

## ***In vitro* micropropagation of *Ananas comosus* L. (Merr.) var. Queen**

**Asit B. Mandal, Aparna Maiti and R. Elanchezhian**

Plant Genetics and Biotechnology Division, Central Agricultural Research Institute, Port Blair-744101, Andamans, India

### **Abstract**

Efficient protocols, developed for micropropagation of pineapple (*Ananas comosus* L.) var. Queen through *in vitro* culture of axillary buds from crowns of mature fruits, slips and suckers (induced from adult plants), are presented in this paper. Axenic buds, initially cultured on MS medium with high auxin: cytokinin ratio, when shifted onto MS with high cytokinin: auxin, showed high percentage of culture establishment (80.6%). Further, the shoot multiplication increased upon subculture on freshly prepared high BAP containing multiplication medium. The cycle was found to be repeatable. MS with 2.5 mg L<sup>-1</sup> BAP, 2.0 mg L<sup>-1</sup> NAA & IBA, 10% CW (coconut water) was found to be the best multiplication medium. *In vitro* grown shootlets were successfully rooted on MS with 0.5 mg L<sup>-1</sup> NAA and 0.5 mg L<sup>-1</sup> IBA. While evaluating different culture systems viz., semi-solid, liquid stationary and liquid shaken, the last one was found to be the most promising and maximum buds could be produced over a period of 6 months. Maximum (12.25±1.20) buds per culture in liquid shaken culture was observed in the fifth passage (each of 30 d duration) in an experiment of 6 months duration. The requirement of BAP in liquid shaken culture medium was almost half of the requirement in semi-solid medium to achieve a maximum multiplication. Pulsing of explants from liquid culture to semi-solid medium enhanced the culture response as evident from higher shoot multiplication rate with all types of explants. The potential of callus cultures in regenerating plantlets was found to be immense. Maximum (14.33±2.78) plantlets per culture were developed through this technique. They performed at par with *in vitro* grown shootlets developed from axillary buds. This offers a route to integrate callus based plantlet development with *in vitro* multiplication to produce more plantlets within a short time, though the risk of somaclonal variation remains, which is sought to be minimized through judicious cultural manipulation.

**Key words:** Pineapple, *Ananas comosus*, *in vitro*, micropropagation

### **Introduction**

Pineapple (*Ananas comosus* L.) fruits provide a cheap source of nutrition and energy. They are in short supply in Bay Islands—a remote Union Territory of the Indian Republic. Growing pineapple in the congenial agroclimate of Bay Islands seems to be the best possible alternative. However, this also is constrained due to non-availability of sufficient planting materials in time. Furthermore, transporting planting materials from different sources of mainland increases the danger of introducing new disease causing organisms, which thrive and spread fast in the hot and humid island conditions. This may lead to disastrous consequences. *In situ* production of plantlets is thus a long felt need. Underpinning all the constraints in purview, *in vitro* micropropagation of pineapple was undertaken with a popular variety, Queen, to evolve an efficient system of plantlet production in Bay Islands, and thereby, at least, partially mitigate the fruit shortage in these Islands. The acreage can be increased in the more availability of plantlets for cultivation and thus pineapple production can be enhanced to meet local demands.

For rapid increase and early release of plantlets, micropropagation through *in vitro* culture is advantageous. Cloning, by *in vitro* culture of axillary buds from crowns of mature pineapple fruits was successfully developed by Zepeda and Sagawa (1981), who estimated 5000 plants could be produced from an explant of a single crown in a year. Wakasa *et al.* (1978) generated large

number of plantlets and reported the presence of somaclonal variation in morphological traits. Further, Wakasa *et al.* (1979) showed that materials derived from syncarps exhibited maximum variation as compared to crown and axillary buds in the field. Mapes (1974) reported the induction of somatic embryos in wild pineapple *Ananas erectifolius*. Successful micropropagation system in India, involving lateral buds, was evolved by Mathews *et al.* (1976). Callus formation at the base of excised axenic buds (Lakshmi Sita *et al.*, 1974) and plantlet regeneration from embryo callus (Srinivasa Rao *et al.*, 1981) had been reported. Indiscriminate use of callus culture associated micropropagation seems to be not advisable owing to inadvertent somaclonal variation. The present work was aimed at improvising multiplication rate already reported by Zepeda and Sagawa (1981) and others in var. Queen (Fig. 1), which the author hopes will be a compatible technology for mass production of planting materials with minimized problems of somaclonal variation.

### **Materials and methods**

A commercial variety of pineapple (*Ananas comosus* (L.) Merr.) viz., Queen, was employed as the source material for the present study. Crowns, from mature fruits, actively growing small suckers and slips induced in field grown adult plants, were made free of leaves. Defoliated whole crowns, suckers and slips were washed in running tap water for 10 min. They were soaked in

5% aqueous Teepol solution for 3-5 min with vigorous shaking and washed, 3-4 times in distilled water. These materials were soaked again, for 5 minutes, in 1% Bavistin solution (carbendazim 50% WP) to remove fungal contamination and were again washed in distilled water. The buds, present in the axils of leaves were excised with a sharp scalpel with minimum parental tissue and were further disinfected by immersing in freshly prepared 0.01%  $\text{HgCl}_2$  solution along with 3-4 drops of Tween 20 as wetting agent in sterile Erlenmeyer flasks *in vacuo* for 5 min. The buds were washed thrice with sterile  $\text{H}_2\text{O}$  and mgdried on sterile tissue paper for about 5 min under laminar airflow. Axenic buds were cultured as specifically mentioned for each experiment hereunder. All experiments were done with a minimum of 25 replicates if not mentioned otherwise.

**Experiment 1. Effect of shifting culture from high auxin: cytokinin to high cytokinin: auxin containing media:** Disinfected buds were cultured on MS medium (Murashige and Skoog, 1962) with 3 mg L<sup>-1</sup> NAA, 0.5 mg L<sup>-1</sup> BAP and 10% coconut water (CW) along with control set initially for 15 days and then shifted to MS medium with 4 different hormonal treatments in combination (containing high cytokinin: auxin ratio) as highlighted in Table 1. Another batch of fresh explants, from crown, slip and suckers were also cultured on MS with high cytokinin: auxin (Table 1) and were subcultured on the same medium composition for comparison with the former set. After approximately two and half months small explants developed into shootlets. The buds developed were counted and the percentage of culture response was worked out.

**Experiment 2. Assessment of multiplication rate over a period of time:** Axenic buds grown *in vitro* on MS medium were shifted onto multiplication medium containing MS with 2.5 mg L<sup>-1</sup> BAP, 2 mg L<sup>-1</sup> of NAA & IBA and 10% CW (best media composition as found in our earlier experiments; unpublished data). At 30 d interval, multiplied shoots were manually separated and individual shoots were subcultured on freshly prepared medium of same composition. Such propagation-separation cycle was continued for a period of 6 months with observation and data recording at monthly interval.

**Experiment 3. Effect of different culture systems on shoot multiplication:** One batch of axenic buds grown *in vitro* on MS medium was transferred onto MS containing various concentrations of BAP (0.5, 2.5, 5 mg L<sup>-1</sup>); NAA & IBA (0.25, 0.5, 2.5 mg L<sup>-1</sup>) and 10% CW either singly or in combinations. In semi-solid system, explants were cultured in 50 ml capacity culture tubes with cotton plugs while in liquid medium, explants were cultured in 150 ml capacity Erlenmeyer flasks with cotton plugs (in both cases same amount of media were used). All the cultures were maintained at 25±2°C under continuous diffused light from cool white fluorescent tubes. In liquid shaken culture, flasks with explants were placed on rotary shakers at 120 strokes per min. Multiplied shoots were subcultured at one-month interval, after manual separation of individual shoots. In case of liquid culture, media were renewed at 15d interval and multiplied shoots were separated and cultured in new flasks at one-month interval.

**Experiment 4. Effect of pulsing:** One set of axenic buds grown *in vitro* in liquid MS medium (liquid shaken culture) was shifted to semi-solid medium containing MS/ half MS composition with various concentrations of cytokinins *viz.*, BAP & kinetin (0.5, 2.5, 5.0 mg L<sup>-1</sup>); auxins *viz.*, NAA & IBA (0.5, 2.5, 5.0 mg L<sup>-1</sup>) with and

without 10% CW, singly or in combinations. Another set of buds was also cultured simultaneously on semi-solid medium of the same composition with different hormones, as mentioned above, from the beginning for comparison with the former. Explants, showing culture response, were counted and number of developed buds per culture was recorded.

**Experiment 5. Exploitation of callus culture in micropropagation:** Two different kinds of explants *viz.*, buds and whole leaves from *in vitro* shootlets (grown on MS) were cultured on MS with different concentrations of NAA (2.5, 5.0 mg L<sup>-1</sup>); IAA (2, 4 mg L<sup>-1</sup>) and BAP (0.5, 2.5, 5.0 mg L<sup>-1</sup>) singly or in combinations with or without 10% CW. Induced calli, after a month, were subcultured on callus maintenance medium (CMM), with very low concentrations of hormones *viz.*, NAA, IAA and BAP (0.5, 1.0 mg L<sup>-1</sup>), with or without 10% CW. After two subcultures of one month duration each, the developed, friable and fluffy yellowish-white calli were shifted onto regeneration medium containing MS with various concentrations of BAP (2.5, 5.0 mg L<sup>-1</sup>); auxins *viz.*, IBA, NAA (0.5, 1.0 mg L<sup>-1</sup>), with and without 500 mg L<sup>-1</sup> casein hydrolysate (CH) and 50 mg L<sup>-1</sup> adenine hemisulfate. Developed plantlets were counted after 40 d. They were rooted on MS with different concentrations of NAA & IBA (0.25, 0.5, 1.0, 2.0 mg L<sup>-1</sup>). A few shootlets were tested for multiplication on MS with 2.5 mg L<sup>-1</sup> BAP, 2 mg L<sup>-1</sup> of NAA & IBA, 10% CW and the number of buds produced per culture was recorded for comparison with the multiplication rate of *in vitro* grown plantlets, derived from axillary buds.

**Experiment 6. Effect of cytokinin and coconut water on plantlet regeneration from callus culture:** A portion of pure calli of var. Queen, obtained from Experiment 5, was put on regeneration media containing MS with 0.5 mg L<sup>-1</sup> IBA, 250 mg L<sup>-1</sup> CH, 50 mg L<sup>-1</sup> adenine sulfate and different concentrations of cytokinin (1, 2, 3, 4 mg L<sup>-1</sup> BAP/kinetin) singly, with and without 10% CW. The cultures were kept under diffused light continuously, at 25±2°C. After 40 d of culture, developed plantlets were separated, counted and recorded as number of buds /culture.

## Results and discussion

Disinfected buds, from three different explants *viz.*, crown, slip and suckers, were initially cultured on MS, supplemented with 3 mg L<sup>-1</sup> NAA and 0.5 mg L<sup>-1</sup> BAP. After 15 d these buds were shifted to culture tubes containing fresh MS, with 10% CW, 2.5 mg L<sup>-1</sup> BAP/kinetin and 0.5 mg L<sup>-1</sup> NAA and IBA, singly or in combinations. Medium and hormonal treatments adopted were the modifications of previous formulations used by Mathews and Rangan, (1981). *In vitro* propagation using axillary buds had been successful earlier also (Mathews *et al.* 1976; Mathews and Rangan, 1979). In our study, within 20-25 d, buds sprouted, subsequently became greenish and swelled up. In 2-2.5 months, these buds gradually developed into small plantlets with 3-5 leaves. Differential culture response (Table I) was observed across explants and over four different treatments. Maximum response (80.6%) was found on MS with 2.5 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup> NAA & IBA, and 10% CW. Minimum response (54.6%) was observed with buds from crown, cultured on MS with 2.5 mg L<sup>-1</sup> kinetin and 10% CW. Such buds when subcultured on freshly prepared multiplication medium containing MS with 2.5 mg L<sup>-1</sup> BAP, 2 mg L<sup>-1</sup> IBA & NAA and 10% CW, which was the best medium composition found in our earlier

experiments; (unpublished data), multiple shoots started emerging and continued over a long time. The increase in the multiplication rate by shifting culture from medium containing high auxin: cytokinin to high cytokinin: auxin had earlier been reported and it has been confirmed in the present experiment also (Fig. 1).

To assess the multiplication rate over a period of six months the Experiment 2 was undertaken on MS with 2.5 mg L<sup>-1</sup> BAP, 2 mg L<sup>-1</sup> NAA & IBA and 10% CW. At the end of each passage of 30 d, developed multiple shoots were separated individually and cultured on freshly prepared medium of the same composition. Mean multiplication of 25 cultures, over a period of six months, is presented in Table 2. A good deal of variation was observed across explants and over passages. The multiplication rate was maximum in the 5th passage, irrespective of explant source, though, increment was marginal in all the cases. Mean

multiplication rate per month per culture was maximum in the buds obtained from sucker (6.28), followed by slip (5.41) and crown (4.75). The plausible expedition may be that the buds from suckers were endowed with more potential for shoot multiplication due to higher nutrient reserves in suckers than available in slips or crowns.

Culture systems, especially semi-solid *i.e.* with agar (0.8%) and liquid medium without agar seems to have profound influences on the multiplication rate. Even in liquid medium, stationary and shaken cultures were found to give differential response (Rangan, 1979). To assess such differential multiplication response, if any, with the variety Queen, Experiment 3 was conducted. Among the 24 treatments, three formulas were found to be promising (Table 3). In the semi-solid medium, the BAP requirement was more than in liquid medium, probably because of

**Table 1. Effect of different cytokinins and auxins on *in vitro* culture response of buds from diverse explants in shifting culture**

Treatment in MS medium	Culture established (%)		
	Crown	Slip	Sucker
2.5 mg L <sup>-1</sup> K + 0.5 mg L <sup>-1</sup> NAA & IBA + 10% CW (v/v)	68.5 (57.2)*	73.4 (59.6)	74.6 (64.0)
2.5 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA & IBA + 10% CW (v/v)	65.2 (51.0)	77.5 (68.4)	80.6 (76.5)
2.5 mg L <sup>-1</sup> K + 10% CW (v/v)	54.6 (43.5)	64.2 (48.6)	64.8 (51.8)
2.5 mg L <sup>-1</sup> BAP + 10% CW (v/v)	60.5 (49.2)	78.5 (68.6)	71.2 (59.5)

\*Figures in parentheses represent percent culture established which were initially cultured and maintained on same media composition

(\*) high cytokinin: auxin supplemented medium

**Table 2. Effect of different BAP concentrations on plantlet multiplication rate plantlets over six passages**

Explant source	Multiplication rate (No. of buds /culture /month)						
	Passage						Mean
	I	II	III	IV	V	VI	
Crown	3.6±0.37	3.8±0.48	4.5±0.85	5.6±0.81	5.8±0.76	5.2±0.41	4.75
Slip	4.4±0.52	4.7±0.61	5.2±0.72	5.8±0.73	6.4±0.91	6.0±0.81	5.41
Sucker	5.3±0.71	5.8±0.64	6.1±0.52	6.5±0.85	7.2±0.86	6.8±0.59	6.28

Multiplication medium contains MS medium with 2.5 mg L<sup>-1</sup> BAP, 2 mg L<sup>-1</sup> NAA and 10% CW.

**Table 3. *In vitro* culture response of buds obtained from crown of var. Queen under different culture system**

Culture system	Promising media composition	Culture Showed multiple shoots (%)	Multiplication rate		
			1st month	Max multiplication rate (No. of buds / month /culture)	Passage No. (30 d)
Semi - solid*	MS + 0.5 mg L <sup>-1</sup> NAA + 5 mg L <sup>-1</sup> BAP + 10% CW	72.0	4.42 ± 0.38	6.5 ± 0.38	5th
	MS + 0.5 mg L <sup>-1</sup> NAA + 5 mg L <sup>-1</sup> BAP.	63.0	4.02 ± 0.46	5.6 ± 0.66	5th
	MS + 5 mg L <sup>-1</sup> BAP + 0.25 mg U' IBA & NAA.	61.0	3.82 ± 0.34	5.2 ± 0.47	6th
Liquid shaken	MS + 2.5 mg L <sup>-1</sup> BAP.	100 (78.0)**	7.10 ± 1.08 (5.64 ± 0.44)	12.25 ± 1.20 (9.6 ± 0.90)	4th 5th
	MS + 2.5 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA.	100 (68.5)	6.40 ± 0.79 (4.89 ± 0.52)	10.60 ± 1.10 (8.9 ± 0.90)	4th 4th
	MS + 2.5 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA + 10% C W (v/v)	98.4 (66)	5.33 ± 0.54 (4.24 ± 0.30)	9.67 ± 0.89 (7.10 ± 0.65)	4th 5th

\* Semi-solid medium was gelled with 0.8% agar.

\*\* Figures within parentheses indicate values in stationary culture.

Fig. 1. *In vitro* culture and multiplication in pineapple: (1). Initial explant from sucker on MS with 3 mg L<sup>-1</sup> NAA, 0.5 mg L<sup>-1</sup> BAP and 10 % CW. (2). After 1.5 month on MS with 2.5 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup> NAA & IBA and 10% CW. (3). After 2.5 month on the multiplication medium (MM) containing MS with 2.5 mg L<sup>-1</sup> BAP, 2 mg L<sup>-1</sup> NAA & IBA and 10% CW. (4). Multiplying buds on MS with 2.5 mg L<sup>-1</sup> BAP after a month interval. (5). Liquid shaken culture containing MS with 0.5 mg L<sup>-1</sup> NAA, 5 mg L<sup>-1</sup> BAP and 10% CW. (6). Multiplying shoots shifted from liquid stationary culture containing MS with 2.5 mg L<sup>-1</sup> BAP to MM. (7). Redifferentiation of calli from whole leaf explant into plantlets on MS with 500 mg L<sup>-1</sup> CH, 50 mg L<sup>-1</sup> adenine hemisulfate, 2 mg L<sup>-1</sup> BAP and 0.5 mg L<sup>-1</sup> IBA. (8). Proliferating buds from callus (obtained from MS with 3 mg L<sup>-1</sup> BAP, +0.5 mg L<sup>-1</sup> BA, 250 mg L<sup>-1</sup> CH, 50 mg L<sup>-1</sup> adenine sulfate) multiplying on MM. (9). Performance of *in vitro* developed plantlets in field.

**Table 4. Effect of pulsing on in vitro micropropagation of buds obtained from different explants in pineapple**

Explant (axillary bud) source	Pulsed *			Non pulsed		
	Best media composition	% culture response	Multiplication rate (No. of shoots /month /culture)	Best media composition	% culture response	Multiplication rate (No. of shoots /month /culture)
Crown	1/2 MS +2.5 mg L <sup>-1</sup> BAP +0.5 mg L <sup>-1</sup> NAA +10% CW(v/v)	86.0	5.6	1/2 MS +2.5 mg L <sup>-1</sup> BAP +2.5 mg L <sup>-1</sup> kinetin +0.4 mg L <sup>-1</sup> IBA	63.0	3.1
Slip	1/2 MS +2.5 mg L <sup>-1</sup> BAP +0.5 mg L <sup>-1</sup> kinetin +10% CW(v/v)	79.5	5.8	MS +2.5 mg L <sup>-1</sup> BAP +0.5 mg L <sup>-1</sup> kinetin +10% CW (v/v)	43.0	2.4
Sucker	Ms +5.0 mg L <sup>-1</sup> BAP +0.5 mg L <sup>-1</sup> NAA +5% CW (v/v)	82.0	6.3	1/2 MS +5 mg U' BAP +2.5 mg L <sup>-1</sup> IBA	54.0	2.9

\**In vitro* grown plantlets were obtained from liquid shaken culture containing MS with 2.5 mg L<sup>-1</sup> BAP were shifted to semi-solid medium.

**Table 5. Potential of callus cultures in plantlet development in pineapple**

Explant	Best callus induction medium (CIM)	Best callus maintenance medium (CMM)	Best regeneration medium (RM)	No. of buds /culture	Best rooting medium	Explant showed response (%)
Calli developed at base of <i>in vitro</i> grown buds	MS +2.5 mg L <sup>-1</sup> NAA +2 mg L <sup>-1</sup> IAA +2.5 mg L <sup>-1</sup> BAP	MS +0.5 mg L <sup>-1</sup> IAA +2 mg L <sup>-1</sup> NAA +10% CW(v/v)	MS +CH 500 mg L <sup>-1</sup> +50 mg L <sup>-1</sup> adenine hemisulfate +2 mg L <sup>-1</sup> BAP+ 0.5 mg L <sup>-1</sup> IBA	14.33±2.78	MS +0.5 mg L <sup>-1</sup> IBA	57.2
Calli developed at base of whole leaf explants	MS +5 mg L <sup>-1</sup> NAA +2 mg L <sup>-1</sup> BAP +10% CW(v/v)	MS +0.5 mg L <sup>-1</sup> BAP +1 mg L <sup>-1</sup> NAA +10% CW.(v/v)	MS +0.5 mg L <sup>-1</sup> NAA +1 mg L <sup>-1</sup> IBA+2.5 mg L <sup>-1</sup> BAP +CW 5%(v/v)	11.56±1.67	MS + 0.5 mg L <sup>-1</sup> NAA + 0.25 mg L <sup>-1</sup> IBA	68.5

uniform distribution of hormones and nutrients in the latter, the former. Percentage of culture response was found to be almost hundred in liquid shaken culture. Maximum multiplication rate in liquid cultures was 7.10 (±1.08) buds in the first month. Only three medium compositions, those performed better than others, were continued for another six months. Maximum multiplication to 6.54 (±0.76) buds per culture was observed on the fifth passage, in case of semi-solid medium on MS with 0.5 mg L<sup>-1</sup> NAA, 5 mg L<sup>-1</sup> BAP and 10% CW, whereas, in shaken liquid culture maximum of 12.25±1.20 buds per culture was observed in the fourth passage on MS with 2.5 mg L<sup>-1</sup> BAP. In case of stationary culture a maximum of 9.67 (±0.89) buds per culture was achieved in fifth passage on the same medium as in shaken culture. Two major conclusions, those emerge from this study are: (1) shaken liquid culture enhanced multiplication rate in the var. Queen, (2) BAP requirement in semi-solid medium was found to be approximately double that of liquid medium.

Clonal propagation, via callus phase is a feasible proposition in

pineapple micropropagation (Mathews and Rangan, 1979; Srinivasa Rao *et al.*, 1981), though developed plants may not be genetically homogenous due to chromosomal instability (Murashige, 1974). As early as 1973, Mapes (1973) reported partial success in inducing differentiation in tissue culture of some bromeliads. It offers ample scope for the selection of desirable types by exploiting genetic heterogeneity, especially, from hybrid seed calli (Srinivasa Rao *et al.*, 1981). To assess the feasibility of callus induction and plantlet production, Experiment 5 was conducted with *in vitro* grown plantlets of var. Queen. Calli were successfully developed at the base of *in vitro* grown small plantlets, as well as, from the base of whole leaf explants (from *in vitro* grown plantlets). They were cultured on semi-solid MS with NAA (0.5, 2.5, 5.0 mg L<sup>-1</sup>); IAA (0.5, 2.5 and 0.5 mg L<sup>-1</sup>) and BAP (0.5, 1.0, 2.5 mg L<sup>-1</sup>) singly or in combinations. For about 15 d, the explants remain quiescent. Afterward fluffy yellowish-white calli developed at the cut end of the explants. 57.2 and 68.5% of shoot and leaf explants produced calli. Such calli were subcultured at least twice before shifting onto

**Table 6. Effect of reduced auxin (NAA), high cytokinin and organic adjuvant CW in regeneration of plantlets from pure callus culture in pineapple var. Queen**

Cytokinin RM* ( mg L)	Hormone level in	Explant showed differentiation(%)	No of buds /culture (in 40 d)
BAP	1.0	79.5 (85.6)**	9.60±1.25 (10.62±1.6)**
	2.0	82.6 (94.2)	8.62±0.81 (9.50±1.3)
	3.0	95.3 (100.0)	10.25±2.50 (11.10±2.3)
	4.0	74.5 (83.2)	7.60±1.67 (8.50±1.2)
Kinetin	1.0	64.4 (75.5)	8.50±2.10 (8.96±0.8)
	2.0	67.6 (71.6)	8.36±1.95 (8.80 ±0.9)
	3.0	72.2 (78.5)	9.25±1.68 (10.62±1.9)
	4.0	74.5 (79.2)	6.20±0.90 (8.35±1.2)
Control	Control	88.6 (92.3)	9.33±1.87 (10.26±1.8)

\*Regeneration medium (RM) contains MS+0.5 mg L<sup>-1</sup> IBA +250 mg L<sup>-1</sup> CH +50 mg L<sup>-1</sup> adenine sulfate +30g L<sup>-1</sup> sucrose +8g L<sup>-1</sup> agar.

\*\*Figures within parentheses indicate per cent plantlets regenerated on RM with additional 10% CW.

regeneration media for plantlet development. While subculturing, occasional bud development and greenish protuberances were visible. Calli from an individual explants were maintained separately. Maximum (14.33±2.78) plants from individual callus were developed from calli developed at the base where as 11.56±1.67 buds were observed from calli from leaf explants (Table 5). This indicates greater potential of the former in regenerating more plantlets than the latter. By repeated subculturing of such calli on medium containing high auxin (3.0 mg L<sup>-1</sup> NAA), the appearance of protuberance like structures was found to be reduced and ultimately pure cultures could be established in the fourth subculture.

To determine the effect of different concentrations of BAP and kinetin and the effect of organic adjuvant CW in regenerating plantlets, parts of pure calli from individual explant from Experiment 5 were cultured on MS with 0.5 mg L<sup>-1</sup>, IBA, 250 mg L<sup>-1</sup>, CH, 50 mg L<sup>-1</sup> adenine sulfate and different concentrations of cytokinin (1, 2, 3, 4 mg L<sup>-1</sup>) BAP / kinetin (modification of Mathews and Rangan 1981) alone. In all the treatments 15 cultures were maintained. Facile regeneration up to 95.6% was observed on MS supplemented with 3 mg L<sup>-1</sup> BAP CW at 10% and it was found to profoundly increase the regeneration percentage (Table 4). It was hundred per cent when used in conjunction with 3 mg L<sup>-1</sup> of BAR It is mentionable that calli from control set (*i.e.* with out hormone) showed plantlet regeneration of 88.6 and 92.3% on RM without and with CW, respectively. Our observation of enhanced plantlet production by shifting culture from high auxin to auxin free media is corroborative to the earlier observations of Nishi *et al.* (Rice) (1968); Carter *et al.* (1968) (Oats); Rangan, (1974) (*Panicum*) Masteller and Holden, (1970) (Sorghum); Rangan, (1976) (*Paspalum*, *Eleusine* and *Pennisetum*). However, CW did not significantly influence the number of plantlets produced from each culture. Such regenerated shoots were employed for *in vitro* multiplication (as performed in Experiment 1) and multiple shoots @ 5.63±0.92 per culture per month were observed. This offers ample scope in integrating callus culture with clonal propagation through axillary bud multiplication. In nutshell the prospect of *in vitro* culture of lateral buds from crowns, slips and suckers of var. Queen is found to be promising. Callus. Mediated clonal propagation is also possible for this variety, but as the risk of somaclonal variation seems to creep in the possibility of occurrence of genetic heterogeneity can not be ruled out which reduces the guarantee of obtaining quality propagules from clonal propagation. But, such propagation, may be found suitable in selection programmes from callus cultures of hybrids for desirable types. Otherwise, stringent field evaluation of *in vitro*

developed plantlets may be done to ascertain the incidence of somaclonal % variation before envisaging such a proposition.

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