Pakistan
Journal of Biological Sciences

The Effect of Different Hormones and Incubation Periods on *in vitro* Proliferation of Pineapple (*Ananas comosus* L.) Merr cv. Smooth Cayenne) Shoot-Tip Culture

Abdelhamid M. Hamad and Rosna Mat Taha
Institute of Biological Sciences, Faculty of Science,
University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract: Seven different hormone treatments, namely 6-benzylaminopurine (BAP) at 2, 3 mg L^{-1} was applied singly and in combination with Indole Acetic Acid (IAA) at 0.18, 0.8 and 1.8 mg L^{-1}, BAP at 3.3 mg L^{-1} in combination with IAA at 1.8 and 3.3 mg L^{-1} and triple combination of BAP at 2.3, IAA at 1.8 and Gibberellic acid (GA_3) at 1.0 mg L^{-1} were tested, over four different incubation periods of 30, 45, 60 and 75 days, for their effect in the proliferation and growth of *Smooth cayenne* pineapple shoot-tip culture. Combined application of BAP at 3.3 and IAA at 1.8 mg L^{-1} induced the highest proliferation of 19 shoots/explant and the highest total of 121 and 125 shoots over 4 cycles of multiplication. Raising the IAA to 3.3 mg L^{-1} resulted in the lowest proliferation and stunted shoots. Incorporation of GA_3 improved the shoot length but caused drastic reduction in proliferation. The other treatments showed an intermediate effect.

Key words: Pineapple, *Ananas comosus, in vitro* proliferation, incubation period length, shoot production

INTRODUCTION

Quick replacement of inferior varieties of pineapple with newly released or introduced ones is a very difficult task. Pineapple is one of the most highly density planted fruit crop and at the same time the fewest propagules producer. Up to 120,000 (Koen *et al.*, 1991) pineapple plants could be planted in one hectare. On average, pineapple plant produce two propagules/year. Following, the traditional method of propagation, it would take 8 years to obtain enough propagules from one mother plant to plant just only a half hectare (Almeida *et al.*, 2002).

Tissue culture has been successfully applied to pineapple. It has the potential to produce millions of propagules per year. However, conflicting rate of multiplication and total plantlets production were reported and contradicting hormone treatments were recommended. A total production of plantlets, for instance, ranged from 40 (Dewald *et al.*, 1988); 280 (Devi *et al.*, 1997); 5000 (Zepeda and Sagawa, 1981); 40000 (Liu *et al.*, 1989); 80000 (Kiss *et al.*, 1995); 100000 (Sripaoraya *et al.*, 2003) from single explant per year. Others reported that starting by 1 (Bhatia and Ashwath, 2002; Vesco *et al.*, 2001); 2 (Soneji *et al.*, 2002); 10 (Drew, 1980); 22 (Firoozabady and Gutterson, 2003); 40 (Fitchet, 1990) and 80 explants (Almeida *et al.*, 2002) a

total of 1 million, 10000, 1.25 million, 15757, 30000 and 161080 shoots could be obtained in 9, 6, 3, 7, 3 and 8 months respectively. In addition, while most of the researchers used combination of BAP and NAA, replacement of NAA of the combination with IAA (Gangopadhyay *et al.*, 2005; Almieda *et al.*, 1997; Hirimburegama and Wijesinghe, 1992), IBA (Boxus *et al.*, 1991) and 2,4-D (Lui *et al.*, 1989) were also recommended for *in vitro* shoot formation of pineapple. Wakasa (1989), Zee and Mune Kala (1992) and Kiss *et al.* (1995) limited the incorporation of NAA to the establishment medium only. In fact, Wakasa (1989) emphasized that continuous presence of NAA on the multiplication medium was detrimental to growth and multiplication of pineapple shoot -tip culture. Almeida *et al.* (1997) suggested the use of BAP and IAA combination but while keeping the IAA constant, used high level (3.0 mg L^{-1}) of BAP for establishment and low level (1.0) for multiplication. Combination of three hormones Kin, NAA plus either IBA (Soneji *et al.*, 2002; Mathew *et al.*, 1976) or IAA (Mathew and Rangan, 1979, 1981) were reported for multiplication of pineapple. Bordoloi and Sarma (1993) omitted NAA and suggested KN, IBA plus CH (Casein hydrolysate). Similarly, combination of BAP, NAA plus either IBA (Srinivasa *et al.*, 1981) or IAA (Cabral *et al.*, 1984) was used for *in vitro* shoot formation of pineapple. Teixeira *et al.* (2006) removed also NAA and

---

Corresponding Author: Professor Dr. Rosna Mat Taha, Institute of Biological Sciences,
Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
Tel: 603-79674372   Fax: 603-79674173

suggested BAP, IAA, IBA combination. Instead of the double or triple hormones treatments, others suggested singly applied Kin (Fotso *et al.*, 2001) and BAP (Be and Debergh, 2006; Sripaoraya *et al.*, 2003; Bhatia and Ashwath, 2002; Almeida *et al.*, 2002) for pineapple multiplication.

In this study, the response of pineapple shoot-tip culture (obtained from fruit crown) to various hormone treatments, at different incubation period length (30, 45, 60 and 75 days) over four repeated cycles of sub-culturing were examined. The objective was to obtain verified figure of multiplication rate and total shoot-bud production in which an acceptable and applicable micropropagation system for pineapple could be established.

MATERIALS AND METHODS

Terminal growth point about 1 cm in size of 25 smooth cayenne pineapple (*Ananas comosus* L. Merr.) were excised from fruit crown, placed in a beaker, washed thoroughly with water and sterilized with Clorox (20% for 25 min). The explants were then rinsed twice in distilled water for 5 min, trimmed to 5 mm and cultured in cylindrical glass jar with a rimmed neck and plastic cover containing 20 mL of hormone free MS medium solidified with 7 g L^{-1} agar. The cultures were transferred to incubation room and kept under 16 h light at 25°C. After one month, contaminant-free cultures were sub-cultured on solidified MS enriched with 2.0 mg L^{-1} BA. The multiple shoot-buds obtained after 1.5 months of incubation were separated into individual bud and re-cultured individually on the same but fresh medium. The process repeated for two times until surplus of stock cultures were obtained.

Then, a total of 280 shoot-buds were (40/treatment) individually cultured on solidified MS media containing either BA(2.3); BA(2.3) with IAA(0.18); BA(2.3) with IAA(0.8); BA(1.3) with IAA(1.8); BA(3.3) with IAA(1.8);

BA(3.3) with IAA(3.8) or BA(2.3) with IAA(1.8) with GA_3(1.0). Every 1, 1 1/2, 2 and 2 1/2 months, 9 cultures of every hormone treatment were used for sub-culturing, counting the number of shoot-buds and measuring the length of the shoot-buds produced. The process was repeated for four times for each of the incubation period. The average number and length of shoots/explant and the total number of shoots produced were calculated and used for evaluation of the different treatments.

The experiments designed as Complete Randomize Block Design (CRBD) and the means significance tested at p = 0.05 by Duncan's multiple range test. This study were conducted at Institute of Biological Science, University of Malaya at KL, Malaysia in years 2000/2002.

RESULTS

Table 1 and 2 showed that at each incubation period, there were different optimal hormone treatments. After 30 days of incubation, the best hormone treatment were BAP(2.3) IAA(0.18); BAP (2.3) IAA(0.8) and BAP (3.3) IAA (1.8). When the length of incubation period was increased to 45 days, the best choice were BAP (2.3) and BA(3.3) IAA(1.8). After 60 and 75 days of incubation those explants cultured on media containing BAP(2.3) IAA(0.8) and BAP(3.3) IAA(1.8) produced the largest number of shoots/explant, respectively. MS media containing BAP (3.3) IAA (1.8) and BAP (2.3) IAA (0.8) were one of the best at all incubation except at 60 and 45 days, respectively. BAP (2.3) was one of the best at two incubation periods, i.e., 45 and 75 days while BAP(2.3) IAA (0.18) was one of the best at only one incubation period which is 30 days and BAP (2.3) IAA (1.8) was one of the best only at 75 days of incubation.

The average number of shoots/explant proliferation potential, (Table 1) and the total shoot produced over four cycles of multiplication, regeneration capacity, (Table 2) showed that different hormone treatments could

Table 1: The effect of hormone treatments and the length of incubation periods on the *in vitro* proliferation (Shoot No./culture) and growth (Shoot length in mm) of pineapple shoot-tip culture

| Hormone treatments (mg L^{-1}) | Shoot No./culture Incubation period length (days) | | | | | Shoot length (mm)/culture Incubation period length (days) | | | | |
	30	45	60	75	Average	30	45	60	75	Average
BAP(2.3)	5[gh]	10[de]	13[bc]	17[a]	11[AB]	6[ef]	7[de]	9[bc]	9[bc]	8[B]
BAP(2.3)IAA(0.18)	7[fg]	8[ef]	13[bc]	12[bcd]	10[B]	6[ef]	7[de]	8[cd]	8[cd]	7[C]
BAP(2.3IAA(0.8)	6[fg]	7[fg]	14[b]	18[a]	11[AB]	7[de]	7[de]	9[bc]	9[bc]	8[B]
BAP (2.3) IAA (1.8)	5[gh]	8[ef]	13[bc]	18[a]	11[AB]	6[ef]	5[f]	8[cd]	10[bc]	7[C]
BAP (3.3) IAA (1.8)	6[fg]	10[de]	12[bcd]	19[a]	12[A]	6[ef]	8[cd]	8[cd]	9[bc]	8[B]
BAP (3.3) IAA (3.8)	5[gh]	7[fg]	8[ef]	11[cd]	7[C]	5[f]	6[ef]	6[ef]	7[de]	6[D]
BAP (2.3) IAA (1.8) GA_3 (1.0)	3[h]	6[fg]	7[fg]	12[bcd]	7[C]	8[cd]	8[cd]	11[a]	11[a]	10[A]
Average	5[DD]	8[CC]	11[BB]	15[AA]		6[DD]	7[CC]	8[BB]	9[AA]	

Data represent mean of 36 explants cultured individually on agar solidified MS medium. Means of the same column followed by the same letter(s) was not significantly different at p = 0.05 as tests by Duncan's multiple range test. Overall average of hormone treatments followed by single and overall of incubation length followed by double capital letter(s)

Table 2: The effect of hormone treatments and incubation periods on the total shoot production of pineapple shoot-tip culture

| Hormone treatments (mg L^{-1}) | Incubation period length (days) | | | |
| | Total shoot production | | | |
	30	45	60	75
BAP(2.3)	384^e	8470^{cde}	25200^{cd}	68000^b
BAP(2.3),IAA(0.18)	1680de	2430de	24570^{cd}	21294^{cd}
BAP(2.3),IAA(0.8)	1134de	2268de	32256^c	78750^b
BAP(2.3),IAA(1.8)	384^e	3430de	27000^{cd}	92160^b
BAP(3.3),IAA(1.8)	1056de	6240^{cde}	13824^{cde}	121125^a
BAP(3.3),IAA(3.8)	360^e	2100de	2520de	10780^{cde}
BAP(2.3),IAA(1.8),GA$_3$(1.0)	54^e	882de	2160de	10080^{cde}

Data represent mean of 36 explants cultured individually on agar solidified MS medium. Means of the same column followed by the same letter(s) do not differ significantly at p = 0.05 as determined by Duncan's multiple range test. Total shoots = average of shoot/treatment at the first sub-culture multiplied by that of the second, third and fourth sub-culture

have equal potential, but different regeneration capacity. For instance, after 75 days of incubation, explant cultured on BAP (2.3); BAP (2.3) plus IAA at (0.8 and 1.8 mg L^{-1}) and BAP (3.3) with IAA (1.8) produced statistically equal number of shoots/explant, but, BAP (3.3) IAA (1.8) resulted in significantly higher total of shoot production. Comparing to single application of BAP (2.3 mg L^{-1}), at each incubation period, incorporation of IAA at 1.8 mg L^{-1} together with BAP, suppressed the BAP effect on the shoot formation, if the explant remained exposed for 45 days. However, if the incubation extended to 60 and 75 or shortened to 30 days, the number of shoots produced in response to incorporation of IAA were not significantly different. Incorporation of IAA at 0.8 mg L^{-1}, on the other hand, enhanced the BAP effect, if the incubation length was 30 or 60 (1 shoot higher), suppressed the effect at 45 (2 shoot less) and have no effect if the explant remained for 75 days in culture. IAA at 0.18 mg L^{-1} produced two more shoots after 30 days but reduced the number of shoots by 2 and 5 after 45 and 75 days of incubation, respectively. Similarly, comparing to BAP(3.3) IAA(1.8), raising IAA concentration to 3.8 mg L^{-1} lowered the shoot production by 1, 3, 4 and 8 if the incubation period were 30, 45, 60 and 75 days respectively. Incorporation of GA$_3$ at 1.0 mg L^{-1} together with BAP(2.3) IAA(1.8), reduced the shoot formation by 2, 2, 6 and 6 if the incubations periods were at 30, 45 and 60 days, respectively but increased the shoots by one at 75 days of incubation.

Table 1 also showed that the length of the proliferated shoots varied as the hormone combination, concentration or the length of incubation period varied. Excluding of GA$_3$, which was the optimal for shoot length at any incubation period, there were different optimal treatments at each incubation length. After 30 days the tallest shoots were those proliferated in medium containing BAP (2.3) and IAA (0.8). However, after 45, 60 or 75 days, the tallest shoots were obtained from media

containing BAP (3.3) IAA (1.8); BAP (2.3) and BAP (2.3) IAA (0.8) or BAP ((2.3) IAA (1.8), respectively. Generally, the length of shoots increased over time. However, the difference between tallest and shortest shoots of all treatments was very small. It did not exceed 5 mm at the most. Comparing to BAP (2.3) IAA (1.8) and BAP (3.3) IAA (1.8), incorporation of GA$_3$ at 1.0 mg L^{-1} to the former promoted the length while raising the IAA concentration of the second to 3.8 mg L^{-1} resulted in shorter shoots. Comparing the length of shoots, cultured in BAP (2.3) alone with those when IAA was added, showed that IAA at 0.18 mg L^{-1} resulted in shorter shoots if the incubation extended to 75 days. Shoots cultured for 30, 45 and 60 days were not significantly different. On the contrary, IAA at 0.8 mg L^{-1} increased the length only if the incubation was shortened to 30 days. No significant differences were observed at longer period. IAA at 1.8 mg L^{-1} on the other hand, resulted in shorter shoots, if the incubation was for 45 and 60 days and longer shoots at 75 days; but no significant difference were observed at short incubation period of 30 days.

DISCUSSION

At each incubation period of 30, 45, 60 and 75 days, there were different optimal hormone treatments for proliferation and growth of pineapple (Table 1). Similar result was reported for rose (Arnold *et al.*, 1995). Although, the different optimal at different incubation period may give a tempting impression that the different treatments effect the shoot proliferation differently; the statistically equal number of shoots/explant of four treatments after 75 days of incubation (Table 1) did not support that impression. The different among treatments might not be due to its ability to induce proliferation but due to slowing or speeding up of shoot development. The different optimal growth at different incubation period imply that no general statement could be made about the best treatment. The choice remains to be a matter of purpose and other management factors. It also articulate the need for developing of a standard for evaluation of *in vitro* proliferation potential and the factors involved in proliferation.

Using either shoots/explant (Table 1) or total shoots produced (Table 2) for treatment comparison would lead to the same conclusion as long as the incubation period did not exceed 60 days. On either basis of comparison (shoot rate or total), singly applied BAP at 2.3 mg L^{-1} and combined application of BAP at 3.3 mg L^{-1} plus IAA at 1.8 mg L^{-1} were the best at 30 and 45 days of incubation and combined application of BAP at 2.3 mg L^{-1} and IAA at 1.8 mg L^{-1} was the best at 60 days of incubation. However, at 75 days of incubation, the two means lead to

two different conclusions. The shoot/explant did not distinguish between treatment BAP (2.3) and BAP (3.3) IAA (1.8), while the total shoot did. The two treatments have statistically different total of shoot production. That is may be because, the shoot/explant reflects the "proliferation potential" of a single explant over four cycles of multiplication while the total shoot production reflect the potential at each single cycle.

Commercially, the main aim of clonal micropropagation is to produce a large number of propagules over short period. That is the total shoot rather than the average shoots/explant. Accepting the average shoot/explant as a tool for evaluation, at 75 day of incubation could lead to choosing of a treatment although its total shoot production were 53125 less than the other. It is too large a number to be ignored. A different total and equal average of shoots/explant were also observed in Paeony (Harris and Mantell, 1991). It seemed that the total number of shoots production rather than the shoots/explant should be emphasized as a mean for evaluation of the factors involved in proliferation.

Working with pineapple, Mathew and Rangan (1979) and Hirimburegama and Wijesinghe (1992) reported that BAP was more effective than Kin or other auxins for pineapple shoot-tip proliferation. However, while Mathew and Rangan (1979) stated that incorporation of IAA merely enhanced the BAP effect, Hirimburegama and Rangan (1979) stated that the IAA at low level did enhance, but at higher level completely blocked the proliferation process. Present results (Table 1) supported their findings. Comparing of the hormone treatments at each incubation period revealed that, IAA could enhance or suppress the BAP effect depending on the concentration of IAA used and the period the explant remained exposed to the treatment. Comparing to single application of BAP at 2.3 mg L^{-1}, presence of IAA at any level (0.18; 0.8; 1.8 mg L^{-1}) for 45 days reduced the BAP effect as measured by number of shoot produced (Table 1). However, when the incubation period was shortened to 30 or extended to 60 days, IAA at low level, increased the number of shoots produced, while at intermediate level have no effect on the proliferation. On the contrary, low level of IAA reduced the proliferation at 75 days. Using different species and explants, other researchers reported similar findings. At constant level of BAP, the proliferation of cotyledonary node segment of Barbatimao (Franca *et al.*, 1995) and inflorescence bud of ginger, *Alpitia prupurata* (Illg and Faria, 1995) decreased as IAA concentration increased. On the contrary, Herrata *et al.* (1990) reported that increasing of IAA enhanced the BAP effect on the proliferation of *Digitollis thapsi* shoot-tip. Kannan and Jasrai (1996), on the other hand, observed that IAA at different concentrations

neither enhanced nor suppressed the BAP effect on the proliferation of *Gmelina arbonea*.

In this study, the number of shoots produced/explant increased as the incubation period length increased (Table 1). Boxus *et al.* (1991) and Hirimburegama and Wijesinghe (1992) reported similar findings. Of all of the treatments, the highest number of shoots formed at each incubation period was 7 at 30 days, 10, 14 and 19 shoots/explant at 45, 60 and 75 days of incubation, respectively. The rate of 7 shoots obtained after 30 days of incubation (Table 1) is double that reported by Zepeda and Sagawa (1981) but less than one third of that reported by Bordoloio and Sarma (1993) using same length of incubation period but different hormone treatment. Similarly, the rate obtained after 45 days of incubation were higher than that reported by Bhatia and Ashwath (2002) and equal to that reported by Sripaoraya *et al.* (2003). The rate of 14 shoots obtained after 60 days is higher than that reported by Be and Debergh (2006) and Almeida *et al.* (1997) but less than that reported by Boxus *et al.* (1991) and the rate of 19 shoots obtained after 75 days of incubation is higher than that reported by Tiexiera *et al.* (2006) and Soneji *et al.* (2002). The differences between our results and their's could be attributed to different in hormone treatments and number of multiplication cycles.

Length of *in vitro* pineapple shoots is very important. Longer shoots could be easily *ex vitro* rooted and acclimatized (DeWald *et al.*, 1988). Furthermore, it could be segmented without losing its proliferation potential (Almeida *et al.*, 2002; Mathew and Rangan, 1979). GA_3 was added to increase the shoot length. However, the improvement in the shoot length did not exceed 5 mm, at the most (Table 1). In addition, the improvement was at the expense of proliferation. Presence of GA_3 caused a loss of about 50% of the proliferation potential. The small gain in shoot length over incubation and the minute differences between treatments may be due to the fact that the figure represent an average of shoots of various ages, those which were just about two weeks with those which were formed 30, 45 or 60 days ago. Moreover, increase in proliferation over time could easily mask the treatment effect on shoot length. Contradict finding about the effect of GA_3 incorporation together with BAP and IAA was reported. Incorporation of GA_3 resulted in stunted, deformed shoots and lower proliferation of rose (Hasagawa, 1980). However, Gulsen and Dumanoglu (1991) noticed that, GA_3 improved both the shoot length and the proliferation of Quince A while Vinterhalter and Neskovic (1992) reported GA_3 have no effect on Quince A *in vitro* culturing. Tchemets *et al.* (1987) reported that the GA_3 effect depends on the concentration used. At 1.0 mg L^{-1}, GA_3 reduced the

proliferation of Mozzard F/21 cherry rootstock while at 4.5 mg L^{-1}, increased the proliferation substantially.

CONCLUSION

The research revealed that not only different hormone treatments were recommended but also a wide variation among the reported rate shoot formation and expected total of propagules production. In addition, the incubation period in the previous studies was fixed at one level, the selections among the treatments were judged solely by the rate of shoot formation and the expected total was not supported by actually conducted subcultures. To our knowledge this is the first report in which the response to different hormone treatments and different incubation periods is being compared by the rate of shoot formation as well as total shoots production. Beside, this study showed that shoot rate formation is not enough for selection among treatments, investigation at different incubation period give the propagator several alternatives to choose from according to his budget, facilities available and the due time of propagules delivery. If 30, 45, 60 or 75 days incubation was adopted, the total shoots obtained after 4, 6, 8 and 10 months would be 1680, 8470, 25200 and 121125 shoots from single explant respectively. Incubation for 30 and 45 days would allow 3 and 2 cycles of production per year and the total expected would be 5140 and 16540, respectively. Starting by 10 explants and using of combination of BAP at 3.3 and IAA at 1.8 and incubation period of 75 days, the production could reach million of shoots per year. It could be said that system of agar solidified MS enriched with combination of BAP at 3.3 mg L^{-1} and IAA at 1.8 mg L^{-1} and incubation for 30, 45 and 75 days and combination of BAP at 2.3 mg L^{-1} plus IAA at 0.8 mg L^{-1} for 60 days incubation resulted in better rate and total than so far reported recommendations for pineapple *in vitro* culture in solid and static liquid system of same incubation period length.

ACKNOWLEDGMENT

REFERENCES

Almeida, W.A.B., G.S. deSantana, A.P.M. Rodriguez and M.A.P. Costa, 2002. Optimization of a protocol for the micropropagation of pineapple. Revista Brasileira de Fruticultura (Brazil), 24 (2) : 296-300.

Almeida, W.A.P., A.P. Matos and A. Sousa, 1997. Effect of benzylaminopurine (BAP) on *in vitro* proliferation of pineapple (*Ananas comosus* L. Merr.). Acta Hortic., 425 (1): 253-242.

Arnold, N.B., M.R. Binns, D.C. Clourier, N.N. Barthakur and R. Pellerin, 1995. Study of the effect of growth regulators and time of plantlets harvest on the *in vitro* multiplication rate of hardy and hybrid tea roses. J. Hortic. Sci., 67 (6): 727-735.

Be, L.V. and P.C. Debergh, 2006. Potential low cost micropropagation of pineapple (*Ananas comosus*). South Afr. J. Bot., 72: 191-194.

Bhatia, P. and N. Ashwath, 2002. Development of rapid method for micropropagation of a new pineapple (*Ananas comosus* L. Merr.). Clone Yeppoon gold. Acta Hortic., 575 (2): 125-131.

Bordoloi, N.D. and C.M. Sarma, 1993. Effect of various media composition on *in vitro* propagation of *Ananas comosus* L. Merr. J. Plant Sci. Res., 9 (1-4): 50-53.

Boxus, P., J.M. Terzi, C.H. Lievens, M. Pylyser, P. Ngaboyamabina and K. Duhem, 1991. Improvement and perspectives of micro-propagation techniques applied to some hot climate plant. Acta. Hortic., 289 (1): 55-64.

Cabral, J.R.S., G.A.P. Cunha and E.M. Rodrigues, 1984. Micopropaca do abacaxizerto. Anais do V II congresso. Brasileiro de Fruticultura, 1: 124-127.

Devi, Y.S., A. Mujib and S.C. Kundu, 1997. Efficient regeneration potential from long term culture of pineapple. Phytomorphology, 47 (3): 255-259.

DeWald, M.G., G.A. Moore, W.B. Shorman and M.H. Evans, 1988. Production of pineapple plant *in vitro*. Plant Cell Rept., 7: 535-537.

Drew, R.A., 1980. Pineapple tissue culture. Un-quartered for rapid multiplication. Queen Land Agric. J., 106: 447-451.

Firoozabady, E. and N. Gutterson, 2003. Cost effective *in vitro* propagation methods for pineapple. Plant Cell Report, 21 (9): 844-850.

Fitchet, M., 1990. Clonal micropropagation of Queen and Smooth cayenne pineapples. Acta Hortic., 275 (2): 261-266.

Fotso, N.D.O., T.A. Tita and N. Niemenak, 2001. Direct *in vitro* regeneration of *Ananas comosus* (L.) Merril var. Cayenne from crowns cultivated in a liquid medium. Fruits-Paris, 56 (6): 415-421.

Franca, S.C., I.B. Duarte, R.M. Moraes and A.M.S. Pereira, 1995. Micro-propagation of *Stryphnodendron polyphythum* (Barbatimao). Plant Cell. Tissue Org. Cult., 42 (3): 291-293.

Gangopadhyay, G., T. Bandyopadhyay, R. Poddar, S.B. Gandopadhyay and K.K. Mukherjee, 2005. Encapsulation of pineapple micro shoots in alginate beads for temporary storage. Curr. Sci., 88 (6): 972- 977.

Gulsen, Y. and H. Dumanoglu, 1991. The effect of sucrose, agar and pH on shoot multiplication and quality in Quince A micropropagation. Acta Hortic., 289 (1): 115-116.

Harris, R.A. and S.H. Mantell, 1991. Effect of stage II sub-culture duration on the multiplication rate and rooting capacity of micro-propagated shoot of Paeony (*Paeonia suffruticosa* Ander.). J. Hortic. Sci., 66 (1): 95-102.

Hasagawa, P.M., 1980. Factors affecting shoot and root initiation from cultured rose shoot-tips. J. Am. Soc. Hortic. Sci., 105 (2): 216-222.

Herrata, M.T., M. Cacho, M.P. Ccorchete and J.F. Turrago, 1990. One step shoot-tip multiplication and rooting of *Digitalis thapsi*. Plant Cell. Tissue Org. Cult., 22 (3): 179-182.

Hirimburegama, K. and L.P.J. Wijesinghe, 1992. *In vitro* growth of *Ananas comosus* L. Merr. (Pineapple) shoot apices on different media. Acta. Hortic., 319 (2): 203- 208.

Illg, R.D. and R.T. Faria, 1995. Micro-propagation of *Alpinia purpurata* from inflorescence buds. Plant Cell. Tissue Org. Cult. 40 (2): 183-185.

Kannan, V.R. and Y.T. Jasrai, 1996. Micropropagation of *Gmelina arborea*. Plant Cell, Tissue Org. Cult., 46 (3): 269-271.

Kiss, E., J. Kiss, G. Gyulai and L.E. Heszky, 1995. A novel method for rapid micro-propagation of pineapple. Hortic. Sci., 30 (1): 127-129.

Koen, I.J., S.F.Du. Plessis and G. Smart, 1991. Effect of planting density on the fertilizer requirement of Queen pineapples. J. S. Afr. Soc. Hortic. Sci., 1 (1): 33-36.

Liu, L.J., E. Rosa-Marquez and E. Lazardi, 1989. Smooth leaf (spinless) red Spanish pineapple (*Ananas comosos* L. Merr) propagated *in vitro*. J. Agr. Univ. Puerto Rico, 73 (4): 301- 311.

Mathew, V.H., T.S. Rangan and Narayanaswamay, 1976. Micropropagation of *Ananas sativus in vitro*. Z. Pflanenphysiol. Bd., 79: S 450-454.

Mathew, V.H. and T.S. Rangan, 1979. Multiple plantlets in lateral bud and leaf explant *in vitro* culture of pineapple. Sci. Hortic., 11: 319-328.

Mathew, V.H. and T.S. Rangan, 1981. Growth and regeneration of plantlets in callus culture of pineapple. Sci. Hortic., 14: 227- 234.

Soneji, J.R., P.S. Rao and M. Mhatre, 2002. Somaclonal variation in micropropagated dormant axillary buds of pineapple (*Ananas comosus* L. Merr.) J. Hortic. Sci. Biotechnol., 77 (1): 28-32.

Srinivasa, N.K., R. Doreswamy and E.K. Chaeko, 1981. Differentiation of plantlets in hybrid embryo callus of pineapple. Sci. Hortic., 15: 235-238.

Sripaoraya, S., R. Marchant, J.B. Power and M.R. Davey, 2003. Plant regeneration by somatic embryogenesis and organogenesis in commercial pineapple (*Ananas comosus* L.). *In vitro* Cell. Dev. Biol. Plants, 39 (5): 450- 454.

Tchemets, A.M., V.A. Smirnov and N.M.A. Bramenko, 1987. Study of effecting of some nutrient medium factors on the development of cherry and sweet sherry rootstock *in vitro*. Acta. Hortic., 212 (2): 595-598.

Teixeira, S.L., J.M. Riberio and M.T. Teixera, 2006. Influence of NaClO on nutrient medium sterilization and on pineapple (*Ananas comosus* cv Smooth cayenne) behavior. Plant Cell Tissue Organ. Cult., 86 (3): 375-378.

Vesco, L.L.D., A.A. Pinto, G.R. Zaffari, R.O. Nodari, M.S. Reis and M.P. Guerra, 2001. Improving pineapple micropropagation protocol manipulation through explant size and medium composition. Fruits, 56: 143-154.

Vinterhalter, B. and M.M. Neskovic, 1992. Factors affecting *in vitro* propagation of quince (*Cydonia oblonga* M.U.). J. Hortic. Sci., 67 (1): 39-43.

Wakasa, K., 1989. Pineapple (*Ananas comosus* L. Merr). Biotechnology in Agriculture and Forestry. P.S. Bajaj (Eds.). Vol. 7. Trees II.

Zee, F.T. and M. Munekata, 1992. *In vitro* storage of pineapple (*Ananas* spp.) germplasm. Hortic. Sci., 27 (1): 57-58.

Zepeda, C. and Y. Sagawa, 1981. *In vitro* propagation of pineapple. Hortic. Sci., 16 (4): 495.