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Antimicrobial Agents alters the *In Vitro* Plant Regeneration in *Solanum nigrum* (L.)

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Summary

Effect of different antimicrobial agents such as bavistin, cefotoxime and kanamycin on shoot regeneration using auxillary bud explnts of *Solanum nigrum* was studied. Axillary buds when cultured on MS medium supplemented with bavistin (200 mg/L) and in combination with BA (1.0 mg/L) showed the formation of multiple shoot was 4.5 and 4.8 respectively. Bavistin in combination with cytokinins showed enhanced shoot formation from the explants. Cefotoxime does not decrease the shooting response but it induced the maximum shoot formation (3.5) with a high frequency of regeneration (88%) from the axillary bud cultures of *Solanum nigrum.*The influence of kanamycin on regeneration of *Solanum nigrum* was evaluated.MS medium supplemented with 50 μM/L kanamycin induced highest number of shoots (3.0) and highest mean shoot regeneration (90%).

Key Words: *Solanum nigrum*, Axillary bud explants, Bavistin, Cefotoxime, Kanamycin, Plant regeneration

Introduction

Mass propagation of plant species through *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology. *Solanum nigrum* is an important herbaceous medicinal plant belongs to solanaeae family. Solancae family comprises a number of plants widely known for the presence of natural products of medical significance mainly steroidal lactones, glycosides, alkaloids and flavonoids. The herb is antiseptic, antidysentric and diuretic used in the treatment of cardiac, skin disease, psoriasis, herpi virus and inflammation of kidney. The fruits and leaves have been traditionally used against various nerve disorders [1]. It has very important gastric ulcerogenic activities [2], and is recommended in ayurveda for the management of gastric ulcers. Most prominent medical properties are the presence of alkaloids solamargin and solosonin which yield solasodine as glycone, solasodine has embryogenic, teratonic and antimicrobial activities [3].

Considering the high economical and pharmacological importance of secondary metabolites, industries are deeply interested in utilizing plant tissue culture technology [4]. In the present study the effect of different antimicrobial agents such as bavistin, cefotoxime and kanamycin on *in vitro* shoot regeneration was evaluated.

Materials and Methods

Collection of Plant material

The plants are collected from Anjaneya Puram, Tirupati, and Andhra Pradesh, India and grown in the nursery of Department of Biotechnology, S.V. University, and Tirupathi.

Surface sterilization

Explants were washed thoroughly under running tap water to remove the traces of dust etc. followed by treatment with 10% teepol/tween-20 for 5 minutes. Then the explants were sterilized in 70% ethanol for a minute, and finally with 0.01% $Hgcl_2$ for 1-2 minutes and washed 3-4 times with sterile double distilled water.

Culture medium

Auxillary bud explants (1-2 cms) were inoculated on MS medium [5] containing 3% sucrose and gelled with 0.8% agar supplemented with various concentrations of antimicrobial agents such as bavistin, cefotoxine and kanamycin. The pH of the medium was adjusted to 5.8 before gelling with agar and autoclaved for 20 minutes at 121^0C for 15 lbs pressure.

Sub culturing

Sub culturing was carried out at regular intervals of thirty days. Visual observations of the cultures were taken for every transfer and the effects of different treatments were quantified on the basis of percentage of cultures showing response.

Culture conditions

The growth room conditions maintained for *in vitro* cultures were 26 ± 2^0C and 60-70% relative humidity, light intensity was 3000 lux with a photoperiod of 18 hrs day light and 6 hrs dark. Each experiment was conducted at least thrice with 20 replicates per treatment

Results

Effect of bavistin on the plant regeneration from axillary bud explants of field grown S. nigrum plants

In axillary bud cultures, all the explants were responded well with bavistin. Maximum frequency of regeneration (85%), shoot number (4.50±0.31) and shoot length (4.33 ± 0.32) was obtained in bavistin (200 mg/L). Bavistin in combination with BA (1.0 mg/L) showed increased regeneration frequency (92%), shoot number (5.5 ± 0.20) with decreased shoot length (4.16 ± 0.33) when compared with bavistin alone supplemented MS medium. (Table-1; Figure-1) Bavistin with TDZ also showed less regeneration frequency (84%), shoot number (3.66 ± 0.33) and shoot length (3.0 ± 0.23) compared with the cultures having only bavistin. Interestingly, the bavistin induced axillary bud cultures are callus free.

Table-1: Effect of Bavistin singly and in combination with plant growth regulators on *in vitro* shoot organogenesis from axillary bud explants of *Solanum nigrum* plants

Bavistin (mg/L)	BA	TDZ	Regeneration frequency (%)	Number of shoots / explant	Length of shoot (cm)	Callus
50	-	-	60	1.50 ±0.31	3.00 ±0.23	-
100	-	-	74	2.00 ±0.23	3.50 ±0.31	-
150	-	-	76	2.66 ±0.33	4.0 ±0.31	-
200	-	-	85	4.50 ±0.31	4.33 ±0.32	-
300	-	-	70	1.50 ±0.31	5.0 ±0.23	-
100	1.0	-	92	5.5 ±0.20	4.16 ±0.33	C++
100	-	1.0	84	3.66 ±0.33	3.0 ±0.23	C++
-	1.0	-	80	6.0 ±0.31	4.5 ±0.31	C+
-	-	1.0	50	2.5 ±0.31	3.00 ±0.23	C+

Observations: After 4 weeks, values are mean ± SE of 20 independent determinants

Figure-1: Shoot initiation and multiplication from auxillary bud explants cultured on
A) MS + Bavistin 200 mg/L (bar 1cm = 1.5 cm)
B) MS + Cefotoxime 50 μm/L (bar 1cm = 1.3 cm)
C) MS + Kanamycin 50 μm/L (bar 1cm = 1.5 cm)
In vitro root organogenesis from regenerated shoots.
D) MS + IBA (0.50mg/L) (bar 1cm = 2.0 cm)
E) MS + IBA (0.50mg/L) (bar 1cm = 1.2 cm)
F) Hardened Plantlet in polybag containing soil and vermiculite in 1:1 ratio (bar 1cm = 2.0 cm)

Effect of cefotxime on the plant regeneration from axillary bud explants of field grown S. nigrum plants

The influence of cefotoxime on regeneration of *Solanum nigrum* was evaluated. The obtained data reveals the influence of cefotoxime on the regeneration of axillary buds of *Solanum nigrum* and the shoot initiation was observed after one week of inoculation. High frequency (88%), maximum shoot number (3.5 ± 0.31) and maximum shoot length (7.3 ± 0.31) was induced at (50 μM/L) cetoxime. At higher concentrations of cefotoxime (120 μM/L) showed decreased frequency (40%), shoot number (1.0 ± 0.16) and shoot length (4.0 ± 0.16) respectively. (Table-2; Figure-1) Cefotoxime at (20-50 μM/L) was considered as optimum concentration for *Solanum nigrum* cultures. In the present study cefotoxime alone stimulated the shoot organogenesis in the axillary bud explants of *Solanum nigrum*.

Table-2: Influence of cefotoxime added to MS medium on regeneration of plantlets from axillary bud explants of *Solanum nigrum*

Cefotoxime (μg/L)	Regeneration frequency (%)	Number of shoots / explant	Length of shoot (cm)	Callus
10	45	1.5 ±0.20	7.0 ±0.16	-
20	52	1.8 ±0.15	9.0 ±0.23	C+
30	58	2.0 ±0.23	5.5 ±0.20	-
40	72	2.5 ±0.20	4.0 ±0.16	-
50	88	3.5 ±0.31	7.3 ±0.31	-
80	87	2.0 ±0.23	6.0 ±0.40	-
100	50	1.5 ±0.20	6.5 ±0.20	-
120	40	1.0 ±0.16	4.0 ±0.16	-

Observations: After 4 weeks, values are mean ± SE of 20 independent determinants

Effect of kanamycin on the in vitro plant regeneration from axillary bud explants of field grown Solanum nigrum plants

The influence of kanamycin on regeneration of *Solanum nigrum* was evaluated. The obtained data reveals the influence of kanamycin on the regeneration of axillary buds. At lower concentrations (10-20 μM/L) light green coloured basal callus was formed. High frequency (90%), maximum shoot number (3.0 ± 0.40) and maximum shoot length (7.0 ± 0.23) was obtained with (50μM/L) kanamycin. The maximum shoot length was recorded in the cultures with 100 μM/L kanamycin, above 50 μM/L kanamycin the regeneration frequencies gradually decreased with increase in concentrations up to 100 μM/L. (Table-3; Figure-1) Higher concentration (above >100 μM/L) tested completely inhibited regeneration.

Table-3: Influence of kanamycin added to MS medium on regeneration of plantlets from axillary bud explants of *Solanum nigrum*

Kanamycin (μg/L)	Regeneration frequency (%)	Number of shoots / explant	Length of shoot (cm)	Callus
10	60	1.0 ±0.16	4.5 ±0.20	C+
20	72	1.5 ±0.20	5.5 ±0.20	C+
30	80	1.8 ±0.15	4.3 ±0.30	-
40	85	2.0 ±0.23	5.0 ±0.40	-
50	90	3.0 ±0.40	7.0 ±0.23	-
80	82	1.8 ±0.16	6.0 ±0.23	-
100	76	1.5 ±0.20	7.3 ±0.31	-
120	50	1.0 ±0.16	5.8 ±0.16	-

Observations : After 4 weeks, values are mean ± SE of 20 independent determinants

Table-4: Effect of auxins IBA, NAA on root induction from *in vitro* regenerated shoots of *Solanum nigrum*

Plant growth regulator (mg/l)	Frequency of root initiation (%)	Mean no. of roots	Mean shoot length (cm)
IBA			
0.25	80	11.0 ± 0.28	3.2 ± 0.18
0.50	95	18.5 ± 0.17	4.5 ± 0.31
0.75	90	15.4 ± 0.22	3.6 ± 0.35
1.00	76	12.8 ± 0.31	2.8 ± 0.17
NAA			
0.25	80	14.5 ± 0.34	3.0 ± 0.28
0.50	90	16.8 ± 0.17	4.2 ± 0.18
0.75	75	12.4 ± 0.22	2.8 ± 0.17
1.00	65	10.6 ± 0.35	1.6 ± 0.35

Observations: After 4 weeks, values are mean ± SE of 20 independent determinants

In vitro derived shoots with a length of (3-5cm) were excised and transferred to MS medium supplemented with different concentrations of auxins such as IBA and NAA (0.1–2.0 mg/l). High rooting frequency (98%) with highest number of roots (18.5 ± 0.17) were obtained in IBA (0.5 mg/L). (Table-4; Figure-1)

Acclimatization and hardening

Well rooted plantlets were separated from the culture tubes, washed and transferred to polybags containing soil + vermiculite in 1:1 ratio for hardening. Finally the hardened plantlets were transferred to field conditions. Rooted shoots showed the maximum percentage of survival.

Discussion

Antimicrobial agents (fungicides and antibiotics) are generally added in plant tissue culture media to control or eliminate contaminating microorganisms that are either present in the original explants or arise as laboratory contaminants.

Several of these agents are reported to effect cell culture growth and the occurrence of various regeneration events.

Bavistin appeared to have much stronger cytokinin-like activities. This is evident from a promotory effect of bavistin on shoot bud regeneration. Bavistin is a systemic fungicide that belongs to benzimidazole family. Benzimidazoles are a group of organic fungicides with systemic action that are extensively used in agriculture. It has been reported that the molecular structure of methyl benzimidazole carbamate or carbendazim (bavistin) has some resemblance to kinetin, adenine and to many other cytokinins based on adenine [6]. Bavistin was found to be the least toxic to plant cells and had a broad – spectrum fungicidal activity [7]. In addition, it has also been demonstrated that these compounds have beneficial effects on the physiology of the plant [8]. One study has shown that bavistin induces differentiation of roots and shoots in calli derived from carrot segment [6]. The explanation that bavistin induces shoot regeneration in *Solanum nigrum* cultures is possibly due to its cytokinin-like activity. Similar results were also reported in *Bacopa monniera* cultures and the shoot regeneration promoting activities of bavistin are results of increased biosynthesis of endogenous cytokinins within the cultures [9]. Usage of bavistin to control the fungal contamination does not show any negative effect on *Solanum nigrum* cultures and further promotes the shoot regeneration.

The antibiotic cefotoxime is reported to promote shoot regeneration from callus cultures of barley [10] and durum wheat [11]. Where as in case of apricot cefotoxime alone does not induce the shoot organogenesis, but in combination with other antibiotics such as timentin and vancomycin stimulated the shoot organogenesis [12]. The chemical structure of this antibiotic does not readily suggest a breakdown product with auxin-like properties [13]. And a different mode of action may have to be sought. Kanamycin sensitivity seems to be very dependent on the explant and species, and a wide range of organogenesis inhibiting concentrations can be found in the literature. Studies conducted in other plant systems have also shown that antibiotics [14] and fungicides [6] promote shoot regeneration. The possible mechanism of stimulatory effect of antibiotics on regeneration may involve generation of stress that makes cells competent for regeneration alternatively the antibiotics may mimic plant growth regulators [13, 15].

Conclusion

In the present investigation usage of antimicrobial agents eliminated the contaminating microorganisms and does not show any negative effect on *Solanum nigrum* cultures and it further promotes the shoot regeneration. Hence, the shoot promoting activities of these antimicrobial agents are very useful in micropropagation and conservation of this very important medicinal plant.

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