1. **Title of research project:** **"Biotechnological strategies for the Production of bioactive compounds from cell cultures of *Alpinia* species"**

2. **Name of PI:** **Dr. Ajay G. Namdeo**

3. **Funding Agency:** **University Grants Commission (UGC)**

4. **Project Reference number/ File number:** **F. No. 42-700/20013 (SR) dtd 22/3/13**

5. **Executive summary of the project along: with output:**

BIOTECHNOLOGICAL STUDIES FOR THE PRODUCTION OF BIOACTIVE COMPOUNDS FROM CELL CULTURES OF *ALPINIA* SPECIES"

1. Callus initiation

Healthy and friable callus is the requirement of suspension culture for mass cultivation of plant cells for any biotechnological approach like precursor feeding and elicitation. Callus consists of undifferentiated mass of cells developed on a semi-solid medium that can be initiated from any viable explants of whole plant. The maintenance of callus cultures depends on adequate supply of nutrients, growth hormones and controlled sterile environment. The cells, under *in vitro* conditions, contained all the genetic information present in parent plant. MS media containing different combination of growth hormones were used for callus initiation from *Alpinia purpurata*. About 200 different media composition was tried for callus initiation from different parts of *Alpinia purpurata* on MS medium supplemented with growth hormones. The growth of callus was observed in each medium and the maximum growth pattern was observed in MS medium supplemented with 2, 4-D +kinetin (2:2) as compare to other media. The results were shown in Table 5.4.1. These compositions were given healthy, green, friable callus within a period of 6 months. The growth of callus was observed as fresh weight (FW) and dry weight (DW) measured every week. Both fresh weight (FW) and dry weight (DW) increased over the period of incubation. Effect of phyto hormones on callus growth is presented in Table 5.4.1. Maximum dry weight of callus (540 mg) was obtained on MS medium supplemented with 2, 4-D + kinetin (2:2) after 6 months of incubation.

Table B1: Effect of phyto hormones on callus growth

Media	Hormones	Conc. (ppm)	FW (mg)	DW (mg)
MS	NAA + BAP +kinetin	1:2:1	1540	120
MS	NAA + BAP	2:2	240	15
MS	NAA + BAP + kinetin	1:2:2	1625	80
MS	BA + kinetin	2:2	550	20
MS	NAA + BA	1:2	650	22
MS	2,4-D +kinetin	2:2	2260	540
MS	2,4-D +BAP	1:2	840	45
MS	NAA + BAP+NAA	2:2:2	75	10

2.

MULTIPLICATION AND ELONGATION OF SHOOT:

2.1 Initiation of shoots

For the initiation of shoots, two week old callus obtained in MS medium supplemented with 2, 4-D (2ppm) + Kn (2ppm) was transferred in various shooting medium. Table 2.1 indicates different growth hormones and their combinations for the initiation of shoots from *Alpinia purpurata* callus. Shoot initiation was observed after one month of incubation. Maximum number (9-11) of shoots was observed in medium with NAA+BA (0.1:3.0) after incubation. Similar shoot initiation was observed in medium supplemented with NAA+BA (0.1:2.0). Shoots were allowed to grow in this medium for one month and then separated and transferred individually in culture flask and bottles for rooting. Fig. 2.1 shows formation of shoots in MS medium containing NAA+BA (0.1:3.0). Maximum shoot multiplication was observed in MS medium containing NAA+BA (0.1:3.0), after two successive sub culturing we get elongated shoot with mature leaves.

Table B2: Effect of NAA and BA on shoot proliferation of *Alpinia purpurata*.

S. No.	Hormones	Conc. (ppm)	Observation
1	NAA+BA	0.1+1	Cell mass was less, less granular callus, no rooting formation.

2	NAA+BA	0.1+2	After sub culturing the callus in same media 2-3 small shoots arises from some test tubes, shorter, thicker shoots about 0.5 mm-2 cm heights.
3	NAA+BA	0.1+3	Superficial callus granular, core compact, more callus, rooting was observed with some test tube.

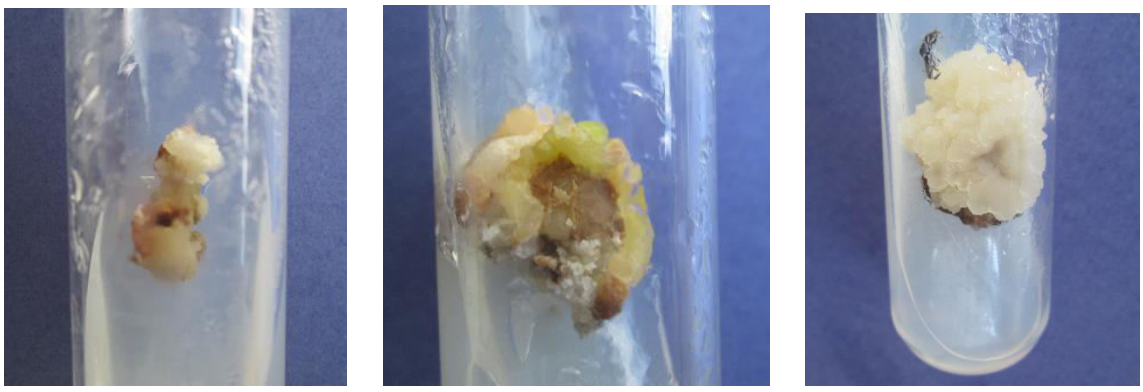

Figure 1: Different Stages of Callus initiation in *Alpinia galanga*

Figure 2: Micropropagation in *Alpinia galanga*

Figure 3: Micropropagation in *Alpinia galanga*

3 Initiation of roots

The shoots with gradual decline in cytokinin and gibberellins and subsequent sub culturing to basal media were exposed to various auxins for root initiation. IAA, IBA and NAA at concentration ranging from 1-4 ppm were used in both MS medium full strength (M_1) and MS medium half strength (M_2). The observations were reported at the end of 15th day and 30th day for number of roots per shoot, frequency of rooting and nature of rooting.

The effect of IAA on root induction from *Alpinia purpurata* shoots using MS rooting media (M_1 and M_2) is presented in Table 3. Fast rooting was observed in all concentration of IAA and the rapid initiation of roots was observed after 2 weeks incubation. However, shoots incubated in MS medium with IBA showed delayed root initiation (3-4 weeks). Fig 3 shows initiation of root from *Alpinia purpurata* shoots in MS medium containing different growth hormone and their combinations. However, the use of IAA (M_1 medium) greatly improved rooting on all shoots with maximum rooting percentage (89.32%) found in MS media containing 3 ppm of Indole

acetic acid (IAA). At higher concentration of IAA (8 ppm and 10 ppm) the percentage of rooting decreased slightly due to negative effect.

Table 3: Effect of IAA on rooting in MS solid medium.

S. No.	Medium	IAA (PPM)	Number of roots/shoot	% rooting	
				After 15 days	After 30 days
1	MS (M_1)	1	2-1,white, long, slender	21	43
2	MS (M_1)	2	3-4, white, long, slender	13.48	76.19
3	MS (M_1)	3	9-11, white, long, slender, green white.	32.34	89.32
4	½ MS (M_2)	1	2-1, roots long, medium, White	----	18.18
5	½ MS (M_2)	2	4-5, shorter thicker, longer, green white	17.64	34.20
6	½ MS (M2)	3	3-4	32.21	45.30
7	½ MS (M2)	4	3-5	21.30	44.57