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Invitro Regeneration And GUS Expression Studies In Ground Nut (*Arachis Hypogaea* L.) Variety ICGV 15311.

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Abstract

Ground nut is an important source of food,edible oil,dietary vitamin, minerals, proteins, animal feed etc. In this research, an efficient of regeneration and Agrobacterium mediated genetic transformation with GUS expression is reported in a commercially important variety ICGV 15311, using embryo axis, Leaf, Half cut embryo axis, Cotyledonary node. Various combinations and concentrations of plant growth regulators are used to obtain multiple shooting and rooting.MS (Murashig and Skoog) medium supplemented with various concentrations of Auxins (IBA,NAA,IAA and 2,4D) and Cytokinins (BAP and KIN).Explants cultured on 2,4 D and KIN expressed only swelling and enlargement without further development.BAP and NAA showed more shoots on embryo axis explants on medium concentration and combination of BAP (2.0 mg/l) and NAA(0.5mg/l).Rooting obtained on IBA (2.0mg/l). The bacterial culture 1.0 OD 600, incubation with 50 µl acetosyringone and co cultivation period of 3 days were found to be efficient for transformation. Tansformed putatives grown on shooting and rooting medium and remained healthy putatives transferred to soil for hardening.

Keywords: Agrobacterium Transformation, GUS expression, Ground nut, Embryo axis, Cotyledonary node, Leaf, Shooting, Rooting.

Keymessage: Invitro Regeneration and GUS expression studies in Ground nut. Multiple shooting medium,Rooting medium was found by different concentrations and combinations of Plant Growth Regulators.

Abbreviations:
MS : Murashig and skoog basal salt medium
BAP : 6-Benzyl amino purine
IBA : Indole 3-butyric acid
IAA : Indole 3-Acetic acid
NAA : 1- Naphthalene Acetic acid
PGRs : Plant growth regulators
RIM : Root Induction medium

SIM : Shoot induction medium
2,4 D : 2,4 –D Dichloro phenoxy acetic acid
KIN : Kinetin
GUS : β – Glucuronidase
OD : Optical Density.

Introduction:

Ground nuts were originally considered to be food for animals ,now became an important source of protein in many developing countries.(Singh and Singh 1991).Ground nut are valued for their high quality oil content. About 2/3rd of the world production of ground nuts is utilized as an edible oil, making it one of the world's leading oil is found in the cotyledons which comprise approximately 72.4% of the kernel (Fedeli et al.,1968 ; Woodroof,19830).
Genetic transformation allows introduction of desired genes across species better (Sharma and Anjaiah 2000).Many successful genetic transformation protocols have been reported in Ground nut via Agrobacterium tumefaciens (Sharma and Anjaiah 2000).
Employing Agrobacterium as a tool for transformation, both tissue cultured callus mediated and in planta protocols have been successfully adopted.Transgenic research has picked up the momentum during last decode and resulted in development of several transgenic lines with novel traits and improved plant performance.

Materials and methods:

Seeds of ground nut cultivar ICGV15311obtained from the International Crops Research Institute for the Semi –Arid Tropics (ICRISAT) Patancheru, Hyderabad, India, were used for present study.

Surface sterilization

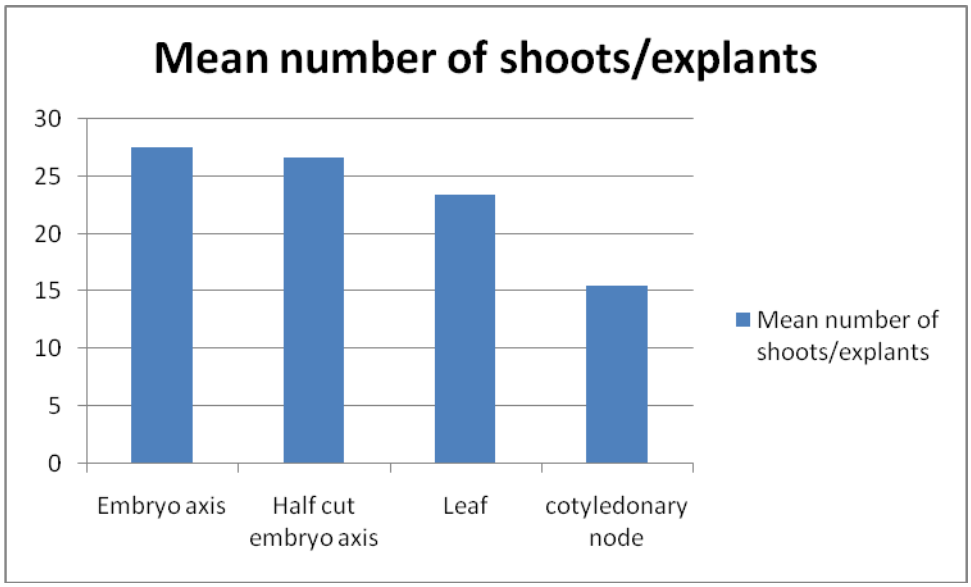
Seeds of ICGV 15311 are washed thoroughly under running tap water for 15 minutes followed by washing 3 to 4 times with sterile distilled water. Then it treated with 70% ethanol soakedfor 30 seconds and washed with sterile distilled water 4 times. After treat it with 10% $Hgcl_2$ by soaking for 10 minutes followed by washing sterile water for 3 to 4 times Soaked in sterile distilled water for 6 hours, after blotted drying inoculate the seeds on MS medium. After 5 days seeds are germinated.

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.

Table 1: Shoot regeneration frequency from different explants of **ICGV 15311** 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	**56**	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	**50**	**49**	**20**
3.00	1.0	41	25	34	18
4.00	1.0	39	45	31	14
5.00	1.0	23	27	28	12
Grand Mean		**27.5**	**26.6**	**23.4**	**15.5**

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



4. 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 2: 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.omg/l	14.5cm	11cm	10.5cm	0.9cm
2	2.0mg/l	12cm	10.8cm	9.8cm	9.2cm
3	2.0mg/l	13cm	10cm	9.5cm	8.1cm
4	2.0mg/l	12cm	10.6cm	0.9cm	7.9cm
5	2.omg/l	10cm	09cm	8.10cm	7.5cm

4. Genetic transformation:

Embryo axis explants were transformed using the optimized parameters and co cultivation media in different batches, each batch approx. 100 explants. After co-cultivation, explants were washed with sterile distilled water blotted dry on a sterile paper and transferred to shoot regenerated medium BAP (2.0mg/l) and NAA (0.5mg/l) with 200mg/l Cefotaxime for 2 weeks. Further they were sub cultured for next 2 weeks in 16/8 h light/dark cycle.

For rooting, elongated shoots of 2-3 cm were transferred to rooting induction medium MS medium supplemented with IBA (2.0 mg/l) with 10mg/l hygromycin. These shoots were also used for grafting on non transformed 1 week old stocks germinated on soilrite. Grafted plants were covered with polyethylene to maintain high humidity, until new leaves emerge. polyethylene covers were removed and the plants were acclimatized to laboratory condition and then transferred to green house conditions for hardening.

5. Confirmation of the Transgene Integration

Transient GUS expression in embryo axis explants just after co cultivation and stable expression in leaves were assessed by using β-Glucuronidase Reporter Gene blue colour formation occurs.

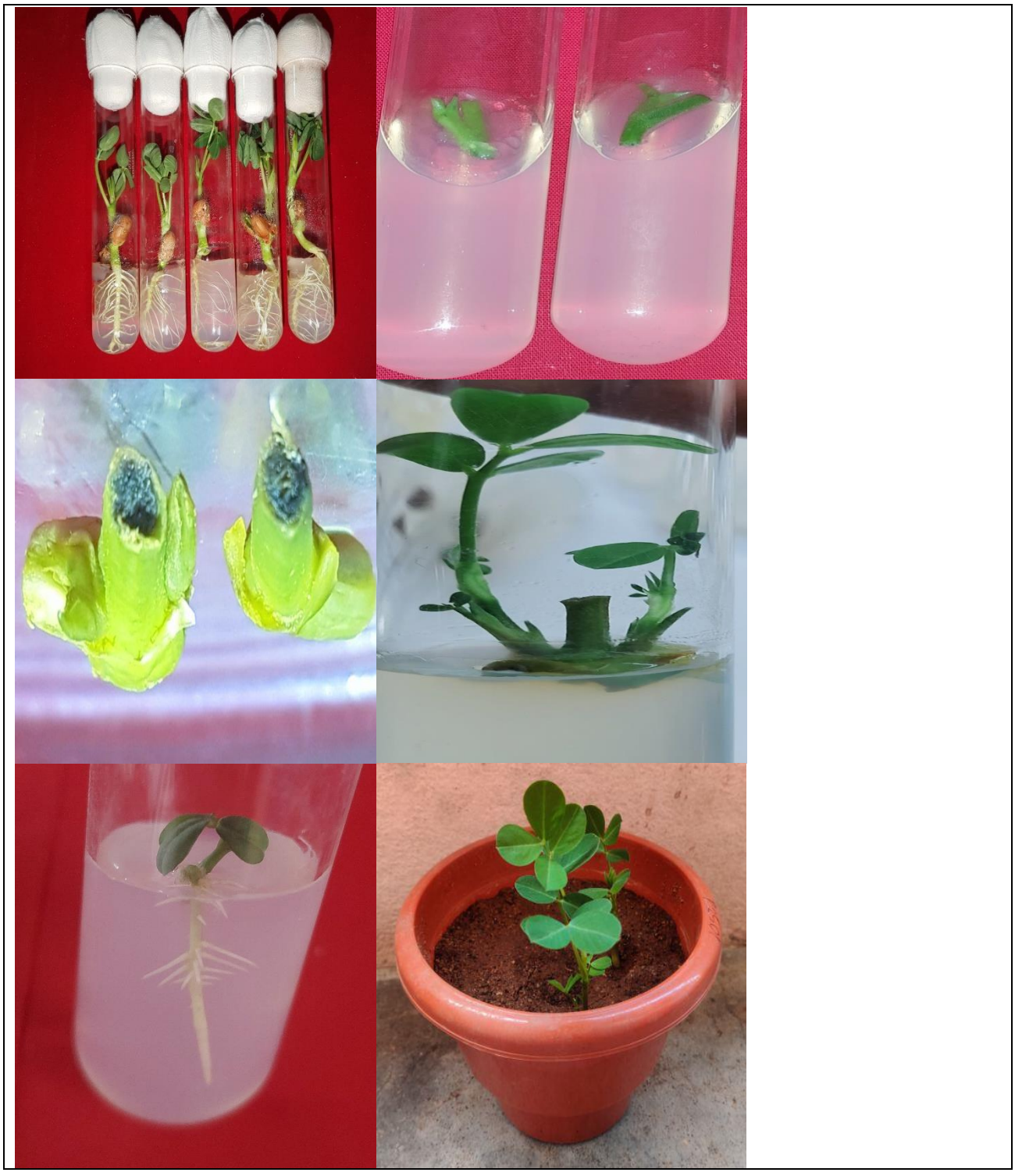


Figure-1: A- Seed germination of selected cultivar ICGV 15311**, B-** Inoculated Embryo axis **C-** Gus expression in embryo axis. **D-** Multiple Shoot induction, **E-**Root induction, **F-** Transformed plant.

Results & Discussion:

It was observed that 90% explants turned green on media inoculated in SIM (MS+BAP 2.0 mg/l) and NAA (0.5mg/l) shown highest shoot growth and elongated shoots of approx.2 cm were separated from each other then cultured on RIM (MS+IBA 2.0 mg/l) where as roots were emerged from 100% shoots. Rooted plantlets were transferred to the soil and covered with transparent polythene to maintain the high humidity. It took 2 weeks to acclimatize the plantlets in the laboratory conditions and then transferred to the green house for further growth.

Conclusion:

An efficient Transformation and regeneration protocol was developed for Indian cultivar of ground nut variety ICGV15311.The shooting induction media MS with BAP (2.0 mg/l) and NAA (0.5mg/l) shown best shoot induction ability. The rooting induction media MS supplemented with IBA (2mg/l) shown good response to rooting. The cultivar ICGV 15311 was transformed using developed method and expression of the transgene was confirmed by GUS histo chemical assay.

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