ZKG INTERNATIONAL ISSN: 2366-1313

HIGHLY EFFICIENT REGENERATION STUDIES OF VARIETIES OF MAIZE (*Zea mays* L.) CULTIVARS P3501 AND P3546

Jyothi Potta[1], Rajender Vadluri[2], Sumankalyan Sadhu[3], Anusha Bairy[4], Vaishnavi Anumula[4], Sujatha D[5], Sreelatha Beemagani[1*]

[1]Department of Microbiology, Chaitanya (Deemed to be University), Himayathnagar (V), Moinabad (M), Ranga Reddy District, Hyderabad-500075(TG)

[2]Department of Biotechnology, Chaitanya (Deemed to be University), Himayathnagar (V), Moinabad (M), Ranga Reddy District, Hyderabad-500075(TG)

[3] Department of Microbiology, Kakatiya University, Hanumakonda-506009(TG).

[4]Department of Biotechnology, Kakatiya University, Hanumakonda-506009(TG).

[5]Department of Botany, Kakatiya University, Hanumakonda-506009(TG).

[1*]**Corresponding Author:** Sreelatha Beemagani

lathahod@chaitanya.edu.in

Professor, Department of Microbiology, Chaitanya (Deemed to be University), Himayathnagar (V), Moinabad (M), Ranga Reddy District, Hyderabad-500075(TG).

ABSTRACT

An efficient in vitro regeneration protocol was established in Maize varieties of P3501 and P3546 seeds, and from explants embryo axis, auxiliary meristem, leaf and cotyledonary node. Various concentrations and combinations of different plant growth regulators (PGRs) were employed to induce multiple shooting and rooting to obtained complete plantlets of Maize. Shoots regeneration was grown on MS medium (Murashige and Skoog) supplemented with 6-Benzylaminopurine (BAP) at 0.2mg/L concentration. Explants were cultured on MS basal (Murashige and Skoog's) medium supplemented with 2.0mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D) + 0.2mg/L 6-benzylaminopurine (BAP) showed good response to shooting in 23 days. For root formation, developed shoots which were in vitro conditions (24±2^{o}C and photoperiod of 16/8hours) transferred to MS medium supplemented with 1.0mg/L Indole-3-butyric acid (IBA) in 15days. Plantlets were transferred to 1:1:1 peat moss, vermiculite and sand and grown in laboratory for maturity. A wide range of cultivars grown, only P3501 was responded excellent with this regeneration system.

Keywords: Maize, embryo axis, auxiliary meristem, leaf, cotyledonary node, shoot regeneration, root regeneration.

Key message: an efficient regeneration protocol was established for Maize. Multiple shooting and rooting medium were found by different concentrations and combinations of plant growth regulators (PGRs).

ISSN: 2366-1313

Introduction

Maize (*Zea mays* L.) is the world's third most important cereal crop after rice (*Oryza sativa*) and wheat (*Triticum aestivum*) [1]. Maize is an important annual cereal crop of the world belonging to the family Poeceae [2]. Maize considered as a staple food in many parts of the world. Globally Maize is known as 'Queen of cereals' [3] because of its highest genetic yield potential among the cereals. Every part of maize plant has economic value [4]. The grains, leaves, stalk, tassels and cob, all be used to produce a large variety of food and non-food products [5]. It is the most versatile crop and it is grown in more than 166 countries across the globe [6]. It is cultivated nearly Haworth production of 1148 million tonnes and productivity of 5823 kg/ha all over the world having wider diversity of soil, climate, biodiversity and management practices contributing 37% in the global grain production (FAOSTAT 2019). India produced 30 million tonnes in an area of 9.9 million hectares in 2020-21 (agricoop.nic.in).

According to Indian Institute of Maize Research, in India, maize is principally grown in two seasons; rain (Kharif) and winter (Rabi) [7]. Kharif maize represents around 83% of maize area in India, while Rabi maize correspond to 17% of maize area [8].

Maize is a tall deep rooted, warm weather annual grass plant. A single long stalk will develop from seed. Long smooth leaves attached at the stem nodes. Seed producing shoots originate from the base of the main stem. The female flowers are borne on the corn which arises at the leaf arial near the mid-point along the stem. A mass of long styles (silks) protrudes from the tip as a mass of silky threads. Most varieties of corn require 100-140days from seedling to ripeness.

In this present investigation, we report a new plant regeneration method for Indian cultivars P3501 and P3546 from explants of embryo axis, auxiliary meristem, leaf and cotyledonary nodes [9][10]; various concentrations and combinations of different plant growth regulators were used for multiple shooting and rooting [11] to obtain a complete maize plantlet. MS (Murashige and Skoog) [12] medium supplemented with different concentrations and combinations of auxins (IAA, IBA, PAA, NAA and 2,4-D) [13] and cytokinins (BAP, TZ, KIN and DPU) [14] were used for this regeneration studies [15][16]. More shoots were obtained from embryo axis on MS medium containing 2.0mg/L 2,4-D and 0.2mg/L BAP and more roots were obtained from embryo axis on MS medium supplemented with 1.0mg/L IBA.

Materials and methods

Plant material

Healthy and mature Seeds of maize cultivars P3501 and P3546 were collected from the Indian Institute of Maize Research (ICAR), Agricultural Research Station, Karimnagar, Telangana, INDIA were used for present study.

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Surface sterilization of plant material

Seeds of two varieties P3501 and P3546 were washed thoroughly under running tap water for 20 minutes followed by washed with sterile water three times. Then it treated with 70% ethanol [17] soaked for 2 minutes and washed with sterile water for five times. Treated with 0.1% mercuric chloride ($HgCl_2$) [19] by soaking for 15 minutes followed by washing with sterile water for five times. After this, the seeds were allowed to dry on sterile blotting paper for 20 minutes [20].

Inoculation

The seeds P3501 and P3546 were inoculated on MS basal medium with the embryo axis [9][10], on the medium surface at the rate of one seed per tube and incubated in the culture room at temperature of 25±2ºC [19]. 5-7days old seedlings were selected to dissect the explant and taken embryo axis, auxiliary meristem, leaf and cotyledonary node were used as explants for direct regeneration [15][16].

For shoot induction, cultivars P3501 and P3546 were studied on MS medium supplemented with various concentrations and combinations of auxins (IAA, IBA, NAA and 2,4-D) [13] and Cytokinins (BAP and KIN) [14].

Explants of embryo axis, auxiliary meristem, leaf and cotyledonary node [9][10] grown aseptically from 7days old seedlings of maize grown on MS medium. Explants grown on MS medium supplemented with various concentration and combinations of phytohormones [21], changes in response of different explants were recorded. All the cultured explants showed expansion followed by swelling and callus initiation.

Multiple shoot regeneration

The explants showed shoot induction which were directly in contact with the medium surface. The shoots were observed on different growth regulators in number and percentage of different explants (embryo axis, axillary meristem, leaf, cotyledonary node) [9][10] for shoot induction for cultivars P3501 and P3546 were tested on MS medium supplemented with various concentrations and combinations of auxins [13]and cytokinins [14].

The shooting media tested with MS medium supplemented with 2,4-D (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L) and BAP (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50mg/L); BAP (0.5 - 5.0mg/L) and IAA (0.05 - 0.50mg/L); 2,4-D (0.5 - 5.0mg/L) and IAA (0.05 - 0.50mg/L); BAP (0.5 - 5.0mg/L) and IBA (0.05 - 0.50mg/L); 2,4-D (0.5 - 5.0mg/L) and IBA (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50mg/L); BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L) and NAA (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50mg/L); and 2,4-D (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L) and NAA (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50mg/L).

Explants of embryo axis, axillary meristem, leaf and cotyledonary node [9][10] were grown aseptically in 23 days on MS basal medium. The explants grown on MS medium supplemented with various concentrations and combinations of auxins [13] and cytokinins [14], the changes in a

ISSN: 2366-1313

response of different explants were recorded.

Explants cultured on MS basal medium with2,4-D (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L) and BAP (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50mg/L) showed best number shoots.

Root regeneration

The response of different explants (embryo axis, axillary meristem, leaf, cotyledonary node) [9][10] for root induction for cultivars P3501 and P3546 were tested on MS medium supplemented with various concentrations and combinations of only auxins.

The rooting media tested with MS medium supplemented with IBA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L), IAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L) and NAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L).

Explants of embryoaxis, axillary meristem, leaf and cotyledonary node were grown aseptically from 20 days old seedlings of maize on MS basal medium. The explants grown on MS medium supplemented with various concentrations and combinations of auxins, the changes in a response of different explants were recorded.

Multiple shoot regeneration

Explants cultured on IAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L) and NAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/L) showed only swelling and enlargement. but failed to germinate into roots. The roots were developed on MS medium supplemented with IBA 1.0mg/L.

Results and Discussion

The rooted shoots were transferred to small pots containing sterile potting mix of peat moss, vermiculite and sand (1:1:1). And covered with polythene bags with small holes to maintain high humidity [15] for 15 days. After 15 days, polythene bags were removed, plantlets were allowed to grow in the in vitro conditions for 5 days. The shoots that acclimatized successfully were transferred to field conditions with 82% survival rate.

Table 1.1 Shoot regeneration frequency from different explants of P3501 on MS medium supplemented with 2,4-D + BAP.

S.No.	Concentration of hormones mg/L		Explants			
	2,4-D	BAP	Embryo axis	Auxiliary meristem	Leaf	Cotyledonary node
1.	0.5	0.05	04	06	-	-
2.	1.0	0.10	10	09	02	-
3.	1.5	0.15	13	08	04	04
4.	**2.0**	**0.20**	**26**	**11**	**06**	**07**
5.	2.5	0.25	15	06	05	02
6.	3.0	0.30	17	07	03	05
7.	3.5	0.35	16	09	01	03

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8.	4.0	0.40	12	07	01	01
9.	4.5	0.45	11	06	01	02
10.	5.0	0.50	07	08	-	-
Grand Mean			13.1	7.7	2.3	2.4

Data scored after 23days of culture initiation grown on the MS medium with growth regulators 2,4-D + BAP and about 50 explants.

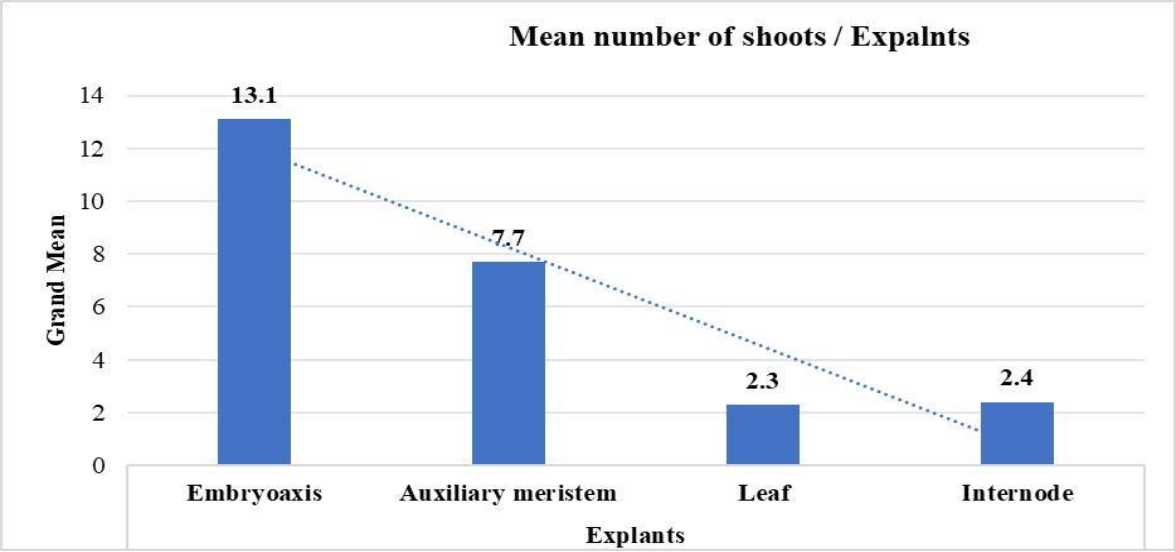

Fig 1.1 In maize variety P3501, highest number of shoot regeneration seen in explant embryo axis on MS medium supplemented with 2,4-D 2.0mg/L and BAP 0.2mg/L (52%).

Table 1.2 Shoot regeneration frequency from different explants of P3501 on MS medium supplemented with BAP + IAA.

S.No.	Concentration of hormones mg/L		Explants			
	BAP	IAA	Embryo axis	Auxiliary meristem	Leaf	Cotyledonary node
1.	0.5	0.05	02	02	-	-
2.	1.0	0.10	06	04	-	-
3.	1.5	0.15	12	09	02	01
4.	2.0	0.20	16	11	06	05
5.	2.5	0.25	17	12	07	05
6.	3.0	0.30	19	12	09	07
7.	**3.5**	**0.35**	**21**	**14**	**10**	**08**
8.	4.0	0.40	13	11	08	04
9.	4.5	0.45	06	09	07	02
10.	5.0	0.50	03	03	-	-
Grand Mean			11.5	8.7	4.9	3.2

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Data scored after 23days of culture initiation grown on the MS medium with growth regulators BAP + IAA and about 50 explants.

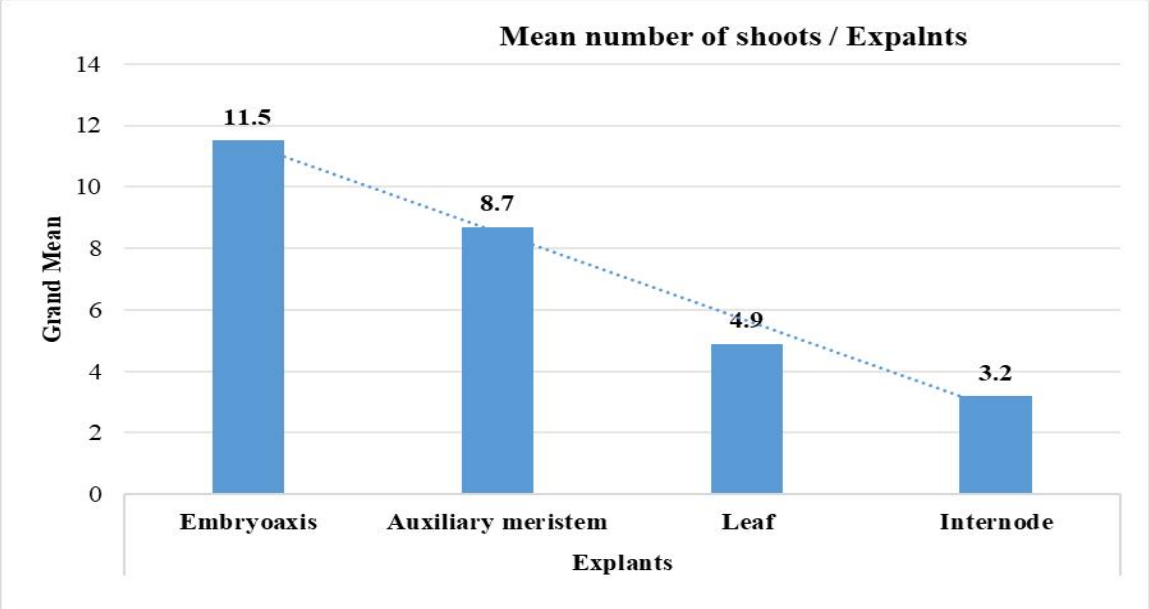

Fig.1.2 In maize variety P3501 highest number of shoot regeneration seen in explants embryo axis on MS medium with BAP 3.5mg/L and IAA 0.35mg/L (42%).

Table 1.3 Shoot regeneration frequency from different explants of P3501 on MS medium supplemented with 2,4-D + IAA.

S.No.	Concentration of hormones mg/L		Explants			
	2,4-D	IAA	Embryo axis	Auxiliary meristem	Leaf	Cotyledonary node
1.	0.5	0.05	04	03	-	-
2.	1.0	0.10	07	05	-	-
3.	1.5	0.15	09	07	03	02
4.	2.0	0.20	13	10	06	04
5.	**2.5**	**0.25**	**16**	**11**	**09**	**08**
6.	3.0	0.30	13	09	07	06
7.	3.5	0.35	11	07	04	04
8.	4.0	0.40	09	05	02	03
9.	4.5	0.45	05	03	01	02
10.	5.0	0.50	02	03	-	-
Grand Mean			8.9	6.3	3.2	2.9

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Data scored after 23days of culture initiation grown on the MS medium with growth regulators 2,4-D + IAA and about 50 explants.

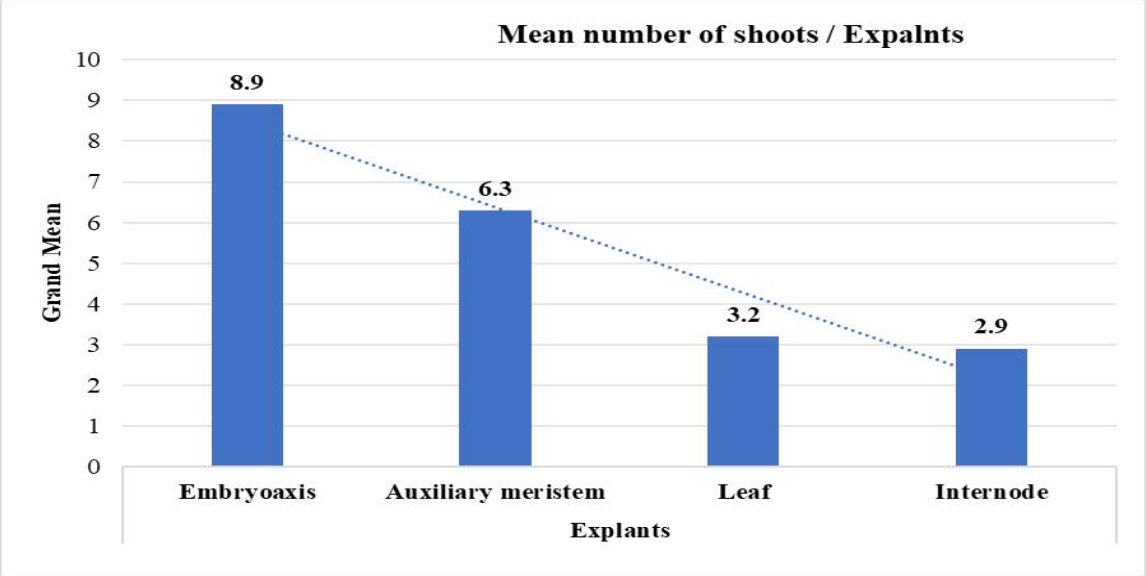

Fig.1.3 In maize variety P3501 highest number of shoot regeneration seen in explants embryo axis on MS medium with 2,4-D 2.5mg/L and IAA 0.25mg/L (32%).

Table 1.4 Shoot regeneration frequency from different explants of P3501 on MS medium supplemented with BAP + IBA

S.No.	Concentration of hormones mg/L		Explants			
	BAP	IBA	Embryo axis	Auxiliary meristem	Leaf	Cotyledonary node
1.	0.5	0.05	03	02	-	02
2.	1.0	0.10	06	03	03	03
3.	1.5	0.15	08	07	06	04
4.	**2.0**	**0.20**	**13**	**09**	**07**	**06**
5.	2.5	0.25	11	07	05	05
6.	3.0	0.30	10	07	06	05
7.	3.5	0.35	08	05	04	04
8.	4.0	0.40	06	04	03	02
9.	4.5	0.45	04	03	02	-
10.	5.0	0.50	03	02	-	-
Grand Mean			7.2	4.9	3.6	3.1

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Data scored after 23days of culture initiation grown on the MS medium with growth regulators BAP + IBA and about 50 explants.

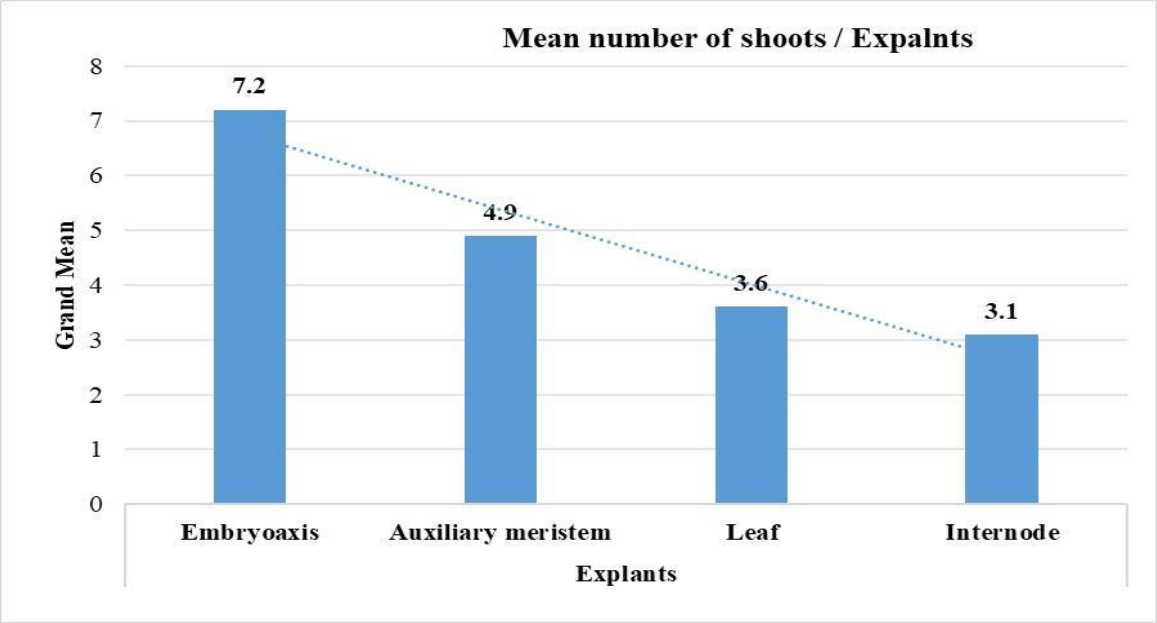

Fig.1.4 In maize variety P3501 highest number of shoot regeneration seen in explants embryo axis on MS medium with BAP 2.0mg/L and IBA 0.20mg/L (26%).

Table 1.5 Shoot regeneration frequency from different explants of P3501 on MS medium supplemented with 2,4-D + IBA.

S.No.	Concentration of hormones mg/L		Explants			
	2,4-D	IBA	Embryo axis	Auxiliary meristem	Leaf	Cotyledonary node
1.	0.5	0.05	02	02	-	-
2.	1.0	0.10	03	02	-	01
3.	1.5	0.15	06	04	02	01
4.	2.0	0.20	08	05	03	03
5.	2.5	0.25	11	07	05	04
6.	**3.0**	**0.30**	**12**	**10**	**06**	**05**
7.	3.5	0.35	10	08	05	03
8.	4.0	0.40	07	05	05	02
9.	4.5	0.45	04	04	03	02
10.	5.0	0.50	03	02	01	-
Grand Mean			6.6	4.9	3.0	2.1

ISSN: 2366-1313

Data scored after 23days of culture initiation grown on the MS medium with growth regulators 2,4-D + IBA and about 50 explants.

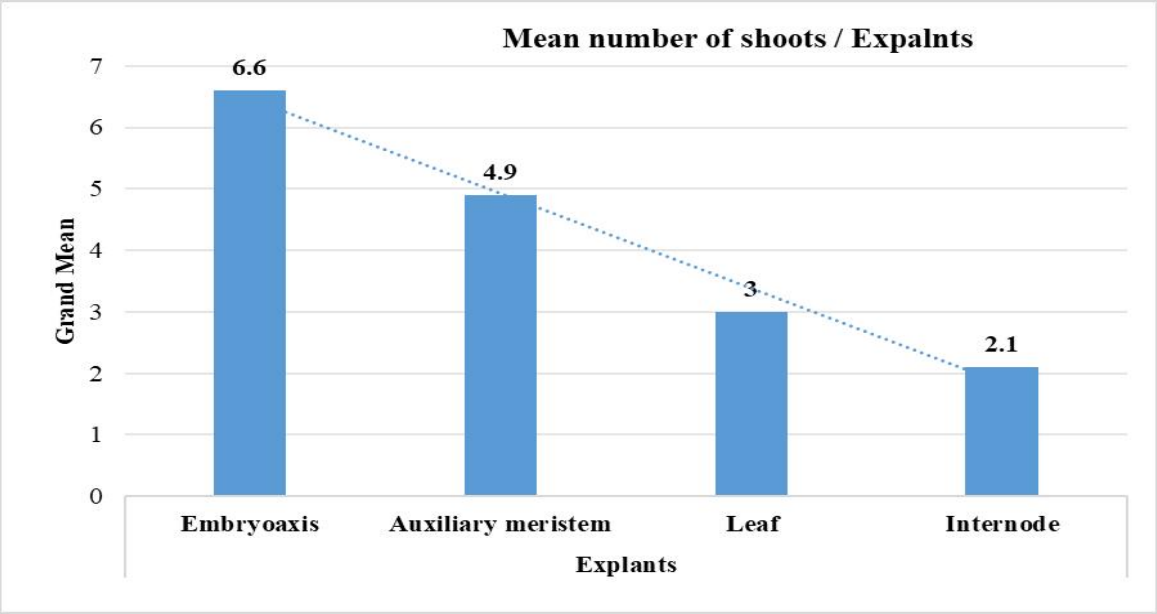

Fig.1.5 In maize variety P3501 highest number of shoot regeneration seen in explants embryo axis on MS medium with 2,4-D 3.0mg/L and IBA 0.30mg/L (24%).

Table 1.6 Shoot regeneration frequency from different explants of P3501 on MS medium supplemented with BAP + NAA.

S.No.	Concentration of hormones mg/L		Explants			
	BAP	NAA	Embryo axis	Auxiliary meristem	Leaf	Cotyledonary node
1.	0.5	0.05	02	02	-	-
2.	1.0	0.10	03	02	-	01
3.	1.5	0.15	05	04	03	01
4.	2.0	0.20	08	06	05	03
5.	**2.5**	**0.25**	**11**	**09**	**06**	**04**
6.	3.0	0.30	07	08	04	03
7.	3.5	0.35	06	06	05	03
8.	4.0	0.40	04	05	03	02
9.	4.5	0.45	03	03	02	01
10.	5.0	0.50	01	01	-	-
Grand Mean			5.0	4.6	2.8	1.8

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Data scored after 23days of culture initiation grown on the MS medium with growth regulators BAP + NAA and about 50 explants.

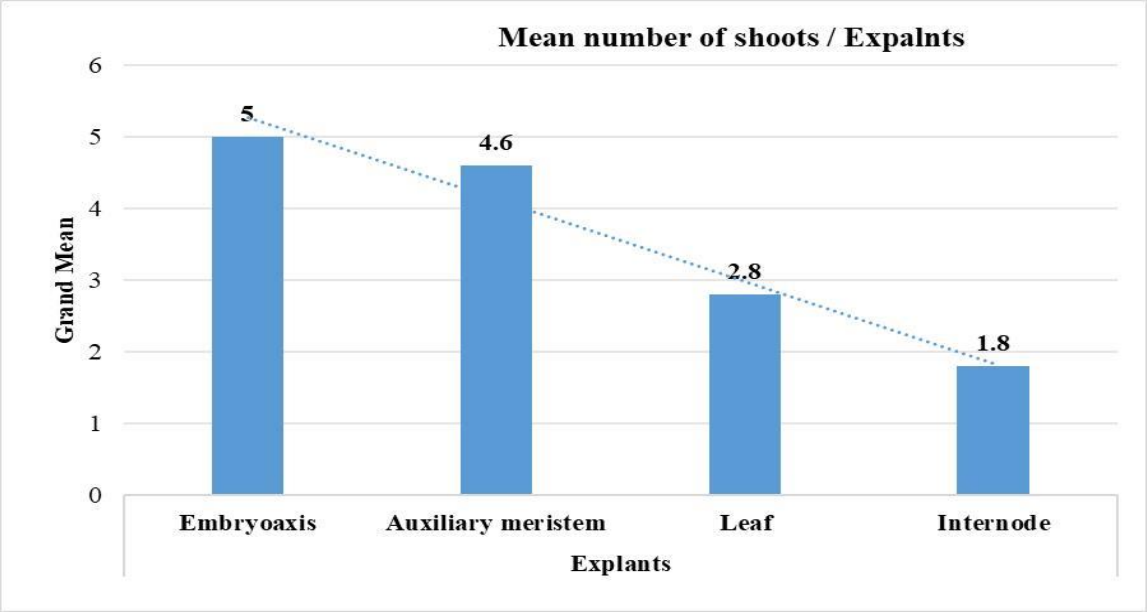

Fig.1.6 In maize variety P3501 highest number of shoot regeneration seen in explants embryo axis on MS medium with BAP 2.5mg/L and NAA 0.25mg/L (22%).

Table 1.7 Shoot regeneration frequency from different explants of P3501 on MS medium supplemented with 2,4-D + NAA.

S.No.	Concentration of hormones mg/L		Explants			
	2,4-D	NAA	Embryo axis	Auxiliary meristem	Leaf	Cotyledonary node
1.	0.5	0.05	02	02	-	-
2.	1.0	0.10	04	04	01	02
3.	1.5	0.15	07	07	03	04
4.	**2.0**	**0.20**	**10**	**08**	**05**	**03**
5.	2.5	0.25	07	07	04	02
6.	3.0	0.30	07	06	03	02
7.	3.5	0.35	05	04	03	01
8.	4.0	0.40	04	03	02	01
9.	4.5	0.45	02	02	01	-
10.	5.0	0.50	01	01	-	-
Grand Mean			4.9	4.4	2.3	1.5

ISSN: 2366-1313

Data scored after 23days of culture initiation grown on the MS medium with growth regulators 2,4-D + NAA and about 50 explants.

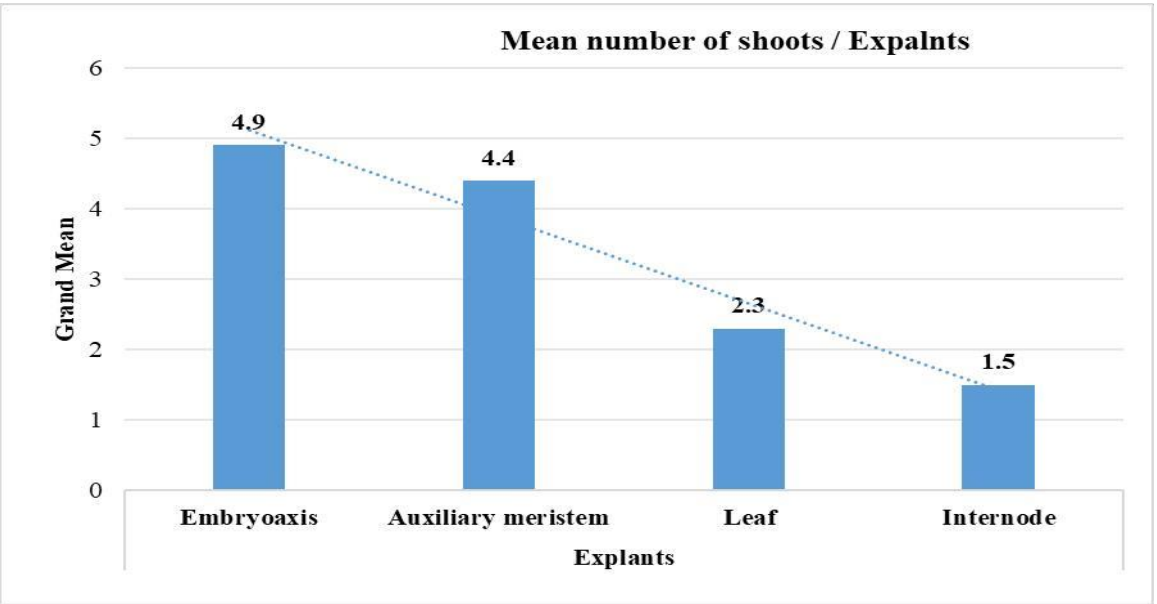

Fig.1.7 In maize variety P3501 highest number of shoot regeneration seen in explants embryo axis on MS medium with 2,4-D 2.0mg/L and NAA 0.20mg/L (20%).

Maize variety P3501, highest number of shoot regeneration seen in explant embryo axis on MS medium supplemented with 2,4-D 2.0mg/L with BAP 0.2mg/L (52%)

Root regeneration

Table 2.1 Root regeneration frequency from different explants of P3501 on MS medium supplemented with IBA 1.0mg/L.

S.No.	Concentration of hormones mg/L	Length of roots from Explants in cm			
	IBA	Embryo axis	Auxiliary meristem	Leaf	Cotyledonary node
1.	1.0	8.0	5.2	1.7	3.6
2.	1.0	8.3	5.9	1.4	3.2
3.	1.0	7.4	5.5	1.6	3.5
4.	1.0	7.1	5.7	1.2	3.4
5.	1.0	6.9	5.3	1.3	3.4
Grand Mean		7.54	5.52	1.44	3.42

ISSN: 2366-1313

Data scored after 15days of culture initiation grown on the MS medium with growth regulators IBA and about 50 explants.

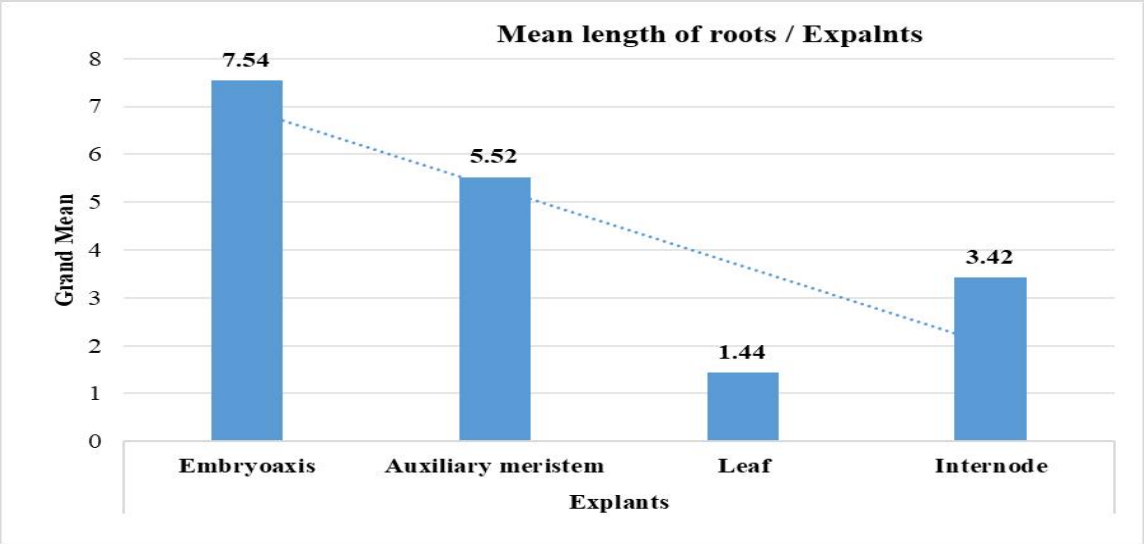

Fig.2.1 In maize variety P3501 maximum length of root regeneration seen in explants embryoaxis on MS medium with IBA 1.0mg/L.

Table 2.2 Root regeneration frequency from different explants of P3546 on MS medium supplemented with IBA 1.0mg/L.

S.No.	Concentration of hormones mg/L	Size of roots from Explants			
	IBA	Embryoaxis	Auxiliary meristem	Leaf	Cotyledonary node
1.	1.0	6.6	4.7	0.4	1.4
2.	1.0	6.2	4.9	0.9	1.9
3.	1.0	6.4	4.5	1.1	1.7
4.	1.0	6.6	4.7	0.8	1.8
5.	1.0	6.5	4.3	0.9	1.9
Grand Mean		6.46	4.62	0.82	1.74

Data scored after 15 days of culture initiation grown on the MS medium with growth regulators IBA and about 50 explants.

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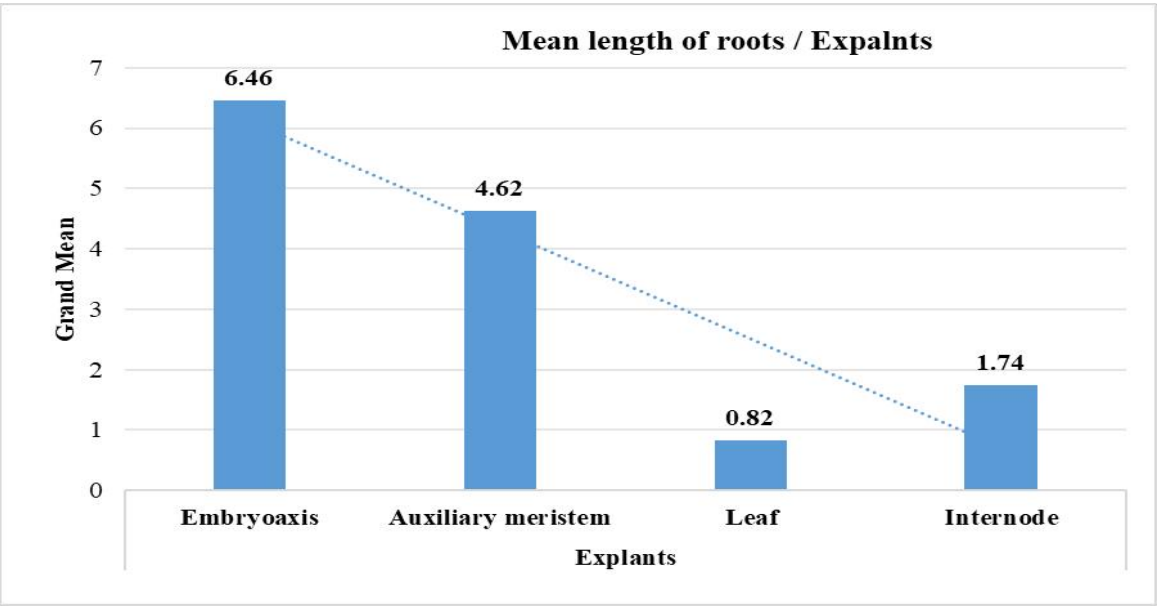

Fig.2.2 In maize variety P3546 maximum length of root regeneration seen in explants embryoaxis on MS medium with IBA 1.0mg/L.

Maize variety P3501, highest number of root regeneration seen in explant embryoaxis on MS medium supplemented with IBA 1.0mg/L

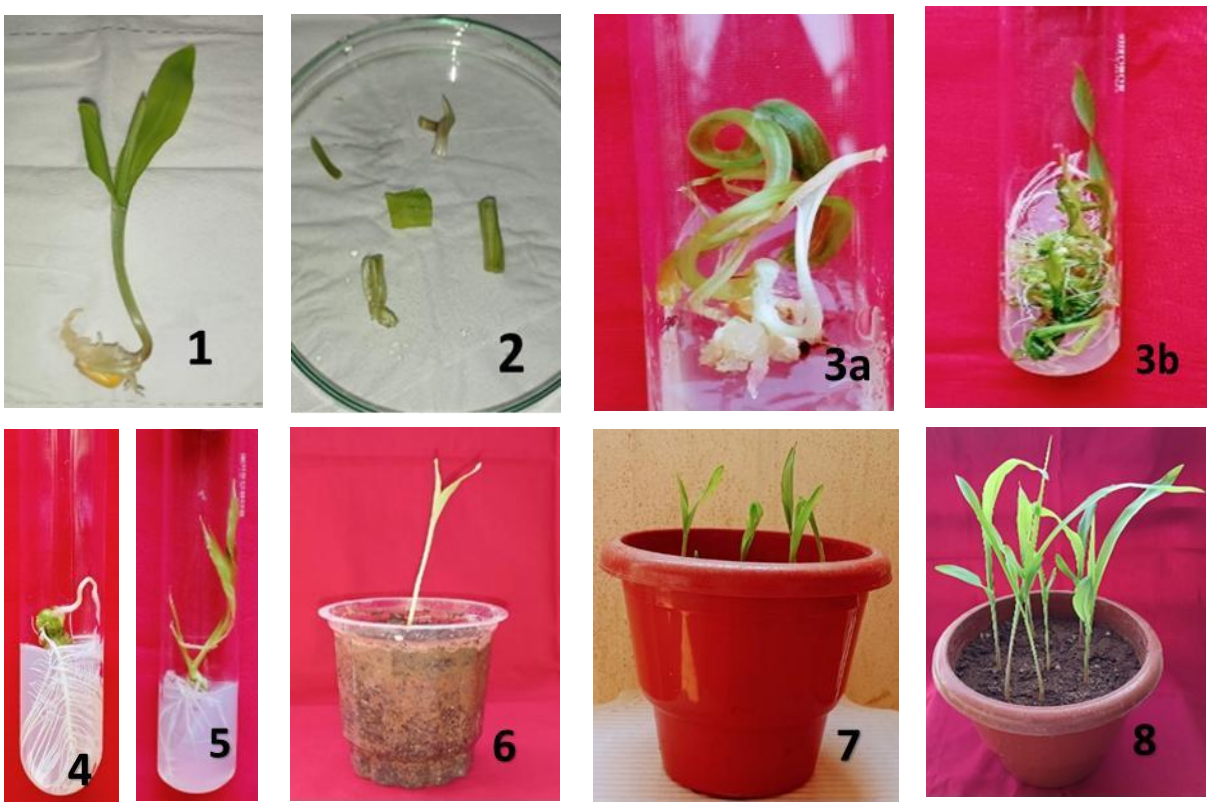

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Figure 3 (1-8): 1. Germinated P3501 seed, **2.** Explants used in regeneration, **3.** Multiple shoot induction, **4.** Root induction, **5.** Plant regeneration, **6.** Acclimatization of regenerated plant,**7.** Transferred plants in to pot, **8.** Survived plant growth in pots.

Conclusion

The tissue culture regeneration studies were carried out with various hormonal concentrations and combinations of different genotypes, explants for establishing maximum regeneration were achieved. The regeneration of maize study revealed that 2,4-D in combination with BAP resulting in higher shoot regeneration frequency among the different genotypes. P3501 genotype proved to be higher frequency of shoot regeneration from all the seedling explants; among the explants embryo axis was found to be highly shoot regenerative.

In this regeneration study that IBA resulting in higher shoot regeneration frequency among the different genotypes. P3501 genotype proved to be higher frequency of root regeneration from all the seedling explants; among the explants embryo axis was found to be highly root regenerative.

Acknowledgement

The author is grateful to the Indian Institute of Maize Research (ICAR), Agricultural Research Station, Karimnagar, Telangana, INDIA for supplying maize germplasm lines and breeding material for the present research.

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