ^{ef} ± 0.028
4	MS + mechanical scarification	16.67 ^{fgh} ± 1.73	0.06 ^{ef} ± 0.017
3	MS + GA (10 mg L ⁻¹) + mechanical	25 ^e ± 1.15	0.05 ^{ef} ± 0.0057
5	MS + GA ₃ (10 mg L ⁻¹) + cold	20 ^f ± 1.73	0.02 ^{ef} ± 0.005
10	Cold (4 ° C for 3 weeks)	24.66 ^e ± 0.88	0.1 ^{ef} ± 0.03
6	MS + GA ₃ (3 mg L ⁻¹) + Kin (5 mg L ⁻¹)	35.71 ^{cd} ± 1.73	0.1 ^{ef} ± 0.0746
7	MS + 2, 4-D (2 mg L ⁻¹) + NaCl (1M)	20 ^f ± 2.3	0.01 ^f ± 0.002
8	MS + 2,4-D (2 mg L ⁻¹) + mechanical scarification	12.5 ^h ± 1.15	0.04 ^{ef} ± 0.01
9	MS + NAA (1 mg L ⁻¹) + BAP (3 mg L ⁻¹)	58.33 ^a ± 1.73	0.12 ^e ± 0.02
11	MS/2+ (NAA 1 mg L ⁻¹ + BAP 3 mg L ⁻¹) + mechanical scarification	32 ^d ± 1.15	0.72 ^c ± 0.04
12	MS/4 +(NAA 1 mg L ⁻¹ + BAP 3 mg L ⁻¹) + mechanical scarification	0 ⁱ	0 ^f
13	MS/4 + (NAA 3 mg L ⁻¹ + BAP 1 mg L ⁻¹) + mechanical scarification	0 ⁱ	0 ^f
14	MS/4 + (NAA 1 mg L ⁻¹ + BAP 3 mg L ⁻¹) + cold	13.36 ^{gh} ± 1.27	1.01 ^b ± 0.055
15	MS/4 +(NAA 3 mg L ⁻¹ + BAP 1 mg L ⁻¹) + cold	17.5 ^{fg} ± 2.3	1.74 ^a ± 0.11
16	MS/2 + (GA ₃ 10 mg L ⁻¹) + cold	0 ⁱ	0 ^f

I: MS: Murashige and Skoog media; GA₃: gibberellic acid; NAA: naphthalene acetic acid; BAP: benzyl amino purine; 2,4D: 2,4-Dichlorophenoxyacetic acid ; ¥: means ± standard deviations; in each column the means with similar English letter are not significant at *P* < 0.01.

2.2. Germination related traits

To determine the optimal parameters of seed germination, the impacts of certain physical and chemical pretreatments on germination rate and germination percent were evaluated. The germination process was observed by emerging the first radicle. The number of seeds germinated in each pre-treatment was recorded every two days. The germination percentage was calculated using the following equation at the end of the germination period (Amoakoh et al. 2017): Germination percentage (GP) = (number of seeds germinated/number of planted seeds) × 100. The germination rate (GR) was calculated as the sum of the percentage of seed germination in each count by the time elapsed since the beginning of the test (Amoakoh et al. 2017). The mathematical expression of this criteria is expressed as $GR = \sum \text{percentage of germination} / \text{days to the final count}$.

2.3. Optimization of callus induction

For callus induction, two different explants (seed and hypocotyl) were used. The hypocotyl (4–6 mm long) and sterile seed explants were cultured in MS basal medium containing 3% (w/v) sucrose and 0.8% (w/v) agar, which supplemented with various combination of PGRs including different concentrations of auxins and cytokinins including (1 mg L⁻¹ NAA + 3 mg L⁻¹ BAP), (3 mg L⁻¹ NAA + 1 mg L⁻¹ BAP), (1 mg L⁻¹ BAP + 2 mg L⁻¹ 2, 4 D) and (2 mg L⁻¹ BAP + 1 mg L⁻¹ 2, 4 D). The pH of the culture media was adjusted to 5.8 before autoclaving for 20 min at a pressure of 1.06 kg/cm². Explants were cultured on each medium with six replicates under 16 h light/8 h dark photoperiod at 25 ± 2°C for two months in the culture chamber. The callus induction percent (CI) and callus growth rate (CGR) were measured. To calculate CI, callus induction was measured using the following equation: $[(n/N) \times 100]$, at the end of two month from the initial of callus induction. In this formulae, “n” represents the total number of callus explants, and “N” is the total number of cultured explants. Callus growth rate was calculated according to the means of callus growth rates (mm day⁻¹) at every 15 days in 2 months period (Golkar et al 2019).

2.4. Methyl jasmonate and putrescine elicitation

Two independent experiments were done to study the effects of different concentration of MeJ and Pu on phytochemical traits of *A. jesdianum*. Methyl jasmonate (Sigma Aldrich, USA) was dissolved in pure ethanol and filtered using a micro filter of 0.22 µ pore size and then added to the MS medium. Putrescine (Sigma-Aldrich, USA) was prepared in doubled distilled water and the pH was adjusted to 5.8. Filter-sterilized (0.22-µm filter) putrescine were added to the MS medium. For elicitation purposes, after two months of callus induction, the friable and white callus as equal fragments were transferred to new MS media containing 3 mg L⁻¹ of BAP and 1 mg L⁻¹ of NAA under different concentrations of MeJ (0, 25, 50, and 100 µM) and Pu (0, 0.5, and 1 mM). All treatments were incubated in the 16 h light/8 h dark photoperiod at 24 ± 2°C in the culture chamber. The concentrations of MeJ and Put were chosen based on some preliminary experimental works and literature review. Different phytochemical characteristics were measured under MeJ and Put elicitation after 4 weeks of incubation.

2.5. Physiological characteristics

Callus relative fresh weight (RFW) was according to this formulae: $RFW = (FW_2 - FW_1) / \text{days}$, where, FW_1 is the fresh weight of the callus at the initiation of the elicitation and FW_2 is the final fresh weight of the callus at the final day post of elicitation process. CGR was calculated at 15, 30, 45 and 60 day post callus transferring to elicitation media.

2.6. Lipid peroxidation assay

The amount of cell membrane damage was determined by measuring malondialdehyde (MDA) as the end product of peroxidation of membrane lipids. For this assay, 0.2 g of fresh calli were homogenized with 3 mL trichloroacetic acid (TCA) (0.1% w/v) (Merck, Com.). The homogenate was centrifuged for 30 min at 4000 rpm. Then the supernatant (5 mL) was collected and mixed with 4 mL of thiobarbituric acid (TBA) (0.5% w/v) and 20% TCA (w/v). Then, the sample was heated at 95°C for 25 min and then placed on ice bath. Its absorbance was measured at 532 and 600 nm with a spectrophotometer. The content of MDA was calculated by an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as $\mu\text{mol g}^{-1} \text{ FW}$ (Heath and Packer, 1968).

2.7. Methanolic callus extract

At first, 0.5 g of completely dried callus were powdered and added to 10 mL of diethyl ether [$\text{C}_2\text{H}_5)_2\text{O}$]. The mixture was well mixed and stored in a refrigerator for 24 h. For complete evaporation of diethyl ether, the supernatant was transferred to dryer. Then 10 mL of 80% methanol were added to that purified supernatant and then filtered using a 0.4 μm filter.

2.8. Determination of total phenolic content

Based on the Folin-Ciocalteu reagent method described by Singleton et al. (1999) total phenolics content (TPC) was determined. In brief, 0.2 g fresh weight of calli were homogenized with 3 mL CH_3OH (Merck, Com) and centrifuged at 4000 rpm for 25 min. Then, methanolic extract (0.5 mL) was mixed with 2.5 mL of Folin–Ciocalteu reagent (Sigma- Aldrich, Com), followed by the addition of 2.0 mL of 7 % Na CO solution. The mixture was disposed in the dark for 90 min at room temperature. The absorbance of the supernatant was recorded at 765 nm against the reagent blank by a spectrophotometer. The amount of TPC was then quantified by the method of calibration curve using gallic acid (GA_3) (Sigma- Aldrich, Com) as standard.

2.9. Determination of total flavonoids and total favonols content

Total flavonoids (TFD) and total flavonols (TFL) contents were determined spectro- photo metrically using the method of Miliauskas et al. (2004). In brief, 0.2 g fresh calli was ground in 3 mL of methanol and centrifuged at 4000-rpm for 25 min. Then, supernatant was used to assay TFD and TFL contents. For estimation of TFL, 0.5 mL of methanolic extract, 0.5 mL of AlCl_3 (2% w/v) solution and 1.5 mL of sodium acetate (5% w/v) were mixed and after 90 min of incubation at room temperature, the absorbance of mixture was measured at 445 nm. For total flavonoid 0.5 mL of methanolic extract, 125 μL of AlCl_3

solution (%10 w/v) and 125 μ L of CH₃COOK (1M) were mixed and the samples were kept at room temperature for 30 min. A spectrophotometer was used to measure the absorbance of the reaction mixture at 415 nm with. The contents of total flavonoids and flavonols were expressed as mg quercetin (QE) equivalents per gram of fresh mass (mg QE g⁻¹ FW).

2.10. Determination of anthocyanin

At the first step, 2 mL acidified methanol (1% HCl) was used for homogenizing the fresh calli (0.2 g) at room temperature (Hara et al. 2003). After one day, the total extract was centrifuged for 25 min at 4000 rpm. Then, the content of anthocyanin was determined by spectrophotometer at wave length of 511 nm based on the extinction coefficient of Raphanusin (33000 M⁻¹ cm⁻¹).

2.11. Determination of photosynthetic pigments

Carotenoids and chlorophyll contents were determined according to the method of Lichtenthaler and Buschmann (2001). Acetone (80%) (Merck, Com.) was used for these pigments extraction from fresh calluses. For carotenoids, absorption spectra of the extracts were measured at 470, 652 and 665 nm and its concentrations were calculated according to the Lichtenthaler equations.

2.12. DPPH radical scavenging activity

Radical scavenging activities of safflower calli were determined by DPPH assay (Golkar and Taghizadeh, 2018). The methanolic extract (20 μ L) was added to 1 mL of 50 μ M DPPH (Sigma, Aldrich) solution in methanol. The prepared extracts ranged from 0 to 250 μ g mL⁻¹. The mixtures were mixed and incubated in the dark condition for 20 min. The reduction of DPPH absorption was measured at 515 nm. The positive control was ascorbic acid (Sigma, Aldrich). The DPPH radical scavenging activity was calculated using the equation: Inhibition concentration (inhibition percentage: IP %) = (absorbance control – absorbance sample)/(absorbance control) $\times$ 100.

2.13. Statistical analysis

This study elicitation section was carried out as a factorial based on a completely randomized design with six replications. The germination and callus induction experiments were carried out as a completely randomized design with six replications. The analysis of variance done using SAS software version 9.3 (SAS Institute, 2011). Then, mean comparisons ($\pm$ standard deviations) were carried out using Fisher's least significant difference (LSD 5%) test.

3. Results And Discussion

3.1 Seed germination

The optimization of germination is considered as a major gap in *A. jesdianum*. To the best of our knowledge, no previous study has reported the optimization in germination through seed in *A. jesdianum*. Some studies have optimized germination in some *Allium* species (Kamenetsky and Gutterman 2000). Some treatments such as scarification and darkness helped break the dormancy of some genus as *A. suworowii*, *A. aflatunense*, and *A. altissimum* at cold temperatures (Kamenetsky and Gutterman 2000). This study, for the first time, evaluated the effects of different treatments to triggering germination of *A. Jesdianum* under *in vitro* culture. Different treatments including mechanical (scarification), chemical (different combination of plant growth regulators), and thermal conditions (cold temperature) were applied to optimize the germination ratio in *A. jesdianum* (Table 1). Evaluation of germination under different treatments showed extensive variation in GP in about 30–45 days after initiating the treatment. There was a statistically significant difference ($P < 0.01$) in GP and GR between different pre-germination treatments (Table S1). According to the data in Table 1, the maximum GP (58.33%) was observed in seeds treated with treatment including MS + NAA (1 mgL^{-1}) + BAP (3 mg L^{-1}). On the other hand, the minimum of GP (0%) was observed in three treatments including 1) [MS/4 + (NAA 1 mgL^{-1} + BAP 3 mgL^{-1}) + mechanical], 2) [MS/4 + (NAA 3 mgL^{-1} + BAP 1 mgL^{-1}) + mechanical], and 3) and [MS/2 + (GA_3 10 mgL^{-1}) + cold] (Table 1) (Table 1). The differences in the GP of *A. jesdianum* seeds suggests the significant impact of various pretreatments on breaking the seed dormancy in this species. The germination rate varied from $1.74 \text{ (mm day}^{-1}\text{)}$ in the treatment of [MS/4 + (NAA 3 mgL^{-1} + BAP 1 mgL^{-1}) + cold] to $0.01 \text{ (mm day}^{-1}\text{)}$ in treatment of MS + 2, 4D (2 mgL^{-1}) + NaCl (1M) (Table 1). The phenomenon of seed dormancy in seeds of some medicinal plants is usually linked with internal factors such as physiological dormancy or physical barriers that cover the seed coat and/or the enclosed embryo (Abbas et al., 2018). Reviewing the previous investigations, different treatments such as mechanical scarification and cold-water stress were found to be effective for germination in seeds of *A. jesdianum*; however, chemical treatments had the most effect on breaking seed dormancy. Therefore, from the above findings, it can be inferred that dormancy of the seeds of *A. jesdianum* was probably associated with the enclosed covering around the embryo. The seeds under control condition gave a similarly high GP (52.17%) but seeds took a long time to germinate, similar to germination in *Parkia biglobosa* (Abbas et al. 2018). It seems that the ecological background of the natural habitat for seed collection region and harvesting time play predominant roles in seed germination capacity in *A. jesdianum*. The optimized protocol could be used for rapid propagation of this plant under *in vitro* conditions.

3.2. Callus induction

Despite the role of *A. jesdianum* as an important medicinal plant, no research has focused on using *in vitro* cultures in this neglected species. In the present investigation, callus initiation from explants (hypocotyl and seeds) were observed within 14–18 days on all medium compositions (Fig. 1). The induced calli grew into yellow-to-greenish color with a semi-friable structure on four different media. The growth of calli were fast after their induction.

Analysis of variance showed a significant effect of treatments on CI and CGR (Table S2). Callus induction in all tested PGRs and explants were effective (Table 1). The effects of different PGRs (2,4-D, NAA and BAP) and explants (seed and hypocotyl) on callus induction (%) and callus growth rate of *A. jesdianum* is presented at Table 2. As seems, this is the first report regarding the optimized protocol for callus induction in *A. jesdianum*. According to Table 2, MS/2 media supplemented with 1 mgL⁻¹ NAA and 3 mgL⁻¹ BAP were recognized as the best combination for highest callus induction (83.92%) in seeds explants of *A. jesdianum* (Table 2), which showed no significant difference with MS/2 media supplemented with NAA (1 mg L⁻¹) + BAP (3 mgL⁻¹) from hypocotyl explants (Table 2). The least value for CI (42.01%) was denoted to MS/2 media which was supplemented by 2 mgL⁻¹ BAP and 1 mgL⁻¹ 2,4-D (Table 2). These findings showed no callus induction on complete MS media for this species. So it could be resulted that lower concentrations of macro and micro elements was better for cells differentiation from explants in this genus. The good response of hypocotyl explants could be due to homogenous cell nature and easy availability of growth substances to each cell of homogenous tissues, hypocotyl explants showed high potential for callus induction. The combined effects of auxins and cytokinins have been considered as essential factors for callus induction in *A. jesdianum*. Comparison of four different treatment implied that higher concentrations of BAP (as cytokinin group) than 2,4D and NAA (as auxins), could be promotive for more frequency in callus initiation and its growth in *A. jesdianum*. This finding was similar to previous reports in *Allium schoenoprasum* L (Zdravkovic´-Korac et al. 2010) and *Allium Ampeloprasum* L. (Monemi et al. 2014). Different with this finding, necessity of higher doses of auxins rather than cytokinins is emphasized for callus induction in other species in Liliaceae family as *Allium sativum* (Dixit et al. 2013) *Allium cepa* (Bekheet et al. 2006) and micropropagation in *Allium neapolitanum* Cirillo (Stelmaszczuk and Kozak 2013). According to reports of Khar et al. (2005), lonely application of 2,4-D (0.5mgL⁻¹) was optimized for maximum callus formation in *Allium cepa* L. These inconsistencies can be due to the effects of many factors on callus induction in *Allium* species such as genus, genotypes, type of explants, medium composition and plant growth used (Stelmaszczuk and Kozak 2013). The highest (0.48 mm day⁻¹) callus growth rate were observed in MS/2 media supplemented with 1 mgL⁻¹ NAA and 3 mgL⁻¹ BAP (Table 2), but the lowest one (0.18 mm day⁻¹) was observed at MS/2 media which was supported by 2 mgL⁻¹ BAP + 1 mgL⁻¹ 2,4D (Table 2). This implied at a positive interactive effects of these concentrations of BAP and 2,4D on used explant for enhancing the callus growth.

Table 2

The effects of combination of different plant growth regulators on the callus-related traits of *A. jesdianum*

Explant	Plant growth regulators treatments	Callus color and texture	CI (%)	CGR (mmday ⁻¹)
Seed	NAA 1 mg L ⁻¹ + BAP (3 mg L ⁻¹) MS/2+	yellow-greenish/friable	¥83.92 ^a ± 8.80	0.48 ^a ± 0.05
Hypocotyl	MS/2+ BAP (1 mg L ⁻¹) + 2,4D (2 mg L ⁻¹)	yellow/ friable	78.33 ^a ± 7.35	0.23 ^{bc} ± 0.1
Hypocotyl	BAP 2 mg L ⁻¹ + 2,4D (1 mg L ⁻¹) MS/2 +	yellow/ friable	42.01 ^b ± 22.77	0.18 ^c ± 0.08
Hypocotyl	MS/2 + NAA (1 mg L ⁻¹) + BAP (3 mg L ⁻¹)	pale- yellow / friable	86.7 ^a ± 8.12	0.23 ^b ± 0.1
¥: means ± standard deviations; in each column the means with similar English letter are not significant at <i>P</i> < 0.01. CI: callus induction; CGR: callus growth rate.				

3.3. Elicitation effects on different studied traits under callus culture

To synthesize SMs under callus cultures, appropriate techniques need to be developed efficiently (Smetanska 2008). It is well known that *in vitro* culture systems may offer much more efficient production of SMs if culture conditions are well optimized (Mulabagal et al. 2004). Among chemical elicitors, MeJ has been used widely for *in vitro* elicitation studies as callus culture systems (Singh and Dwivedi 2018), but rare studies demonstrated the effects of Pu elicitation on SMs under callus culture (Sudha et al. 2003). The effects of different concentrations of MeJ and Pu on callus-related traits, MDA content, different phenolic compounds, photosynthetic pigments (chlorophyll and carotenoids), and antioxidant activity were evaluated after elicitation. According to the analysis of variance (ANOVA) results, there was a statistically significant difference for all studied traits under elicitation (Table S3).

3.3.1. Callus growth traits

When studying the callus growth, the responses of relative fresh weight and callus growth rate were evaluated to different doses of MeJ and Pu. Overall, a decrease in RFW was observed in response to most of the treatments compared to the control (Table 3). The least content for RFW (0.60) was observed at 100 µM concentration of MeJ rather than control (0.42) (Table 3). The stressor effects of both used elicitors (especially at higher concentrations) reduced the RFW. This is probably due to the inhibitory effects of elicitors on cell growth and capacity of cell osmotic adjustment, which increase the requirement for maintaining turgor of the growing cells, consuming energy, and decrease in callus growth

(Golkar et al. 2019). Similarly, higher concentrations of other elicitors phenylalanine and mevalonic acid in *Zingiber officinale* (El-Nabarawy et al. 2015) and chitosan in safflower (Golkar et al. 2019) reduced the fresh weight of the callus. The CGR showed variation from 0.53 (mm day⁻¹) at 0.5 mM Pu to 0.17 (mm day⁻¹) under 100 µM MeJ (Table 3). For CGR, all elicitations resulted in a significant reduction compared to the control, except for 0.5 (mM) of Pu (Table 3). This result indicates the beneficiary effects of lower concentrations of Pu (0.5 mM) on motivating callus growth. Such a response is consistent with findings of cell growth and callus diameter in *Hypericum perforatum* (Abdollahipoor et al. 2017). It is well established that Pu stimulates not only callus cell extension but also its cell division. The nonsignificant effects of MeJ on callus growth were also reported on cultures of *Zanthoxylum stenophyllum* Hemsl (Biondi et al. 2004). Elicitation by these elicitors changed the color of calli to yellow-to-pale brown and pale brown after the elicitation period. The calli under Pu were more turbid. The elicitation process diminishes the friable structure of the calluses to an approximate extent.

Table 3

The mean comparison for different studied traits of *A. jesdianum* under callus elicitation (methyl jasmonate and putrescine)

Elicitors						
Traits	Control	MeJ (25 μ M)	MeJ (50 μ M)	MeJ (100 μ M)	Pu (0.5 mM)	Pu (1 mM)
Callus related traits						
CGR	0.42 ^b ±0.02	0.39 ^b ± 0.02	0.19 ^d ± 0.02	0.17 ^d ± 0.03	0.53 ^a ± 0.003	0.31 ^c ± 0.01
RFW	1.52 ^a ± 0.08	1.12 ^b ± 0.055	0.8 ^c ± 0.06	0.60 ^d ± 0.027	0.72 ^{dc} ± 0.002	0.64 ^d ± 0.02
Lipid peroxidation						
MDA (μ mol g ⁻¹ FW)	0.18 ^c ± 0.007	0.21 ^c ± 0.05	0.33 ^b ± 0.014	0.47 ^a ± 0.018	0.26 ^{bc} ± 0.033	0.53 ^a ± 0.05
Secondary metabolites						
TPC(mg GAEg ⁻¹ FW)	2.86 ^d ± 0.22	3.79 ^b ± 0.10	6.02 ^a ± 0.20	2.899 ^{cd} ± 0.38	3.54 ^{bc} ± 0.06	3.01 ^{cd} ± 0.20
TFD (mg QEg ⁻¹ FW)	0.20 ^d ± 0.01	0.33 ^{bc} ± 0.02	0.52 ^a ± 0.043	0.39 ^b ± 0.02	0.62 ^a ± 0.048	0.22 ^{cd} ± 0.007
TFL(mg QEg ⁻¹ FW)	0.24 ^c ± 0.008	0.31 ^b ± 0.01	0.39 ^a ± 0.01	0.26 ^c ± 0.008	0.27 ^c ± 0.009	0.25 ^c ± 0.02
Ant (μ mol g ⁻¹ FW)	2.43 ^d ± 0.41	8.99 ^a ± 0.53	7.46 ^b ± 0.22	5.59 ^c ± 0.29	2.93 ^d ± 0.3	2.41 ^d ± 0.1
Photosynthetic pigments						
ChlT (mg g ⁻¹ DW)	0.15 ^b ± 0.01	0.17 ^b ± 0.01	0.27 ^a ± 0.03	0.16 ^b ± 0.01	0.17 ^b ± 0.003	0.29 ^a ± 0.007
Car (mg g ⁻¹ DW)	0.031 ^b ± 0.01	0.038 ^b ± 0.04	0.059 ^a ± 0.01	0.035 ^b ± 0.007	0.045 ^{ab} ± 0.005	0.029 ^b ± 0.002

I: CGR: callus growth rate; RWC: relative water content; MDA: malondialdehyde, TPC: total phenolics content; TFD: total flavonoids, TFL: total flavonols; Ant: Anthocyanin; ChlT: Total chlorophyll; Car; carotenoids; DPPH: 2, 2-diphenyl-1-picrylhydrazyl. *, **: significantly different at the 5% and 1% probability levels, respectively.

3.3.2. Lipid peroxidation

During the elicitation process, some processes such as lipid peroxidation of membranes lead to enhancing antioxidant enzyme activity and the activation of SMs production (Das and Roychoudhury 2014). Lipid peroxidation produces MDA as a sign of oxidative damage to scavenging ROS under elicitation (Das and Roychoudhury 2014). Callus evaluation revealed a significant increase in MDA content under treatments of MeJ and Pu. This increase varied from $0.18 \mu\text{mol g}^{-1}$ FW in the control to $0.53 \mu\text{mol g}^{-1}$ FW in the samples treated with 1 mM Pu (Table 3). The MDA content showed a 2.94-fold increase ($P < 0.05$) in the calluses exposed to 1 mM (Pu) compared with the control cultures. In agreement with these previous studies, exposure to MeJ significantly increased the MDA content in *Panax ginseng*. (Ali et al. 2007) through suspension culture and other elicitors such as chitosan (Golkar et al. 2019) through callus culture. According to the results, MDA content under 1 mM (Pu) was not significantly different from the control (Table 3), indicating participating effects of Pu in sustaining membrane integrity in plant cells (Yiu et al. 2009). It has been reported that exogenous application of Pu reduces the severity of oxidative damage in *Allium fistulosum* by increasing the antioxidant capacity of the plant (Yiu et al. 2009). This may suggest that a higher concentration (> 0.5 mM Pu) could act as a stressor agent on calli cells by increasing the MDA content significantly.

Plants employ a different variety of defense response pathways in response to environmental stresses such as elicitor exposure at cellular levels (Ramirez-Estrada et al. 2016). These cellular responses lead to the more production and accumulation of several SMs such as phenolics and their derivatives (Namdeo 2007; Ramirez-Estrada et al. 2016; Erb and Kliebenstein 2020). In *Allium* species, some studies have reported elicitation effects on some SMs and their content in the whole plant (Pirbalouti 2019; Alwan et al. 2020). However, to the best of our knowledge, no study has evaluated changes in SMs of *A. jesdianum* at a cellular level. According to the findings, using MeJ and Pu resulted in a significant increase in TPC compared to control (Table 3). The highest ($6.02 \text{ mg GAEg}^{-1}$ FW) and the least ($2.86 \text{ mg GAEg}^{-1}$ FW) contents of TPC were observed at treatments of 50 mM MeJ and control treatments, respectively (Table 3). In callus cultures treated with 25, 50, and 100 μM of MeJ, the TPC showed 1.32-, 2.10-, and 1.01-fold increases compared to control, respectively (Table 3). When treated with 50 μM MeJ, callus cultures induced maximum corresponding TPC levels of $6.02 \text{ mg GAEg}^{-1}$ FW, while 100 μM MeJ resulted in inhibited levels with the value $2.89 \text{ mg GAEg}^{-1}$ FW on callus culture (Table 3). Similarly, the enhancing effect of lower concentrations of MeJ on total phenolics induction was reported on other species through callus cultures of *Zanthoxylum stenophyllum* (Biondi et al. 2004) and suspension cultures in *Artemisia absinthium* L. (Ali et al. 2015), *Ajuga bracteosa* (Saeed et al. 2017) *Polygonum multiflorum* (Thiruvengadam, et al. 2016), and *Thevetia peruviana* (Mendoza et al. 2018).

The callus treated with 0.5- and 1-mM Pu indicated 1.23- 1.05-fold increases in TPC content, respectively (Table 3). The effect of *in vitro* elicitation of Pu on TFC accumulation has been rarely reported in literature review; however, the results showed its positive effect on increasing some phenolic acids (e.g., ferulic, caffeoylquinic, and sinapic acids) in *Gardenia Jasminoides* L. by the concentration of 0.5 mg L^{-1} of it (Al-Maameri et al. 2020). The TFD content showed a significant increase compared to the control (0.2 mg QE g^{-1} FW) up to $0.52 \text{ (mg QE g}^{-1} \text{ FW)}$ under 50 mM of MeJ (Table 3). For Pu elicitation, only the lower

concentration level (0.5 mM) showed a significant difference with control (Table 3). This finding was like the positive effects of MeJ increase in the total flavonoids of *Hypericum perforatum* (Wang et al. 2015) and *Thevetia peruviana* (Mendoza et al. 2018) through callus culture and cell suspension culture of *Taxus baccata* (Jalalpour et al. 2014) and *Ajuga bracteosa* (Saeed et al. 2017). Under *in vitro* elicitations, an increase in TFD content suggested higher rates of its synthesis and accumulation. This can be attributed to the possible positive effects of elicitors on the enhanced expression of the genes, contributing to the synthesis pathway of total flavonoids (e.g., phenylpropanoid) (Talukder et al. 2016).

The content of TFL showed variation from 0.24 (mg QEg⁻¹ FW) in control to 0.39 (mg QE g⁻¹ FW) under 50 µM MeJ (Table 3). No significant differences were observed between the two levels of Pu with control for the TFL content. It may be concluded that Pu elicitation did not provide adequate efficiency for increasing TFL in calluses of *A. jesdianum* and thus other concentrations should be applied. Reviewing the literature shows no study on the elicitation effects of Pu (as a compound in polyamines) on TFD and TPC under callus culture. Polyamines as putrescine had a significant effect on the potential of plant regeneration and accumulation of flavonols, hydroxybenzoic, and hydroxycinnamic acid derivatives in *Cucumis anguria* L. (Thiruvengadam and Chung 2015). Similarly, the effect of PAs increased the amount of withanolides in *Withania somnifera* plants that were regenerated under *in vitro* conditions (Sivanandhan et al. 2011). In jojoba plants, putrescine (50 ppm) significantly increased total phenolics content, total flavonoids, tannin content, and antioxidant activity (Taha et al. 2015). Ghareib and Hanfy Ahmed (2005) reported that putrescine foliar application (1–2 ppm) improved total phenolic compounds in *Pisum sativum* shoots (Gharib and Hanafy Ahmed 2005).

Anthocyanins are a group of water-soluble flavonoids that are produced at one end of the flavonoid biosynthesis pathway in the cytoplasm and enter the cells actively or separately by glutathione pump (Castañeda-Ovando et al. 2009). AnthocyaninS production is affected under different environmental elicitors (Castañeda-Ovando, et al. 2009). In the present study, elicitation by MeJ and Pu increased the *in vitro* accumulation of anthocyanin in the *A. jesdianum* calli (Table 3). Its values varied from 2.43 µmol g⁻¹ FW at control condition to 8.99 µmol g⁻¹ FW at 25 mM MeJ. Higher concentrations of MeJ (50 and 100 µM) showed a descending trend for Ant rather than 25 µM (MeJ) (Table 3). The results of this study are consistent with those of Ram et al. (2013) and Suan See et al. (2011), who reported that the quantity of anthocyanin accumulation depends on MeJ concentration in *Rosa hybrida* and *Melastoma malabathricum*, respectively. The additional result obtained indicated that a concentration of 50 µM MeJ was the best that could induce the highest pigment production and was used in this study.

According to Suan See et al. (2011), biosynthesis and accumulation of anthocyanin could be stimulated by MeJ elicitation via induction of particular enzymes such as catalyzes. Besides, synthetic pathways for anthocyanin biosynthesis or increase the gene(s) expression of this specialized metabolite biosynthesis are triggered by MeJ elicitation (Ram et al. 2011). The concentration of 0.5 mM Pu led to a 1.21-fold increase in anthocyanins rather than control, but the higher concentration (1 mM Pu) did not have any significant effect compared to the control (Table 3). This finding was similar to the elicitation effects of Pu on anthocyanin production of *Daucus carota* under callus culture (Sudha et al. 2003). Therefore, at

higher concentrations, both elicitors had an inhibitory or non-significant effect on Ant content in *A. jesdianum*. Among these two elicitors, MeJ acted as a better inducer of anthocyanin biosynthesis in *A. jesdianum* callus cultures than Pu. The novel results from the present study show that *callus* cultures of *A. jesdianum* under elicitation could be considered as an efficient way for these valuable pigments production.

A variety of environmental factors, as elicitors, affect the synthesis and accumulation of phytochemical compounds such as phenolic compounds, which are the best oxygen radical scavengers (Dias et al. 2016). The significant increase in phenolics content may be compromised by the inducing effect on triggering effects of MeJ and Pu on signal transduction pathways. These effects, in turn, speed up enzyme catalysis and lead to the formation of specific compounds such as flavonoids, flavonols, and anthocyanins in *A. jesdianum*.

Totally, the medium concentration of MeJ (50 μM) and a lower concentration of Pu (0.5 mM) showed an enhancement effect on the contents of phenolics, flavonoids, flavonols, and anthocyanin. This result could be attributed to higher production of trans-cinnamate as a product of L-phenylalanine deamination by phenylalanine ammonia-lyase enzyme and more conversion of trans-cinnamate to different SMs under lower concentrations of elicitors (Alrawaiq and Abdullah 2014). In this regard, the following factors have significant effects on the trends of the elicitation process: the differences in chemical characteristics of elicitors, its preparation source and concentration, exposure time of elicitation, age and type of explant for culture, growth regulations in media culture and nutrition composition, and its interaction with plant species and genotypes (Namdeo 2007; Narayani and Srivastava 2017). Considering that the production of plant SMs is strongly controlled by signaling events and environmental factors, a fine and one-by-one analysis might be required to obtain a deeper understanding of the type of their modulation effects in different plant species at the callus level.

3.3.4. Photosynthetic pigments

Information about photosynthetic pigments (chlorophyll and carotenoids) action under *in vitro* elicitation provides useful information about their efficiency on photochemical processes at this stage. According to the findings, these two elicitors provided an effective strategy for enhanced production of these pigments under callus cultures of *A. jesdianum*. Total chlorophyll content demonstrated a significant increase under 50 μM MeJ (0.27 mg g^{-1} DW) and 1 mM Pu (0.29 mg g^{-1} DW) (Table 3) compared to the control (Table 3). The highest (0.27 mg g^{-1} DW) and the least (0.002 mg g^{-1} DW) contents for ChlT were for 50 μM (MeJ) and 1 mM Pu, respectively (Table 3). This increase under 50 μM MeJ could be due to chlorophyllase activity stimulation under MeJ elicitation (Hanaka et al. 2015). Few studies have shown the changes of carotenoids content by cell tissue culture strategies. The effects of MeJ have been evaluated on the callus culture of *Cleome rosea* (de Rocha et al. 2015); nevertheless, no studies have been done about Pu elicitation on carotenoids content in callus culture. In carotenoids, elicitation by MeJ at 50 μM showed a significant increase (1.9-fold) compared to control (Table 3). Other concentrations of MeJ and Pu elicitation showed no significant difference with control (Table 3). According to our

literature, no study demonstrated elicitation effects of Pu on carotenoids content in callus level. According to this finding, selected dosages of Pu in these studies did not stimulate the increase in carotenoids at the callus level. Although the responses of accumulation of carotenoids were dependent on both MeJ and Pu concentrations, more studies are needed to find the exact effects of Pu on activation of carotenoids pathway under cellular level.

.3.5. DPPH activity

To neutralize the effect of stresses caused through the elicitation process, the plant cells employ antioxidative activities in different ways such as enhancement of SMs (Golkar et al. 2019; Nabi et al. 2021). In this study, antioxidant activity was determined as DPPH radical scavenging activity. The highest (86.40%) and the least (66.31%) radical scavenging activities were found in callus cultures treated with 100 mM MeJ and control conditions, respectively (Fig. 2). Callus cultures treated with 50 mM and 100 mM of MeJ showed no significant difference for reactive scavenging ability through the DPPH method. The DPPH activity under two different concentrations of Pu showed no significant effect for DPPH activity compared to control conditions (Fig. 2). It was observed that *A. jesdianum* calluses had a dose-dependent DPPH radical scavenging activity under MeJ elicitation (Fig. 2). This result suggests *A. jesdianum* is a good free radical scavenger under elicitation. Similarly, the increase in antioxidant activity of callus cells (e.g., DPPH method) was observed under MeJ elicitation in different cell culture techniques (Ali et al. 2007; Ho et al. 2020).

3.4. Correlation between different traits

The simple correlation among studied traits is presented in Table 4. A negative and significant correlation was found between RWC with TFD (-0.46**) and TFL (-0.35**). The lipid peroxidation content as MDA showed a negative and significant correlation with RWC (-0.64**) and CGR (-0.53**) (Table 5). This finding shows the importance of cell wall integrity on retaining cell water content and its growth under cellular conditions. The antioxidant activity (DPPH method) showed positive and significant correlation with TFL (0.43**), TPC (0.33**), and ChIT (0.37**). The antioxidant activity through the DPPH method was found to be mainly TPC (0.33**) and TFL (0.43**), depending on callus cultures treated with different elicitors. Furthermore, a positive correlation between TPC and TFL with DPPH suggests that the elicitation process increased the antioxidant activity of *A. jesdianum* through more accumulation of TPC and TFL in callus culture.

Table 4

The simple correlation coefficients for different morpho- biochemical traits under elicitation by chitosan and gibberellic acid.

	RFW	CGR	DPPH	TFD	TFL	TPC	ChIT	Car	MDA	Ant
RFW ^I	1									
CGR	0.39*	1								
DPPH	-0.31*	-0.59**	1							
TFD	-0.46**	0.04	0.17	1						
TFL	-0.35*	-0.45**	0.43**	0.32*	1					
TPC	-0.09	-0.26	0.33*	0.04	-0.2	1				
ChIT	0.16	-0.44**	0.37*	0.05	0.17	0.61**	1			
Car	-0.09	0.24	-0.2	0.17	-0.03	0	-0.16	1		
MDA	-0.64**	-0.52**	0.28	0.05	0.15	-0.11	-0.31*	-0.02	1	
Ant	0.87**	0.18	-0.25	-0.46**	-0.29	0.16	0.43**	-0.19	-0.66**	1
I: RFW: relative fresh weight; CGR: callus growth rate; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; TFD: total flavonoids, TFL: total flavonols; TPC: total phenolics content; ChIT: Total chlorophyll; Car: Carotenoids; MDA: malondialdehyde; Ant: Anthocyanins. *, **: significantly different at the 5% and 1% probability levels, respectively.										

4. Conclusion

Plant cell cultures have been perceived as an attractive method for producing SMs under controlled conditions. This study optimized seed germination, callus induction, and the effects of two different elicitors (MeJ and Pu) on the enhancement of different phenolics and callus related traits in *A. jesdianum*, by the first. The findings showed higher efficiency of MeJ than Pu for increasing different secondary metabolites and antioxidant activity in calli of *A. jesdianum*. The superiority of Pu elicitation was increasing callus growth and the contents of two studied pigments (carotenoids and chlorophyll). This study suggests evaluating other different concentrations of Pu for callus elicitation. The concentration of 50 µM MeJ showed the best selective dose for enhancing the contents of phenolic compounds in *A. jesdianum* through callus culture. This study could lead to introducing a new way to improve the production of the beneficial phytochemical compounds from undifferentiated callus cells in the threatened medicinal plant of *A. jesdianum*.

Abbreviations

BAP : 6-Benzylaminopurine; **2,4-D**: 2,4-Dichlorophenoxyacetic acid; **FW**: Fresh weight; **DW**: Dry weight; **NAA**: α -Naphthaleneacetic acid; **MS**: Murashige and Skoog; **MeJ**: Methyl jasmonate; **Pu**: Putrescine; **TPC**: total phenolics content; **TFD**: total flavonoids; **TFL**: total flavonols; **DPPH**: 2,2-diphenyl-1-picrylhydrazyl.

Declarations

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Declarations

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Figures

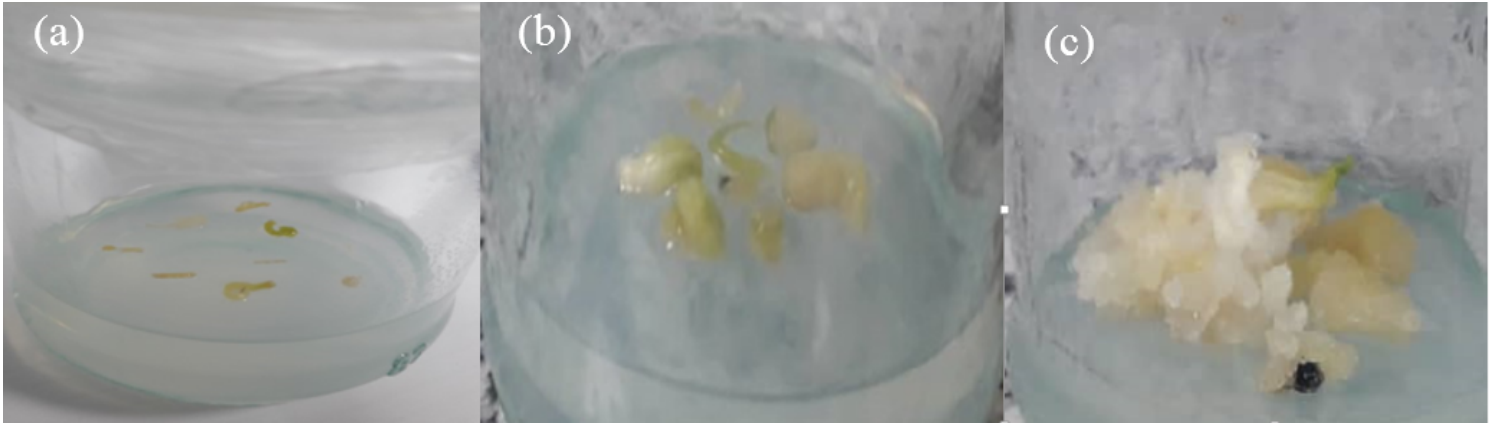


Figure 1

Hypocotyl culture (a) and callus induction from hypocotyl (b) and seed (c) explants of *A. jesdianum* on Murashige and Skoog medium supplemented with 1.0 (mg L⁻¹) 2,4- D+1.0 (mg L⁻¹) Kin (a), 1 (mg L⁻¹) NAA+ 0. 5 (mg L⁻¹) BAP (b).

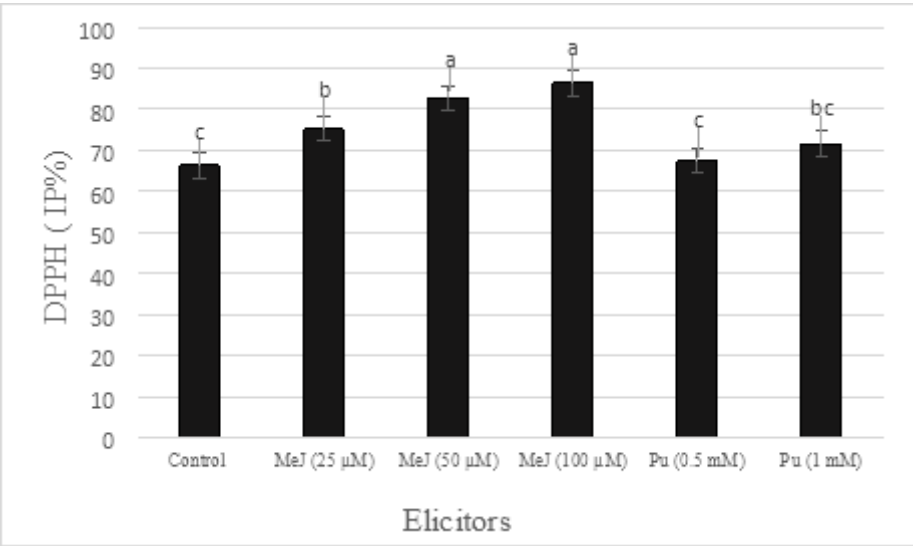


Figure 2

The means for DPPH activity (± standard deviation) in different elicitors (methyl jasmonate and putrescine) under in vitro callus culture in *A. jesdianum*.