International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(3) March -2016

EXOGENOUS SUPPLY OF SALICYLIC ACID RESULTS INTO BETTER GROWTH OF BANANA PROPAGULES UNDER *IN VITRO* CONDITIONS

Anil Kumar Singh., Trushali Tala., Manali Tanna., Hetal Patel., Prashant Sudra., Dhaval Mungra., Dhaval Nirmal., Tannu Khatnani and Preetam Joshi

THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 3, pp. 9488-9493, March, 2016

International Journal of Recent Scientific Research

RESEARCH ARTICLE

EXOGENOUS SUPPLY OF SALICYLIC ACID RESULTS INTO BETTER GROWTH OF BANANA PROPAGULES UNDER *IN VITRO* CONDITIONS

Anil Kumar Singh., Trushali Tala., Manali Tanna., Hetal Patel., Prashant Sudra., Dhaval Mungra., Dhaval Nirmal., Tannu Khatnani and Preetam Joshi*

Department of Biotechnology, Shree M and N Virani Science College, Rajkot (India) 360005

ARTICLE INFO

Article History:

Received 05th December, 2015
Received in revised form 08th January, 2016
Accepted 10th February, 2016
Published online 28st March, 2016

Keywords:

Salicylic acid, banana tissue culture, carbohydrates, proteins, growth parameters

ABSTRACT

Salicylic acid (SA) induced changes in *in vitro* growth and biochemical characteristics were studied in banana micropropagules. Banana shoots were cultured on standard banana multiplication medium supplemented with different concentration of SA. Treatment of SA was given in two different ways; a) SA was incorporated in medium prior to autoclaving and, b) filter sterilized SA was added after autoclaving the medium (post autoclaving). No significant changes were noted in growth when mode of SA application was altered. SA at low concentration proved to put forth positive effect on shoot growth and biomass production while the higher concentration of SA confirmed retardation in growth. Similarly, total chlorophylls, proteins, carbohydrates and phenolics contents were also increased significantly, when micropropagules were grown on standard banana multiplication medium supplemented with low concentration of SA. The study draw to close that SA, at low concentration encourages *in vitro* growth and biochemical activities in banana micropropagules even so added prior to autoclaving.

INTRODUCTION

Salicylic acid chemically falls under the bizarre category of plant phenolics compound which contain aromatic ring along with hydroxyl group and its derivatives. SA plays a significant role in growth and development, ethylene biosynthesis, flower induction, respiration and stomatal behavior (Raskin 1992). In recent years SA has gained focus in research due to its role as an endogenous signal mediating molecule in plant defense responses against pathogens (Lu *et al.*, 2016). . It has also been established that SA plays a role during the plant response to abiotic stresses such as drought, chilling, heavy metal toxicity, heat, and osmotic stress. Role of SA in biotic and abiotic stress tolerance, both *in vitro* and *in vivo* condition, has been investigated in several plants (Czajkowski *et al.*, 2015; Sánchez-Rojo *et al.*, 2015; Multu *et al.*, 2013; Agami and Mohamed, 2013; Hayat *et al.*, 2009; Tirani *et al.*, 2013). Role of SA as plant growth regulator has been envisaged in past and possible use of SA as growth regulator in plant tissue culture has been confirmed (Raskin, 1992; Gasper *et al* 1996).

SA can regulate *in vitro* growth of plants by modifying physiological and biochemical functions. Incorporation of SA in nutrient medium induces resistance against *Dickeya solani* infection in potato cultures (Czajkowski *et al.*, 2015). Addition of SA in medium also resulted in higher accumulation of secondary metabolites in *Cistus heterophyllus* (López-Orenes *et al.*, 2013). Likewise, multiple shoot induction and higher accumulation of andrographolide (a potential cancer therapeutic agent) has been observed in *Andrographis paniculata*, when cultured on SA supplemented nutrient medium (Zaheer and Giri, 2015). Galal (2012) reported that the exogenous application of SA in the culture media had a positive effect and good response on callus growth, shoot multiplication and root proliferations in the tree *Ziziphus spina*. Banana plantlets under the influence of SA, performed better in *in vitro* conditions, when challenged to water stress (Bidabadi *et al.*, 2012). Salicylic acid has also proved promotory role in *in vitro* regeneration of *Hibiscus acetocella* and *H .moschentos* (Sakhanokho and Kelly, 2009). It has also been reported that SA induces somatic embryogenesis in *Plumbago rosea* (Komaraiah *et al.*, 2004) and carrot (Hosseini *et al.*, 2009). Although promotory role of SA in enhanced photosynthetic pigments accumulation in wheat seedlings and *Brassica napus*, has been reported (Hayat *et al.*, 2005; Ghai *et al.*, 2002), but contrary to this Moharekar *et al.* (2003) has

Corresponding author:* **Preetam Joshi
Department of Biotechnology, Shree M and N Virani Science College, Rajkot (India) 360005

advocated that higher concentration of SA results in decline in photosynthesis and induction of oxidative stress in wheat and moong.

Banana is considered to be one of the oldest and important staple fruit crop of many developing countries including India. It is widely grown in the tropics and subtropics in all types of agricultural systems. Along with its comprehensive use and high economic worth, banana is counted as top ranked fruit crop of India in terms of annual production (Bairwa *et al.*, 2015). Tissue culture technology has been commercially exploited as one of the few exercises of in vitro propagation of economically important plants. This technology has proved better over traditional methods in banana cultivation with respect to finest, true to type and disease free plant production (Saraswathi *et al.*, 2016). Demand for tissue cultured banana is growing rapidly along with rise in global production, more over tissue-cultured banana plants have become an integral part of commercial banana production. The micropropagation protocol for banana has already been reported by several workers (Banerjee and Langhe., 1985; Swamy *et al.*, 1983; Wong, 1986). The present investigation was undertaken to study the stimulatory effect of salicylic acid on micropropagules of banana under in vitro condition to improve its proliferation and multiplication efficiency.

MATERIALS AND METHODS

Shoot cultures of banana were established according to the protocol described by Banerjee and Langhe (1985). After initial establishment of cultures, the shoots were multiplied on Murashige and Skoog's (1962) medium containing 3.0 mg l^{-1} BAP, IAA 0.01 mg l^{-1}, 0.8% agar and 3.0% sucrose. Regular sub- culturing was done on every three weeks interval. Cultures were kept under standard growth room conditions which was maintained 28±2°C temperature and a 16 h light/ 8h dark cycle providing 45µ mol m^{-2} s^{-1} photon flux density. We tried a range of salicylic acid concentration (5.0-100 mg l^{-1}) to study its regulatory effects. SA was added into standard shoot multiplication medium along with the PGR mentioned as above.

Two different ways were adopted to incorporate the SA. In first approach, SA was incorporated in the nutrient medium prior to autoclaving (pre-autoclaving) while in the second case, filter sterilized SA was added in the medium following the autoclaving (post –autoclaving), when the temperature of medium was brought down to about 50°C. Each culture bottle contained ca.50 ml of semi-solid medium and they were caped tightly to avoid contamination. The pH of medium was always adjusted to 5.8 before autoclaving. Each culture bottle was aseptically inoculated with a cluster of single shoot (ca. 1.5 cm) and kept under standard growth room conditions already mention above. The SA treated shoots were further sub-cultured on the same SA containing fresh medium at a gap of every three weeks up to six cycle *i.e.* a period of 126 days. All the above treatments were repeated thrice and three replicates were set for each experiment. At the end of experiment the micropropagules were taken out of culture bottle and were subjected to measurement of various growth parameters and biochemical analysis.

Measurement of growth parameters

Total shoot number, average shoot length and the biomass production in terms of fresh and dry weight was measured. For measurement of biomass (fresh weight and dry weight), propagules obtained from each treatment were taken out and the fresh weight was measured using an electronic top pan balance. For dry weight calculation, after measuring the fresh weight those fresh shoots were kept in an oven at 62˚C for 48 hrs for drying.

Biochemical analyses

***Chlorophyll contents*:** The chlorophyll contents was calculated as per the method described by Arnon (1949). For this, 500 mg of shoots (grown on SA containing medium) were weighed and grounded in pestle and mortar with 80 % acetone under dark conditions. Extracts were centrifuged at 10,000 rpm and the supernatant was used to measure absorbance on spectrophotometer (UV-Vis Schimadzu, Japan) at three different wavelengths (663, 652 and 645 nm). Concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using following formulae:

$$\text{Total Chlorophyll (mg g}^{-1}\text{)} = \frac{20.2\times A_{645} + 8.02\times A_{663}}{a\times 1000\times W}\times V$$

$$\text{Chlorophyll a (mg g}^{-1}\text{)} = \frac{12.7\times A_{663} - 2.69\times A_{645}}{a\times 1000\times W}\times V$$

$$\text{Chlorophyll b (mg g}^{-1}\text{)} = \frac{22.9\times A_{645} - 4.68\times A_{663}}{a\times 1000\times W}\times V$$

V= Volume of the extract in ml
W= Fresh weight of the sample (leaf) in g
a= Length of light path in cell (1 cm)

***Total phenols*:** The phenol contents was measured as per the method described by Mahadevan (1975) using Folin Ciocalteu's reagent. For this purpose, 500 mg of shoots (grown on SA containing medium) were weighed and crushed in pestle and mortar in 70 % methanol. The extract was centrifuged at 10,000 rpm for 15 minutes. The clear supernatant was used for quantitative determination of total phenol contents. For each reaction 500 µl methanolic extract was taken in a test tube and to this was added 1.0 ml suitably diluted (1:1 ratio of reagent and DDW) Folin Ciocaltaeu's reagent followed by 2.0 ml of Na_2CO_3 (20% w/v) solution. The test tubes were heated in boiling water bath with interval shaking for about 1.0 min. Tubes were subsequently cooled under running tap water. The blue colored product was diluted to 25 ml by adding DDW and the per-cent transmittance was measured at 650 nm in a spectrophotometer (UV-Vis Sc himadzu, Japan). The total phenol concentration in each sample was calculated with the help of already prepared standard curve using different concentrations (10-100 µg) of caffeic acid.

***Total carbohydrates*:** Quantitative estimation of total carbohydrate content was carried out as per the method described by Tandon (1976). SA treated *in vitro* derived propagules were homogenized in 0.1 M phosphate buffer (pH 7.0) and the homogenates were centrifuged at 10,000 rpm for 15 min. For each reaction 15 µl of supernatant was mixed with 4.0 ml of 0.2% Anthrone reagent (in conc. H_2SO_4) and placed in water bath for five minutes. The absorbance was recorded at 610 nm wave length. The total carbohydrate contents were determined using standard curve prepared from various concentrations of glucose.

***Total Protein*:** Quantitative estimation of total protein was performed as per Bradford's method (1976). One ml of the suitably diluted crude tissue extract (the supernatant) was mixed with 5.0 ml of Coomassie Brilliant Blue G–250 dye (Bradford reagent) and transmittance of the resultant solution (coloured complex) was read with Spectrophotometer (UV-Vis Schimadzu, Japan) at 595 nm. The amount of protein was determined using standard curve prepared from various concentrations of albumin protein.

For all the above analysis, three replicates were used and each reaction was repeated thrice. Suitable blanks were maintained wherever required. Statistical analyses was done to check the validity of data.

RESULTS

In the present study, addition of salicylic acid (SA) in standard banana multiplication medium during culture conditions resulted into significant change in growth pattern of banana micropropagules. It has also been observed that mode of application of SA in nutrient medium did not affect much on in vitro growth of shoots; particularly physical parameter such as shoot length, shoot number, fresh weight and dry weight were remained almost same. However, the biochemical parameters such as chlorophyll contents and total phenol contents did reveal noteworthy difference when mode of incorporation of SA was altered.

Contrary to this total carbohydrate contents remained unchanged when compared for two different modes of SA treatments. For control, banana cultures, which were grown on simple MS medium and supplemented with prescribed PGR's without the incorporation of SA, were taken as a reference. The shoot number and average shoot length were recorded less in case of SA treatment where low concentration of SA (5.0 mg l^{-1}) was incorporated in medium. Raise in SA beyond 5.0 mg l^{-1} (*i.e.* 10 and 25 mg l^{-1}) resulted into steady increase in both shoot number and shoot length but these values did not go beyond those obtained with control plantlets. Addition of more SA further than 25 mg l^{-1} showed significant decrease in above growth parameters (Table 1).

Similarly to shoot number and shoot length, the same effect on biomass accumulation (in terms of fresh weight and dry weight) was also observed, when shoots were grown on various concentration of SA. At low concentration of SA, fresh weight and dry weight were recorded low which steadily improved at higher concentration of 10 and 25 mg l^{-1} but again this increase did not comparable to observation with control. Moreover, further increase in SA concentration proved exceedingly toxic for cultures which was observed in terms of low biomass accumulation (Table 2). Noticeably, we find that when SA was added before autoclaving the medium, the total chlorophyll content increased in a plodding manner corresponding to increased amount of SA, but that is only upto 25 mg l^{-1} concentration. Further increase in the SA concentration resulted in decrease of total chlorophyll, though it still remained comparable to control. Contrary to this, when filter-sterilized SA was incorporated after autoclaving the medium, no significant change in total chlorophyll content was recorded at 25 mg l^{-1} or less concentrations. However, considerable increase in total chlorophyll was recorded at 50 mg l^{-1} concentration of SA in the same treatment condition (Table 3). Gradual increase in total phenol, carbohydrates and protein was observed with initial concentrations (5.0 and 10.0 mg l^{-1}) of SA, when added in the medium before autoclaving. Addition of more SA resulted in decline of all these bio-molecules; although it still remained higher than their respective controls (Table 3).

Table 1 Effect of salicylic acid on in *in vitro* growth of banana micropropagules

Mode of SA application	Salicylic acid concentration mg l-1	No. of shoots Mean ± SD	Length of shoots (cm)±SD	Fresh weight (g)±SD	Dry weight (g)±SD
Pre-autoclaving	0	15.23±2.36	4.62±0.45	14.12±2.35	3.19±0.050
	5	09.36±1.22	2.81±0.23	5.36±1.29	1.21±0.020
	10	18.45±3.25	3.31±0.11	7.65±1.21	1.75±0.011
	25	22.45±3.69	4.56±0.21	9.94±1.65	2.17±0.012
	50	11.47±2.88	2.61±0.15	4.53±0.96	0.92±0.006
	75	09.78±1.05	1.67±0.11	3.69±0.23	0.42±0.021
	100	06.63±1.04	1.62±0.14	2.98±0.12	0.64±0.009
Post-autoclaving (Filter sterilized)	0	16.56±5.32	4.87±0.21	13.87±1.29	3.04±0.032
	5	08.29±2.12	2.96±0.19	5.98±1.91	1.20±0.012
	10	17.32±4.65	4.19±0.32	6.66±3.45	1.48±0.010
	25	22.45±4.21	4.69±0.42	11.45±5.20	2.53±0.021
	50	10.41±3.64	3.09±0.21	3.90±2.87	0.81±0.006
	75	09.36±1.23	2.91±0.13	2.96±3.65	0.59±0.001
	100	06.66±1.09	1.93±0.62	2.87±0.21	0.52±0.002
SE		3.86	0.81	1.21	0.0212
CD5%		12.12	2.32	3.85	0.0786
CD1%		14.24	3.66	5.5	0.0953
CV		30.21	32.18	42.26	6.51

SE Standard Error; CD Critical Difference; CV Coefficient of variation SD Standard Deviation

Table 2 Effect of salicylic acid on chlorophyll contents in banana micropropagules grown under *in vitro* conditions

Mode of SA application	Salicylic acid concentration mg l^{-1}	Total chlorophyll content (mg g^{-1} FW± SD)	Chlorophyll a content (mg g^{-1} FW± SD)	Chlorophyll b content (mg g^{-1} FW± SD)
Pre-autoclaving	0	0.25±0.0042	0.11±0.0021	0.12±0.0022
	5	0.32±0.0202	0.17±0.0125	0.16±0.0100
	10	0.37±0.0212	0.19±0.0020	0.16±0.0092
	25	0.38±0.0236	0.22±0.0095	0.17±0.0111
	50	0.23±0.0195	0.13±0.0068	0.10±0.0045
	75	0.24±0.0068	0.12±0.0036	0.08±0.0032
	100	0.24±0.0042	0.10±0.0009	0.12±0.0022
Post-autoclaving (Filter sterilized)	0	0.21±0.0041	0.10±0.0019	0.11±0.0036
	5	0.19±0.0042	0.11±0.0103	0.09±0.0022
	10	0.17±0.0039	0.07±0.0008	0.10±0.0013
	25	0.15±0.0026	0.06±0.0007	0.09±0.0026
	50	0.16±0.0019	0.07±0.0006	0.09±0.0009
	75	*	*	*
	100	*	*	*
SE		0.0066	0.0035	0.0041
CD5%		0.0191	0.0112	0.0131
CD1%		0.0258	0.0142	0.0173
CV		4.66	5.01	5.22

* Inadequate sample tissue *SE Standard Error; CD Critical Difference; CV Coefficient of variation SD Standard Deviation*

Table 3 Effect of salicylic acid on total phenol, carbohydrates and protein contents in banana micropropagules grown under *in vitro* conditions

Mode of SA application	Salicylic acid concentration mg l^{-1}	Total Phenol content (mg g^{-1} Fresh tissue)± SD	Total carbohydrate content (mgg $^{-1}$Fresh tissue)± SD	Total Protein content (mgg $^{-1}$Fresh tissue)± SD
Pre-autoclaving	0	1.02±0.03	65.6±2.82	60.6±2.36
	5	1.32±0.07	82.21±3.26	78.21±3.66
	10	1.78±0.22	92.26±4.21	89.88±4.56
	25	1.61±0.09	65.85±2.74	55.69±3.64
	50	1.12±0.14	86.78±3.62	79.95±5.82
	75	1.11±0.12	112.28±4.61	103.25±4.98
	100	1.08±0.08	135.02±4.12	120.45±4.65
Post-autoclaving (Filter sterilized)	0	1.05±0.05	57.56±2.32	50.65±3.23
	5	1.27±0.06	53.45±2.66	45.85±2.12
	10	0.46±0.01	41.85±8.5	35.76±3.74
	25	0.53±0.04	86.22±4.69	76.36±2.88
	50	0.67±0.02	140.45±5.24	127.84±4.33
	75	*	*	*
	100	*	*	*
SE		0.0657	8.5665	7.8975
CD5%		0.190	22.2436	21.3542
CD1%		0.335	32.3412	30.6512
CV		09.55	17.32	16.25

* Inadequate sample tissue *SE Standard Error; CD Critical Difference; CV Coefficient of variation SD Standard Deviation*

On the other hand, post-autoclaving addition of SA did not show responses comparable to that when SA was incorporated before autoclaving. Although there was Slight increase in total chlorophyll contents at 5.0 mg l^{-1} SA, but its higher concentration resulted in quick decline which remained always lesser than control. There was an enhancement in total carbohydrate contents with increasing concentrations of SA, when added after autoclaving. It was therefore, concluded that the method of incorporation of SA did not make much disparity in terms of growth parameter. Although, the biochemical parameters differed significantly. It was also noted that initial low concentrations of SA (*i.e.* 5.0 and 10.0 mg l^{-1}), irrespective of its mode of treatment negatively affected both growth as well as biochemical parameters.

At the same time as, increase in SA concentration to a moderate level improved the growth conditions but this again showed negative drift ahead of optimum concentration.

DISCUSSION

Salicylic acid has been reported to play a very important role in the regulation of plant growth, development, interaction with other organisms and in the responses to biotic and abiotic stresses (Hayat *et al.*, 2010; Yusuf *et al.*, 2012; Rivas-San Vicenta and Plasencia, 2011). There are only few reports of regulatory role of SA in growth of micropropagules, morphogenesis of shoot and somatic embryogenesis under *in vitro* conditions (Babel *et al.*, 2014; Komaraiah *et al.*, 2004; Galal, 2012; Ram *et al.*, 2013).. Hence, the present study was attempted to envisage the promotory role of exogenously

supplied SA on *in vitro* growth and development of micropropagules of banana. There are reports where filter sterilized SA was used to study its modulatory role under *in vitro* conditions in *Cistus heterophyllus* and *Rosa hybrida* (López-Orenes *et al.*, 2013; Ram *et al.*, 2013). Similar mode of incorporation of SA in nutrient medium was adopted by Handro *et al.* (1997) and Bidabadi *et al.* (2012) to study stem elongation and water stress studies under *in vitro* conditions. Contrary to this many workers used to add SA prior to autoclaving of medium for different studies under *in vitro* conditions (Komaraiah *et al.*, 2004; Sakhanokho and Kelley, 2009; Galal, 2012). However, there is only a single report of comparison of these two modes of SA treatment during tissue culture (Babel *et al.*, 2014). We here discovered that the mode of application of SA in the growth medium did not have much influence on shoot growth and biomass production in banana during culture conditions. Even though the biochemical parameters such as total phenols, chlorophyll pigments, total protein and carbohydrates did show variation between two treatments, this may be due to change of pH of medium which was distorted due to incorporation of SA after autoclaving the medium. From these observation we put forth that SA could be incorporated in the medium before autoclaving to elicit desired responses. Positive response of adding SA in terms of shoot proliferation and biomass accumulation to resist water stress under *in vitro* condition in banana has been reported (Bidabadi *et al.*, 2012). In the current study, when the lower concentration of SA was supplemented with standard multiplication medium, it resulted in increased growth of shoots, their length and increase in fresh and dry weight of banana micropropagules. However, higher concentrations of SA negatively affected these parameters. Our results are in agreement with report of Babel *et al.* (2014) where the same observation were recorded for *Chlorophytum borivilianum* cultures. According to Hosseini *et al.* (2009), higher concentration of SA support synthesis of growth retarding hormone ethylene, which do not favor somatic embryogenesis in carrot. In our case also may be accumulation of ethylene with increasing concentration of salicylic acid proved inhibitory for *in vitro* shoot multiplication and elongation. In the this case also, possibly the higher concentration of synthesized ethylene with increased concentration of salicylic acid is responsible for growth retardation. The similar results has also been reported in *Ziziphus spina christi* tissue culture propagules Galal (2012).

When shoots of this tree were grown on medium supplemented with low level of SA, improvement in shoot bud proliferation was observed, while the higher concentration adversely affected the growth. Similarly Hayat *et al.* (2005) has also reported low level of SA result into higher pigment synthesis, higher biomass production, greater leaf number and high nitrate reductase activity in wheat seedling, while its higher concentration adversely affected the growth. In case of *Chlorophytum borivilianum*, when shoots were grown on medium supplemented with low level of SA, photosynthetic pigments, total carbohydrates, total phenol and biomass increased significantly while, the higher concentration discouraged (Babel *et al.* 2014). In present case also the total chlorophyll, carbohydrates, protein, phenol and biomass increased with lower level of SA, at the same time as the higher concentration resulted in depressed growth and less accumulation of these biomolecules. Khodary (2004) has reported enhanced levels of carbohydrate and photosynthetic pigments in low SA treated and salt stressed maize plants. It can therefore, be concluded that lower concentrations of SA under in vitro condition in banana resulted into enhancement of growth and augment important physiologically biochemical activities while higher concentration suppress the growth.

Acknowledgement

Authors are thankful to Department of Biotechnology (DBT) - New Delhi, for providing partial financial support under its DBT-Star College Scheme. Authors are also thankful to Gujarat State Biotechnology Mission (GSBTM, Gandhinagar) for financial assistance under FAP-2014 (GSBTM/MD/ PROJECT/SSA/1400/2014-15).

References

Agami, R. A. and Mohamed, F.G. 2013. Exogenous treatment with indole-3-acetic acid and salicylic acid alleviates cadmium toxicity in wheat seedlings. Ecotoxicol. Environ. Safety, 94:164-171.

Arnon, D.I. 1949. Copper enzyme in isolated chloroplasts: polyphenoloxidase in Beta vulgaris. Plant Physiol., 24:1-15.

Babel, P., Devpura, V., and Purohit, S. D. (2014). salicylic acid induced changes in growth and some biochemical characteristics in in vitro cultured shoots of *Chlorophytum borivilianum* sant. et fernand.. Intern. J. of Recent Sci. Res., 5(4):774-779.

Bairwa, K. C., Singh, A., Jhajaria, A., Singh, H., Goyam, B. K., Lata, M., and Singh, N. 2015. Conventional sucker with tissue culture banana production in Central India-A case study. *African Journal of Agricultural Research*, 10(14):1751-1755.

Banerjee, N., and de Langhe, E. 1985. A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of Musa (banana and plantain). Plant Cell Reports, 4(6):351-354.

Bidabadi, S.S., Mahmood, M., Baninasab, B. and Ghobadi, C. 2012. Influence of salicylic acid on morphological and physiological responses of banana shoot tips to in vitro water stress induced by polyethylene glycol. *Omics Journal*, 5:33- 39.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2):248-254.

Czajkowski, R., van der Wolf, J. M., Krolicka, A., Ozymko, Z., Narajczyk, M., Kaczynska, N., and Lojkowska, E. 2015. Salicylic acid can reduce infection symptoms caused by *Dickeya solani* in tissue culture grown potato (*Solanum tuberosum* L.) plants. *European Journal of Plant Pathology*, 141(3):545-558.

Galal, A. 2012. Improving effect of salicylic acid on the multipurpose tree Ziziphus spina- christi (L.) Willd tissue culture. Am. J. Plant Sci., 3:947-952.

Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, D. M., and Thorpe, T. A. (1996). Plant hormones and plant growth regulators in plant tissue culture. In Vitro

Cellular & Developmental Biology-Plant, 32(4):272-289.

Ghai, N., Setia, R.C., Setia, N., 2002. Effects of paclobutrazol and salicylic acid on chlorophyll content, hill activity and yield components in *Brassica napus* L. (cv. GSL-1). Phytomorphology, 52:83–87.

Handro, W., Mello, C.M., Manzano, M.A. and Floh, E.I.S. 1997. Enhancement of stem elongation and flower bud regeneration by salicylic acid. R. Bras. Fisiol. Veg., 9:139-142.

Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A. 2010. Effect of exogenous salicylic acid under changing environment. Environ. Exp. Bot., 68:14-25.

Hayat, S., Fariduddin, Q., Ali, B. and Ahmad, A. 2005. Effect of salicylic acid on growth and enzyme activities of wheat seedlings. Acta. Agron. Hung., 53:433-437.

Hayat, S., Masood, A., Yusuf, M., Fariduddin, Q.and Ahmad, A. 2009. Growth of Indian Mustard (*Brassica juncea* L.) in response to salicylic acid under high temperature stress. Braz. J. of Plant Physiol., 21:187-195.

Hosseini, S.S., Mashayekhi, K. and Alizadeh, M. 2009. Ethylene production and somatic embryogenesis of carrot explants as affected by salicylic acid treatments. American Eurasian J Agric. Environ. Sci., 6:539-545.

Khodary, S.F.A. 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int. J. Agric. Biol., 6:5-8.

Komaraiah, P., Jogeswar, G., Ramakrishna, V.S. and Kavikishor, P.B. 2004. Acetylsalicylic acid and ammonium-induced somatic embryogenesis and enhanced plumbagin production in suspension cultures of *Plumbago rosea* L. In Vitro Cell Dev. Biol.- Plant, 40:230-234.

López-Orenes, A., Martínez-Moreno, J. M., Calderón, A. A., and Ferrer, M. A. 2013. Changes in phenolic metabolism in salicylic acid-treated shoots of *Cistus heterophyllus*. Plant Cell, Tissue and Organ Culture, 113(3): 417-427.

Lu, H., Greenberg, J. T., and Holuigue, L. 2016. Editorial: Salicylic Acid Signaling Networks. Frontiers in plant science, 7.

Mahadevan, A. 1975. Methods in physiological plant pathology. Sivakami Publication, Madras (India).

Moharekar, S. T., Lokhande, S. D., Hara, T., Tanaka, R., Tanaka, A., and Chavan, P. D. 2003. Effect of salicylic acid on chlorophyll and carotenoid contents of wheat and moong seedlings. Photosynthetica, 41(2): 315-317.

Multu, S., Karadagoglu, O., Atici, O. and Nalbantoglu, B. 2013. Protective role of salicylic acid applied before cold stress on antioxidative system and protein patterns in barley apoplast. Biol. Plant., 57:507-513.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15:473-497.

Ram, M., Prasad, V.K., Singh, K.S., Hada, S.B. and Kumar, S. 2013. Influence of salicylic acid and methyl jasmonate elicitation on anthocyanin production in callus culture of *Rosa hybrida* L. Plant Cell Tiss. Org. Cult., 113:459-467.

Raskin, I. 1992. Role of salicylic acid in plants. Annual Review of Plant Physiol., 43:439-463.

Rivas, S.V. and Plasencia, J. 2011. Salicylic acid beyond defense: its role in plant growth and development. J. Exp.Bot., 62:3321-3338.

Sakhanokho, F.H. and Kelley, Y.R. 2009. Influence of salicylic acid on in vitro propagation and salt tolerance in *Hibiscus acetosella* and *Hibiscus moscheutos* (cv 'Luna Red'). Afr. J. Biotechnol., 8:1474-1481.

Sánchez-Rojo, S., Cerda-García-Rojas, C. M., Esparza-García, F., Plasencia, J., Poggi-Varaldo, H. M., Ponce-Noyola, T., and Ramos-Valdivia, A. C. 2015. Long-term response on growth, antioxidant enzymes, and secondary metabolites in salicylic acid pre-treated *Uncaria tomentosa* microplants. Biotechnol. letters, 37(12):2489-2496.

Saraswathi, M. S., Uma, S., Kannan, G., Selvasumathi, M., Mustaffa, M. M., and Backiyarani, S. 2016. Cost-effective tissue culture media for large-scale propagation of three commercial banana (*Musa* spp.) varieties. *The Journal of Horticultural Science and Biotechnology*, 91(1):23-29.

Swamy, R. D., Rao, N. S., and Chacko, E. K. 1983. Tissue-culture propagation of banana. Scientia Horticulturae, 18(3), 247-252.

Tandon ,P. 1976. Further studies on the process of gall induction on *Ziziphus* and the factors involved. Ph.D. Thesis, Jodhpur University, Jodhpur, India.

Tirani, M.M., Nasibi, F. and Kalantari, H.K.M. 2013. Interaction of salicylic acid and ethylene and their effects on some physiological and biochemical parameter in canola plants (*Brassica napus* L.). Photosynthetica, 51:411-418.

Wong, W. C. 1986. *In vitro* propagation of banana (Musa spp.): initiation, proliferation and development of shoot-tip cultures on defined media. Plant cell tiss. and organ culture, 6(2), 159-166.

Yusuf, M., Fariduddin, Q., Varshney, P. and Ahmad, A. 2012. Salicylic acid minimizes nickel and /salinity – induced toxicity in Indian mustard (*Brassica juncea*) through an improved antioxidant system. Environ. Sci. Pollut.Res., 19:8-18.

Zaheer, M., and Giri, C. C. 2015. Multiple shoot induction and jasmonic versus salicylic acid driven elicitation for enhanced andrographolide production in *Andrographis paniculata*. Plant Cell Tiss. and Organ Culture, 122(3), 553-563.

****

How to cite this article:

Anil Kumar Singh *et al*.2016, Exogenous Supply of Salicylic Acid Results Into Better Growth of Banana Propagules Under *In Vitro* Conditions. *Int J Recent Sci Res.* 7(3), pp. 9488-9493.

TSSN 0976-3031