Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 3314-3318
Received: 30-06-2020
Accepted: 24-08-2020

Lokhande TK
College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Deshmukh VD
College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Kharde AV
College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Talekar BK
College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Corresponding Author:
Deshmukh VD
College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Effect of different concentrations of ba and naa on callus formation from cotyledonary leaf explants of wild Brinjal (*Solanum incanum* L.)

Lokhande TK, Deshmukh VD, Kharde AV and Talekar BK

Abstract
Different concentrations and combinations of BA and NAA hormones were used in MS medium to observe callus induction from cotyledonary Leaf Explants of Wild Brinjal (*Solanum incanum* L.) The rate of callus formation varied in different treatments used. Explants were cultured on MS media supplemented with different combinations and BA (0.0 mg/l, 0.5 mg/l, 1.0 mg/l, 1.5 m/l) in combination with NAA (0.2 mg/l, 0.4 mg/l,0.6 mg/l,0.8mg/l) with three replications. BA (1.5mg/l) in combination with NAA (0.2 mg/l) recorded highest result in days for callus initiation, fresh weight of callus and diameter of the callus. The protocol in the study might be useful for the production of disease free and healthy plant materials and also it would be useful for genetic transformation of eggplant using biotechnological approach.

Keywords: 6-Benzyl amino purine (BAP), α-Naphthalene acetic acid (NAA), *Solanum incanum*, regeneration

Introduction
Wild brinjal (*Solanum incanum* L.) belongs to family *Solanaceae* and *genus Solanum.* Scientific name of wild brinjal is *Solanum virum. Solanum incanum* commonly seen in Africa, Middle East and South Africa that belongs to subgenus Leptostemonum and Melongena section. Sirajudheen Anwar (2018) [9]. It is also known as bitter garden egg, bitter apple, snake apple, sodom apple, thorn apple, etc. In India, it is found in moderate and tropical region. Specially in Punjab, Bengal, South India, Orissa and Maharashtra
Solanum incanum is bushy herb up to 3 m tall (Abebe H. 2014) [1] with spines on the stem/stalk and calyces and with velvet hairs on the leaves. Flowers pale to deep blue, mauve or purple. The leaves are alternate egg shaped in outline with broad end at base (ovate) with slightly wavy margins (especially on young leaves) with a grey-green upper surface and a green- white lower surface. It is common as a weed, around houses, in overgrazed grassland & on roadside. It is also found at forest edges and in bush land and grassland from sea-level up to 2500 m altitude. It is considered an indicator for low-fertility soil. (Mwaura *et al.* 2011) [6].
It is used as medicinal plant. The crop is cultivated on small family farms and considered to be important source of nutrition and cash income for many resource poor farmers. Half mature fruits of wild brinjal are dried and alkaloids are extracted from it. Among all alkaloids found in wild brinjal solasodine content is up to 3%. The fruits are known for being low in calories and having a mineral composition beneficial for human health. They are also rich source of potassium, magnesium, calcium and iron. Its fruits help to lower the blood cholesterol level. In India, it is also used for the treatment of diabetes, bronchitis, asthma, dysuria, dysentery and anticancer activity.
Plant Tissue Culture provided better application for *in vitro* mass multiplication of plant. Micropropagation has many advantages over conventional breeding methods of vegetative propagation which suffer from several limitations. In micropropagation the multiplication rate is greatly increased, it also permits the production of pathogen-free materials. *In vitro* culture is not only applicable for plant conservation and propagations but also for secondary metabolite manipulation and production by considering above point effect on callus induction of wild brinjal (*Solanum incanum* L.) by using different concentrations of BA and NAA were studied.

Materials and Methods
Experimental site: The experiment was conducted in Department Plant Biotechnology of MGM College of Agricultural Biotechnology Gandheli, Aurangabad.

Experimental details
Statistical design:-Factorial Randomized Block Design. (FRBD) No. of treatment combinations:-16 (Combinations of 4 levels of BA and 4 levels of NAA).
No. of replications:-03

Treatment Details

Levels of BA in combination with NAA

Symbol of BA and NAA	Concentrations of BA and NAA (mg/l)
B_1N_1	0+0.2
B_1N_2	0+0.4
B_1N_3	0+0.6
B_1N_4	0+0.8
B_2N_1	0.5+0.2
B_2N_2	0.5+0.4
B_2N_3	0.5+0.6
B_2N_4	0.5+0.8
B_3N_1	1.0+0.2
B_3N_2	1.0+0.4
B_3N_3	1.0+0.6
B_3N_4	1.0+0.8
B_4N_1	1.5+0.2
B_4N_2	1.5+0.4
B_4N_3	1.5+0.6
B_4N_4	1.5+0.8

Preparation of media:
Murashige and Skoog (MS) medium was commonly used for all the experiments. Murashige and Skoog basal medium was supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar with different levels of BA and NAA combination. The pH adjusted at 5.6 to 5.8 before autoclaving.

Collection of Plant material
Seeds (*Solanum incanum L.*) were collected from field of MGM Agricultural Biotechnology, Gandheli.

Explants selection and preparation
The seeds obtained from the field was carefully drawn and soaked in water for 2 hours and seeds were sown in pot in the laboratory. After 30 to 35 days cotyledon was emerge out and used as explants. The explants were washed in running tap water for half hour and the unwanted waste materials and microbes was surface sterilized. The explants were washed with tween-20 for 10 minutes and again washed with running tap water for half hour. For further sterilization explants were transferred in the laminar air flow. Then it was washed with 70% ethanol for few seconds. The explants were washed with double distilled water and further with the 0.1% mercuric chloride. The explants was further rinsed five times with sterile double distilled water and then inoculated on a Murashige and Skoog basal medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar and different combination levels of BA and NAA.

Inoculation on micropropagation media
After 20 to 30 days, sterilized cotyledonary explants were surface sterilized and inoculated on micropropagation media supplemented with different concentrations of BAP (0, 0.5, 1.0 and 1.5mg/l) in combination with NAA (0.2, 0.4, 0.6 and 0.8mg/l). Add 3% sucrose and 0.8% agar and adjust the pH 5.6 to 5.8 before autoclaving. Explants were incubated at 25^0 c temperature with 16/8 hour photoperiod at 3000 lux light intensity.

Result and Discussion
Days required for callus initiation by using different Concentrations of BA and NAA from cotyledonary leaf explants of wild Brinjal (*Solanum incanum* L.) presented in Table 1.

Days required for callus initiation

Table 1: Combined effect of BA and NAA on days required for callus initiation.

	Days required for callus initiation
Levels of BA	
B_1	13.666
B_2	10.500
B_3	13.333
B_4	15.750
SE±	0.459
C.D at 1%	1.378
Levels of NAA	
N_1	14.000
N_2	12.416
N_3	13.166
N_4	13.666
SE±	0.459
C.D at 1%	1.378
Interaction (BA X NAA)	
SE±	0.915
C.D at 1%	2.746
GM	13.270

a. Effect of BA
Treatment 0.5 mg/l (B_2) recorded (10.500) number of days for callus initiation over other three treatment. B_1 (0 mg/l) and B_3(1.0 mg/l) were at par with each other and significantly superior over B_4.

b. Effect of NAA
Treament0.4 mg/l (N_2) were found significantly superior over N_1 (0.2mg/l), N_3 (0.6mg/l), N_4 (0.8mg/l). Treatment N_1, N_3, N_4 were not significantly differ from each other.N_1 found least over rest of the treatment of NAA.

Table 2: Interactive effect of BA and NAA on days required for callus initiation.

NAA/BA	N_1 (0.2mg/l)	N_2 (0.4mg/l)	N_3 (0.6mg/l)	N_4 (0.8mg/l)	Mean (n=4)
B_1(0mg/l)	15.333	13.000	12.333	14.000	13.666
B_2(0.5mg/l)	12.666	11.000	**8.333**	10.000	10.499
B_3(1.0mg/l)	13.666	12.666	14.333	12.666	13.332
B_4(1.5mg/l)	14.333	13.000	17.666	18.000	15.749
Mean (n=4)	13.999	12.416	13.166	13.666	GM=13.311

CD - 2.746

Treatment 0.5 mg/l BA + 0.6 mg/l NAA(B_2N_3) recorded highest result in (8.333) number of days for callus initiation and the second highest result found at treatment 0.5mg/l BA+ 0.8 mg/l NAA(B_2N_4) were recorded fresh weight(10) and (12.666) of callus found superior over rest of the all treatment. Treatments B_1N_1 was found significantly superior over B_1N_3, B_2N_2, B_2N_3 and B_2N_4. Results were shown in Table 2.
Treatment B_3N_3, B_4N_1 and B_4N_2 found superior over B_2N_2, B_2N_3 and B_2N_4. B_3N_1, B_4N_2 and B_1N_2 found best result over $B_2N_3$and B_2N_4. Treatment $B_2N_3$and B_2N_4were at par with each other. Earlier researchers reported similar type of work in *Solanaceae* family plants. Such as, Ray *et al*. (2010) [7], Shivaraj G and Srinath Rao (2010) [8], Jamil *et. al.* (2013) [5],

Bhatti *et al.* (2014) [2], Chakravarthi *et al.* (2010) [4] studied different concentrations and combinations of hormones i.e. BA and NAA were used in MS medium for callus formation.

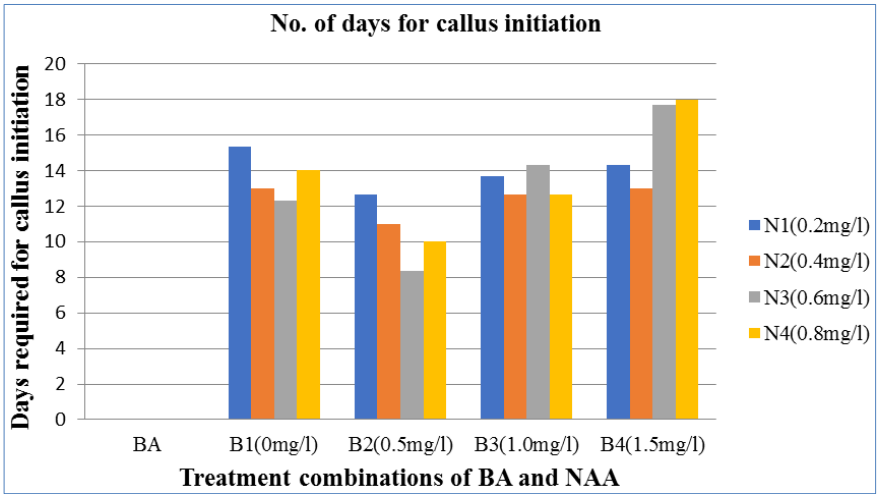

Fig 1: Fresh weight of callus by using different Concentrations of BA and NAA from cotyledonary leaf explants of wild Brinjal (*Solanum incanum* L.) presented in Table 3.

Table 3: Effect of BA and NAA on Fresh weight of callus (gm)

	Fresh weight of callus (gm)
Levels of BA	
B_1	0.743
B_2	1.691
B_3	2.615
B_4	2.056
SE±	0.055
CD at 1%	0.167
Levels of NAA	
N_1	2.155
N_2	1.838
N_3	1.645
N_4	1.488
SE±	0.055
CD at 1%	0.167
Interaction (BA X NAA)	
SE±	0.111
CD at 1%	0.335
GM	1.778

Effect of BA

Treatment B_3 (1.0 mg/l BA) recorded highest fresh weight (2.615) of callus over other treatments of BA. The second highest recorded at treatment B_4 found significantly superior over B_2 and B_1.However, treatment B_2also found significantly superior over B_1 treatment.

Effect of NAA

Treatment N_1 (0.2 mg/l NAA) were significantly superior over B_1, B_2, B_3. However, treatment B_2 was also found significantly superior over B_3 and B_4. Treatment B_3 and B_4 were at par with each other.

Table 4: Interactive effect of BA and NAA on fresh weight of callus:

NAA/BA	N_1(0.2mg/l)	N_2(0.4mg/l)	N_3(0.6mg/l)	N_4(0.8mg/l)	Mean(n=4)
B_1(0mg/l)	0.863	0.796	0.726	0.606	0.748
B_2(0.5mg/l)	1.836	1.756	1.640	1.563	1.706
B_3(1.0mg/l)	2.676	2.636	2.596	2.540	2.612
B_4(1.5mg/l)	3.243	2.133	1.616	1.233	2.056
Mean (n=4)	2.154	1.830	1.645	1.486	GM=1.781

CD-0.335

Treatment 1.5mg/l BA+0.2mg/l NAA (B_4N_1) is significantly superior over rest of all the treatments. However, B_3N_1, B_3N_2, B_3N_3, B_3N_4were second highest significantly superior over rest of the treatment tried. B_4N_4were also found significantly superior over B_1N_1, B_1N_2, B_1N_3, B_1N_4.

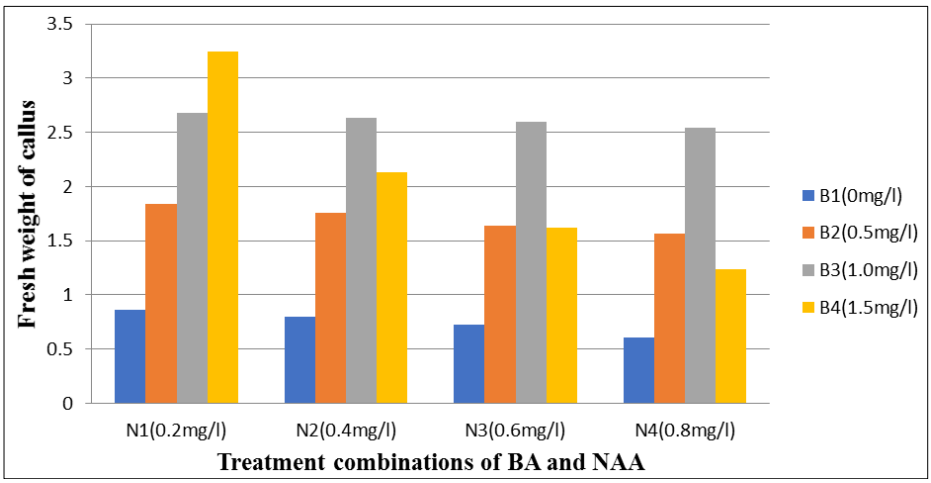

Fig 2.

Diameter of callus

Table 4: Data represented in table shown diameter of callus.

	Diameter of callus (mm)
Levels of BA	
B_1	4.683
B_2	8.396
B_3	11.825
B_4	14.303
SE±	0.385
CD	1.155
Levels of NAA	
N_1	10.625
N_2	9.953
N_3	9.503
N_4	9.116
SE±	0.385
CD	1.155
Interaction (BA X NAA)	
SE±	0.766
CD	2.300
GM	9.801

Effect of BA

Treatment B_4 (1.5 mg/l) recorded highest diameter over rest of the all other treatment. Treatment B_3 (1.0 mg/l) were found significantly superior over B_2 and B_1. B_2 (0.5 mg/l) were significantly found superior over B_1(0 mg/l).

Effect of NAA

Treatment N_1 (0.2 mg/l) found significantly superior over treatment N_4 (0.8 mg/l) however; Treatment N_1, N_2 and N_3 were at par.

Table 5: Interactive Effect of BA and NAA on diameter of callus.

NAA/BA	N_1(0.2mg/l)	N2(0.4mg/l)	N_3(0.6mg/l)	N_4(0.8mg/l)	Mean(n=4)
B_1(0 mg/l)	5.333	4.433	4.633	4.333	4.683
B2(0.5mg/l)	8.833	8.666	8.200	7.866	8.391
B_3(1.0mg/l)	13.033	12.166	11.333	10.766	11.824
B_4(1.5mg/l)	15.300	14.566	13.866	13.500	14.308
Mean(n=4)	10.624	9.957	9.508	9.116	GM=9.801

CD-2.300

For interactive effect of BA and NAA on diameter of callus Treatment B_4N_1(1.5 mg/l+0.2mg/l), B_4N_2(1.5 mg/l+0.4 mg/l), B_4N_3(1.5 mg/l+0.6mg/l) and B_4N_4(1.5 mg/l+0.8 mg/l) were at par with each other and found significantly superior over B_3N_3, B_3N_4, B_2N_1, B_2N_2, B_2N_3, B_2N_4, B_1N_1, B_1N_3, B_1N_2 and B_1N_4. B_3N_1(1.0mg/l+0.2mg/l), B_3N_3(1.0mg/l+0.6mg/l), B_3N_4(1.0 mg/l+0.8 mg/l) were at par with each other and found significantly superior over B_2N_1, B_2N_2, B_1N_1,B_2N_3, B_1N_3, B_1N_2 and B_1N_3. B_2N_1, B_2N_2, B_2N_3, B_2N_4 were found at par and significantly superior over B_1N_1, B_1N_2, B_1N_3 and B_1N_4.

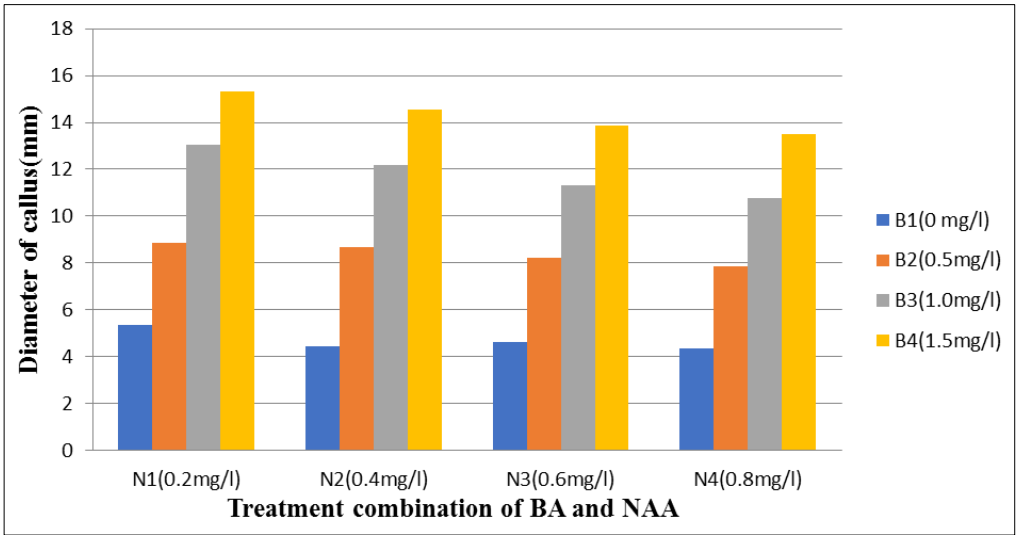

Fig 3.

Conclusion

Experiment was laid out in Factorial Randomized Blocked Design with four treatments and explants were cultured on MS media supplemented with different combinations and BA (0.0 mg/l, 0.5 mg/l, 1.0 mg/l, 1.5 m/l) in combination with NAA (0.2 mg/l, 0.4 mg/l,0.6 mg/l,0.8mg/l) with three replications. BA (1.5mg/l) in combination with NAA (0.2 mg/l) recorded highest result in days for callus initiation, fresh weight of callus and diameter of the callus as compared to other concentrations of BA and NAA.

The interactive effect of BA and NAA on days for callus initiation was at 0.5 mg/l BA+ 0.2 mg/l NAA in 8 days after inoculation.

The interactive effect of BA and NAA on fresh weight of callus was at 1.5 mg/l BA+0.2 mg/l NAA five weeks after inoculation.

The interactive effect of BA and NAA on diameter of callus was found at 1.5 mg/l BA +0.2 mg/l NAA five weeks after inoculation.

References

1. Abebe H, Gebre T, Haile Dane A. Phytochemical Investigation on the Roots of *Solanum Incanum*, Hadiya Zone, Ethiopia. Journal of Medicinal Plants Studies 2014;2(2):83-93.
2. Bhatti K, Jamil M, Tufail M. Direct organogenesis (shoot and root) of Eggplant (*S.melongena L*).World Applied Sci. J 2014;30(3):317-321.
3. Bose TK, Some MG, Kabir K. *Vegetable crops*, 2nd edn (Noya Prokash, Kalyani, India), 1993, 281.
4. Chakravarthi VN, Vijaya I, Uttam A, Goparaju, Yechuri. Effect of genotype, explants and hormonal concentration on *in vitro* response of Eggplant. J Sci. Bio. 2010;2(3):77-85.
5. Jamil M, Parvaiz M, Tufail M, Harshd J, Hussain S, Imtiaz S. Callogenesis, regeneration of shoot and root of Brinjal (*S.melongena L.*). World Applied Sci. J 2013;26(8):1039-1045.
6. Mwaura L, Anjarwalla P, Ofori DA, Stevenson PC, Smith P, Jamnadass R. Pesticidal Plant Leaflet, *Solanum incanum* L, 2011, 01-03.
7. Ray BP, Hassan L, Sarker SK. Plant regeneration from seedling derived explants through callus of Eggplant (*S.melongena L.*). J Krishi Foundation 2010;8(2):98-107.
8. Shivaraj G, Srinath R. Rapid and efficient plant regeneration of eggplant (*S.melongena L.*) from cotyledonary leaf explants. Ind. J of Biotech. 2011;10:125-129.
9. Sirajudheen Anwar. Pharmacological Investigation of *Solanum incanum* against *P. falciparum*, *L. infantum*, *T. cruzi and T. brucei*: A Role of Antioxidant Effect and Clinical Overview. *Biomedical & Pharmacology Journal*, 2018;11(2):653-660.