International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://theaspd.com/index.php

Studies on Effect of Different Concentrations and Combinations of Plant Growth Regulators on Induction of Callus and Micropropagation of Physalis Angulata, an Important Medicinal Herb

Ritika Kumari[1*], Niti Kiran[1], Kadambini Das[1], Deepak Kumar[2], Neelam Kumari[1], Gaurav Pandey[1], Poonam Sinha[1], Ranjana Kumari[1]
[1]University Department of Botany, Babasaheb Bhimrao Ambedkar Bihar University, Muzaffarpur, Bihar 842001, India
[2]Ram Dayalu Singh College, Babasaheb Bhimrao Ambedkar Bihar University, Muzaffarpur, Bihar 842001, India
***Corresponding Author:** Ritika Kumari
Email: ritikasingh1909@gmail.com

ABSTRACT:
In the present study experiments were done to select best concentrations and combinations of BAP, KN, NAA and 2,4-D for induction of callus, generation of microshoots and rooting in the tissue culture raised plantlets in Physalis angulata an important medicinal herb. The combinations and concentrations used for callus induction were (BAP, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 mg/l + 2,4-D 0.5, 1.0 and 1.5 mg/l) respectively. For regeneration of shoots from the nodal explant, the combinations and concentrations used were (BAP, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/l, KN, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l, NAA 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) respectively. From the table-1 (Graph-1), it may be noted that highest percentage of response for callusing was in the MS medium supplemented with 2.0 mg/l BAP + 1.0 mg/l 2,4-D, which was 58.64, when 0.25 mg/l BAP alone was supplemented in MS medium the percentage of response for callusing was the lowest which was 14.62 only. Similarly, highest percentage of response for microshoots generation in the nodal explants was in MS+ 2.0 mg/l BAP+ 1.0 mg/l KN+ 1.0 mg/l NAA which was 79.30. In this medium, the number of shoots per explant was also the highest that was 13.8 respectively. When BAP alone was supplemented in MS medium at 1.0 mg/l the percentage response was 63.50 and number of shoots 8.7. When the concentration of BAP was increased from 2.0 mg/l there was gradual reduction in the percentage response for microshoots regeneration on the nodal explants. This was also true for KN and NAA as MS + 4.0 mg/l BAP + 3.0 mg/l KN + 3.0 mg/l NAA the percentage of response for microshoots regeneration on nodal explants was only 52.80 and number of shoots was 8.2 respectively. Well grown microshoots were cultured in rooting medium which was supplemented with different concentrations of NAA, IBA and IAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg/l). It was noted that maximum percentage of response for rooting was observed in MS + 3.0 mg/l IBA which was 88.7 and the mean root number was 5.8 and the mean length was 3.4 cm respectively. In case NAA and IAA the higher response for rooting was in MS+ 2.0 mg/l NAA (76.8) and MS+ 2.0 mg/l IAA (82.7). The number and length of the branches were also higher in above medium.
***KEY WORDS:** Physalis angulata, Medicinal herb, Callus induction, Nodal explants, Plant Growth Regulators.*

INTRODUCTION:

Physalis angulata of the family Solanaceae is a wild herbaceous plant. At Muzaffarpur this plant is found during April to July in the rice field. It grows in bunch and sometimes entire field may be covered with this plant. One peculiar feature of this plant was noted that none of the grazing animals like goat, cow, or other, were grazing this weed although they were in abundance. Calyx of this plant unites to form a balloon like structure inside which the fruit develops. The plant has medicinal properties and traditionally it is used to cure arthritis, diarrhea, dysentery, skin diseases. It also act as an anti leishmanial agent. It has anti inflammatory property. It has anti diabetic property. It is also used to cure bacterial infection. It is also used to cure kidney, liver and gall bladder problems. (Silva, 2005, Melissa et al; 2005). Its antitumor property is due to presence of physalin compounds. Physalin B and F have been reported to have antitumor properties (Auton et al; 1987; Chiang et al; 1992; Sunayama et al; 1993).

Tissue culture studies and micropropagation of different medicinal plants have been done by different workers. Some of them are being mentioned here. Chan et al; (2003) reported micropropagation of member of Aracaceae. Fragua set al; (2004) in Ficuscarica L.

Pati et al; (2005) reviewed the micropropagation of rose plant. Ali et al; (2007) did in vitro study on micropropagation of Caladium bicolor. Neibaur et al; (2008) observed effect of different concentrations of auxins and cytokinin on callus induction and plant regeneration in Paspalum vaginatum. Nhut et al; (2010) developed highly efficient protocol for in vitro propagation of ornamental plant. Ray et al; (2011); reported regeneration of brinjal in vitro. Sayyed et al; (2013) reported effect of different concentrations of NAA and BAP on micropropagation of Alstroemeria. Arab et al; (2014) studied effect of nutrient media, different cytokinin types and their concentrations on in vitro multiplication of G.N. 5, hybrid almond peach root stock. Kavbiahi (2015) observed effect of different concentration of BAP and α-Naphthalene acetic acid on micropropagation of Begonia rex. Bhatti et al; (2017) reported effect of 2,4-D and NAA on callus induction in date palm and Medjool. Aslam et al; (2020) observed in vitro regeneration potential of white lupin (Lepinus albus) from cotyledenary nodes.
Keeping all these ideas in mind the present work was done to observe impacts of plant growth regulator for callusing, micro shoots production and rooting in it in vitro in Physalis angulata an important medicinal plant.

MATERIALS & METHODS:

Culture Medium:

Required amounts of MS basal medium were taken from the stock solutions for the preparation of 1 L medium in a 1L Borosil conical flask. The volume was made 1L by adding required amount of Distilled water. In this 30 gm Sucrose was dissolved. In another 1 L conical flask, 8 gm of agar powder was dissolved in 500 ml distilled water by boiling slowly. Then both the solutions were mixed. Then it was added with different concentrations of BAP alone separately. The pH was adjusted to 5.8 by adding 1N NaOH/HCl. In the other medium different concentrations of BAP + 2,4-D was added. Medium was dispensed in 250 cc conical flask 40 ml each. It was plugged with cotton plug wrapped with muslin cloth. All the flasks containing culture medium were sterilized by autoclaving them at 121^0C at 15 lb pressure for 20 minutes. Then they were allowed to cool and after two days were used for induction of callus.
Similarly, medium for regeneration of microshoots on nodal explant, medium was prepared by adding different concentration of BAP alone and with five different concentrations of KN and NAA. For rooting MS Medium was supplemented with different concentrations of NAA, IBA and IAA separately. In all the media, inoculation was done after two days of preparation.

Preparation of Explants:

Healthy plant of Physalis angulata was collected from the campus of B.R.A. Bihar University. In the laboratory the healthy and young leaves were separated with the help of surgical blade. All the selected leaves were surface sterilized in running tap water for 50 min, in order to remove dust particles from the surface. Then they were washed with distilled water, followed by washing with detergent (tween 20). This was followed by washing with distilled water. The explants were treated with 0.1% $HgCl_2$ concentration for 2-3 minutes. Above explants were rinsed with freshly prepared distilled water thrice having 3 minutes duration this was done to remove even a trace of chemical adhered on the surface of the explants. They were then wrapped in pre-sterilized and moist muslin cloth and stored at low temperature in freeze. This explant was sued for induction of callus. All the above procedures were followed for the preparation of nodal explants. Nodal explants were used for regeneration of microshoot. Leaf explants 0.5 to 1X1 cm were used for inoculation. All inoculations were done in the aseptic conditions of Laminar Air Flow Chamber. Inoculated culture tubes were incubated in culture room at 26 ± 1^0C temperature, 3000 lux of light provided by white cool fluorescent tube light (Philips) and 16 hr photoperiod. All the cultures showing contamination were discarded after autoclaving. All experiments were done in triplicate containing 15 cultures in each cycle.

RESULT AND DISCUSSION:

Leaf explant cultured in MS + BAP alone or MS+ BAP+ 2,4-D remained green and started swelling on 7^{th} day of inoculation. However, callusing on cut ends started on 14^{th} day of inoculation. Although callusing was noted on all the culture medium that was MS + BAP alone or MS + BAP + 2,4-D,. However, maximum percentage of callusing was in MS + 2.0 mg/l BAP+ 1.0 mg/l 2,4-D that was 58.64. The next highest percentage of callusing was observed in MS + 2.5 mg/l BAP + 1.0 mg/l 2,4-D that was 46.34. When MS + 0.25 mg/l BAP alone was used, the percentage of callusing was 14.62 only. Similarly, lowest percentage of callusing was observed in MS + 4.0 mg/l BAP + 1.5 mg/l 2,4-D. The percentage of response was only

28.02. At lower percentage the texture of callus was Brown and compact while the texture of callus in case of MS + 2.0 mg/l BAP + 1.0 mg/l 2,4-D was yellow green and friable. Data for callusing have been placed in table-1 (Graph-1).

Internodal explants were inoculated in MS + BAP alone or MS+BAP+KN+NAA respectively for regeneration of shoots. The data obtained have been presented in table-2 (Graph-2&3). From the table-2 (Graph-2&3), it may be noted that percentage of response for regeneration of shoots were in all the culture conditions but the highest percentage of response was in MS+2.0 mg/l BAP+ 1.0 mg/l KN+ 1.0 mg/l NAA which was 79.30. It was followed in MS+ 2.5 mg/l BAP + 1.5 mg/l KN + 1.5 mg/l NAA. Lowest percentage of response for regeneration of shoot was observed in MS+ 0.25 mg/l BAP alone. It was further noted that increasing concentrations of BAP 4.0 mg/l, KN 3.0 mg/l, NAA 3.0 mg/l had no promising impact on the regeneration of microshoots on nodal explant of Physalis angulata.

Number of shoots per explant was also noted in different culture conditions. Here again higher number of shoots (13.8) was found in the medium where the highest percentage of response for micro shoots regeneration was found. Lowest number of shoots was noted in the culture conditions where minimum percentage for regeneration was observed. In case of number of shoot also the increasing concentrations of BAP + KN+ NAA had no promising role. It was noted that the number of shoots on nodal explant cultured in MS + 0.25 mg/l, BAP was 5.6, while in MS + 4.0 mg/l BAP + 3.0 mg/l KN + 3.0 mg/l NAA, the number of branches were 8.2 only.

Well grown plantlets were cultured in rooting medium. Here the condition was MS + 5 different concentrations of NAA, MS + 5 different concentrations of IBA and MS + 5 different concentrations of IAA. Rooting was noted in MS + all the concentrations (0.5, 1.0, 1.5, 2.0, 2.5 mg/l) of NAA. Here highest percentage of response was in MS + 2.0 mg/l NAA that was 76.8 and mean number of roots was 3.6, while mean length was 2.8 cm. Lowest percentage of response 50.6 was in MS + 0.5 mg/l NAA and the mean number of shoot was 1.6 and length 1.2 cm respectively.

In case of MS + different concentrations of IBA (1.0, 1.5, 2.0, 3.0, 3.5) although rooting was in all the culture conditions but highest percentage of response for rooting 88.7 was noted in MS + 3.0 mg/l IBA, the mean number of roots was 5.8 and the length was 3.4 cm respectively. This was followed in MS + 2.0 mg/l IBA where the percentage of response was 75.3 and mean number of roots was 4.6, and the length was 2.6 cm respectively. In case of MS+ IAA, rooting was noted at all the concentration of IAA but the highest percentage of response was observed in MS + 2.0 mg/l IAA, which was 82.7, the mean number of roots was 4.8 and length was 3.2 respectively. Comparatively the best rooting medium was MS + 3.0 mg/l IBA, followed by MS + 2.0 mg/l IAA. In case of NAA, similar concentration was less promising for rooting in Physalis angulata.

DISCUSSION:

In the present study, experiments for induction of callus from leaf explants, multiple shoot on nodal explants, and rooting in plantlets regenerated through tissue culture were done. Here callus formation was noted in all the culture conditions but higher percentage was observed in MS + 2.0 mg/l BAP + 1.0 mg/l 2,4-D. Present findings are in agreement with the findings of Mastuti and Munawarti (2017), Bhatti et al; (2017). Dar et al; (2021) and Pramono et al; (2021). Similarly, microshoots were regenerated on nodal explants. Here also highest response was noted in MS + 2.0 mg/l BAP + 1.0 mg/l KN + 1.0 mg/l NAA. Above findings corroborate with the findings of Balaraju et al; (2008); Mohapatra et al; (2008); Zulkarnain and Neliyati (2017); Key et al; (2022). Although rooting in the tissue culture raised plantlets were observed in all the culture conditions but higher percentage of response, higher number of roots and root lengths were obtained in MS + 3.0 mg/l IBA. Above findings are also supported by the findings of Mastuti and Munawati (2017), Saleem et al; (2022) and Zinal et al; (2023). It may be concluded that for callusing the best explant is the leaf segments, and for shoot regeneration the best explant is the nodal segment of Physalis angulata L. Similarly, the synergistic effect of the cytokinins and auxin is more promising for shoot regeneration, than that of the alone cytokinin.

ACKNOWLEDGEMENTS:

The authors gratefully acknowledge the Head of Department of Botany, B.R.A. Bihar University, Muzaffarpur for providing facilities during this research work.

PHOTO PLATE-1:


Fig-1


Fig-2

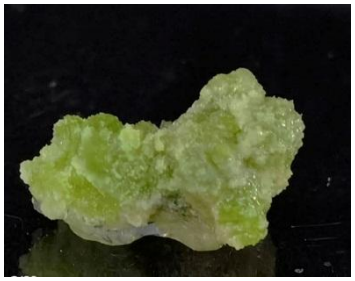


Fig-3

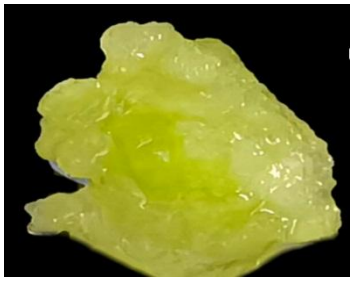


Fig-4


Fig-5

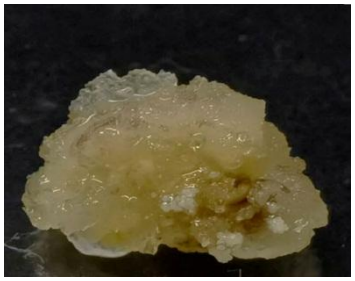


Fig-6


Fig-7

Explanation of Callus:
Fig-1: MS+4.0 mg/l BAP+ 1.5 mg/l 2,4-D
Fig-2: MS+1.0 mg/l BAP
Fig-3: MS+1.5 mg/l BAP+ 0.5 mg/l 2,4-D
Fig-4: MS+2.0 mg/l BAP+ 1.0 mg/l 2,4-D
Fig-5: MS+2.5 mg/l BAP+ 1.0 mg/l 2,4-D
Fig-6: MS+3.0 mg/l BAP+ 1.0 mg/l 2,4-D
Fig-7: MS+3.0 mg/l BAP+ 1.5 mg/l 2,4-D

PHOTO PLATE-2:


Fig-1

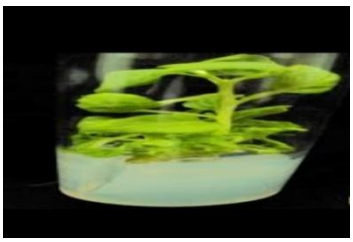


Fig-2


Fig-3


Fig-4


Fig-5


Fig-6

Fig-7

Explanation of Regeneration of shoots:
Fig-1: MS+2.0 mg/l BAP+ 1.0 mg/l KN + 1.0 mg/l NAA
Fig-2: MS+2.5 mg/l BAP+ 1.5 mg/l KN + 1.5 mg/l NAA
Fig-3: MS+3.0 mg/l BAP+ 2.0 mg/l KN + 2.0 mg/l NAA
Fig-4: MS+1.5 mg/l BAP+ 0.5 mg/l KN + 0.5 mg/l NAA
Fig-5: MS+1.0 mg/l BAP
Fig-6: MS+0.5 mg/l BAP
Fig-7: MS+0.25 mg/l BAP

Table-1 Induction of callus from leaf explants of Physalis angulata.

S.N.	Concentration of Plant Growth Regulators (mg/l)		Percentage Response	Texture of Callus
	BAP	2,4-D		
1	0.25	00	14.62	BC
2	0.5	00	16.78	YGC
3	1.0	00	27.86	YGF
4	1.5	0.5	38.55	YGF
5	2.0	1.0	58.64	YGF
6	2.5	1.0	46.34	YGF
7	3.0	1.0	37.18	WF
8	3.5	1.5	39.08	WC
9	4.0	1.5	28.02	WC
BC = Brown Compact, YGC = Yellow Green Compact, YGF = Yellow Green Friable, WF = White Friable, WC= White Compact.				

Table-2 Development of shoots from Internodal explant of Physalis angulata

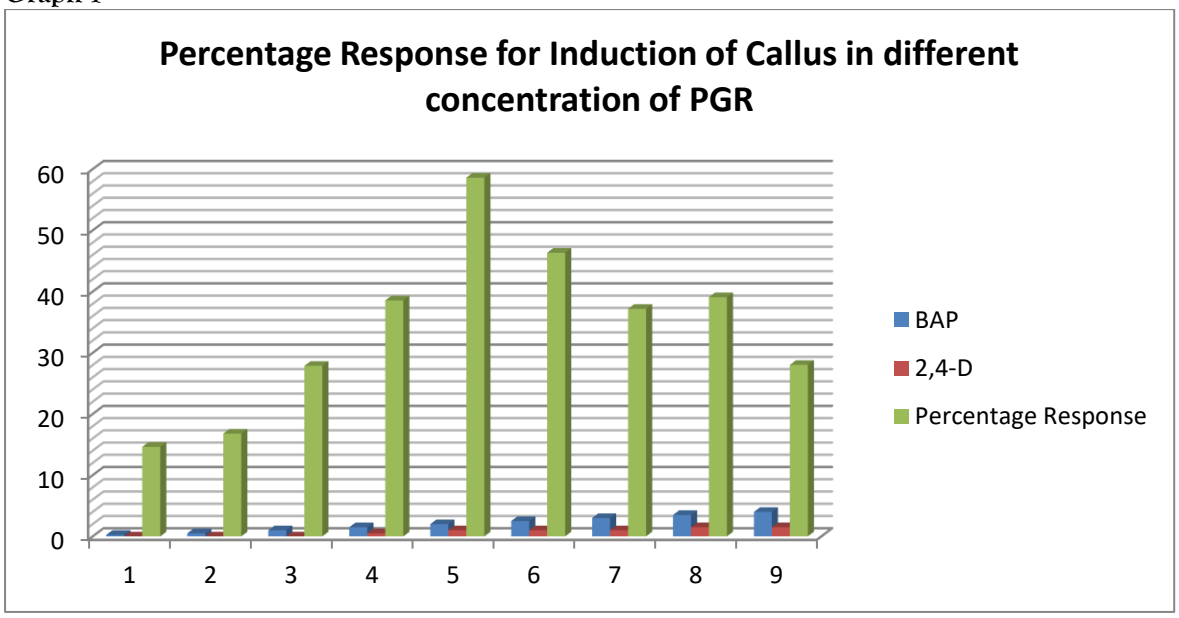
S.N.	Concentration of Plant Growth Regulators (mg/l)			% Response	No. of Shoots/ explants, Mean+ SD
	BAP	KN	NAA		
1	0.25	00	00	44.42	5.6 ± 0.208
2	0.5	00	00	56.60	7.2 ± 0.229
3	1.0	00	00	63.50	8.7 ± 0.235
4	1.5	0.5	0.5	68.70	10.4 ± 0.268
5	2.0	1.0	1.0	79.30	13.8 ±0.247
6	2.5	1.5	1.5	75.20	10.2 ± 0.292
7	3.0	2.0	2.0	71.60	9.4 ± 0.318
8	3.5	2.5	2.5	56.50	8.6 ± 0.305
9	4.0	3.0	3.0	52.80	8.2 ± 0.234

Table-3 Induction of roots in different concentration of auxins.

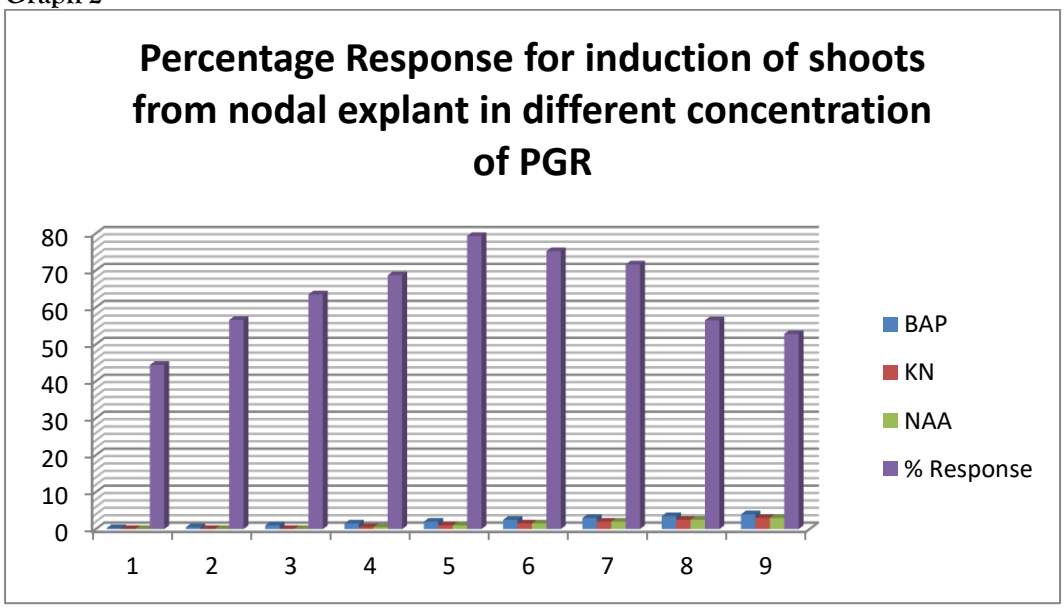
Plant Growth Regulator (mg/l)	% Response	Mean root numbers Mean ± SD	Mean root length (cm) Mean ± SD
NAA			

0.5	50.6	1.6 ± 1.08	1.2 ± 0.96
1.0	68.5	2.4 ± 1.60	1.3 ± 1.25
1.5	70.6	2.8 ± 1.55	2.6 ± 1.26
2.0	76.8	3.6 ± 1.77	2.8 ± 1.35
2.5	52.0	1.4 ± 1.44	1.4 ± 1.21
IBA			
1.0	60.4	2.8 ± 1.12	1.8 ± 0.23
1.5	74.5	3.3 ± 1.27	2.4 ± 0.48
2.0	79.8	4.6 ± 1.45	2.6 ± 0.49
3.0	88.7	5.6 ± 1.19	3.4 ± 0.60
3.5	75.3	3.6 ± 0.97	2.2 ± 0.41
IAA			
0.5	55.8	1.8 ± 0.97	1.3 ± 0.91
1.0	68.4	3.8 ± 1.06	1.6 ± 0.98
1.5	73.6	4.5 ± 1.16	2.6 ± 1.14
2.0	82.7	4.8 ± 1.11	3.2 ± 1.19
2.5	78.5	4.2 ± 1.17	2.2 ± 0.96

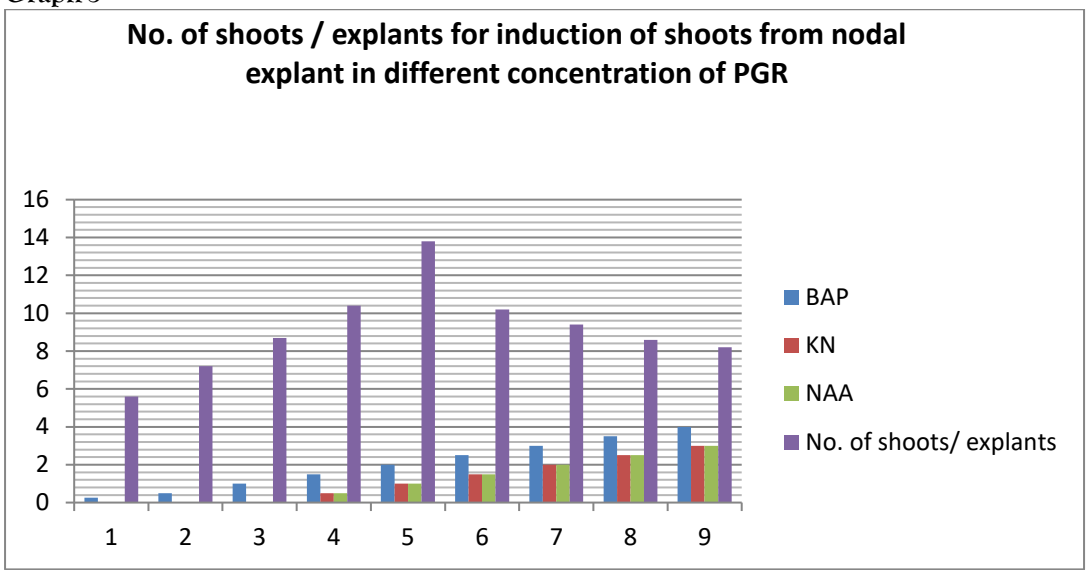
Graph-1



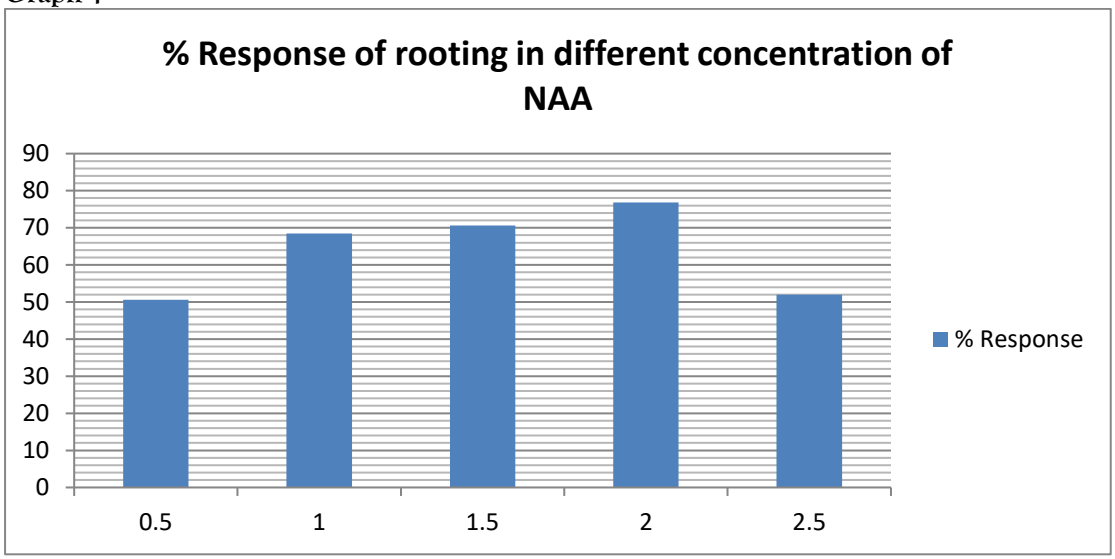
Graph-2



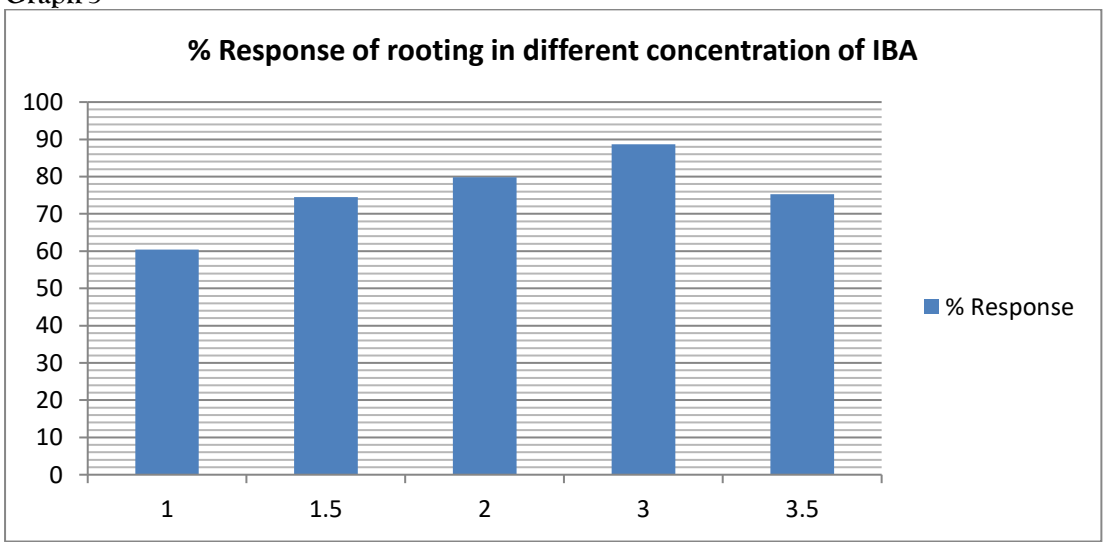
Graph-3



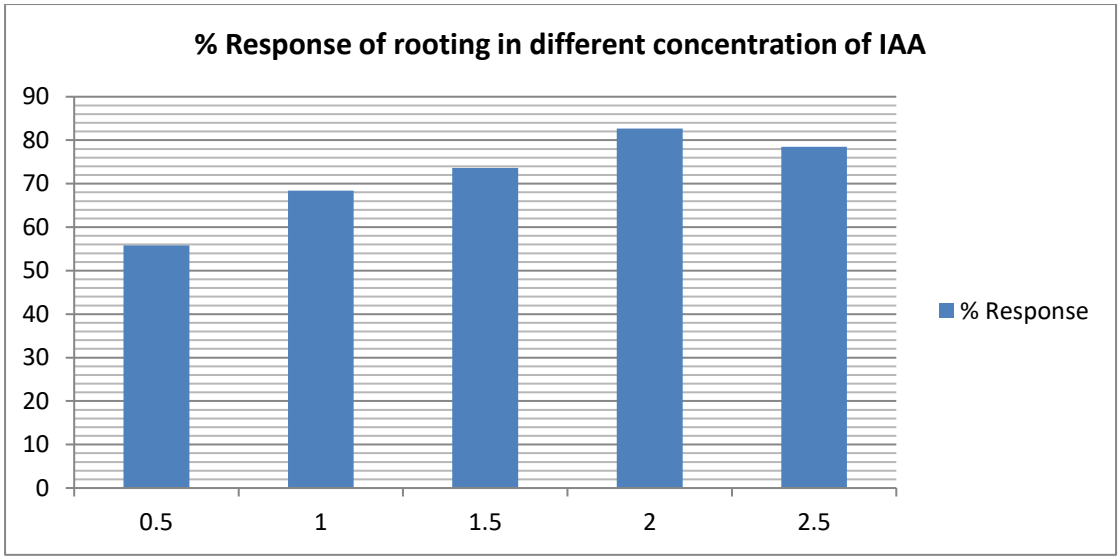
Graph-4



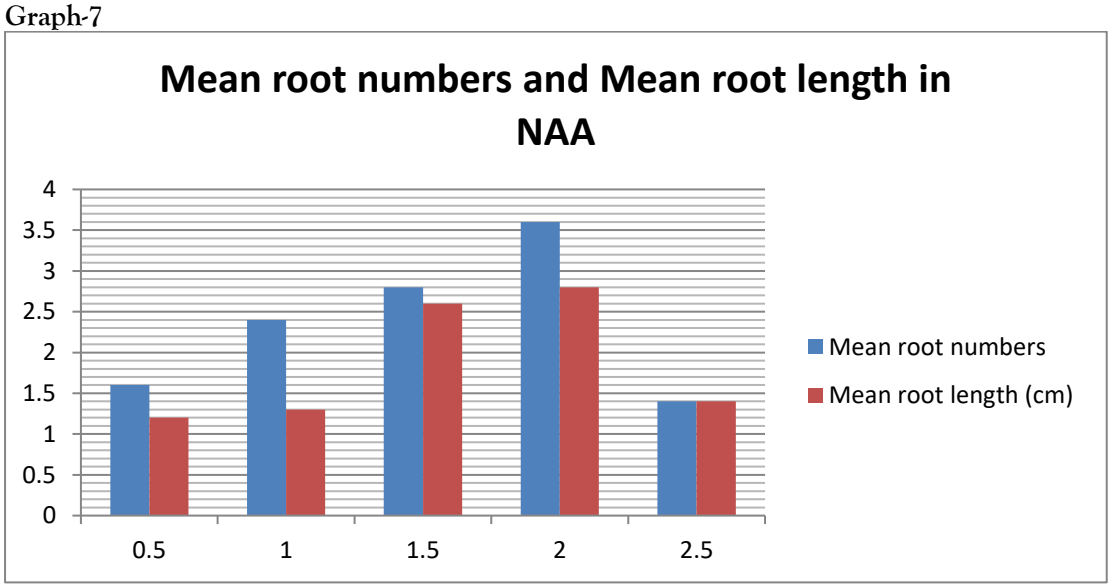
Graph-5



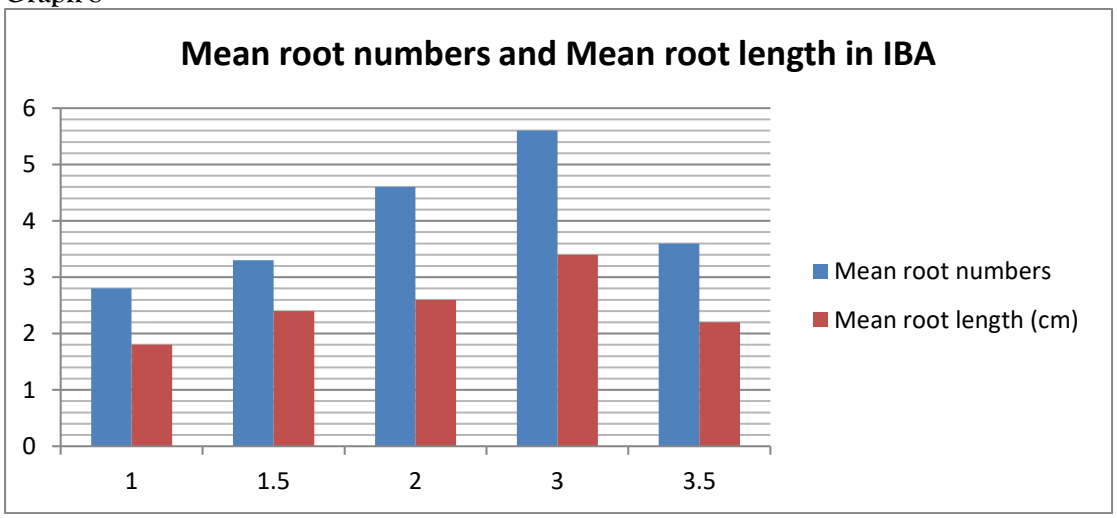
Graph-6



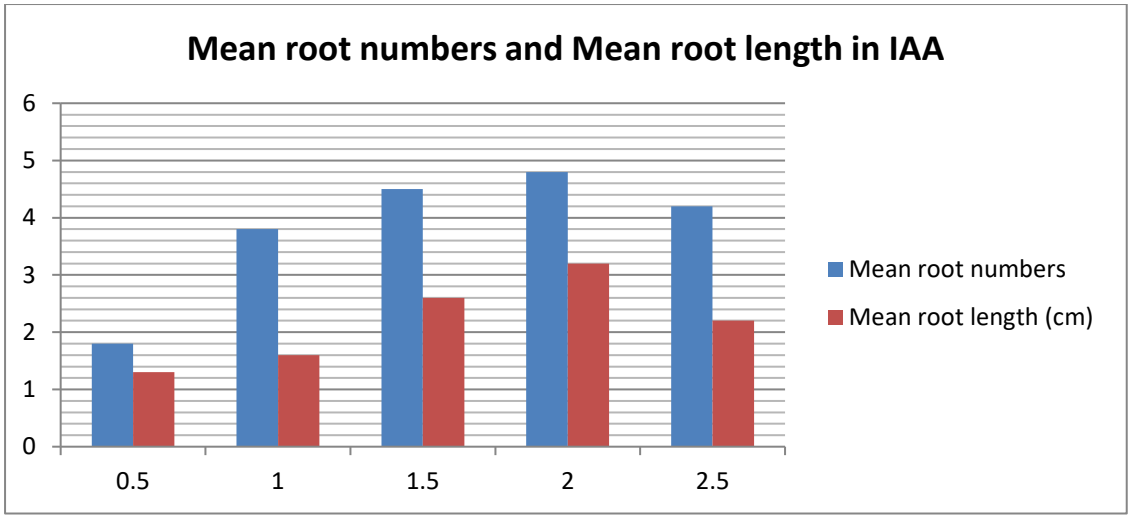
Graph-7



Graph-8



Graph-9



ABBREVIATIONS:

MS = Murashige and Skoog
BAP = 6, Benzyl Amino Purine
2,4-D = 2,4-Diechlorophenoxy Acetic Acid
NAA = Naphthalene Acetic Acid
KN = Kinetin
IBA = Indole Butyric Acid
IAA = Indole Acetic Acid

REFERENCES:

1. **Ali A; Munawar, A. and Naz. S. (2007):** An in vitro study on micropropagation of Caladium bicolor. Int. J. of Agricult. and Biol. 9(5): 731-735.
2. **Arab, M.M., A. Yadollahi, A. Shojaeiyan, S. Shokri and S.M. Ghosh(2014):** Effects of nutrient media, different cytokinin types and their concentrations on in vitro multiplication of G.N. 5 (hybrid among peach) vegetative root stock. J. of Genetic Engineering and Biotechnology 12: 81-87.
3. **Aslam, M.M. Karanja, J.K. Zhag, Q., Lin, H., Xza T. Aktar K; Xu, W. (2020):** In vitro regeneration potential of white lupin (Lupinus albus) from cotyledonary nodes. Plants 9: 318-328.
4. **Balaraju, K., Agastian, P., Preetam raj J.P., Arokiyaraj S., Ignacimuthu, S. (2008):** Micropropagation of Vitexagnus castus (Verbenaceae) a valuable medicinal plant. In vitro Cell Dev. Biol. 44: 436-441.
5. **Bhatti, A., Singh, D. Garg S. and Shivalingam P. (2017):** Effect of 2,4-D and NAA on callus induction in date palm and Medjool. Int. J. of Farm Sciences 7: 132-136.
6. **Chan, L., Tan, C.M. and Chew, G.S. (2003):** Micropropagation of the Aracaceae ornamental plants. Acta Horticulture 606: 383-390.
7. **Dar, S.A., I.A. Nawachoo, S. Tyub, A.N. Kamilli (2021):** Effect of plant growth regulators on in vitro induction and maintenance of callus from leaf and root explants of Atropaacum inata Royal ex Lindl. Biotechnology Reports 32: 1-5.
8. **Fraguas, C.B., Pasqual, M; Dutra, LF. And Cazzeta O. (2004):** Micropropagation of Fig (Ficus carica L.) Plant. In vitro Cell Development and Biology Plant 40: 471-474.
9. **Gikloo Elahi, B.M., Knorowchahli (2012):** Effect of explant types, Plant Growth Regulators on indirect organogenesis in Silybum marianum. Afr. J. Biotechnol. 37: 9023-9027.
10. **Kaviani, B. (2015):** Some useful information about micropropagation of Begoniarex Putz. By 6-benzylamine (BA) and α-Naphthalene acetic acid (NAA). Int. J. of Biosciences 6(5): 8-15.
11. **Kay, T.O. A.M., Mon., M.Y. Hatwe, K.S.,O, W.T. H.tet, Lone Kyi, W.W. Win (2022):** Effect of different concentration and combinations of BAP and NAA on micropropagation of Anthurium (Anthurium andraeanum L.) Red. J. of Scientific and Innovative Research 11(2): 25-30.
12. **Mastuti Retno and A. Munawarti (2017):** In vitro morphogenesis response of various explants in Physalis angulata L. The Journ of Exp. Life. Sci. 7(2): 102-105.
13. **Mohapatra H., Bari D.D., Rath S.P. (2008):** In vitro regeneration of medicinal plant Centella asiatica. Biol. Plant 52(2): 339-342.
14. **Murashige, T. and Skoog, F. (1962):** A revised medium for rapid growth and bioassay with tobacco tissue culture. Physilogia Plant 15: 473-497.
15. **Neibaur I., Gallo M. and Altpeter F. (2008):** The effect of auxin type and cytokinin concentration on callus induction and plant regeneration frequency from immature inflorescence segments of Paspalum vaginatum. In vitro Cell Dev. Biol. Plant 44: 480-486.
16. **Nhut, D.T; Hai, N. T. and Phan, M.X. (2010):** A highly efficient protocol for micropropagation of Begonia tuberosus. In: Jain S.M. Ochatt (eds) Protocol for in vitro propagation of ornamental plants. Springer Protocols Humana Press. (15-20).
17. **Pramono, P.A., N. Harijati and Wahyu W. (2021):** Effect of the combination of NAA and BA on callus induction from hypocotyls explants in black cumin (Nigella sativa L.). IOP Conf. Ser. Earth and Environ. Sci. 743(1): 012013

18. **Ray, B.P., L. Hassan and K. Nasiruddin (2011):** In vitro regeneration of Brinjal (Solanum melongena L.) Bangladesh J. Agri. Res. 36(3): 397-406.
19. **Saleem Yasmeen, Emad, M.Z., A. Ali, Shagufta Naz. (2022):** Synergistic effect of different plant growth regulators on micropropagation of sugar cane (Saccharum officinarum L.). Agriculture 12: 1-15.
20. **Pati, P.K., Rath, S.P. Sharma M., Sood A. and P.S. Ahuja (2005):** In vitro propagation of rose- a review. Biotechnology Advance 94-114.
21. **Seyyed R.S., B. Kaviani and N.P., Dechkoai (2013):** The effect of different concentrations of NAA and BAP on micropropagation of Alstroemeria. European J. of Experimental Biology 3(5): 133-136.
22. **Z. Zinal; A. Anwar, Gustian, Fitriwati, and R.Y. Yunita (2023):** The effect of several concentrations of BAP and source of explants to Gambier shoot induction (Uncaria gambier (Hunter)Roxb. Earth and Environ. Sci. 1160-012021: 1-8.
23. **Zulkarnian and Neliyati (2017):** The effect of NAA and BAP on Tissue culture of Tangkit pine apple (Ananas comosus L.). Biospecies 10(1): 1-10.