# *In vitro* callus induction from different explants of *Gymnema hirsutum* Wight & Arn- an important Anti-diabetic medicinal plant.

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**Abstract**

An efficient in vitro callus induction protocol was developed for the first time in Gymnema hirsutum Wight & Arn . which is a potent antidiabetic plant.shoot tips and nodal explants were inoculated on MS medium fortified with auxins (2,4-D and NAA) and cytokinins (kinetin) different concentrations ranging from 1.0 to5.0 mg/l in alone or in combinations for rapid callus induction. Callus was obtained in both explants in all the concentrations. These explants failed to produce callus on MS medium without growth regulators. The highest callus induction , light/dark green with campact/friable nature and growth rate (fresh and dry weights) was observed on MS media containing 2,4-D(3.0mg/l) + NAA(1.0 mg/l) followed by 2,4-D and NAA at 3.0 mg/l. but kinetin is less effective for callus induction than the 2,4-D and NAA . The higher concentrations of 2,4-D (4.0 and 5.0 mg/l) callus was turned to brown colour and necrotic . It is concluded that, in addition to the plant material, callus may also used as a supplement raw material to obtain secondary metabolites for the pharmaceutical industry.

Keywords : Gymnema hirsutum , plant growth regulators 2,4-D , NAA and KIN . callus induction.

**Introduction**

Indian is geography rich for its biodiversity of medicinal herbs, but these medicinal plants species decline very rapidly in last few decades. After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing system like Ayurveda, siddha and unnani. This is because of the adverse effect of the synthetic drugs. Herbal drugs play an important role in health care program,especially in developing countries. So there is a need for doing extensive research mostly on traditional medicines.

Gymnema hirsutum , Wight & Arn (Asclepidaceae) . has potent antidiabetic properties. It is being used in ayurvedic and homeopathic system of medicine from time immorial (Dixit and pandey, 1984, Kapoor 1977, Mitra et al 1995 ). The word gymnema has been derived from Hindu word ‘Gumar’ a destroyer of sugar . This species is woody of climber of tropical and subtropical regions (Anonymous, 1997 ) . The bioactive compound found in the leaves of G hirsutum is commonly known as ‘Gymnemic acid’. This substance inhibits glucose absorption in small intestine and decreases high glucose levels in blood (Shimizu et al, 1997). This plant is also helpful in the treatment of asthma, eye complaints, inflammation, family planning and snake bite (Uniyal 1993; Selvanayagam et al, 1995 ) It is fast disappearing and or threatened with extension due to its indiscriminate collection , over exploritation of natural resources for commercial

purposes and to meet the requirements of the pharmaceutical industry . The attempt for its production and conventional propagation is hampered due to its poor seed viability, low rate of germination and poor rooting ability o of vegetative Cuttings. Tissue culture offers an alternative propagation method which accelerates large scale multiplication , improvement and conservation of plant. There are a number of studies on Gymnema sylvestre callus induction and regeneration of plantlets , pharmacological activity and phyto chemistry by invitro technique (Reddy et al 1988; Komalavalli and Rao 1997; 2000 ; Kumar et al 2002, Gopi and vatsala 2006, Kanetkar et al 2006, Komalavalli et al 2007, Upendra kumar et al 2010, Kaushalya and Senavath 2013 and Sunita et al 2014, Neverth less, there are no reports available on Gymnema hirsutum. Invitro callus induction and biomass.

Considering the high economical medicinal and pharmacological importance of secondary metabolites, industries are deeply interested in utilizing plant tissue culture technology for large scale production of these substances. Hence present investigation was undertaken to develop standard protocol to study callus induction by using different plant growth regulators using nodel and shoot tip explants of Gymnema hirsutum.

**Material and Methods**

The young seedlings were collected from higher altitudes of Kolli hills, of Namakkal district, Tamil Nadu, with the Assistance of Prof. Ramachandran, Anna University, Tamil Nadu. These collected seedlings were grown in the Andhra University, Botany experimental farm, Visakhapatnam. The disease free shoot tip and nodal explants were collected from two months old healthy plants. The explants were excised and the contaminants were washed under running tap water for 45 minutes, followed by washing in the liquid detergent tween-20(few drops/100ml) and then rinsed with running tap water. Later they were surface sterilized with 1% fungicide (w/v) for 30 min, followed by rinsing in 1% Mercuric chloride ($H_gcl_3$) for five minutes and washed thrice with sterile distilled water and followed by 70% alcohol.

Full strength media were prepared with various concentrations of 3% sucrose. The medium was gelled with 0.8% agar and PH was adjusted 5.8 using 0.1 NAOH or 0.1 NHcl solution before autoclaving 25 ml of medium was taken in each culture vessel and capped tightly. Then the cultures were autoclaved at $121^0$c for 15 minutes .

The surface sterilized explants of shoot tips and nodal were inoculated under aseptic conditions in laminar air flow chamber. the cultures were incubated at 25°c ± 2°c under fluorescent light . The aseptic shoot tips and nodal were excised and inoculated on to the M.S medium with different concentrations of 2,4-D,NAA and KIN separately ranging from 1.0 mg/l-5.0mg/l and in combinations of different concentrations ranging from1.0 - 5.0mg/l 2,4 D, + NAA with cytokinin (kinetin). The cultures were incubated at 25°c ± 2°c for 16 hrs photo- period under white Fluroscent light intensity of 40 – 60 µ mol $m^{-2}$ $s^{-1}$ Each experiment had to replicates and repeated atleast three times. The percentage of callus formation was recorded after three weeks. The callus induction frequency was determined as follows :

$$\text{Callus induction (\%)} = \frac{\text{No of explants showing callus}}{\text{No of explants inoculated}}$$

For callus growth rate of explants of shoot tips and nodal were placed on MS medium fortified with 2,4-D , NAA and kinetin (1.0 – 5.0 mg/l) and sub culture after three weeks Growth of G. hirsutum was measured in terms of fresh and dry weight . Fresh weights of cells / callus were taken after removing the excess of moisture on the surface using blotting paper . Dry weight of callus was determined by drying in a hot air oven at 60°c for 24 hrs .

The percentage of survival explants and creating calli, its structure, colour, texture percentage response and initiation time were determined for 3 weeks and tabulated for detailed study. The data per culture has subjected to standard deviations and mean separation was carried out by using computer software

**RESULTS**

For callus induction, shoot tips and nodal explants were collected from the two months old field grown plants. The sterilized explants were inoculated on M S medium hormonal free and M S medium agumented with different concentrations of auxins (2,4 – D, and NAA) alone or incombinations ranging from 1.0 – 5.0 mg/l gave varied callusing responses.Callus induction was achived in both explants in all the harmone concentrations alone or incombinations with varied responses such as percentage of explant response, percentage of callus induction , colour, texture, fresh and dry weights of the callus. These explants failed to produce callus on MS medium lack of growth regulators (tables 1-4)

**Effect of 2,4-D**

MS medium supplemented with 2,4-D at the range of 1.0 to 5.0 mg/l induce the callus formation in both explants of shoot tips and nodal segments with in 15-20 days . The concentration 2,4-D increases the callus formation also increased significantly upto 3.0 mg/l

Maximum cell proliferation was obtained in shoot tip explants (86%) and nodal segments (83%) with dark green compact callus.The fresh weight of shoot tip explant(947± 0.36mg)

and nodal explants (880±0.30mg) was recorded at the concentrations of 3.0 mg/l. Higher concentrations of 2,4-D (4.0 and 5.0 mg/l) was gradually declined and turns hard texture with brown colour appears was noticed which leads to necrosis later (table 1 and 2 ; Fig 1 and 2)

**Effect of NAA**

MS medium augmented with NAA at the range of 1.0 to 5.0 mg/l induced the callus formation in both explants of shoot tips and nodes with in 20days . The concentration of NAA increases the callus formation also increased upto 3.0 mg/l with light green compact nature of callus .The Callus induction frequency of shoot tips (82%) and nodes (80%). The fresh and dry weights also increased upto 3.0 mg/l NAA and decreased beyond 3.0 mg/l of NAA. Higher concentrations of NAA (4.0 and 5.0 mg/l) were less effective on callus induction in both explants.

**Effect of kinetin (KIN)**

MS medium supplemented with various concentration of KIN (1.0 – 5.0 mg/l) was found less effective in inducing the callus formation in both explants of shoot tips and nodal segments. Concentration of KIN increases the callus formation is drastically declined, with friable whitish – green callus and fresh and dry weights were also decreased the concentration of KIN increased (Table 1 and 2) (Fig 4)

Table-1: Effect of plant growth regulators (PGRs) on callus induction and callus gowth of shoot tips of G.hirsutum (Mean±SE)

| Plant growth regulators (PGRs) | Concentra-tion Of PGRs (mg/l) | Explant Response (%) | Percenta-ge (%) for callus induction( mean±SE) | Texure of callus | Callus colour | Fresh weight of callus (Mean±SE) (mg) | Dry weigh of callus (Mean±SE) (mg) |
|---|---|---|---|---|---|---|---|
| MS | -- | -- | -- | -- | -- | -- | -- |
| MS+2,4-D | 1.0 | 76±0.26 | 69±0.36 | Compact | Light green | 721±0.26 | 64±0.15 |
| | 2.0 | 83±0.37 | 75±0.40 | Compact | Light green | 830±0.28 | 68±0.18 |
| | 3.0 | 90±0.76 | 86±0.45 | Compact | Dark green | 947±0.36 | 83±0.21 |
| | 4.0 | 76±0.50 | 66±0.30 | Compact | Brown | 680±0.21 | 60±0.12 |
| | 5.0 | 70±0.45 | 60±0.21 | compact | Brown | 610±0.19 | 54±0.09 |
| MS+NAA | 1.0 | 70±0.30 | 62±0.41 | Friable | Light green | 612±0.17 | 56±0.18 |
| | 2.0 | 68±0.46 | 64±0.26 | Friable | Whitish green | 630±0.19 | 59±0.10 |
| | 3.0 | 86±0.57 | 82±0.12 | Friable | Dark green | 867±0.25 | 76±0.40 |
| | 4.0 | 70±0.59 | 60±0.26 | Friable | Light green | 609±0.17 | 54±0.10 |
| | 5.0 | 60±0.35 | 53±0.30 | Friable | Pale green | 575±0.14 | 46±0.12 |
| MS+Kinetin | 1.0 | 45±0.14 | 38±0.16 | Friable | Whitish green | 460±0.13 | 32±0.06 |
| | 2.0 | 40±0.18 | 35±0.26 | Friable | Whitish green | 420±0.12 | 27±0.04 |
| | 3.0 | 36±0.27 | 28±0.34 | Friable | Light green | 380±0.11 | 22±0.03 |
| | 4.0 | 26±0.17 | 23±0.27 | Friable | Whitish green | 340±0.09 | 19±0.01 |
| | 5.0 | 20±0.12 | 16±0.30 | Friable | Whitish green | 300±0.08 | 13±0.01 |

Mean of twenty replicates (20×3)

Table-2: Effect of plant growth regulators (PGRs) on callus induction and callus growth of nodal explants of G.hirsutum (Mean±SE)

| Plant growth regulators (PGRs) | Concentration Of PGRs (mg/l) | Explant Response (%)(Mean ±SE) | Percentage (%) for callus induction( mean±SE) | Texure of callus | Callus colour | Fresh weight of callus (Mean±SE) | Dry weigh of callus (Mean±SE) |
|---|---|---|---|---|---|---|---|
| MS | -- | -- | -- | -- | -- | -- | -- |
| MS+2,4-D | 1.0 | 62±0.23 | 57±0.24 | Compact | Yellowish green | 620±0.21 | 6±0.16 |
| | 2.0 | 78±1.33 | 72±0.36 | Compact | Light green | 745±0.24 | 63±0.21 |
| | 3.0 | 87±0.28 | 83±0.40 | Compact | Dark green | 880±0.30 | 74±0.18 |
| | 4.0 | 60±0.33 | 60±0.25 | Compact | Pale green | 610±0.19 | 54±0.16 |
| | 5.0 | 56±0.25 | 54±0.36 | compact | Yellowish green | 580±0.16 | 45±0.10 |
| MS+NAA | 1.0 | 58±0.26 | 53±0.16 | Friable | Pale green | 560±0.12 | 42±0.16 |
| | 2.0 | 65±0.29 | 64±0.15 | Friable | Yellowish | 660±0.14 | 56±0.18 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | green | | |
| | 3.0 | 83±0.40 | 80±0.26 | Friable | Dark green | 810±0.20 | 69±0.22 |
| | 4.0 | 65±0.32 | 60±0.20 | Friable | yellowish green | 610±0.16 | 53±0.13 |
| | 5.0 | 60±0.22 | 52±0.16 | Friable | Pale green | 560±0.14 | 42±0.09 |
| MS+Kinetin | 1.0 | 36±0.19 | 22±0.16 | Friable | Whitish green | 260±0.11 | 19±0.03 |
| | 2.0 | 30±0.12 | 19±0.76 | Friable | Pale green | 230±0.09 | 13±0.02 |
| | 3.0 | 25±0.10 | 16±0.36 | Friable | Pale green | 200±0.06 | 11±0.02 |
| | 4.0 | 16±0.08 | 12±0.70 | Friable | Pale green | 165±0.03 | 8±0.01 |
| | 5.0 | 10±0.02 | 7±0.20 | Friable | Pale green | 110±0.02 | 4±0.01 |

Mean of twenty replicates (20×3)

**Effect of 2,4-D + NAA**

MS medium with growth regulators (2,4-D(1.0 to 5.0mg/l and NAA 1.0 mg/l on shoot tips and nodal explants were cultured for callus callus induction. Synergistic effects of growth regulators to produce profuse callus formation and higher callus induction frequency in shoot tip (98%)and nodes (92%) with compact light/dark green callus upto 3.0 mg/l 2,4-D+1.0mg/l NAA. The concentrations increases (4.0 to 5.0 mg/l 2,4-D)+1.0 mg/l NAA callus formation ,fresh and dry weights gradually declined in both explants (Table 3 and 4).Similarly, MS medium supplemented with NAA (2.0 to 5.0 mg/l) +2,4-D(1.0 mg/l the explants were cultured, the results were depicted Table 3 and 4( Fig 5).

**Effect of 2,4-D+kinetin**

MS media with growth regulators (2,4-D 1.0 to 5.0 mg/l) and KIN (1.0 mg/l), on shoot tips and nodal explants of G.hirsutum were cultured for callus induction . Moderate amount of callus, with compact light green/dark green calli was noticed from explants upto 3.0mg/l (2,4-D)+1.0mg/l(KIN). The higher concentration 4.0 and 5.0 mg/l 2,4-D+1.0mg/l(KIN) decreased callus formation with compact yellowish green colour. The values of frequency of callus induction, colour,texture of callus, and fresh and dry weights of the callus was given in table 3and 4. Fig Similarly MS media supplemented with kinetin concentration ranging from (2.0mg to 5.0mg/l) +2,4-D(1.0mg/l). the explants were cultured callus formation and other characters are given in table 3 and 4.

**Effect of NAA + kinetin**

MS medium supplemented with NAA (2.0 to 5.0 mg/l + kinetin (1.0mg/l), and kinetin concentrations ranging from 2.0 to 5.0 mg/l + 1.0 mg/l NAA, The explants of nodes and shoot tip explants were cultured,

the results of callus induction, colour and texture of the callus, fresh and dry weights are depicted in table 3 and 4.

**Effect of Kinetin + 2,4+D/NAA**

MS medium containing with concentrations of KIN (1.0 to 5.0mg/l) + 2,4-D (1.0mg/l/NAA 1.0mg/l) was found less effect in callus induction, fresh and dry weights in both explants (Table 3 and 4 ; Fig 6). Among the plant growth regulators auxins (2,4-D,NAA) with Kinetin (KIN) the explants were culture on the best media MSmedium supplemented with 3.0mg/l (2,4-D)+1.0mg/l(NAA).The highest frequency of callus induction,fresh and dry weights were recorded in both explants of G.hirsutum.

Table 3: Effect of plant growth regulators (PGRs)in combination on callus induction and callus growth of shoot tips of G.hirsutum(Mean±SE)

| Plant growth regulators (PGRs) | Concentration Of PGRs (mg/l) | Explant Response (%)(Mean ±SE) | Percentage (%) for callus induction (mean±SE) | Texure of callus | Callus colour | Fresh weight of callus(Mean±SE) | Dry weightof callus (Mean±SE) |
|---|---|---|---|---|---|---|---|
| MS | -- | -- | -- | -- | -- | -- | -- |
| MS+2,4-D+NAA | 1.0+1.0 | 80±0.27 | 76±0.37 | Compact | Light green | 780±0.17 | 68±0.07 |
| | 2.0+1.0 | 85±0.36 | 86±0.42 | Compact | Dark green | 940±0.15 | 76±0.16 |
| | 3.0+1.0 | 100±0.42 | 98±0.47 | Compact | Dark green | 1110±0.26 | 90±0.22 |
| | 4.0+1.0 | 79±0.55 | 75±0.58 | Compact | Brown | 780±0.22 | 66±0.08 |
| | 5.0+1.0 | 76±0.61 | 73±0.62 | Compact | Brown | 755±0.21 | 64±0.12 |
| MS+2,4-D+KIN | 1.0+1.0 | 72±0.62 | 67±0.45 | Compact | Light green | 720±0.31 | 62±0.11 |
| | 2.0+1.0 | 78±0.70 | 72±0.49 | Compact | Light green | 820±0.17 | 66±0.17 |
| | 3.0+1.0 | 86±0.26 | 80±0.36 | Compact | Light green | 920±0.22 | 71±0.19 |
| | 4.0+1.0 | 75±0.35 | 68±0.27 | Compact | yellowish green | 750±0.30 | 63±0.15 |
| | 5.0+1.0 | 70±0.45 | 65±0.20 | Compact | Yellowish green | 680±0.33 | 58±0.08 |
| MS+NAA +2,4-D | 2.0+1.0 | 72±0.52 | 68±0.51 | Compact | Lightgreen | 710±0.26 | 66±0.07 |
| | 3.0+1.0 | 80±0.43 | 74±0.70 | Compact | light green | 860±0.20 | 69±0.09 |
| | 4.0+1.0 | 68±0.45 | 63±0.27 | Friable | Light green | 700±0.36 | 58±0.06 |
| | 5.0+1.0 | 64±0.36 | 60±0.20 | Friable | Yellowish green | 670±0.27 | 56±0.06 |
| MS+NAA +KIN | 1.0+1.0 | 66±0.26 | 63±0.15 | Friable | Light green | 680±0.13 | 58±0.08 |
| | 2.0+1.0 | 69±0.20 | 67±0.12 | Compact | Light green | 720±0.19 | 60±0.07 |
| | 3.0+1.0 | 85±0.19 | 84±0.30 | Compact | Light green | 870±0.15 | 69±0.12 |
| | 4.0+1.0 | 68±0.14 | 59±0.25 | Friable | Pale green | 620±0.10 | 56±0.10 |
| | 5.0+1.0 | 64±0.10 | 56±0.16 | Friable | Pale green | 600±0.12 | 52±0.17 |
| MS+KIN +2,4-D | 2.0+1.0 | 50±0.16 | 45±0.19 | Friable | Yellowish green | 520±0.14 | 42±0.08 |
| | 3.0+1.0 | 42±0.12 | 32±0.14 | Friable | Pale green | 380±0.12 | 30±0.06 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 4.0+1.0 | 30±0.10 | 25±0.12 | Friable | Pale green | 330±0.08 | 23±0.05 |
| | 5.0+1.0 | 27±0.08 | 22±0.09 | Friable | Pale green | 300±0.06 | 19±0.03 |
| MS+NAA | 2.0+1.0 | 46±0.14 | 38±0.16 | Friable | Pale green | 400±0.11 | 35±0.07 |
| | 3.0+1.0 | 40±0.10 | 32±0.09 | Friable | Pale green | 360±0.09 | 28±0.05 |
| | 4.0+1.0 | 29±0.08 | 26±0.07 | Friable | Pale green | 320±0.07 | 24±0.03 |
| | 5.0+1.0 | 26±0.05 | 20±0.05 | Friable | Brown | 290±0.05 | 19±0.01 |

Mean of Twenty replicates(20×3)

Table 4:Effect of plant growth regulators (PGRs)in combination on callus induction and callus growth of nodal explants of G.hirsutum(Mean±SE)

| **Plant growth regulators (PGRs)** | **Concentration Of PGRs (mg/l)** | **Explant Response (%)** | **Percentage (%) for callus induction (mean±SE)** | **Texure of callus** | **Callus colour** | **Fresh weight of callus Mean±SE (mg)** | **Dry weigh of callus Mean±SE (mg)** |
|---|---|---|---|---|---|---|---|
| MS | -- | -- | -- | -- | -- | -- | -- |
| MS+2,4-D+NAA | 1.0+1.0 | 66±0.13 | 60±0.10 | Compact | Light green | 640±0.26 | 58±0.16 |
| | 2.0+1.0 | 80±0.17 | 78±0.60 | Compact | Light green | 800±0.30 | 72±0.16 |
| | 3.0+1.0 | 96±0.26 | 92±0.70 | Compact | Dark green | 990±0.70 | 89±0.26 |
| | 4.0+1.0 | 63±0.30 | 62±0.25 | Compact | Brown | 670±0.55 | 59±0.18 |
| | 5.0+1.0 | 52±0.14 | 58±0.32 | compact | Brown | 620±0.32 | 54±0.16 |
| MS+2,4-D+KIN | 1.0+1.0 | 64±0.26 | 58±0.25 | Friable | Light green | 620±0.30 | 56±0.17 |
| | 2.0+1.0 | 70±0.41 | 74±0.32 | Friable | Light green | 756±0.41 | 65±0.24 |
| | 3.0+1.0 | 82±0.56 | 76±0.17 | Compact | Dark green | 890±0.50 | 73±0.36 |
| | 4.0+1.0 | 58±0.60 | 55±0.26 | Friable | Yellowish green | 590±0.26 | 53±0.15 |
| | 5.0+1.0 | 54±0.15 | 52±0.30 | Friable | Pale green | 560±0.21 | 46±0.12 |
| MS+NAA+KIN | 1.0+1.0 | 59±0.70 | 56±0.27 | Friable | Pale green | 620±0.26 | 60±0.19 |
| | 2.0+1.0 | 66±0.60 | 65±0.16 | Friable | Light green | 680±0.29 | 70±0.28 |
| | 3.0+1.0 | 76±0.17 | 81±0.20 | Friable | Light green | 840±0.31 | 58±0.17 |
| | 4.0+1.0 | 66±0.12 | 58±0.16 | Friable | Yellowish green | 590±0.29 | 54±0.13 |
| | 5.0+1.0 | 62±0.16 | 54±0.10 | Friable | Pale green | 570±0.16 | 52±0.14 |
| MS+NAA+2,4-D | 2.0+1.0 | 67±0.20 | 63±0.21 | friable | Pale green | 650±0.32 | 58±0.16 |
| | 3.0+1.0 | 75±0.35 | 70±0.28 | compact | Light green | 860±0.36 | 71±0.22 |
| | 4.0+1.0 | 64±0.55 | 64±0.12 | friable | Pale green | 670±0.28 | 50±0.12 |
| | 5.0+1.0 | 62±0.60 | 60±0.09 | friable | Pale green | 637±0.16 | 45±0.08 |
| MS+KIN+2,4-D | 2.0+1.0 | 45±0.11 | 40±0.11 | Friable | Pale green | 420±0.22 | 36±0.11 |
| | 3.0+1.0 | 32±0.12 | 29±0.10 | Friable | Pale green | 360±0.18 | 28±0.09 |
| | 4.0+1.0 | 28±0.10 | 24±0.09 | Friable | Pale green | 310±0.17 | 22±0.06 |
| | 5.0+1.0 | 24±0.14 | 20±0.05 | friable | Brown | 289±0.15 | 18±0.05 |
| MS+KIN+NAA | 2.0+1.0 | 40±0.16 | 34±0.15 | Friable | Pale green | 360±0.16 | 33±0.10 |
| | 3.0+1.0 | 30±0.10 | 26±0.09 | Friable | Pale green | 340±0.12 | 26±0.05 |
| | 4.0+1.0 | 24±0.09 | 20±0.07 | Friable | Pale green | 286±0.10 | 18±0.04 |
| | 5.0+1.0 | 20±0.06 | 14±0.03 | friable | Brown | 220±0.08 | 16±0.02 |

Mean of twenty replicates (20×3)

**Callus formation from nodal segments of *G. hirsutum*:**



Fig-1 Fig-2.Non embyogenic(pale green) Fig-3.Non embyogenic(pale green)

Fig-1.Nodal explants
Fig-2. Callus after 3rd weeks
Fig-3. Callus after 5th week

**Callus formation from shoot tips *G. hirsutum*:**

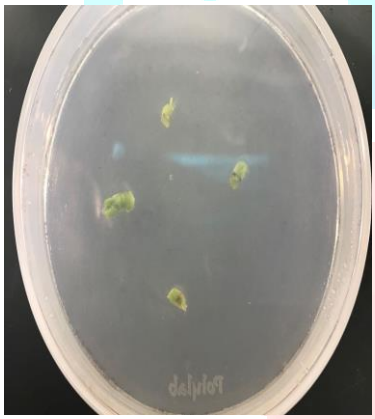
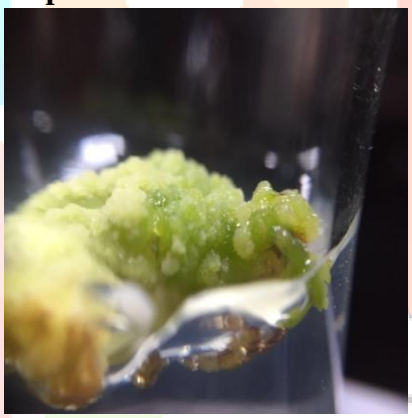


**Fig-4** **Fig-5.** **Fig-6**

Fig-4. Shoot tip explants
Fig-5. Callus after 3rd weeks(Non embryogenic(pale green)
Fig-6. Callus after 5th week(Non embryogenic(pale green)

DISCUSSION:

Plant tissue culture has been identified as an excellent surrogate method to overcome the problem connected with utilization and conservation of medicinal plants . A callus culture system offers many advantages as a model system for several biological investigations. Even callus has proved better for the synthesis of secondary metabolites in several cases. M.S basal medium without growth regulators did not show any promising result in both explants of shoot tips and nodes of G.hirsutum became necrotic after one

week only. Similar results were also reported by carelline, and Lupi (1987) Abdul Latif and Khalafahal(2008) in cotton and Gavahne and Mukundan(2010) in Tinospora cordifolia.

In the present study callus has been successfully induced from shoot tips and nodal explants inoculated on MS medium supplemented with auxins (2,4-D and NAA) and cytokinin (KIN) concentration ranging from (1.0 to 5.0 mg/l) either alone or incombinations with varied callus induction frequency, colour,texture, fresh and dry weights (Table 1-4) Explants produced callus in almost all concentrations. From the tables it is clear that, there was gradual increase in all the parameters considered here along with the increasing concentrations of the 2,4-D and NAA at 3.0 mg/l. individually, but dramatically decreased when kinetin tested.Maximum frequency of the callus induction was observed in combinations of 2,4 D (3.0 mg/l) with NAA (1.0 mg/l) or NAA (3.0mg/l) with 2,4-D(3.0 mg/l). Callus was clumps of unorganized Parenchymetous tissues formed by vigorous proliferation by the mitotic cell division from the small explants in culture showing no polarity. Callus initiated from the cut portions of the explants, where cells at the cut ends undergo mitosis, which leads to callus formation. It may be due to wound reaction or effect of endogenous growth regulator .The texture of callus varied according to the nature of auxins (2,4-D and NAA) and cytokinin (kinetin) ratio Similar observations were also reported by Martin(2002) in Holostemma adakudin. Among the both explants, shoot tips, were more response than nodal segments. 2,4-D is a systhetic harmone which play a significant role in alone for induction of callus in the present study in both explants or in combination of 2,4-D + NAA further enchanced the profuse callus showed green/dark green, compact/fragile callus.This may be due to their role in DNA synthesis and mitosis. The similar results were also reported by Skoog and Miller 1957 and also in other medicinal plants such as Ipomohea abscura (Mungola etal 2009,Abrus prectorius(Hassan etal 2009)Withania somnifera (saritha and naidu,2007). Nagendra Prasad et al(2006) and in Gymnema sylvestre (upendra kumar et al (2010) The colour is being mainly influenced by the location of phenolic secondary metabolites in cells.If the accumulation of the phenotics in the cytoplasm it undergoes oxidation of polymerization and oxidised products appear brown. Similar observations also reported by Lucas et al (2000) . MS medium fortified with NAA + KIN produce light green callus with friable nature in both explants .Similar results were also reported by Shivanna et al (2009) in Biophytum Senesitivum leaf explants .

The calli growth rate from both explants showed maximum increase in first 3rd week subculture than the subsequent subculture. This may be due to less totipotent and reduces mitotic activity as the calli tissues are getting older.Similar results were recorded in gymnema sylvestre (Gopi and vatsala,2006) in Capsicum annuum L (Aniel kumar et al (2010). Tissue culture studies for callus induction have been reported by several workers in different explants.Present findings corroborate with findings of the Gavahne and mukundan (2010) in Tinospora Cordifolia.

CONCLUSION :

The protocol established for the first time for the induction of calli in both explants of shoot tip and nodal segments of Gymnema hirsutum an important antidiabetic plant may be exploited at commercial scale . The secondary metabolites synthezied by these calli in vitro may serve the need of different pharmaceutical companies and the species shall be conserved in its natural population at one hand while the pharmaceutical industries shall get these metabolites without any interruption.

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