Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 2 (6) May 2013: 89- 94

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com

ORIGINAL ARTICLE

Methyl Jasmonate and Salicylic Acid Effects on the Dopamine Production in Hairy Cultures of *Portulaca oleracea* (Purslan)

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ABSTRACT
Elicitors could be used as enhancers of plant-secondary-metabolite synthesis and could play an important role in biosynthetic pathways to enhanced production of commercially important compounds. In this work Purslan hairy roots were cultured for 4 weeks in 250 ml Erlen shake flasks containing ½ MS liquid medium, then the effect of different concentrations of Methyl Jasmonate (MJ) elicitor and Salicylic acid (SA) was investigated on accumulative dopamine in these hairy roots. The different concentrations used for MJ were 0 (control) 100,150, 200 Mmol, and for SA were 0 (control) 125,250 and 500 Mmol. When the final growth phase was completed the treatments were added after 48 h of finishing a 28-day period. It is found that different concentrations of MJ, especially 100 Mmol concentration are an important factor regarding the increase of producing dopamine secondary metabolite, so that 100 Mmol concentration makes the dopamine 4.3 fold times more than treatment control. On the other hand, there could be no significant effect of the different concentrations of SA, on dopamine accumulation compared to the control treatments.
Key words: *Purslan, hairy root, methyl jasmonate, salicylic acid, dopamine*

Received 12.04.2013 Accepted 05.05.2013

INTRODUCTION

Medicinal plants have been considered as the most important curative sources for thousands of years [1]. They are treated as a source of many new diseases and ¼ of drugs contain herbal essences or blends which are made from plants [2].
As biotechnology progresses, natural herbal achievements can be vital sources for chemical drugs in different aspects. Medicinal plant tissue culture has been introduced as a suitable method to produce valuable secondary metabolites [3]. Recently hairy roots culture has been introduced as a constant source to produce secondary metabolites. Hairy roots are made by *Agrobacterium rhaizogenes*. The main advantage of hairy roots culture is that, it has a high potency to produce secondary metabolites in contrast with basic plants [4].
Elicitation has been proved to be effective way to increase secondary metabolite production in hairy roots cultures. A number of elicitors and precursors such as MJ and SA have been used successfully for enhancing production of secondary. Jasmonic acid and its related compounds (all called JA signals here) have long been observed to be transducers of elicitor signals for the production of plant secondary metabolites [5]. Salicylic acid (SA) is a well-known inducer of plant systematic acquired resistance (SAR) in plant–pathogen interaction, but it is not a universal inducer for production of plant defensive metabolites. In *Rubia cordifolia* cultures, both MJ and SA strongly induced anthraquinone phytoalexin production [6].
purslan is herbaceous and succulent plant, which it has cultured in south of Iran as vegetable [7]. It has abilities of the anti-bacterial, -virus, -antherasis, -caducity, -diabetes, and enhancing immunity. Recent studies indicated that the consumption of purslan may help to reduce the occurrence of cancer and heart diseases [8]. purslan contains a variety of bioconstituents, including catecholamines, l-noradrenaline, dopamine, l-dopa, α-amyrin, β-amyrin, and portuloside A. Dopamine (4-(2-aminoethyl)-1,2-benzenediol), which has stimulating effect on the nervous system and used for treatment of Parkinson's disease, congestive heart failure, and myocardial dysfunction [9]. In this work, the effect of MJ elicitor and SA on dopamine accumulation in Purslan hairy roots has been investigated.

MATERIALS AND METHODS

Plant Material

The purslan seeds were collected from medicinal plant Bu-Ali Sina garden of Hamedan city, Iran. Seeds were surface- sterilized in 70% ethanol for 45 seconds, then washed with sterile water for several times. After this stage, Seeds were transferred to 2% sodium hypochlorite solution for 10 min and then rinsed three times with sterile distilled water. For seedling, produce the seed transfer to sterilized Petri dishes with wet filter paper. After Two weeks old from the growth of seedling, cotyledon leaves were used as explants.

Bacterial Strains and Hairy Root Induction

Wild type *Agrobacterium rhzogenes* strain ATCC 15834 for induction of hairy roots was used. The strain was provided from Karaj Biotechnological Research Center. In the beginning, cotyledon leaves were cut from the plant and then they were soaked for 10 minutes in LB medium (Yeast extract 5 g/l, Tripton 10 g/l, NaCl 10 g/l, and agar 15 g/l) containing *Agrobacterium*. For drying of explants, sterilized filter paper was used. After 2 days co-cultivation of explants with *A. rhizogenes* at 24 ˚C in the 1/2 MS medium and dark condition, the explants were transferred on to 1/2 MS medium containing 300 mg/l cefotaxime (filter-sterile, added to the medium) to kill the residual *Agrobacterium*. Controls consisted of explants treated similarly except that they were not co-cultivated with *A. rhizogenes*.

Confirmation of Transformation and Isolation Genomic DNA

Genomic DNA from hairy roots and normal roots (control) of purslan was isolated according to the Cai et al [10]. Plasmid DNA from *A. rhizogenes* strain ATCC 15834 was extracted as described by Sambrook et al [11] by alkaline lysis method. To confirm transformation, PCR analysis was performed by using of specific primers for *rolB* gene amplification.

Extraction and Isolation of Dopamine From Hairy Roots

Dopamine was extracted according to Chen et al, [8]. To find the exact amount of dopamine, Kaniver HPLC system (Berlin, Germany) with (25 cm × 4.6mm) C18 column and UV detector was used. The mobile phase was 0.02 *M* KH_2 PO_4 solution (95%), Acetonitrile (5%), PH 3.0, 280 nm for detection wave length. Dopamine was obtained from Caspian Tamin pharmacy company (Gilan, Iran).

Preparation of Elicitors

Two elicitor including methyle jasmonate, filter- sterilized (with a purity of 95%, purchased from Sigma) with the concentration of 0 (control), 100, 150, 200 Mmol and also Salicylic acid (purchased from Sigma) with the concentration of 0 (control), 125, 250 and 500 Mmol were used on four-week old hairy roots to evaluate their effects dopamine production. Ethanol (96%) and NaOH (0.1 N) were used to dissolve MJ and SA respectively.

Statistical Analysis

Elicitors were used to evaluate the amount of dopamine 4 weeks after culturing hairy roots. Treated roots with MJ and SA were harvested 48 h after finishing a 4-week period. Untreated roots with elicitors were used as control treatment. All the experiments were repeated three times. To draw chart, excel 2007 was used. Significance was determined by analysis of variance (ANOVA) using SAS software (Version 9.1). The mean of the treatment were compared using Duncan´s multiple range test at the level of 5%.

RESULTS AND DISCUSSION

Hairy roots were induced from cotyledon leaves explants of purslan when infected with *A.rhizogenes* (Figures a&b). The hairy roots from leave explants were thin with faster growth rate and they have branches unlike normal roots. Roots have some features such as quick growth, a lot of lateral branches and showed hormone autotrophy.

The main advantage of using hairy root cultures is due to their ability to grow in defined basal media without supplementation of phytohormones and due to their differentiated nature, they show genetic stability and tend to produce high levels of secondary metabolites characteristic of the species [12]. Hairy root cultures for produce secondary metabolite in large number of Pharmaceutical plants such as *Digitalis lanata* [13], *Papaver somnifera* and *Opium poppy* [14], *A*rtemisia *annua* [15], *Solanum aviculare* [16], *Panax ginseng* [17] *Datura stramonium* [18] and many others has been done so far.

The presence of the *rolB* gene in the hairy roots was tested by PCR amplification of the DNA using *rolB* forward and reverses primers. *A. rhizogenes* served as the positive control and DNA from the non-transformed seedlings roots served as the negative control (Figure 2).

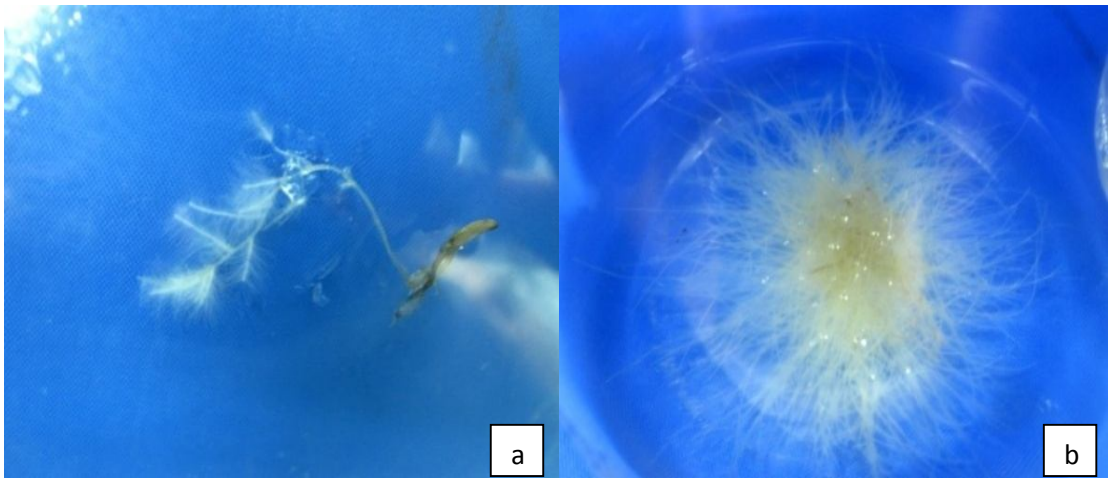

Fig 1: hairy root induction (a) and extension of them in 1/2 MS liquid medium (b).

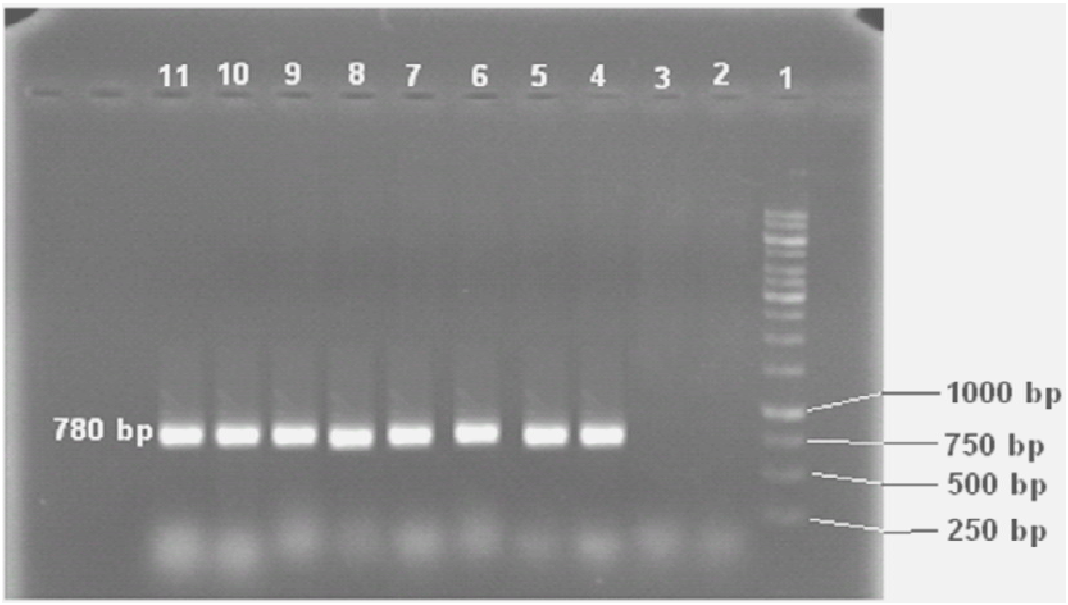

Fig 2: PCR amplification of a 780 bp fragment of the rolB gene using hairy root derived DNA. Lane 1 = molecular weight marker (1000 bp ladder); lane 2-7= transformed roots; lane 8&9 = none transformed roots (negative control) and lane 10&11= *Agrobacterium rhizogenes* DNA (positive control).

The presence of the rolB gene in the hairy root lines was tested by PCR amplification of the DNA using rolB forward and reverses primers. *A. rhizogenes* (colony PCR) served as the positive control and DNA from the non-transformed seedlings roots served as the negative control. All transformants showed presence of the 780 bp rolB amplified product and no rolB gene activity was found in control tissue (Figure 2).

The results of MJ treatment showed that, the different concentrations of this compound had significant effect on dopamine production. MJ at the concentration of 100 Mmol had the highest effect (about 4.3 times of original amount). SA treatment had no significant effect on the amount of Dopamine compared to the control (Figures 3&4).

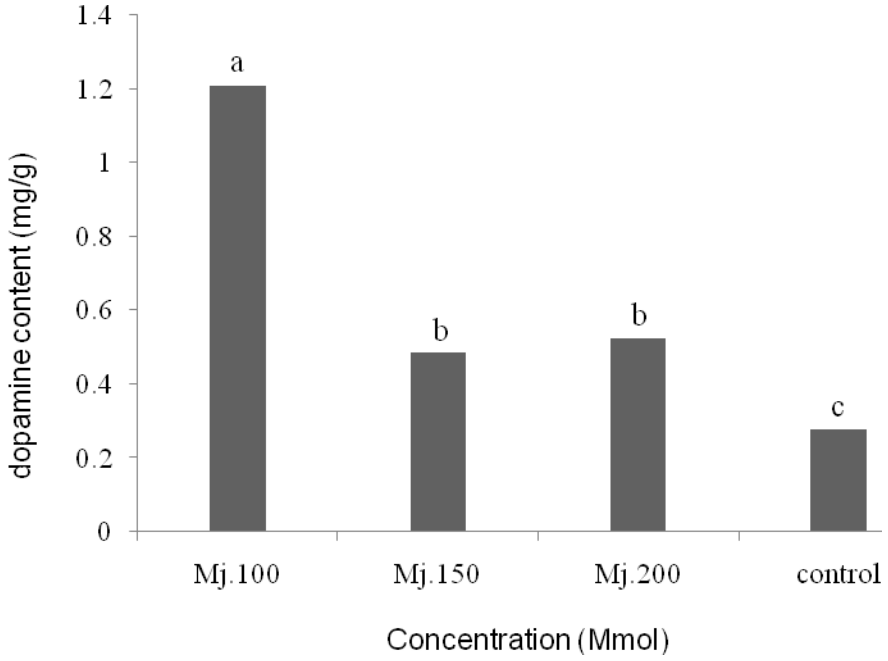

Fig 3: The effect of different concentration of MJ on amount of dopamine
Data are means of three measurements. The mean of the treatments were compared using Duncan´s multiple range test at the level of 5% and columns with the same letter are not significantly different.

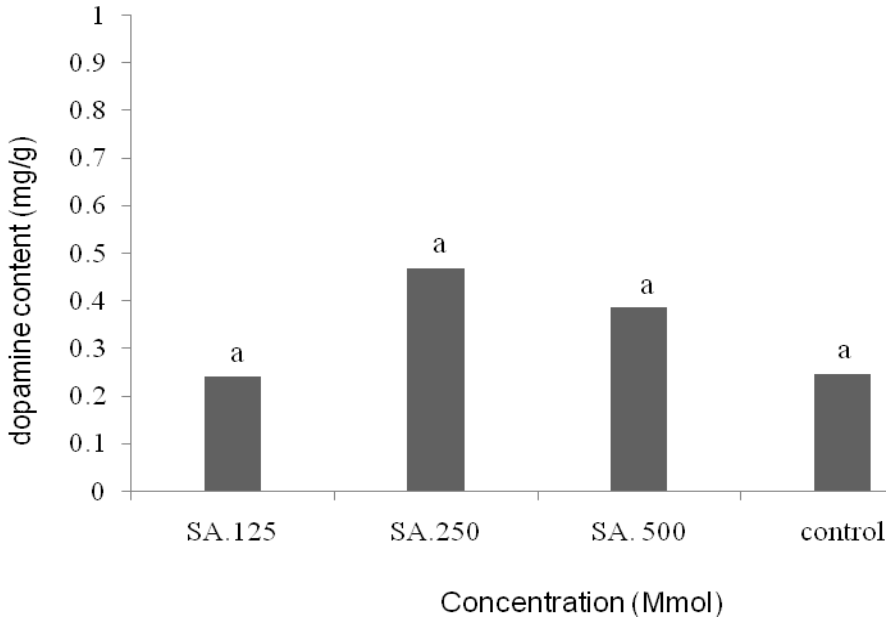

Fig 4: The effect of different concentration of SA on amount of dopamine.
Data are means of three measurements. The mean of the treatments were compared using Duncan´s multiple range test at the level of 5% and columns with the same letter are not significantly different.

In the past years, numerous strategies such cell line selection, medium optimization, cell immobilization, the use differentiated cells, elicitation and more recently metabolic engineering have been developed to improve the productivity of plant cell culture [19]. The accumulation of secondary metabolite in plants is part of the defense response against pathogenic attack, which is triggered and activated by elicitors, the signal compounds of plant defense responses [20]. A number of elicitor

and precursors such as methyl jasmonate have been used successfully for enhancing production of secondary metabolites such as taxoids, baccatins, ginsenosides during cell culture of many plant species [21]. Salicylic acid and jasmonic acid (JA) are seen as the key signals for defense gene expression (22). It was generally thought that SA regulates resistance to fungal, bacterial, and viral pathogens (23), whereas JA induces the production of various proteins via the octadecanoid pathway that provides plants with resistance against insects (24). SA and JA, as well as synthetic mimics, can be applied exogenously to plants to induce the same metabolic changes that lead to resistance as induced by pathogens and insects (25). Therefore, the treatment of biotic and/or biotic elicitors has been a useful strategy to enhance secondary metabolite production in hairy root cultures. In the present study, it was found that addition of MJ into the culture medium enhanced the production of dopamine. Apparently, MJ was found to suitable production of dopamine compared to SA, and the results for MJ were quite promising. Effect of different concentrations of MJ on the amount of dopamine is quite evident, So that the amount of dopamine is reduced with increasing concentrations of MJ. Thus, it can be concluded that the use of appropriate amount of signal compounds can increase the productivity of dopamine in hairy root cultures of purslan.

CONCLUSION

The effect of MJ and SA on the accumulation and biosynthesis of dopamine by hairy root cultures of purslan was investigated. Elicitation resulted for MJ in about 4.3 time higher amounts of non-elicitated (none treated with MJ) hairy roots. The highest yield was achieved by 100µm concentration of MJ after 48 hours of finishing a 28-day period. SA shows that is not appropriate elicitor to increases dopamine in this study. MJ exerted a high stimulating effect on the production of dopamine in purslan hairy roots. The results from this study indicate that MJ elicitation strategy was quite useful to improve the yield of dopamine in *in vitro* hairy root cultures of purslan.

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How to Cite this Article
Y.Ahmadi M. , K. Piri, B.Bahramnejad, P. Habibi. (2013). Methyl Jasmonate and Salicylic Acid Effects on the Dopamine Production in Hairy Cultures of *Portulaca oleracea* (Purslan). *Bull. Env. Pharmacol. Life Sci.,* Vol 2 (6): 89-94.