



Investigating The Impact of Salicylic Acid On *Atropa Accuminata*: A Comprehensive Analysis

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Abstract- This study delves into the intricate interplay between salicylic acid and *Atropa accuminata*, shedding light on the effects of this compound on the physiological aspects of the plant. *Atropa accuminata*, a member of the Solanaceae family, has been recognized for its diverse pharmacological potential due to its rich alkaloid content. Salicylic acid, a well-known plant hormone, has garnered attention for its role in diverse aspects including growth regulation.

The experiment involved exposing *Atropa accuminata* plants to varying concentrations of salicylic acid and monitoring their responses over a defined period. Parameters such as growth rate, callus development and root formation were meticulously assessed. The results showcased a nuanced relationship between salicylic acid concentration and plant response. Lower concentrations of salicylic acid seemed to stimulate growth and enhance growth on nodal explant producing callus in 90% cultures within 25 days. However, it also induced rooting of invitro regenerated shoots at optimum concentration of 0.5mg/liter in terms of maximum culture response (80%), number of days (15) and number of roots (12+-2). This comprehensive analysis provided insights into the intricate crosstalk between salicylic acid concentrations, callus production, shoot regeneration and rooting of invitro regenerated shoots.

Keywords: *Atropa*, Salicylic Acid, Callus, Nodal Explant, Shoot Regeneration.

1. INTRODUCTION

Due to its captivating attributes and extensive potential in the realm of medicine, *Atropa accuminata*, a member of the Solanaceae family, maintains a significant place in botanical inquiry. This perennial plant species, often known as "Deadly Nightshade," has long piqued the interest of scholars, herbalists, and medical practitioners due to its high alkaloid content and fascinating physiological consequences. *Atropa accuminata*'s unusual bell-shaped blossoms and glossy black berries have also contributed to its attractiveness, as has its historical relationship with folklore and traditional medicine. *Atropa accuminata*'s chemical makeup is noteworthy, since it contains a variety of alkaloids such as atropine, hyoscyamine, and scopolamine. These alkaloids have long piqued the interest of researchers due to their potential usefulness in pharmacological and medical situations. Atropine, for example, has been utilised for its anticholinergic properties, whilst scopolamine has been used as an antiemetic and to treat motion sickness. Because of the varied pharmacological actions of these alkaloids, *Atropa accuminata* is being researched as a possible source of new medicines and therapeutic agents. *Atropa accuminata* demonstrates fascinating reactions to numerous environmental conditions, including stressors and growth regulators, in addition to its chemical composition. Salicylic acid is one such growth regulator that has received a lot of interest in

plant research. Salicylic acid is a plant hormone that plays an important role in modulating plant responses to biotic and abiotic stresses, as well as in plant growth, development, and defence mechanisms. Given *Atropa accuminata*'s potential as a source of pharmacologically relevant chemicals, studying the effects of salicylic acid on this plant species may yield new insights into its growth patterns, secondary metabolite synthesis, and stress responses.

Literature Review

According to Cutt et al. (1992), exogenous delivery of SA influenced a wide range of processes in plants, including stomatal closure, seed germination, fruit output, and glycolysis. Furthermore, some of the impacts were caused by other phenolic chemicals. Furthermore, some of the effects have been demonstrated to be caused by other general chemical features of SA.

Raskin (1992) identified salicylic acid's distinct and particular regulatory activities. SA is a water-soluble antioxidant molecule that controls plant development. It also plays a role in biotic stress tolerance in wheat, such as drought tolerance.

Leon et al. (1993) conducted the studies that confirmed that SA is mostly synthesised from benzoic acid (BA). TMV infection increased the activity of the enzyme responsible for converting benzoic acid into SA by four to five fold. TMV infection has been shown to increase BA 2-hydroxylase activity, and the synthesis of BA is the rate limiting step in SA generation. Within four



hours of administration, the glycoside (BA) was produced from exogenously provided radiolabeled. SA. Bergman et al. (1994) proposed that salicylic acid is a critical signalling molecule with various effects on biotic stress tolerance.

According to Tissa Senaratna (2000), physiologically active quantities of SA and its derivatives can impart stress tolerance in the plants *Phaseolus vulgaris* and *Lycopersicon esculentum*. SA demonstrated improved resistance to heat, cold, and drought stressors. As a result, they have a wide range of practical applications in agriculture, horticulture, and forestry.

Breusegem et al., (2001) studied the role of SA as a signalling molecule in modifying plant response to environmental stress.

Kunga ta Lee (2001) evaluated the effect of SA on tropane alkaloid synthesis and the reactions of altered root cultures of *Atropa belladonna* to SA stress. The addition of 0.2mM SA to the roots had no influence on tropane alkaloid release into the medium up to 35%. As a result of varied reactions to SA stress depending on SA concentration, the converted root of *Atropa* shows diverse features.

Fariduddin et al., (2003) conducted a research to assess the effect of exogenously administered salicylic acid in plants and discovered that SA functions as a possible non-enzymatic antioxidant, controlling a variety of plant physiological processes. It has also been discovered to operate as a signalling component in a variety of plant stress responses, including ozone exposure and pathogen infection. SA was also shown to be involved in the activation of the stress-induced antioxidant system, the stimulation of blooming in many plants, ion control absorption by roots, and stomatal conductivity. The foliar application of SA resulted in an increase in pigment content in *Brassica napus*, as well as an increase in net photosynthetic rate, internal carbon dioxide concentration, and water usage efficiency.

Exogenous application of SA, according to Gunes & Alpasalan et al., (2005), may contribute in the control of physiological processes in plants such as stomatal closure, ion uptake, and transport in maize.

Fatma Abd El-Lateef Gharib (2006) discovered that supplying SA exogenously to Basil and Mayoram boosted growth and oil output by enhancing photosynthetic and nutritional content absorption. Because of the increased output of phenolics compared to eugenol, it may serve as a novel source of antioxidant phenolics. SA therapy increased the levels of free amino acids, spermidine, proline, and total polyamines.

Li et al., (2006) has revealed that in plants the complex interactions of signalling networks for self resistance against pathogen attack is known to be regulated and modulated by different PGRs viz SA.

Abdelnassergalal (2012) explored in vitro shoot multiplications from *Ziziphus spina-christi* shoot tips.

Lower cytokinin concentrations were superior to higher ones for lateral bud proliferation. The use of relatively low amounts of SA exogenously improved the proliferation efficiency of *Z. spina-christi* cells. These findings were consistent with previous research that found that exogenous administration of SA increased cellular development and somatic embryogenesis in Coffee arabica cultures.

According to Ahmad et al. (2016), SA promoted the formation of phenolic acids in in vitro callus cultures of Milk Thistle. The administration of SA at 10 mg/l resulted in root differentiation of callus.

Malgorzata et al. (2017) discovered that TIBA, fluridone, and SA at 30 M, 40 M, and 125 M concentrations, respectively, completely reduce somatic embryo formation in *C. delgadii*. Both TIBA and fluridone had a significant impact on endogenous IAA, ABA, and cytokinin levels, as well as hormone ratios. SA treatment causes changes in IAA content and the IAA/CKs ratio, which are notably relevant to the SE caused.

2. METHODOLOGY

Methodology involves a set of methods, principles and rules for regulating a given hypothesis. The current study was carried out at the Plant tissue culture laboratory, Department of Botany. The parameters studied were effect of different concentrations of salicylic acid on *in vitro* propagation of *Atropa acuminata*. The sterilized aseptic conditions are required for invitro micro propagation techniques. Accordingly, we can divide the experimental conduct into three steps.

1. Preliminary Experimental Stage

This encompasses the cleansing and sterilization of glassware, as well as the formulation of the stock solution.

2. Washing and sterilization of glassware

By Sterilization we mean the process of eliminates, removal, killing, or deactivation of all forms of life and other biological agents present in a specific location is referred to as sterilization. The glassware used in the research was purchased from 'Borosil' since it is thermo-resistant. Culture vials, flasks, measuring cylinders, glass bottles, beakers, petri plates, pipettes, and other items are included. It was cleaned extensively with running tap water after being immersed in detergent solution overnight. It was then washed with distilled water 2-3 times. Washed glassware was oven dried before being autoclaved at 121°C and 15 lbs for 15-40 minutes.

3. Preparation of stock solution

Stock solution was prepared in sterilized glass bottles having suitable closure. This was done according to the composition of M S basal medium given by Murashige



and Skoog [1962]. The procedure for preparation of stock solution is given below:

Stock solution I [Macro Salts]

Constituents	[g/l]
Ammonium-Nitrate [NH_4NO_3]	6.5
Potassium-Nitrate [KNO_3]	9.0
Calcium-Chloride [$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$]	4.4
Magnesium sulphate [$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$]	3.4
Potassium Di-hydrogen Phosphate [KH_2PO_4]	1.2

Through the addition of double-distilled water, the final volume was augmented to 100 ml.

Stock II [Micro Salts]

Constituents	g/l
Potassium-Iodide [KI]	0.083
Boric-Acid [H_3BO_3]	0.62
Manganese Sulphate [$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$]	2.23
Zinc Sulphate [$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$]	0.86
Sodium molybdate [Na_2MOO_4]	0.025
Cupric Sulphate [$\text{CuCl}_2 \cdot 5\text{H}_2\text{O}$]	0.0025
Cobalt-Chloride [$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$]	0.0025

The volume was adjusted to reach a final total of 500 ml.

Stock III [Iron Source]

Constituents	g/l
Ferrous sulphate [$\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$]	2.78
$\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$	3.7

Final volume was made up to 500ml.

Stock Solution IV [Inositol]

Constituents	Amount [g/l]
Inositol	5

Final volume was made up to 250ml.

Stock Solution V [Vitamins]

Constituents	Amount [g/l]
Niacin.	0.50
Pyridoxine HCl. (vitamin B6)	0.50
Thiamine HCl. (vitamin B1)	0.01
Glycine.	0.20

Final volume was made up to 500ml.

After preparation and labelling, stock solutions were stored in a deep freeze chamber.

3. EXPERIMENTAL PHASE

Preparation of Media

Media preparation is a critical and essential step in tissue culture, and its importance cannot be overstated. The media composition is tailored to the individual tissue under culture, with distinctions originating from the media's compatibility with various tissue types, eventually regulating growth results. For media preparation, all the procedures were carried out in neat and clean media preparation room.

Materials and Apparatus required:

- 1) Murashige and Skoog (MS) medium (Stock solution)
- 2) Distilled water
- 3) Sucrose
- 4) Agar
- 5) Sodium hydroxide
- 6) Hydrochloride acid
- 7) Beaker and test tubes
- 8) Spatula
- 9) Volumetric flask
- 10) Autoclave
- 11) Sterile tube
- 12) Electronic balance
- 13) Magnetic stirrer
- 14) PH meter

Method

- 12 ml of Different MS media stock solution were added slowly into the beaker as per the following ratio:
- Medium-ist for MS Medium [100ml].

Stock Solutions	Volume (ml)
Stock I [Macro Salts]	10ml
Stock II [Micro Salts]	0.5 ml
Stock III [Iron source]	0.5 ml
Stock IV [Inositol]	0.5 ml
Stock V [Vitamins]	0.5 ml
Total volume	12 ml

- A beaker is filled with 50 ml of distilled water.
- 3 grams of sucrose are introduced.
- The pH is adjusted to 5.8.
- Subsequently, 0.8 grams of agar are incorporated into the beaker.
- The hormone is introduced.
- The media's volume is brought to 100 ml using a volumetric flask, achieved by adding 38 ml of distilled water.
- Subsequently, the media undergoes autoclaving.
- Using a laminar flow hood, the molten medium is carefully dispensed into sterile culture tubes, filling each tube to approximately one-third of its height. Following this, each tube is appropriately labeled.

- Preparation is conducted for each of the following media:

- ✓ MS basal medium (without hormones).
- ✓ MS basal with different concentration of SA.

To explore the effects of different salicylic acid concentrations on *Atropa acuminata*, six different salicylic acid concentrations within the MS medium were used: 0.2 M, 0.3 M, 0.4 M, 0.5 M, and 0.6 M, with MS basal media serving as the control.

4. STERILIZATION OF EXPLANT

In the present work nodal cutting explant was used. Plant material was collected from the Botanical garden, University of Kashmir. For sterilization, the explants were washed thoroughly in a beaker by running tap water for 30 minutes. Labolene was added in order to remove the dirt and other solid particles. Then explants were treated in surfactant tween-20 to remove loose spores, bacteria and other debris.

The explants were rinsed thoroughly with sterile distilled water to remove traces of surfactant. After this the explants were transferred in a laminar airflow and treated with sodium hypo-chloride for a period of 3 minutes. Finally, they were rinsed several times with sterilized water in order to remove the traces of disinfectant. The sterilized plantlets were then used for inoculation.

Inoculation under aseptic conditions:

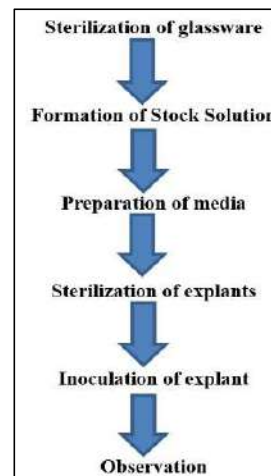
Before going for inoculation under aseptic conditions. The sterilization of laminar airflow was done through UV radiations within 20 to 25 minutes. Also the surface sterilization was done with 70% ethyl alcohol. Forceps, needles, scalpel blade were flame sterilized inoculation. Proper care was taken into consideration for avoiding condemnation of cultures by hand sterilization and covering of mouth and nose with mask. After inoculation the culture vials were recapped and sealed with parafilm and placed in culture room.

Incubation/Maintenance of cultures:

Inoculation of culture vials were made in the culture room under controlled conditions by keeping them on culture racks for providing photoperiod of 1500-2500 lux for 18 hours. Air conditioner was used to maintain temperature around 22± 40°C. Cultures regeneration were observed periodically and %age of response was recorded for determination of different concentration of salicylic acid on *Atropa accuminata*.

Data analysis:

The experiments were repeated thrice and the effects of different concentrations of SA were observed and quantified.



Schematic diagram of methodology adopted in the present work

5. *IN VITRO* RESPONSE FROM NODAL EXPLANTS

Callus production

The callus was produced on nodal explant with different concentrations of salicylic acid viz 0.2mg/ltr, 0.3mg/ltr, 0.4 mg/ltr, 0.5mg/ltr, and (0.6mg/ltr) on MS medium. The effective production of callus in 90% cultures within 25 days was obtained on MS medium containing SA having concentration of 0.5mg/ltr. Observation of Callus production from nodal explant is shown in table 1.

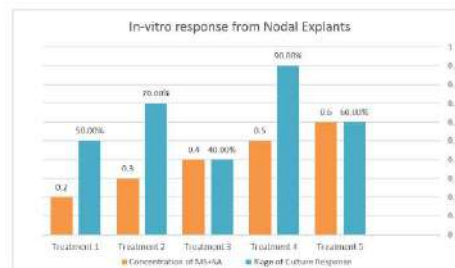
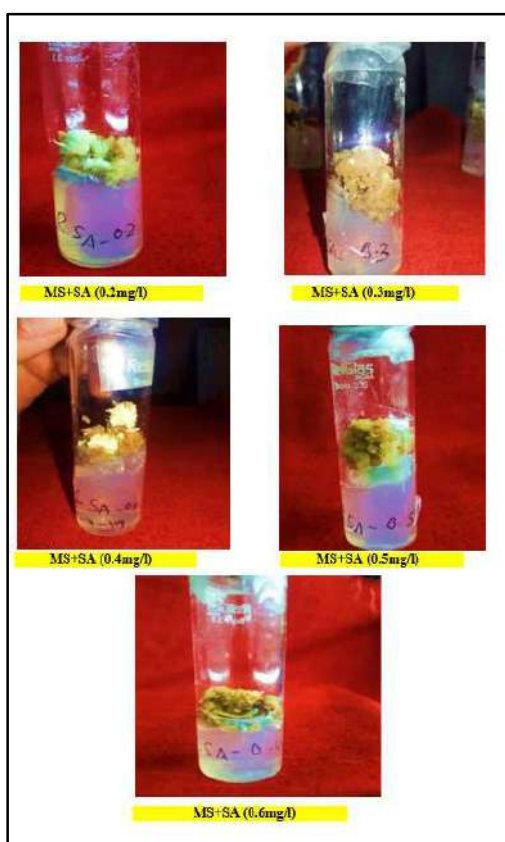


Fig. Culture Response with supplemented SA of different Concentrations

Treatments	No. of days for callus regeneration	Texture and colour of callus	% cultureresponse
MS Basal	-	-	-
MS+SA(0.2mg/l)	14	Hard and light greenish	50
MS+SA(0.3mg/l)	21	Hard and yellowish	70
MS+SA(0.4mg/l)	32	Hard and light brown	40
MS+SA(0.5mg/l)	25	friable and greenish	90
MS+SA(0.6mg/l)	29	Soft and brownish	60

(N=10 replicates per treatments)

Table 1



6. Shoot regeneration

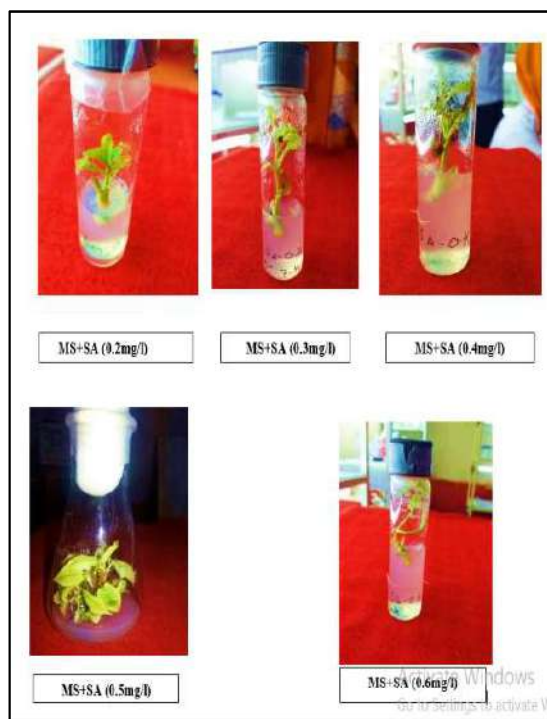
Nodal callus when supplemented with SA of different concentrations produced shoots. Maximum culture response 90 % was obtained on 0.5mg/l within 20 days. However MS medium fortified with SA of concentration, 0.2mg/ltr, 0.3mg/ltr, 0.4mg/ltr and 0.6mg/ltr also produced shoots with culture response 50% , 70% , 40% and 60% within 20 , 33 , 27 and 30 days.

Observation of shoot regeneration from nodal explant is shown in table 2 below.

Treatments	No. of days	% culture response
MS Basal	-	-
MS+SA(0.2mg/l)	20	50
MS+SA(0.3mg/l)	33	70
MS+SA (0.4mg/l)	27	40
MS+SA(0.5mg/l)	20	90
MS+SA(0.6mg/l)	30	60

(N=10 replicates per treatment)

Table 2.



Initiation of roots regenerated in vitro:

During the present study, it was observed that different concentrations of SA proved to be efficient for development of roots in the shoots regenerated through in vitro culture from nodal explants. Out of different treatments, the optimum concentration in terms of maximum culture response (80%), no of days (15) and no of roots (12±2) was found to be obtained at 0.5mg/ltr.





Conclusion

Finally, the study of the effect of salicylic acid on *Atropa Accuminata* has offered useful insights into the complicated connection between this plant species and exogenous salicylic acid administration. We observed significant physiological and biochemical changes in *Atropa Accuminata* in response to various salicylic acid concentrations through a series of carefully prepared tests and studies. It is crucial to note, however, that the effects of salicylic acid were dose-dependent, with greater amounts occasionally producing negative results. This highlights the importance of careful dose assessment when using salicylic acid as a possible growth regulator or stress reliever in agricultural and ecological situations.

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