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Analysis of the differential effects of methyl jasmonate on induction of adventitious roots and antioxidant potential in *Artimisia scoparia*

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Abstract

Artemisia scoparia is an important medicinal plant, having plenty of pharmacological applications. In this study the comparative effects of auxins and elicitors were investigated on the induction, biomass formation and antioxidant potential of adventitious roots (AR) in leaf explants on solid MS media, followed by studying the growth kinetics in suspension cultures. It was observed that among the different auxins employed in vitro, NAA showed highest induction frequency (57%) when employed at 1.0mg/L on solid MS media. Within the elicitors, 0.5mg/L Me-J inducted maximum roots induction frequency (58%), while 1.0mg/LPAA resulted in 43% induction frequency. During AR suspension cultures, in response to 1.0mg/L NAA, shorter lag and stationary phases of 8 and 4 days respectively were observed in the growth curve. The log phase was quite longer consisting of 24 days, wherein highest biomass (7.36g/L; DW) was accumulated on 28 day of the growth curve. Within the different growth phases, day 20 (log phase) was observed to accumulate higher antioxidant activity in the AR suspension cultures than other growth stages. Lesser antioxidant activity was determined in the stationary phase of cell cycle. Highest antioxidant potential (87%) was recorded in the AR suspension cultures raised in presence of 0.5mg/L Me-J. This protocol has the potential for commercial production of antioxidants enriched biomass of *A. scoparia*.

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Introduction

Artemisia L. (Asteraceae) is a large, diverse, and widely distributed genus with more than 500 species. This genus comprises of bitter, aromatic herbs or shrubs, which are famed for the medicinal chemical constituents in their essential oils (Movafeghi *et al.,* 2010). In Pakistan Artemisia species are distributed in arid and semiarid areas of Baluchistan, KPK, Northern Punjab & Kashmir (Ashraf *et al.,* 2010). Artemisia species have been shown to be effective in the treatment of a number of diseases including malaria, cancer, hepatitis, and for infections caused by bacteria, fungi, and viruses (Tan *et al.,* 1998). In traditional ethno medicinal uses, many species of the genus Artemisia have been highlighted for being active as anti-hypoglycemic, anti- hypolipidamic, anti- diuretic, ant-ulcer & anti-inflammatory (Yousaf *et al.,* 2019). *Artemisia scoparia* is one of the medicinally significant species of the genus. It is a faintly scented, slender branched and biennial herb (Movafeghi *et al.,* 2010). The plant extracts of its different parts have shown multiple biological activities for instance anti-cholesterolemic, anti-pyretic, anti-septic, anti-bacterial, cholagogue, diuretic, purgative, vasodilator & anti-asthemic (Fang *et al.,* 2003; Singh *et al.,* 2009). Further, the essential oils derived from *A. scoparia* have proven positive effects as insecticidal and anti-microbial (Kaur *et al.,* 2012). Phytochemistry of *A. scoparia* has shown presence of diverse medicinally active compounds such as flavonoids, phenolics, coumarins and essential oils (Yousaf *et al.,* 2019). Furthermore the major bioactive compounds constitute scoparoic acid, scoparone and artemilanosterol, those have been reported as the main insecticidal and antimalarial compounds, respectively from *A. scoparia* (Fang *et al.,* 2003;Sharma and Ali, 1998). Wild grown medicinal plants are affected for quality production of plant biomass and medicinal metabolites by several factors including the different geographic climates, environmental conditions, pests' manifestation, nutrients deficiency and pesticide contamination (Khan *et al.,* 2013). Healthy plant biomass enriched with potent medicinal products for pharmaceutical preparation can be produced by using plant cell culture technologies (Kazmi *et al.,* 2019a; Khan *et al.,* 2019a). Among the different plant cell culture technologies, adventitious roots (AR) culture technology has so many advantages over the other cell culture types, for the commercial production of plant biomass in large quantity under controlled, aseptic *in vitro* growth conditions, round a year in any season and in limited time and space.

AR are considered as the efficient bio factories for the production of a variety of medicinally important compounds in bulk (Ali *et al.,* 2019; Saeed *et al.,* 2017). Due to the faster growth rate, genetic fidelity, easy maintenance and robustness AR culture is the most preferred strategy for production of more biomass with sustainable metabolites in limited time and space (Khan *et al.,* 2017; Ali *et al.,* 2019; Saeed *et al.,* 2017). Further, it discourages the production of toxic chemicals like opines, as produced normally in case of hairy roots culture (Khan *et al.,* 2015b). AR culture technology can be manipulated for enhancing the yield of biomass and phytochemicals by adopting several *in vitro* stress inducing strategies including elicitation, temperature, light, UV irradiation, pathogen attack and herbicide treatment etc (Khan *et al.,* 2019b; Kazmi *et al.,* 2019b).

Through *in vitro* application of chemical elicitors such as methyl jasmonate (Me-J), different metabolic & physiological pathways in the plant cell can be switched on for the expression of pharmacologically active metabolites through upregulation of the distinct genes involved in the metabolic reactions (Kazmi *et al.,* 2019b; Khan *et al.,* 2017; Ali *et al.,* 2019; Saeed *et al.,* 2017). During exposure of plant cell to any elicitor, stress conditions are initiated which activate plant cell's NADPH oxidase enzyme system, generate reactive oxygen species (ROS), nitrogen species and influence accumulation of secondary metabolites (Rani *et al.,* 2017; Saeed *et al.,* 2017). In the present study for the first time, effects of elicitors including methyl jasmonate (Me-J) and phenyl acetic acid (PAA) in comparison to auxins were investigated on adventitious roots growth

parameters and antioxidant potential in the medicinal plant *A. scoparia*.

Materials and methods

Plant material and sterilization

Seeds of field grown plantlets of *A. scoparia* were collected from Swat (Khyber Pakhtunkhwa). The seed were surface sterilized to produce contamination-free germplasm as a source of explants, to be used in induction and proliferation of adventitious roots. During surface sterilization, seeds were first rinsed in 70% ethanol and then with mercuric chloride solution ($HgCl_2$), and finally washed with distilled water for three times. The sterilized seeds were incubated on MS (Murashige and Skoog, 1962) medium, containing 3.0% sucrose and 0.8% agar. Conditions of growth room were 16 h. light & 8 h. dark photoperiod with light intensity of ~40µM m-2 sec-1, 25±1°C temperature and 70% relative humidity.

Preparation of explants and induction of adventitious roots (AR)

After one month the healthy germinated seedlings were taken from the flasks. Leaf, stem and root sections were excised from the germinated seedlings and were cut into appropriate sizes of 0.5cm, 0.3cm and 0.4cm respectively. These explants were surface sterilized by treatment with 70% ethanol followed by mild treatment with 0.5% mercuric chloride solution ($Hg\ Cl_2$), and in last washing with sterile distilled water. In order to find suitable type of explant for AR formation in *A. scoparia*, the leaf, stem and root explants were cultured on MS solid medium, supplemented with 0.5mg/L α-naphthalene acetic acid (NAA) and maintained under growth room conditions for a month.

Evaluation of the effects of auxins in comparison to elicitors on AR growth parameters on solid MS media

Leaf explants were selected for subsequent experiments on induction and biomass formation of AR in *A. scoparia*. Different doses 0.5-1.5mg/L of the auxins, 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthalene acetic acid (NAA) and Indole-acetic acid (IAA) and the elicitors methyl jasmonate (Me-J) and Phenyl acetic acid (PAA) were used for induction of AR in explants. Leaf explants were cultured on MS media containing 3.0% sucrose (w/v) and solidified at 0.8% (w/v) agar in 150ml conical flask, supplemented with the varying levels of auxins and elicitors. The pH of media was adjusted at 5.8 followed by autoclaving at 121°C for 20 min at 1. atm pressure. MS medium devoid of any auxin and elicitor (MS0) was used as control treatment. Data on growth parameters during AR growth was recorded as (i) Percent explants forming AR, (ii) Number of roots per explant (mean), (iii) Biomass formation in fresh weight and dry weight (g/L). For measurement of dry weight, fresh weight harvested from each culture flask was oven dried at 30°C for 8 hrs.

Growth dynamics of AR suspension cultures in liquid MS media

Based on the higher growth responses on sold MS media, 0.5mg/L Me-J, 1.0mg/L PAA and NAA were used in suspension cultures to study their impacts on AR biomass proliferation and antioxidant potential. First an inoculum culture was developed by harvesting 10g of fresh AR formed *in vitro* on solid MS media and was transferred into liquid MS medium containing 1.0mg/l of NAA and placed on gyratory shaker (110rpm) at room temperature. Two weeks old inoculum culture was subsequently used in further experiments, wherein inoculating 2g fresh root suspension in each flask (250ml) containing 50ml MS medium, 30 g/l sucrose and 0.5mg/L Me-J or 1.0mg/L NAA or PAA. The pH of media was adjusted at 5.8, followed by autoclaving at 121°C for 20 min at 1.atm pressure. The cultured flasks were placed on gyratory shaker (110 rpm) at room temperature. Data concerning the growth kinetics and accumulation of biomass (Dry biomass) was recorded after every 4 days for a period of 44 days.

DPPH° free radical scavenging assay (FRSA)

Antioxidant potential of the AR samples established either on solid MS media or in liquid media was determined by the method of Yousaf *et al.,* (2019). Approximately, 20-30mg of each powdered sample derived from the *in vitro* AR cultures was weighed followed by maceration in 0.5ml methanol (80%) for 10 min, and finally centrifuged at 4,000 rpm for 4

min to obtain crude extract. Further, 0.5ml diluted test sample was added to 0.5ml of 2000µ mol/l of a DPPHº solution for initiation of the reaction.

The reaction mixture in the test tube was kept for 20 min at room temperature, and subsequently the absorbance of the reaction mixture was measured at 520nm using UV–Visible spectrophotometer (Agilent 8453, CA USA). The antioxidant potential of each biological sample was calculated as% DPPH free radical scavenging activity (FRSA)

Statistical analysis

Mean values from the triplicate data were taken, where also one-way ANOVA at significant level $P<0.05$ was determined using computer software Graph pad Prism 5.01 and statistics 8.1.

Results

Effects of explant type, plant growth regulators (PGRs) and elicitors on growth parameters of adventitious roots

In initial experiments, viable seeds of *A. scoparia* were *in vitro* germinated on MSo media (MS media having no plant growth regulator) to provide continual supply of aseptic germplasm as a source of active and aseptic explants. After a month, healthy seedlings were developed which were taken from the cultured flasks to prepare different types of explants for initiation of adventitious roots (AR) *in vitro* cultures. For selection of the suitable type of explant for induction of adventitious roots (AR), three different explants from leaf sections, stem and root portion were cultured on solid MS media supplemented with 0.5mg/LNAA. Wherein, highest adventitious (AR) induction frequency (49%) was observed in leaf explants, moderate response (32.5%) in stem explants while it was lowest (18%) in case of root explants (Fig. 1).

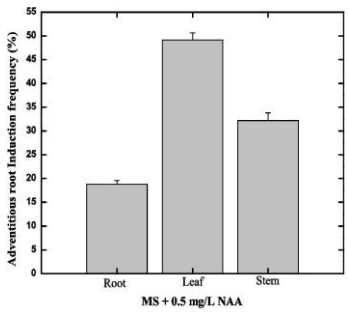

Fig. 1. Effects of explant type on solid MS media containing 0.5mg/L NAA on adventitious root formation in *A. scoparia*. Data are the mean values of triplicates culture flasks.

Thereafter, the leaf explants were selected to be used further in the subsequent experiments for AR formation on solid MS media containing varying levels of PGRs and elicitors. In context, to evaluate the impacts of PGRs and elicitors on induction of adventitious roots in leaf explants, it was observed that among the different auxins employed *in vitro*, NAA showed highest induction frequency (57%) when employed at 1.0mg/L on MS solid media (Fig. 2.a). Among the other auxins, 0.5mg/L 2,4-D resulted in the lowest induction frequency (22%) when compared with IAA (36% at 0.5mg/L). Within the elicitors, 0.5mg/L Me-J inducted maximum roots induction frequency (58%), while 1.0mg/LPAA resulted in 43% induction frequency (Fig. 2.a). It is worth mentioning here that the mode of AR formation was direct induction in explants. Moreover, emergence of AR in explants was observed at the cut ends of explants inoculated in culture flaks after six days of culture cultivation. At all the levels employed, both 2,4-D and Me-J resulted in a significant decline in AR induction frequency when tested at higher levels i.e above 0.5mg/L. Whereas, NAA, PAA and IAA responded optimally at 1.0mg/L and above this concentration a decrease in growth parameters of adventitious roots (AR) was observed for each of these PGRs respectively.

Maximum number of adventitious roots per explant (22.3) were recorded in explants cultivated on MS media supplemented with 0.5mg/L Me-J (Fig. 2.b). This was followed by NAA which resulted in (16.6) roots per explant when added to MS media at 1.0mg/L. The other auxins such as 2,4-D and IAA produced less number of roots per explant, respectively. Similarly, PAA resulted in a moderate response (14.2 roots), when employed at 1.0mg/L on solid MS media (Fig. 2.b). In present study MSo (MS media having no PGR or elicitor) was used as a control treatment. It was observed that on MSo media leaf explants were unable to induce and proliferate adventitious roots. Biomass formation in the cultured flasks in response to the applied PGRs and elicitors was recorded as fresh

biomass and dry biomass. Higher fresh biomass (38.2g/L) was recorded in the adventitious roots grown on solid MS media supplemented with 0.5mg/L Me-J (Fig. 2.c).

It was followed by 1.0mg/L NAA, producing 25.2g/L fresh biomass and 1.0mg/L PAA resulting in 22.2g/L fresh biomass. Further, it was observed that 2,4-D was comparatively less efficient in AR biomass formation in this study (Fig. 2.c). Among the auxins, NAA showed the utmost higher response for dry biomass formation, while Me-J as an elicitor resulted in higher level of dry biomass in the present study. AR grown in presence of Me-J produced varying levels of dry biomass i.e 4.5g/L, 3.4g/L and 1.3g/L when employed at 0.5, 1.0 and 1.5mg/L, respectively (Fig. 2.d). Dry biomass was observed in a linear fashion, coinciding with the fresh biomass at each PGR and elicitor treatments with exception of NAA. In contrast to data on fresh biomass, PAA resulted in higher levels of dry biomass (3.2g/L), when compared with NAA which produced less biomass (1.5g/L) (Fig. 2.d). Similarly IAA also showed higher response than NAA in dry biomass formation.

Effects of plant growth regulators (PGRs) and elicitors on antioxidant potential of adventitious roots grown on solid MS media

Data in fig. 3 shows the assessment of the antioxidant potential through DPPH free radical scavenging activity in the AR cultures raised in vitro on solid MS media at different hormonal and elicitor treatments. It can be seen that almost in all AR samples, considerable antioxidant potential was observed in response to PGRs and elicitors. Highest activity (88%) was recorded in the AR raised at 1.5mg/L Me-J.

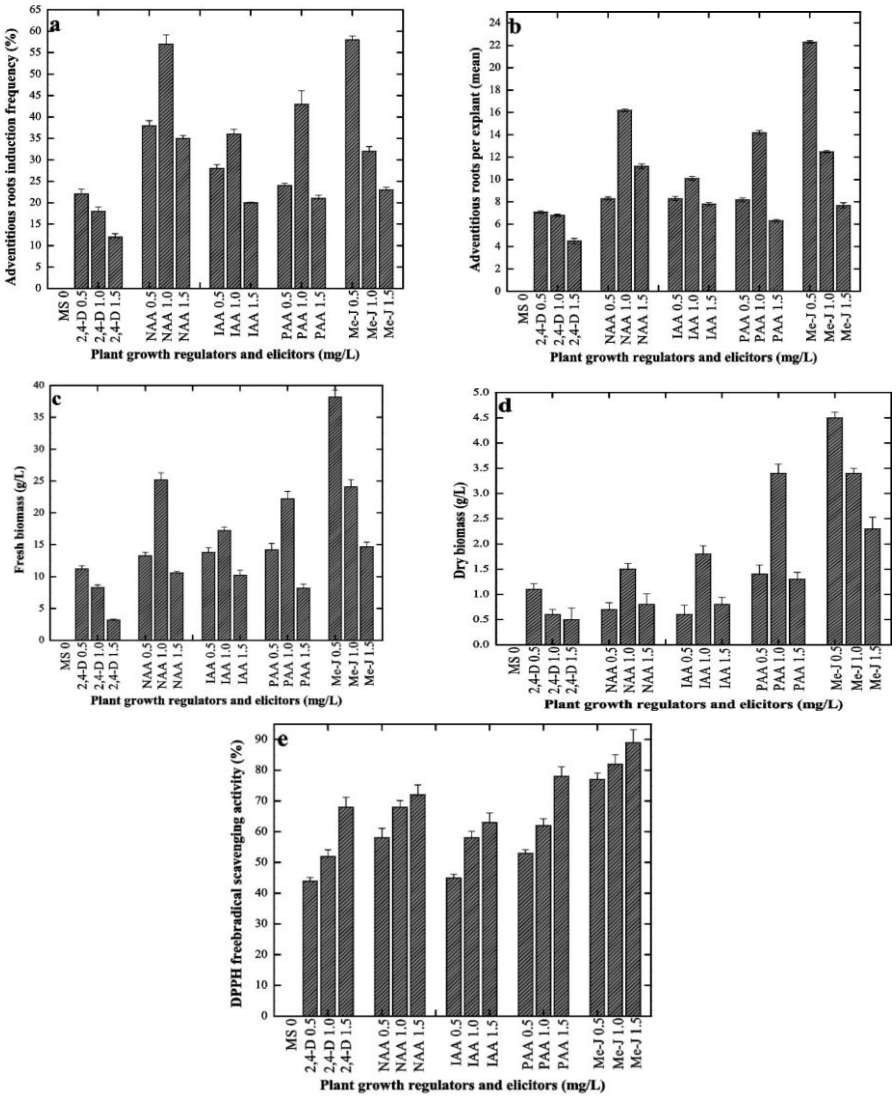

Fig. 2. Effects of auxins and elicitors on adventitious roots growth parameters in *A. scoparia* on MS media. a: induction frequency, b: number of roots, c: fresh biomass, d: dry biomass, e: antioxidant potential.

PAA and NAA resulted in a comparative similar response in the antioxidant activity. However, NAA showed higher activities when compared with other auxins such as IAA and 2,4-D. It is worth mention that unlike data on the growth parameters of adventitious roots, higher levels of the PGRs or elicitors enhanced the antioxidant potential in the root cultures. At higher level such as 1.5mg/L, each auxin and elicitor resulted in higher DPPH free radical scavenging activity in the AR samples. Antioxidant activity of 68%, 70% and 65% were recorded in the AR samples grown on solid MS media supplemented with 1.5mg/L of either 2,4-D, NAA and IAA respectively (Fig. 2e). Nonetheless, the highest value of the free radical scavenging activity (78%) was recorded in the adventitious roots harvested from cultured flasks in response to 1.5mg/L PAA. Thus an overall higher although significantly not different antioxidant potential was observed in AR grown in presence of elicitors, when compared with the PGRs.

Growth kinetics of adventitious roots suspension culture

Suspension cultures are usually used to enhance biomass and accumulation of secondary metabolites, especially in medicinal and aromatic plants. In this study the AR grown on solid media were aseptically transferred to liquid media supplemented with 1.0mg/L NAA, 1.0mg/L PAA or 0.5mg/L Me-J. The concentration of these growth regulators were selected on the basis of their optimal growth responses on solid media. Growth kinetics were studied to assess the impacts of the elicitors in comparison with auxin on biomass accumulation in total 44 days culture period. In presence of 1.0mg/L NAA, shorter lag and stationary phases of 8 and 4 days respectively were observed in the growth curve. The log phase was quite longer consisting of 24 days, wherein highest biomass (7.36g/L) was accumulated on 28 day of the growth curve during AR suspension culture (Fig. 4). Growth of adventitious roots in suspension cultures in presence of 1.0mg/L PAA, revealed shorter log phase of 12 days and longer stationary phase of 16 days in the cell cycle. It was observed that AR suspension cultures accumulated highest biomass (6.83g/L) on day 24 of the growth curve (Fig. 5). Interestingly the potential of Me-J for biomass accumulation in suspension cultures was significantly lower than PAA and NAA. In response to 0.5mg/LMe-J, longer log phase (28 days) and shorter stationary phase (4 days) were observed respectively. Highest biomass (4.83g/L) was accumulated on day 20 of the growth curve (Fig. 6). Compared with initial inoculum (2.35g/L), almost fourth fold increase in biomass was observed in AR suspension cultures established *in vitro* at 1.0mg/LNAA. However, biomass was incremented 3 fold and 2 fold in response to 1.0mg/LPAA and 0.5mg/LMe-J respectively.

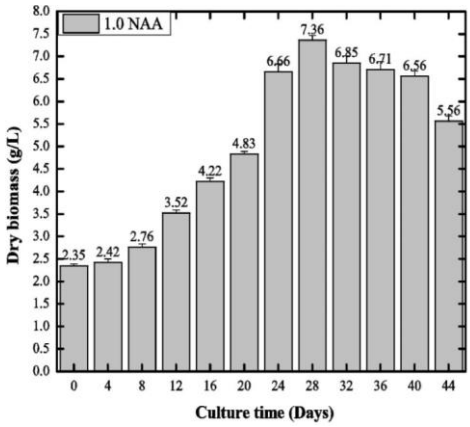

Fig. 3. Estimation of biomass accumulation in the AR suspension cultures in response to 1.0mg/L NAA.

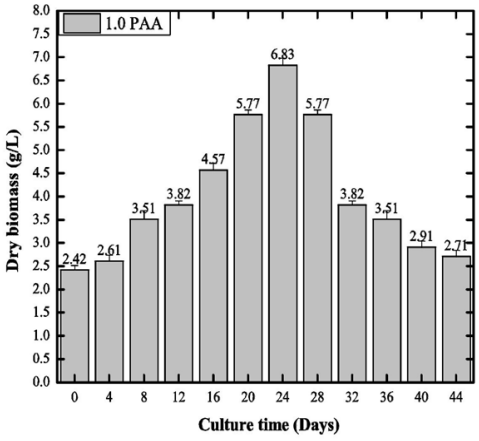

Fig. 4. Estimation of biomass accumulation in the AR suspension cultures in response to 1.0mg/L PAA.

Antioxidant activity of adventitious roots grown in suspension cultures

During bioprocessing of adventitious roots in shake flasks, AR were harvested from the different distinct growth phases of growth cycle to investigate the impacts of hormonal elicitors on the antioxidant potential in AR suspension cultures. For this purpose plant samples of adventitious roots were harvested from the different growth phases including inoculum (day 0), lag phase (day 8), log phase (day 20) and stationary phase (day 44) in the growth curve. The antioxidant activity of the AR samples raised in vitro in response to 1.0mg/L NAA or PAA and 0.5mg/L Me-J respectively was determined through DPPH free radical scavenging activity. Among the different growth phases, day 20 (log phase) was observed to accumulate higher antioxidant activity in the AR suspension cultures than other growth stages. Lesser activity was determined in the stationary phase of cell cycle (Fig. 6). Highest DPPH free radical scavenging activity (87%) was recorded in the AR suspension cultures raised in presence of 0.5mg/L Me-J. PAA and NAA resulted in 75% and 70% antioxidant activity, respectively in the AR suspension cultures in the log phase. Interestingly, the antioxidant potential was found in direct correlation with biomass accumulation during suspension cultures in the present study. Moreover, a sequential increase in the antioxidant activity in response to all the hormonal elicitors was observed in the adventitious roots with progression from one growth phase to another, until the stationary phase which resulted in a significant decline in the activity (Fig. 6).

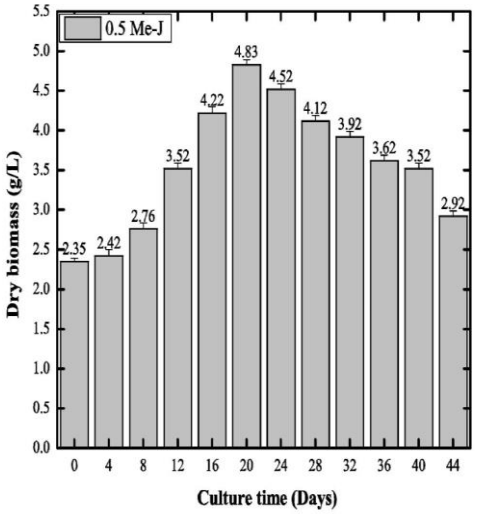

Fig. 5. Estimation of biomass accumulation in AR suspension cultures in response to 0.5mg/L Me-J.

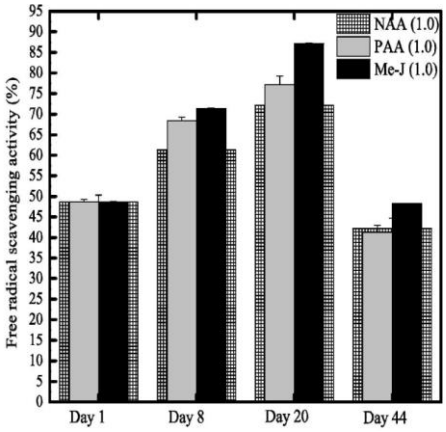

Fig. 6. Evaluation of antioxidant potential in the AR suspension cultures harvested on day 1, day 8, day 20, and day 44. Values are the mean of triplicates.

Discussion

The overall objective of this investigation was to assess the effects of explant type, auxins and elicitors on *in vitro* AR induction, biomass formation and antioxidant potential in *A. scoparia*. Among the different explants tested, leaf explants resulted in higher induction of adventitious roots. The choice of suitable explant is the determining factor for successful optimization of *in vitro* AR regeneration in any medicinally important plant species (Saeed *et al.,* 2017). The different morphogenetic responses by different explants are due to the different physiological and metabolic responses of an explant to the same growth conditions (Khan *et al.,* 2019b). Regarding the impacts of PGRs and elicitors on induction of AR in leaf explants on solid MS media, it

was observed that among the different auxins, NAA was more effective in AR induction (57%), mean number of roots (16.6) and biomass (25.2g/L; fresh biomass) when employed at 1.0mg/L on solid MS media. Among the notable auxins, NAA is reported to play an important role in initiation and establishment of AR (Yan *et al.,* 2014; Khan *et al.,* 2017; Ali *et al.,* 2019). Plant cells can rapidly uptake NAA, mostly through the cut ends in the explants, PH trapping mechanism or through influx carriers. It has been highlighted by Khan *et al.,* (2017) that uptake of NAA is six times faster than other employed PGRS in the tobacco explants. Moreover, MS salts and light does not cause degradation of NAA in the culture growth media (Weathers *et al.,* 2005).

Lower response in AR growth parameters by 2,4-D in our study linked its morphogenetic behavior as multiplicative, and not the regenerative in explants during *in vitro* growth (Khan *et al.,* 2015a) . Similarly, the higher potential of NAA compared with the other auxins such as 2,4-D, IAA and IBA in growth parameters of AR, has been reported in several research studies in various medicinal plants (Sivanesan and Jeong 2009; Praveen *et al.,* 2009; Khan *et al.,* 2015b).

Interestingly significant increases in AR growth attributes was observed in leaf explants, cultivated on solid MS media supplemented with 0.5mg/L Me-J. Wherein, the highest roots induction frequency of (58%), mean number of roots (22.3) and fresh biomass (38.2g/L) were recorded in explants after four weeks of culture period. PAA also resulted in considerable growth parameters during adventitious rooting on solid MS medium, in this study. Though less responsive than Me-J and NAA in induction of AR in explants, PAA resulted in higher levels of dry biomass (3.2g/L), when compared with NAA which produced less biomass (1.5g/L). The growth stimulating effects of Me-J and PAA as chemical elicitors in AR cultures are not well explored. Rather, Me-J is reported for lower growth response in many medicinal plants to influence biomass formation. However, recently both Me-J and PAA have been reported for their positive effects on induction and biomass accumulation of AR in a high valued medicinal plant *Ajuga bracteosa* (Saeed *et al.,* 2017). Both Me-J and PAA can initiate a cascade of different metabolic events in plant cell as a result of the stress condition induced by these elicitors, consequently resulting in induction of adventitious rooting and biomass formation in explants (Khan *et al.,* 2017). Within the different levels of PGRs and elicitors tested *in vitro* on solid MS media, it was observed that higher doses of both PGRs and elicitors resulted in a drastic decline of growth parameters during adventitious rooting. At all the levels employed, both 2,4-D and Me-J resulted in a significant decline in AR induction frequency when tested at higher levels i.e above 0.5mg/L. Whereas, NAA, PAA and IAA responded optimally at 1.0mg/L and above this concentration a decrease in growth parameters of adventitious roots (AR) was observed for each of these PGRs, tested respectively. The adventitious rooting capacity in explants can be ceased at higher doses of PGRs, as they might inhibit AR formation and might promote callus organogenesis in explants (Kollarova *et al.,* 2004) Further over dose and high concentration of auxins in medium may inhibit cell elongation, slows down growth and development of roots, and can cause root apical dormancy (Chao *et al.,* 2006). At higher doses, the elicitors can inhibit growth and can cause cell necrosis and cell death if maintained for prolonged time during in vitro cultures, especially Me-J (Khan *et al.,* 2017; Saeed *et al.,* 2017; Kazmi *et al.,* 2019b). Determination of the optimal level of the PGRs or elicitors at which the plant cells can grow efficiently and result in higher biomass formation during AR cultures, is a crucial and important factor for the establishment of successful protocol, that can be exploited for commercial production of healthy AR biomass (Khan *et al.,* 2015b). It is worth mentioning that in present study, the level of contamination during culturing, sub-culturing and maintenance of AR cultures was lower (data not shown). Since the source of explants was the *in vitro* germinated seedlings that might be the reason for lesser contamination during *in vitro* cultures. Compared with the explants derived from wild the grown plants, the *in vitro* raised seedlings can provide an aseptic source of explants, those can

prevent contamination in the cell cultures (Ali *et al.,* 2018a). Nonetheless, considerable antioxidant potential through DPPH° free radical scavenging activity was observed in AR samples raised *in vitro* on solid MS media in response to PGRs and elicitors. Highest antioxidant potential was recorded in the AR raised at 1.5mg/L Me-J. Further, PAA and NAA resulted in a comparative similar response in the antioxidant activity. However, NAA showed higher activity when compared with other auxins such as IAA and 2,4-D. It is worth mention that unlike data on the growth parameters of adventitious roots, higher levels of the PGRs or elicitors enhanced the antioxidant potential in the root cultures. At higher level such as 1.5mg/L, each auxin and elicitor resulted in higher DPPH° free radical scavenging activity in the AR samples. The antioxidant potential in plant tissues is the function of low molecular weight compounds produced in plant cell to mitigate the harsh stress conditions surrounding the plant cell. These compounds are collectively called as secondary metabolites. The major classes of these secondary metabolites are phenolic acids, flavonoids, terpenes, and alkaloids etc, which are of pharmaceutical interest in preparation of a variety of phyto medicines (Ali *et al.,* 2018b). Generally elicitors acting as stress inducers are more potent to induce and stimulate the antioxidant potential in plant cells (Kazmi *et al.,* 2019a; Saeed *et al.,* 2017). Based on the higher growth responses, 1.0mg/L NAA or PAA and 0.5mg/L Me-J were selected for bioprocessing of AR suspension cultures in the liquid media. Growth curves were established for the selected hormonal elicitors in 44 days culture period for biomass accumulation. Growth dynamics in suspension cultures were monitored to evaluate the impacts of the selected hormonal elicitors on biomass accumulation with progression of different growth stages with passage of time in the culture (days). Interestingly the potential of Me-J for biomass accumulation in suspension cultures was significantly lower than PAA and NAA. Longer log phase (28 days) and shorter stationary phase (4 days) were observed respectively in AR cultures raised in presence of 0.5mg/L Me-J. Compared with initial inoculum (2.35g/L), almost fourth fold increase in biomass was observed in AR suspension cultures raised *in vitro* at 1.0mg/LNAA. However, biomass was incremented 3 fold and 2 fold in response to 1.0mg/LPAA and 0.5mg/L Me-J respectively. The bioprocessing of AR in liquid media is favorable than solid media for obtaining maximum biomass (Khan *et al.,* 2017). Multiple factors like more stable PH, a reduced endogenous hormone gradient, lesser or no media polarity and a lesser effect of toxins are involved in suspension culture to improve *in vitro* growth and proliferation (Wu *et al.,* 2008; Khan *et al.,* 2017). During bioprocessing of adventitious roots in shake flasks, AR were harvested from the different distinct growth phases of growth cycle to investigate the impacts of hormonal elicitors on the antioxidant potential in AR suspension cultures. Highest DPPH° free radical scavenging activity (87%) was recorded in the AR suspension cultures raised in presence of 0.5mg/L Me-J. PAA and NAA resulted in 75% and 70% antioxidant activity, respectively in the AR suspension cultures in the log phase. Interestingly, the antioxidant potential was found in direct correlation with biomass accumulation during suspension cultures in the present study. The role of Me-J in inducing secondary metabolism of plants is well reported in literature and is considered to reprogram cell metabolism and cell cycle progression (Khan *et al.,* 2017; Kazmi *et al.,* 2019b). Different classes of medicinally active metabolites such as phenolics, flavonoids, alkaloids, tannins and essential oils are generated upon the transcription signaling of this compound as revealed in many studies (Kazmi *et al.*, 2019a; Kazmi *et al.,* 2019b). DPPH° free radical scavenging assay is a robust, easy and reliable testing method to determine the antioxidant activity in any plant samples and has been preferred over other antioxidant evaluation assays for its optimal operation and promising authenticity (Khan *et al.,* 2013). Plant cell naturally contains antioxidants having the ability to scavenge the reactive oxygen species (ROS) or free radicals, which are produced as a consequence of stress condition (Khan *et al.,* 2017; Kazmi *et al.,* 2019b). Generally, the antioxidant potential of a plant sample is directly proportional to the presence of total phenols and flavonoids. Plant generates a variety of defense metabolites in response

to stress stimuli such as phenolic acids, flavonoids, alkaloids etc, having benefits in pharmaceutical, natraceauticals and food industries (Saeed *et al.,* 2017; Mohammad *et al.,* 2019).

Conclusions and future prospects

In conclusion, an easy and efficient method of adventitious roots (AR) induction and biomass formation was developed for the first time in *A. scoparia*. Leaf explants cultivated on MS solid media supplemented with 0.5mg/L Me-J resulted in higher growth parameters during AR culture growth. However, NAA at 1.0mg/L resulted in highest biomass accumulation (7.36g/L; DW) in suspension culture. Moreover a significantly higher antioxidant activity was observed in AR samples grown in presence of auxins and elicitors. Thus a suitable model system was optimized to study the effects of auxins and elicitors on biomass formation and antioxidant activity through AR cultures. Molecular aspects need to be elucidated to determine the putative genes involved in the process of elicitation, to answer how elicitation influence biomass accumulation and production of secondary products in AR culture system. This protocol has potential for commercial production of useful secondary metabolites; those can be exploited for pharmaceutical purposes.

Conflict of interests

There is no conflict of interests from any of the authors listed in this article.

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