



**AN EFFICIENT *IN VITRO* REGENERATION STUDIES OF COMMERCIALY  
IMPORTANT GROUND NUT (*ARACHIS HYPOGAEA* L.) CULTIVARS ICGV15311,  
ICGV15287, ICGV13074**

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**ABSTRACT**

Highly efficient *In vitro* regeneration protocol was established of ground nut varieties ICGV 15311, ICGV 15287, ICGV13074 seeds from explants, embryo axis, Half cut embryo axis, leaf and cotyledonary node, various concentrations and combinations of different plant growth regulators (PGR<sub>s</sub>) were employed to introduce multiple shooting and rooting to obtain a complete plantlets of ground nut on Murashige and skoog (MS) medium supplemented with different concentrations of auxins (IAA, NAA, IBA and 2,4 -D) and cytokinins (KIN and BAP). High concentrations of cytokinins and low concentrations of auxins were used for shoot regenerated. The shoot multiplication was influenced by genotype explants type and growth regulators. The explants cultured on KN and 2,4-D Showed on swelling and enlargement without further development. The combinations of BAP and NAA produced more shoots was obtained from embryo axis on a medium containing BAP (2.0mg/L) and NAA (0.5mg/L). Rooting of regenerated shoots was achieved on a medium augmented with IBA (2.0mg/L). Rooted plantlets were successfully transferred to soil and grown in a green house to maturity. ICGV 15311 were responsive with this regeneration system.

**KEYWORDS:** Embryo axis, Ground nut, Cotyledonary node, Leaf, Shoot regeneration, Root regeneration.

**Keymessage:** An efficient *In vitro* regeneration protocol was established for ground nut. Multiple shooting and Rooting medium was found by different concentrations of PGR<sub>s</sub>.

**INTRODUCTION**

Ground nut (*Arachis hypogaea*.L) belongs to the family Leguminosae, sub family papilionidae, tribe Aeschnomenae, sub tribe stylosanthinae, genus Arachis and species hypogaea (Isleib et al 1994). The genus name Arachis derived from a -rachis (Greek meaning without spine) in reference to the absence of erect branches. The species name hypogaea term derived from hypo-ge (Greek, meaning below earth) that grows downward in to the earth so that the pod develops underground. It is also known as pea nuts, earth nut, monkey nuts, manila nut, panda, unpredictable legume and energy capsule (sheshadri, 1962). The cultivated ground nut is an ancient crop of the new world, which is originated in South America (southern Bolivia) where it was cultivated as early as 1000 BC. Dissemination of the crop to Africa, Asia, Europe and the Pacific islands occurred presumably in the 16<sup>th</sup> and 17<sup>th</sup> centuries with the

discovery voyages of the Spanish, Portuguese, British and Dutch (Kraovickas, 1969, 1973; Gregorferal 1980; Hammons 1982; Isleib et al 1994). Ground nut was introduced in to India by the Magellan expedition around 1519 (SubbaRao 1909). Ground nut varieties ICGV 15311, ICGV15287, ICGV13074 seeds from explants Embryo axis, Half cut embryo axis, Leaf and Cotyledonary node. various concentrations and combinations of different plant growth regulators are used for multiple shooting and rooting to obtain a complete ground nut plantlets on MS (Murashige and skoog) medium supplemented with different concentrations of auxins (IAA, NAA, IBA and 2,4-D) and cytokinins (Kin and BAP). More shoots are obtained from embryo axis on a medium containing BAP (2.0 mg/L) and NAA (0.5 mg/L) and more roots are obtained from embryo axis supplemented with 2.0 mg/L IBA on MS medium.

**MATERIALS AND METHODS**

**Plant material**

Seeds of ground nut cultivars ICGV15311, ICGV15287, ICGV13074 were obtained from the International Crops

Research Institute for the Semi –Arid Tropics (ICRISAT) Patancheru, Hyderabad, India, were used for present study.

#### Surface sterilization and plant material

Seeds of three varieties are washed thoroughly in running tap water for 15 minutes followed by washing 3 to 4 times with sterile water. Then it treated with 70% ethanol soaked for 30 seconds and washed with sterile water 4 times ,after treat it with 10% Hgcl<sub>2</sub> by soaking for 10 minutes followed by washing sterile water for 3 to 4 times.

**Inoculation:** Explants (Embryo axis, Half cut embryo axis, Leaf, Cotyledonary node) were inoculated in the

shooting medium (MS basal BAP with NAA, IBA and IAA) of different concentrations.

#### RESULTS AND DISCUSSION

The tissue culture regeneration studies were carried out with various hormonal combinations and concentrations of different explants, genotypes and parameters for establishing maximum regeneration were achieved. The study revealed that BAP in combination with NAA resulting in higher shoot regeneration frequency among the different genotypes ICGV 15311 proved to be superior with higher frequency of shoot regeneration from all the seedlings explants. Among the explants Embryo axis was found to be highly regenerative. The shoots produced roots of good quality on MS medium supplemented with 2.0 mg/L IBA and remained healthy on transfer to soil.

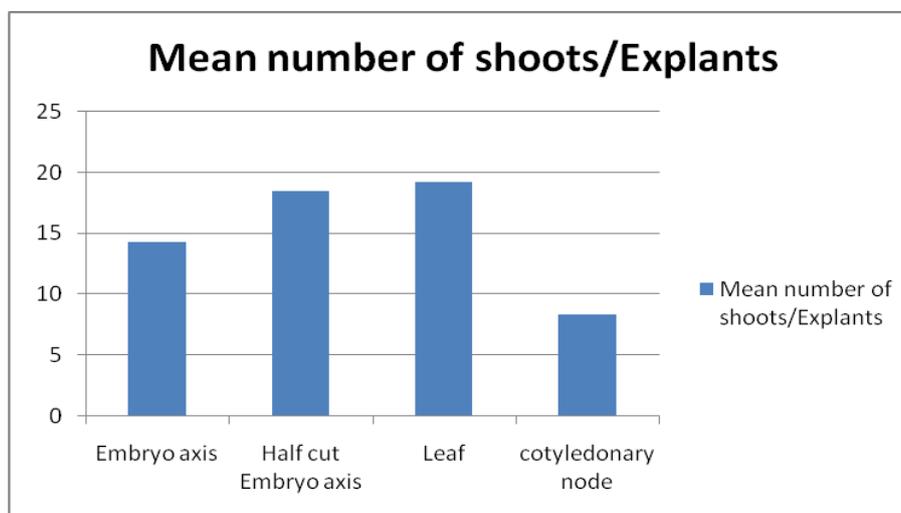
**Table 4.1. A: Shoot regeneration frequency from different explants of ICGV15311 on MS medium supplemented with BAP+IAA.**

Concentration of Hormones (mg/l)		Explants			
BAP	IAA	Embryo axis	Half cut embryo axis	Leaf	Cotyledonary node
1.0	0.5	6	9	8	5
2.0	0.5	10	14	10	3
3.0	0.5	12	16	13	6
4.0	0.5	11	15	15	4
5.0	0.5	13	18	20	9
1.0	1	15	<b>27</b>	21	8
2.0	1	17	24	26	11
3.0	1	18	25	23	10
4.0	1	20	21	27	13
5.0	1	<b>21</b>	16	<b>29</b>	<b>15</b>
<b>Grand Mean</b>		<b>14.3</b>	<b>18.5</b>	<b>19.2</b>	<b>8.4</b>

Data scored after 14 days of culture initiation grown on the medium with BAP +IAA growth regulators and about 80 explants.

The Ground nut variety ICGV15287 in which maximum number of shoot induction is seen in explants embryo

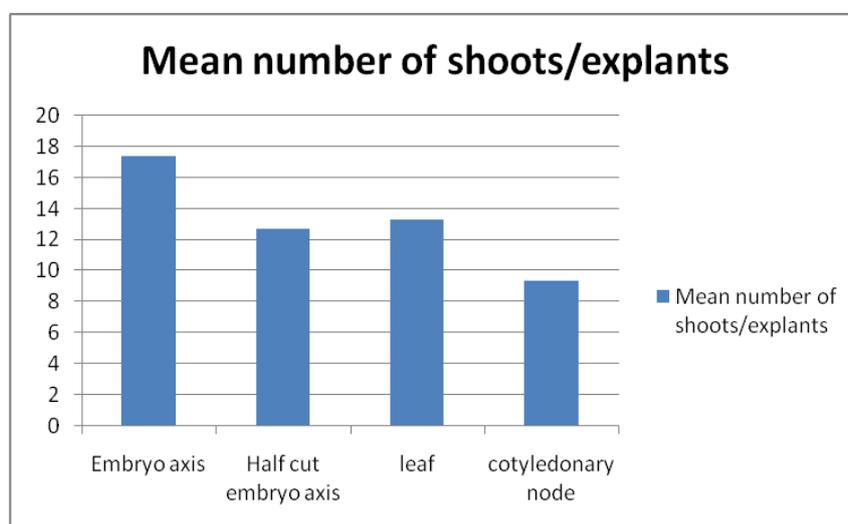
axis on medium BAP4.0 with IAA 0.5mg/l(26.2%),next leaf with medium BAP 5.0 with IAA 0.5mg/l(21.2%) followed by Half cut embryo axis on medium BAP 5.0 with IAA 1.0mg/l(20%) and finally Cotyledonary node as explants on the medium supplemented with BAP 1.0 with IAA 1.0mg/l(18.7%).



**Table 4.1.B: Shoot regeneration frequency from different explants of ICGV 15287 on MS medium supplemented with BAP+IAA.**

Concentration of Hormones(mg/l)		Explants			
BAP	IAA	Embryo axis	Half cut embryo axis	Leaf	Cotyledonary node
1	0.5	11	14	10	4
2	0.5	14	12	11	4
3	0.5	20	13	14	5
4	0.5	<b>21</b>	11	16	7
5	0.5	19	11	<b>17</b>	9
1	1	18	<b>16</b>	14	<b>15</b>
2	1	15	14	15	12
3	1	17	13	13	13
4	1	19	12	12	14
5	1	20	11	10	11
<b>Grand mean</b>		<b>17.4</b>	<b>12.7</b>	<b>13.3</b>	<b>9.4</b>

Data scored after 14 days of culture initiation grown on the medium with growth regulators and about 80 explants.



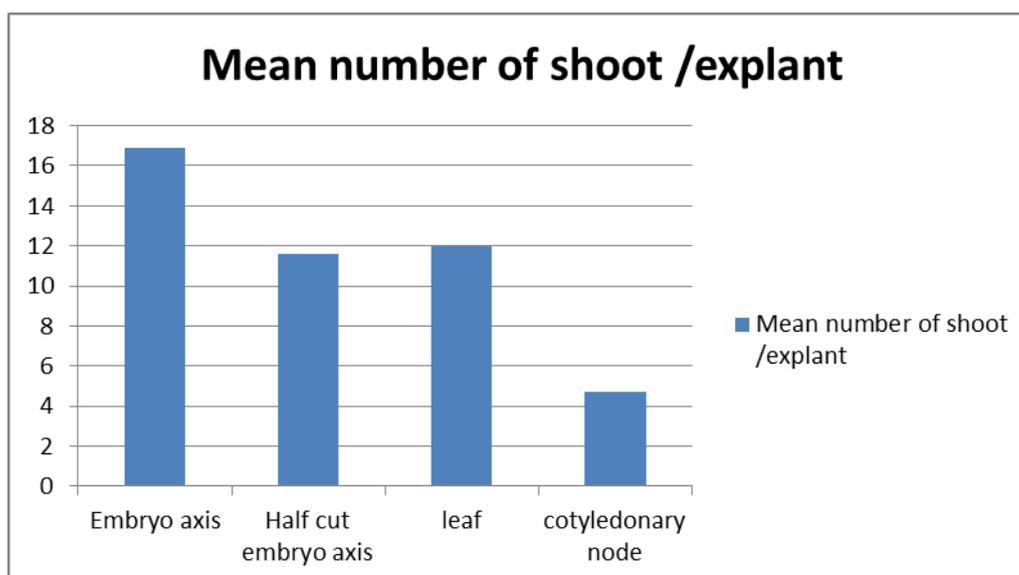
Ground nut variety ICGV13074, maximum shoot frequency is seen in explants embryo axis grown on medium BAP 2.0 with IAA 1.0mg/l(36.2%) next highest shoot regeneration is seen in explants leaf with medium

BAP 1.0 and IAA 1.0(22.5%) followed by explants Half cut embryo axis on medium BAP 1.0 and IAA 1.0mg/l(18.7%) and lastly Cotyledonary node as explants grown on medium BAP 4.0 and IAA 1.0mg/l(8.7%).

**Table 4.1.C Shoot regeneration frequency from different explants of ICGV13074 on MS medium supplemented with BAP+IAA.**

Concentration of Hormones(mg/l)		Explants			
BAP	IAA	Embryo axis	Half cut embryo axis	Leaf	Cotyledonary node
1	0.5	5	7	6	3
2	0.5	15	9	8	5
3	0.5	13	10	9	2
4	0.5	12	11	7	4
5	0.5	10	11	8	5
1	1	17	<b>15</b>	<b>18</b>	4
2	1	<b>29</b>	14	16	6
3	1	24	14	17	5
4	1	19	12	16	<b>7</b>
5	1	25	13	15	6
<b>Grand Mean</b>		<b>16.9</b>	<b>11.6</b>	<b>12</b>	<b>4.7</b>

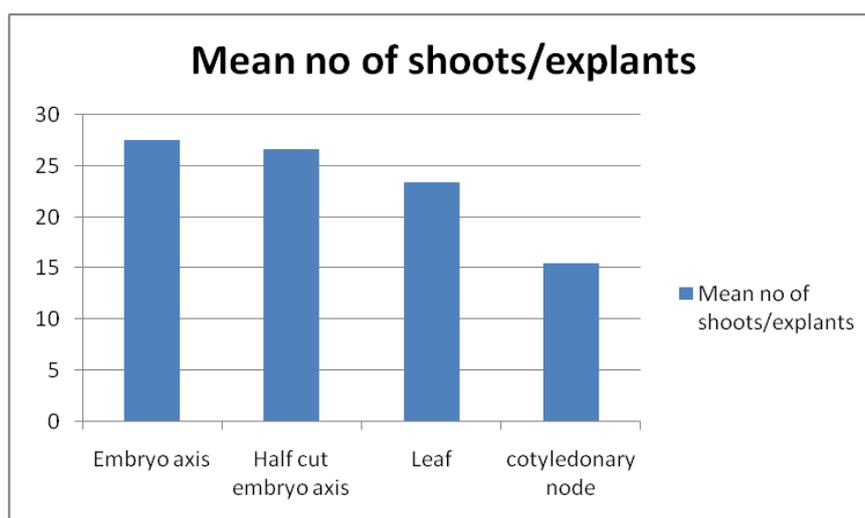
Data scored after 14 days of culture initiation grown on the medium with growth regulators and about 80 explants.



**Table 4.2.A: Shoot regeneration frequency from different explants of ICGV15311 on MS medium supplemented with BAP+NAA.**

Concentrations of Hormones(mg/l)		Explants			
BAP	NAA	Embryo axis	Half cut Embryo axis	Leaf	Cotyledonary Node
1.00	0.5	10	13	11	18
2.00	0.5	<b>56</b>	16	13	16
3.00	0.5	15	17	12	14
4.00	0.5	13	19	11	16
5.00	0.5	18	24	9	12
1.00	1.0	12	<b>50</b>	<b>49</b>	<b>20</b>
3.00	1.0	41	25	34	18
4.00	1.0	39	45	31	14
5.00	1.0	23	27	28	12
<b>Grand Mean</b>		<b>27.5</b>	<b>26.6</b>	<b>23.4</b>	<b>15.5</b>

Data scored after 14 days of culture initiation grown on the medium with growth regulators and about 80 explants.



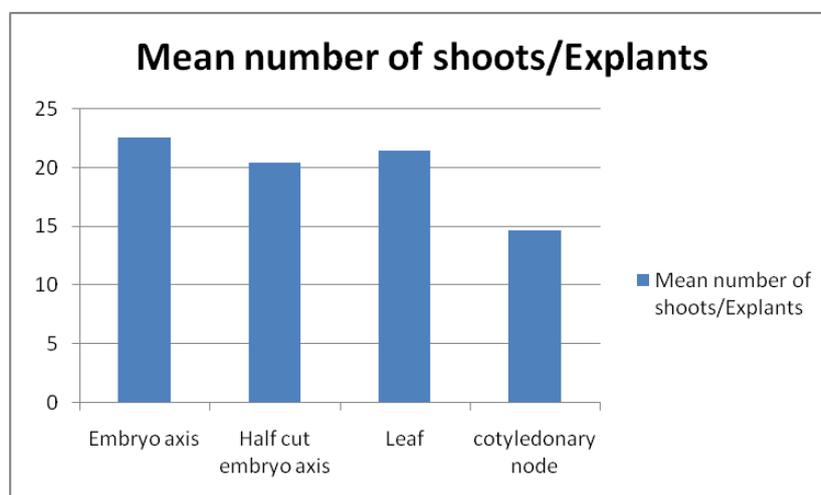
Ground nut variety ICGV15287 in which maximum number of shoot regeneration is seen in explants embryo axis on medium BAP 1.0 and NAA 1.0mg/l(41.2%),next half cut embryo axis as explants grown on medium BAP

1.0 and NAA 1.0mg/l(37.5%) and finally cotyledonary node as explants, medium is supplemented with BAP 1.0 and NAA 1.0mg/l(25%).

**Table 4.2.B.: Shoot regeneration frequency from different explants of ICGV15287 on MS medium supplemented with BAP+NAA.**

Concentrations of Hormones(mg/l)		Explants			
BAP	NAA	Embryo axis	Half cut embryo axis	Leaf	cotyledonary node
1.0	0.5	12	13	15	10
2.0	0.5	19	14	17	12
3.0	0.5	20	16	18	13
4.0	0.5	16	15	18	15
5.0	0.5	19	17	19	11
1.0	1.0	<b>33</b>	<b>31</b>	<b>30</b>	<b>20</b>
2.0	1.0	29	28	27	16
3.0	1.0	31	25	26	17
4.0	1.0	27	24	23	18
5.0	1.0	20	21	22	15
<b>Grand mean</b>		<b>22.6</b>	<b>20.4</b>	<b>21.5</b>	<b>14.7</b>

Data scored after 14 days of culture initiation grown on the medium with growth regulators and about 80 explants.



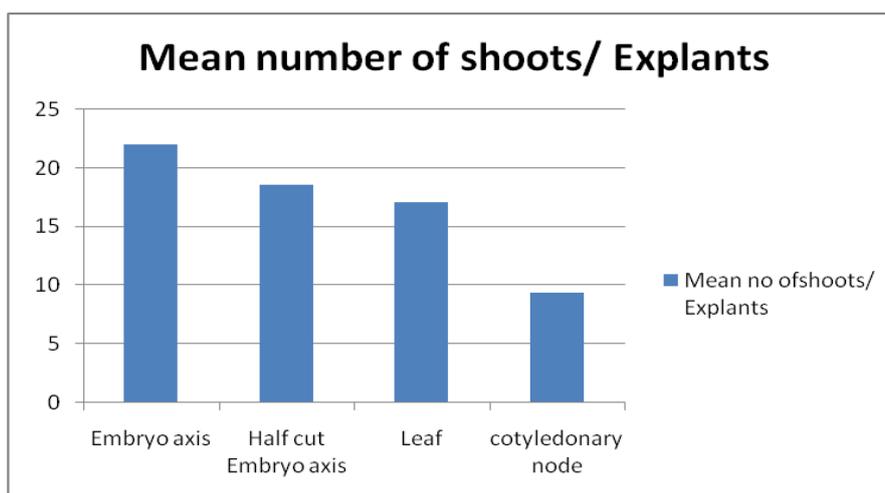
Ground nut variety ICGV15287 in which maximum number of shoot regeneration is seen in explants embryo axis on medium BAP 1.0 and NAA 1.0mg/l(41.2%),next half cut embryo axis as explants grown on medium BAP

1.0 and NAA 1.0mg/l(37.5%) and finally cotyledonary node as explants, medium is supplemented with BAP 1.0 and NAA 1.0mg/l(25%).

**Table 4.2.C.: Shoot regeneration frequency from different explants of ICGV 13074 on MS medium supplemented with BAP+NAA.**

Concentration of Hormones (mg/l)		Explants			
BAP	NAA	Embryo axis	Half cut Embryo axis	Leaf	Cotyledonary node
1.0	0.5	14	10	12	8
2.0	0.5	11	12	12	6
3.0	0.5	16	13	10	5
4.0	0.5	17	13	9	4
5.0	0.5	19	15	10	4
1.0	1.0	<b>39</b>	<b>29</b>	<b>27</b>	<b>15</b>
2.0	1.0	24	26	25	13
3.0	1.0	30	24	23	12
4.0	1.0	27	22	21	14
5.0	1.0	23	22	22	13
<b>Grand mean</b>		<b>22</b>	<b>18.6</b>	<b>17.1</b>	<b>9.4</b>

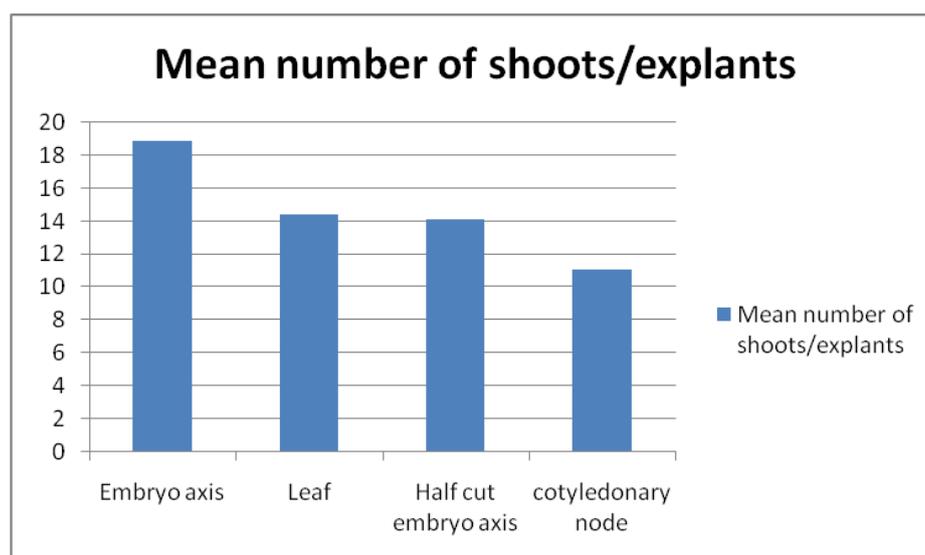
Data scored after 14 days of culture initiation grown on the medium with growth regulators and about 80 explants.



**Table 4.3.A.: Shoot regeneration frequency from different explants of ICGV 15311 on MS medium supplemented with BAP+IBA.**

Concentrations of Hormones (mg/l)		Explants			
BAP	IBA	Embryo axis	Leaf	Half cut embryo axis	Cotyledonary node
1.0	0.5	13	11	9	7
2.0	0.5	17	14	12	5
3.0	0.5	21	15	14	8
4.0	0.5	22	13	18	9
5.0	0.5	19	16	<b>20</b>	10
1.0	1.0	15	13	12	9
2.0	1.0	18	15	13	6
3.0	1.0	17	13	15	10
4.0	1.0	22	16	11	13
5.0	1.0	<b>25</b>	<b>18</b>	17	<b>15</b>
<b>Grand mean</b>		<b>18.9</b>	<b>14.4</b>	<b>14.1</b>	<b>11.1</b>

Data scored after 14 days of culture initiation grown on the medium with growth regulators and about 80 explants.



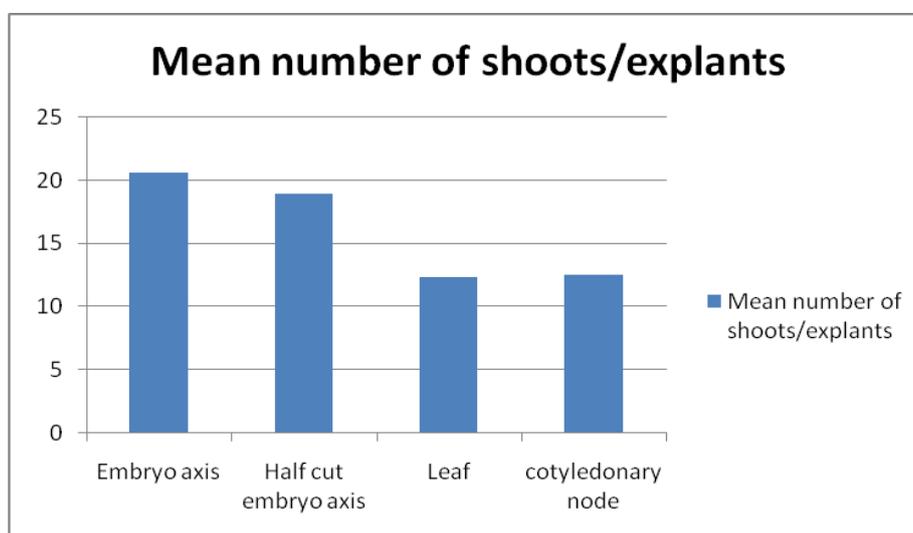
Ground nut variety ICGV 15287, in which highest number of shoot regeneration is seen in explants embryo axis in medium BAP 2.0 and IBA 1.0mg/l(36.2%),next maximum shoot frequency is observed in half cut embryo axis on medium BAP 5.0 and IBA

0.5mg/l(27.5%), followed by leaf explants medium BAP 1.0 and IBA 1.0 mg/l(22.5%),finally explants cotyledonary node on medium BAP 1.0 and IBA 0.5mg/l(20%).

**Table 4.3.B.: Shoot regeneration frequency from different Explants of ICGV 15287 on MS medium Supplemented with BAP + IBA.**

Concentration of Hormones (mg/l)		Explant			
BAP	IBA	Embryo axis	Half cut embryo Axis	Leaf	Cotyledonary node
1.0	0.5	17	18	12	<b>16</b>
2.0	0.5	15	19	11	14
3.0	0.5	20	20	9	15
4.0	0.5	24	21	7	14
5.0	0.5	21	<b>22</b>	8	12
1.0	1.0	22	21	<b>18</b>	13
2.0	1.0	<b>29</b>	19	17	12
3.0	1.0	16	18	15	10
4.0	1.0	19	17	14	11
5.0	1.0	23	15	13	8
<b>Grand Mean</b>		<b>20.6</b>	<b>19</b>	<b>12.4</b>	<b>12.5</b>

Data scored after 14 days of culture initiation grown on the medium with growth regulators and about 80 Explants.



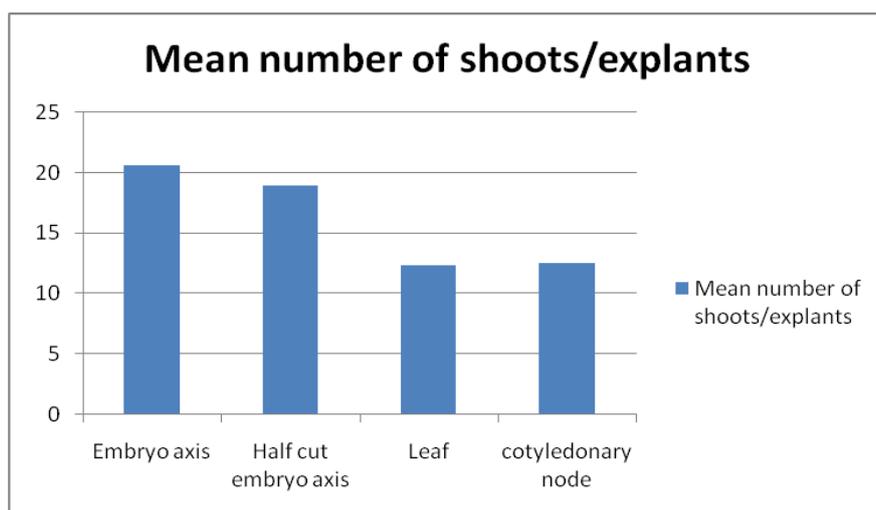
Ground nut variety ICGV 15287, in which highest number of shoot regeneration is seen in explants embryo axis in medium BAP 2.0 and IBA 1.0mg/l(36.2%), next maximum shoot frequency is observed in half cut

embryo axis on medium BAP 5.0 and IBA 0.5mg/l (27.5%), followed by leaf explants medium BAP 1.0 and IBA 1.0 mg/l(22.5%), finally explants cotyledonary node on medium BAP 1.0 and IBA 0.5mg/l(20%).

**Table 4.3.C.: Shoot regeneration frequency from different explants of ICGV 13074 on MS medium supplemented with BAP+IBA.**

Concentration of Hormones (mg/l)		Embryo axis	Half cut Embryo axis	Leaf	Cotyledonary node
BAP	IBA				
1.0	0.5	16	14	10	13
2.0	0.5	19	12	8	13
3.0	0.5	14	12	9	11
4.0	0.5	22	13	7	12
5.0	0.5	25	13	6	11
1.0	1.0	20	<b>19</b>	18	<b>17</b>
2.0	1.0	19	17	16	16
3.0	1.0	<b>26</b>	18	17	15
4.0	1.0	18	17	15	16
5.0	1.0	23	15	<b>19</b>	14
<b>Grand mean</b>		<b>20.2</b>	<b>15</b>	<b>12.5</b>	<b>13.8</b>

Data scored after 14 days of culture initiation grown on the medium with growth regulators and about 80 explants.



**Rooting:** Explants (Embryo axis, Half cut embryo axis, leaf, Cotyledonary node) grown on shooting medium were inoculated in the rooting medium (MS basal with IBA, IAA, NAA).

**Table 4.2.A.: Rooting Regeneration in ICGV15311 on MS+IBA 2.0mg/l.**

Growth Regulators		Size of Roots from Explants			
S. No	IBA	Embryo axis	Half cut embryo axis	Leaf	Cotyledonary node
1	2.0mg/l	<b>14.5cm</b>	<b>11cm</b>	<b>10.5cm</b>	0.9cm
2	2.0mg/l	12cm	10.8cm	9.8cm	<b>9.2cm</b>
3	2.0mg/l	13cm	10cm	9.5cm	8.1cm
4	2.0mg/l	12cm	10.6cm	0.9cm	7.9cm
5	2.0mg/l	10cm	09cm	8.10cm	7.5cm

In the ground nut variety ICGV15287, maximum size of roots are seen in explants Embryo axis (13cm), leaf (11.7cm), cotyledonary node (9.6cm).

**4.2. B. Rooting Regeneration in ICGV15287 on MS +IBA 2.0mg/l.**

S. No	Growth regulators		Size of Roots from Explants		
	IBA	Embryo axis	Half cut embryo axis	Leaf	Cotyledonary Node
1	2.0mg/l	<b>13.9cm</b>	12.5cm	11.5cm	<b>9.6cm</b>
2	2.0mg/l	12.5cm	<b>13cm</b>	<b>11.7cm</b>	9.4cm
3	2.0mg/l	11.7cm	12.7cm	10.9cm	8.4cm
4	2.0mg/l	10.4cm	11.9cm	9.4cm	8.2cm
5	2.0mg/l	10.9cm	12.7cm	10.2cm	7.6cm

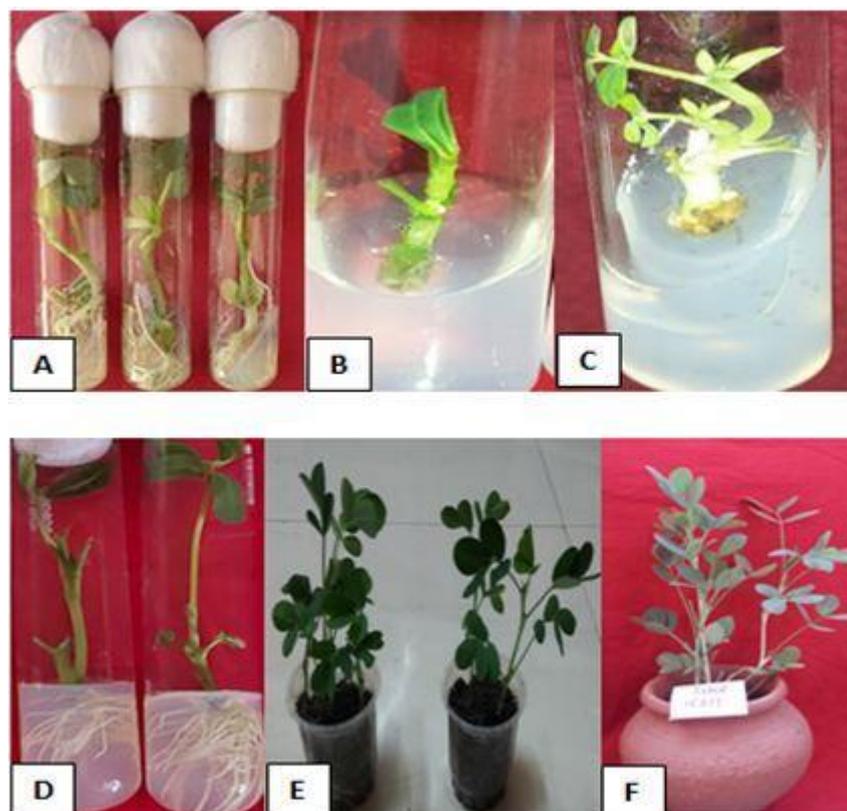
The Ground nut variety ICGV 13074 maximum size of roots are seen in explants. Embryo axis (12.8cm) followed by half cut embryo axis (12cm), leaf (11.9cm) and lastly cotyledonary node (9.5cm).

**4.2. C. Rooting Regeneration in ICGV13074 on MS+IBA 2.0mg/l**

S.NO	Growth Regulators		Size of Roots from Explants		
	IBA	Embryo axis	Half cut embryo axis	Leaf	Cotyledonary node
1	2.0mg/l	<b>12.8cm</b>	10.2cm	<b>11.9cm</b>	<b>9.5cm</b>
2	2.0mg/l	12.5cm	10.2cm	11.5cm	9.0cm
3	2.0mg/l	11.2cm	12cm	10.7cm	8.7cm
4	2.0mg/l	10.5cm	<b>11.9cm</b>	10.5cm	8.2cm
5	2.0mg/l	8.1cm	10.6cm	9.2cm	7.9cm

The rooted shoots were transferred to small pots containing sterile vermiculite and were covered with polythene cover for 5 to 7 days to maintain high humidity. After 7 days, polythene cover is removed for

gaseous exchange, plantlets were allowed to grow in the in vitro condition for 3 to 4 days. The shoots that acclimatized successfully were transferred to field conditions.



**Figure 1(A-F): A. Seed germination of ICGV15311 cultivar, B. Shoot induction of selected cultivar, C. Multiple shoot induction from embryo axis, D. Rooting & Plant regeneration of ICGV15311, E. Acclimatization of regenerated plant, F. Transferred plant in to pot.**

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