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Effect of media type, hormone treatments and incubation periods on the *in vitro* shoot formation and growth of pineapple (*Ananas comosus* L Merr) cv. Smooth cayenne.

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Abstract

Combination of six different media (CH, DP, LS, MS, MT and N) and two hormone treatments (BAP alone at 2.25 mg.l^{-1} and combination of BAP at 3.25 plus IAA at 1.75 mg.l^{-1}) were tested for its effect on the shoot formation and shoot length and weight of Smooth cayenne pineapple over three different incubation periods (30, 60 and 75 days). The optimum media varied according to the parameter used for assessment and hormone treatment. After 30 days of incubation, the highest shoot formation (7 shoots) obtained in MT enriched with BAP alone and LS enriched with combination of BAP and IAA while after 60 days the highest shoot formation (9 shoots) obtained in CH, MS and MT media enriched with BAP alone. After 75 days, CH medium enriched with combination of BAP and IAA resulted in the highest shoot formation (14 shoots). For production of tallest and heaviest shoots shorter incubation of 30 days and MT medium enriched with combination of BAP and IAA was the best choice (11mm, 0.36 g). MS which is the most commonly used medium was at all parameters and irrespective of the hormone treatments and incubation periods inferior than the other media. Recommendation of certain medium type for pineapple should be restricted to the intended length of incubation period and the growth parameter of major interest.

Key words: Pineapple; Media types; Incubation periods; Ananas comosus.

Introduction

Successful *in vitro* propagation of pineapple has been reported by several researchers. However, almost in all cases the attention was focus on testing the effect of hormone types, concentrations and combinations. For better establishment as well as multiplication there were contrasting reports regarding the type and strength of the media. Mathew *et al.* (1976) and Soneji *et al*. (2002) used Nitch medium and Sripaoraya *et al*. (2003) recommended MT while Firoozabady and Gutterson (2003) suggested MS at half salt plus B5 vitamins and Merceir *et al.* (2003) used Kundson salt plus high glycine for establishment of pineapple culture. But all of them used MS for multiplication. Bordoloi and Sarma (1993) found no differences between establishing of pineapple in MS and SH medium. But MS was better for multiplication. MS at half salt but full vitamins (Teng, 1997; Das and Bhowmik, 1997) and at full strength (Khan *et al*., 2004) in solid state was reported for both stages. Fitchet (1990) used solid MT medium for establishment and liquid MT for multiplication. Fernando (1986) used MS salt and White vitamins for both stages, but static liquid culture for establishment and agitated culture for multiplication and Teixeira *et al*. (2006) used the same media in static liquid culture for multiplication. Liquid MS medium at full strength (Kofe and Adachi, 1993) and half strength (Omokolo *et al*., 2001) were use for both stages of establishment and multiplication. Media types, MS and B5 (Bhatia and Ashwath, 2002), MS and N6 (Kiss *et al*., 1995) MS, B5 and AZ (Liu *et al*., 1989), MS, SH, W and B5 (Bordoloi and Sarma, 1993) were compared during multiplication. However, while the first two researchers recommended respectively B5 and N6 over MS, both of the second two recommended MS over B5, SH and W media. Different strengths of MS were compared during multiplication in conventional solid system (Khan *et al*., 2004) and during elongation stage in bioreactor system (Pereze *et al*., 2004). In both, full strength MS was better than half strength. The objective of this study was to compare the effect of six media each enriched with two different hormone treatments (BAP alone at 2.25 mg/l and combination of BAP at 3.25 mg/l and IAA at 1.75 mg/l) on the in vitro shoot formation, shoot length and weight of Smooth cayenne pineapple over three different incubation periods (30, 60 and 75 days).

Materials and Methods

Media preparation and culturing

Six different media, CH (Cheng, 1978), DP (Dutcher and Powel, 1972), LS (Linsmair and Skoog, 1979), MS (Murashige and Skoog, 1962), MT (Murashige and Ticker, 1969) and N (Nitch, 1972) were prepared from stock solutions (Table, 1). A volume of 1100 ml of each type of medium were prepared and divided equally into two beakers. BAP (6-benzyleaminopurine) at 2.25 $mg.l^{-1}$ was added to one of the beakers and combination of BAP at 3.25 plus IAA (indole acetic acid) at 1.75 $mg.l^{-1}$ was added to the other beaker. The pH adjusted to 5.7 and the content of each beaker dispensed into 27 glass jars (15 x 5 cm) 20 ml per each jar. Agar (0.14 g) was added to each jar to solidify the medium at rate of 7 grams of agar per liter. The jars were covered with autoclave-able plastic lids and the media autoclaved at 121 ^{O}C and 1.5 kg/cm for 25 minutes. Shoots obtained from stock culture was individually cultured one shoot per jar. The jars were then arranged into three different shelves in incubation room and kept under constant temperature of 25 ^{O} C and photoperiod of 16 hours of light provided by cool white fluorescent lamps

Data collection and analysis

After 30, 60 and 75 days of incubation, nine jars of each hormone-media combinations (3 jars from each shelf) were taken out for data collection. The multiple shoots complex of each jar was first weighted and then separated into individual shoots for counting the shoots and measuring of their length. Average weight of shoot computed by dividing the weight over the number of shoots and average shoot length computed by summation of length of all shoots and dividing by the number of shoots. Shoot count and average length and weight of the shoots of each three jars of the same treatment (combination of media type, hormone enrichment) divided by three and considered as one replicate. Data were subjected to ANOVA analysis using SPSS statistical package No.11 and the significance of the means was tested by Duncan Multiple Range Test at $p \leq 0.05$.

Results

Three ways analysis of variance (Table, 2) did not detect any significant differences between the different media on the shoot formation per explant ($p \leq 0.29$) and total shoot weight per explant ($p \leq 0.38$). However, the media induced an independent ($p \leq 0.059$) and dependent significant difference via interaction with incubation periods ($p \leq 0.00001$) and hormone ($p \leq 0.021$) and collective interaction with both of hormones and incubations ($p \leq 0.009$) in the average shoot length. The average shoot weight was also under independent influence of media ($p \leq 0.03$) but the dependent influence was only via collective interaction of all of the three factors together ($p \leq 0.03$). No significant difference were detected between the two hormone treatments on shoot formation. Hormones induced no independent significant effect on shoot formation and did not interact with the incubation period. However, the hormones affected the shoot length through interaction with media ($p \leq 0.02$) and effect the length and weight of shoot via collective interaction with media and incubation periods ($p \leq 0.009$ and $p \leq 0.03$). The shoot formation and total shoot weight was affected mainly by the incubation periods. Both of the media and hormones neither had independent nor dependent effect through interaction with each other or with the incubation periods on the shoot formation and total weight per culture. The incubation periods was the only factor that had significant independent effect on all of the four parameters. The shoot length and weight was under interaction effect of incubation periods with hormones and both of the shoot length and weight was under collective interaction of all of the three factors. Contrasting analysis, on the other hand, showed that CH medium resulted in significantly lower shoot weight ($p \leq 0.0115$) and higher shoot formation ($p \leq 0.0746$) than the other media while MS and MT resulted in highest shoot weight ($p \leq 0.0235$).

Mean separation indicated significant difference on shoot formation, length and weight among the different combinations. At incubation of 30 days, MT enriched with

BAP at 2.25 $mg.l^{-1}$ and LS enriched with BAP at 3.25 $mg.l^{-1}$ plus IAA at 1.75 $mg.l^{-1}$ resulted in the highest shoot formation (7 shoots). However, MT enriched with BAP at 3.25 $mg.l^{-1}$ plus IAA at 1.75 $mg.l^{-1}$ resulted in the tallest (11 mm) and heaviest (0.36 g) shoots. When the incubation was extended to 60 days, the best medium for shoot formation was CH enriched with BAP alone (10 shoots) or combination of BAP plus IAA (9 shoots) while N medium enriched with BAP alone induced tallest (10 mm) and heaviest (0.21 g) shoots of all combinations. After 75 days of incubation, explants cultured on CH medium enriched with BAP at 3.25 $mg.l^{-1}$ plus IAA at 1.75 $mg.l^{-1}$ produced the highest shoots formation (14 shoots) while explants cultured on MT enriched with combination of the two hormones resulted on the tallest (10 mm) and heaviest (0.22 g) shoots. Explants cultured on CH and N media produced the most stunted (6 mm) and lightest (0.10 g) shoots. Overall hormones and media, incubation for 75 days resulted in more (10 shoots) and longer (8 mm), but lighter (0.15 g) shoots weight while incubation for 30 days resulted in the fewest shoot formation (4 shoots). Intermediate results obtained after 60 days of incubation (8 shoots).

Discussion

MS medium which is the most commonly used medium for tissue culture was not the best medium for highest shoot formation and growth of Smooth cayenne pineapple. The results (Table, 3) showed that at any hormone treatment and incubation period there was other media which was better than MS. The optimum medium type for pineapple varied according to incubation period, hormone enrichment and the growth parameter and the performance of the medium could be reversed from being superior to inferior by using of different hormone enrichments. After incubation for 30 days, MT medium enriched with BAP alone and LS media enriched with combination of BAP and IAA resulted in the highest shoot formation (7 shoots). When MT enriched with combination of BAP and IAA instead of BAP alone and LS enriched with BAP alone instead of combination of BAP and IAA these two media resulted in lowest shoot formation (3 and 4 shoots). After 60 days, CH enriched with either BAP alone or with combination of BAP and IAA and MT and MS media enriched with BAP alone resulted in highest shoot formation (9 shoots). When MT and MS enriched with combination of BAP and IAA instead of BAP alone these two media resulted in lowest shoot formation (5 and shoots).The performance of CH medium was not reversed by hormone treatments. After 75 days, CH medium enriched with combination of BAP and IAA and N medium enriched with BAP alone resulted in highest shoot formation (14 and 12 shoots respectively). When NN medium enriched with combination of BAP and IAA and CH enriched with BAP alone the two media resulted in lowest shoot formation (7 and 11 shoots). The MS was also not advantageous for length and weight of shoot per explant and total shoots weight per explant. For production of tallest and heaviest shoots, MT medium enriched with combination of BAP and IAA and incubated for 30 days was the best choice (11mm, 0.36 g). LS enriched with BAP and incubated for 75 days resulted in higher shoot weight per explant and total shoots weight per explant than MS medium. MT enriched with BAP and IAA and incubated for 75 days resulted also in higher total shoot weight per explant but equal shoot weight per explant as MS medium. It is clear that suggestion of certain medium type for pineapple should be in accordance with the intended incubation period, hormone enrichment and the growth parameter of major interest.

Superiority of medium type seemed to relate to incubation periods and hormone treatments more than the medium original components. Media with high salt and organic components (MT, LS) may only required in case of short incubation period (30 days). For longer incubation (60 and 75 days), medium with low macro element and iron but high amino acids and vitamins (CH medium) and medium with low macro element and without vitamins, and amino acids and had no calcium, cobalt chloride and potassium (N medium) showed superior performance than MS and other media. After 30 and 75 days incubation and enrichment with BAP alone, N medium resulted in more shoot than MS. However after 60 days and enrichment with BAP alone and 75 days and enrichment with combination of BAP and IAA, MS was better than N and all other media type except CH medium. In both incubations, CH medium was better than MS. The results of media contrasting (CH vs. MS, MT, LS) and (CH vs. DP, LS, MS, MT, N) indicated that low iron (6.0 $mg.l^{-1}$) and Na EDTA (7.2 $mg.l^{-1}$) and high myo-inositol (250 $mg.l^{-1}$) of the CH promoted more shoot formation than the high iron (27.9 $mg.l^{-1}$) and Na EDTA (37.3 $mg.l^{-1}$) of the DP, LS, MS, MT, N media but decreased the shoot weight. Similar, contrasting media with half and full macro elements (CH, N vs DP, LS, MS, MT) and between media with and without glycine, nicotinamide and pyridoxine (MS, MT vs. CH, DP, LS, N) indicated that the macro elements had no effect on the shoot formation but increased the shoot weight. All other contrasting (Table, 2) of media with high and standard vitamins (MT vs MS), high and no vitamins (MT vs DP, N) and standard and no vitamins (MS vs DP, N) and media with and without calcium (N vs CH, DP, LS, MS, MT) indicated that addition of vitamins and calcium was neither essential for formation nor weight of the shoots. Variance analysis (Table, 2), on the other hand, showed that hormones and media neither has independent effect nor significant interaction. The shoot formation was mainly under the influence of incubation period and collective interaction of all of these three factors together (incubation, medium and hormone). Since no significant interaction was detected between any two of these three factors, the collective interaction of the three factors that induced significant different in shoot formation is most likely due to changes in chemical environment resulted from selective uptake and release of substance during the explants growth. On other word, superiority of one medium over the other determined by the types of these changes that occurred during incubation period more than the type and amount of the original salt and organic components of the medium. From Comparison of the components (Table, 1) and results of MS, CH and N media (Table, 3) we may expect that new medium consist of ½ strength macro and microelements and 1/3 iron and 1/6 Na EDTA of that of MS and no calcium and organic components would be very appropriate for multiplication of pineapple. It may result in higher shoot formation and on the same time reduce the cost of multiplication medium.

References.

Bhatia, P. and N. Ashwath. 2002. Development of a rapid method for micropropagation of a new pineapple (*Ananas comosus* (L) Merr. Clone Yeppoon gold. *Acta Hort*.575: 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 Research*. 9: 50- 53.

Cheng, T.Y. 1978. Clonal propagation of woody species through tissue culture techniques. *Proc. Int. Plant Prop. Soci*. 28: 139-

Das, R. K. and G. Bhowmik. 1997. Some somaclonal variants in pineapple (*Ananas comosus* (L) Merr) plants obtained from different propagation techniques. *Inter. J.Trop.Agric*. 15 (1-4): 95- 100.

Dutcher, R. D. and L. E. Powel. 1972. Culture of apple shoots from buds *in vitro*. *J. Am. Soc. Hort. Sci*. 97: 511-

Fernando. K. 1986. *In vitro* propagation of Muritus pineapple. *Trop. Agriculturist*. 142: 7- 19.

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

Fitchet, M. 1990. Clonal propagation of Queen and Smooth cayenne pineapples. *Acta. Hort*. 275: 261- 266.

Linsmaier, E. M. and F. Skoog. 1979. Organic growth factor requirements of tobacco tissue culture. *Physiol. Plant*. 18: 100- 127.

Khan, S., A. Nasib, B. A. Saeed. 2004. Employment of *in vitro* technology for large scale multiplication of pineapples (*Ananas comosus*). *Pak. J. Bot.* 36 (3): 611- 615.

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

Kofi, O. F and T. Adachi. 1993. Effect of cytokinins on the proliferation of multiple shoots of pineapple *in vitro*. *SABRAO Journal*, 25(1): 59- 69.

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

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

Murashige, T. and D. P. H. Tucker. 1969. Growth factor requirement of citrus tissue culture. In: Proc. 1st Int. Citrus Symp. Ed. Chapman, H. D. pp 1155 – 1161, Univ. Calif. Riverside Publ.

Mercier, H., B. M. Souza, J. F. Kraus, R. M. Hamasaki and B. Sotta. 2003. Endogenous auxin and cytokinin contents associated with shoot formation in leaves of pineapple cultured in vitro. *Braz. J. Plant Physiol.*15 (2): 107–112.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue. *Physiol. Plant*. 15: 473- 497.
Nitch, J.P. 1972. Hapliod plant from pollen. *Z. Pflanzenzuecht*. 69: 3

Omokoio, N.D., M. A. Tita and N. Niemenak. 2001. Direct in vitro regeneration of *Ananas comosus* L. Merr. Var. Cayenne from crowns cultivated in a liquid medium. *Fruits*, 56: 415- 421.

Pereze, A., L. Napoles, C. Carvajal, M. Hernandez and C. Lorenzo. 2004. Effect of sucrose, inorganic salts, inositol and thiamine on protease excretion during pineapple culture in temporary immersion bioreactors. *In vitro Cell. Dev. Biol. Plant* 40: 311- 316.

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

Sripaoraya, S.T., M. Robert, B. Power, J, Davey and R. Michael. 2003. Plant regeneration by somatic embyogenesis and organogenesis in commercial pineapple (*Ananas comosus* L) *In vitro Cell. Devel. Biol. Plant*. 39 (5): 450–454.

Teixeira, S. L., J. M. Ribeeiro and M. T. Teixeira. 2006. Influence of NaCIO on nutrient medium sterilization and on pineapple (*Ananas comosus* cv. Smooth cayenne) behavior. *Plant Cell Tiss. Org. Cult*. 86: 375- 378.

Teng, W. L.1997. An alternative propagation method of *Ananas* through nodule culture. *Plant Cell Report*. 16; 454- 457.

Table (1). Minerals and organic components (mg/l) of different media.

Compounds (mg/l)	**Media Type**					
	CH	DP	LS	MS	MT	N
Macroelement						
NH_4No_3	825	1650	1650	1650	1650	725
KNo_3	950	1900	1900	1900	1900	950
$CacL.2H_2O$	220	440	440	440	440	0
$MgSo_4.7H_2O$	185	370	370	370	370	185
$KH2Po_4$	85	170	170	170	170	88
Iron sources						
NaEDTA	7.2	37.3	37.3	37.3	37.3	37.3
$FeSo_4.7H_2O$	6	27.8	27.8	27.8	27.8	27.8
Microelements						
$MnSo_4.4H_2O$	11.4	44	22.25	22.25	22.25	25
$ZnSo_4.7H_2O$	10.6	17	8.6	8.6	8.6	10
H_3Bo_5	6.2	12	6.2	6.2	6.2	10
KI	0.8	0	0.83	0.83	0.83	0
$NaMoO.2H_2O$	0.4	0.5	0.25	0.25	0.25	0.25
$CuSo_4.5H_2O$	0.02	0.05	0.025	0.025	0.025	0.025
$CocL.6H_2O$	0.02	0	0.025	0.025	0.025	0
Vitamines and amino acids						
Thiamine-Hcl	2.5	0	0.4	0.1	10	0
Nicotinamide	0	0	0	0.5	5	0
Pyridoxine-Hcl	0	0	0	0.5	10	0
Isoinositol	250	0	100	100	100	0
Glycine	0	0	0	2	2	0
Carbohydrate						
Sucrose	30000	30000	30000	30000	50000	20000

CH (Cheng, T.Y. 1978); DP (Dutcher, R. D. and L. E. Powel. 1972); LS (Linsmair, E. M. and F. Skoog. 1979); MS (Murashige, T. and F. Skoog. 1962); MT (Murashige, T. and D. P. H. Tucker. 1969); N (Nitch, J.P. 1972).

Table (1). Significant of main and interaction effect of the media, hormone and incubation periods on shoot formation and growth of Smooth cayenne pineapple.

Factors	**df**	**Shoot / explant**			
		Number	Length (mm)	Weight (g)	Total wt.(g)
			p values		
Incubation periods	2	5.71E-13 **	0.0217 *	0.0058 **	5.27E-09 **
Hormones	1	0.3984	0.3109	0.5417	0.9131
Media	5	0.2893	0.0592 *	0.0324 *	0.3844
Contrast					
CH vs DP, LS, MS, MT, N	1	0.0746	0.1042	0.0115 *	0.3528
CH,DP, **LS** N vs MS, MT	1	0.7848	0.1459	0.0235 *	0.1677
CH, N vs DP, LS, MS, MT	1	0.3005	0.0263 *	0.0122 *	0.0836
MS vs MT	1	0.8936	0.2720	0.5372	0.4851
MS vs DP, N	1	0.7573	0.8736	0.4129	0.4631
MT vs DP, N	1	0.8772	0.1544	0.1274	0.1253
N vs CH, DP, LS, MS, MT	1	0.6288	0.2283	0.5136	0.2047
Incubation*Hormones	2	0.5059	0.4570	0.8311	0.8869
Incubation*Media	10	0.3129	0.00007 **	0.0893	0.8323
Hormone*Media	5	0.1402	0.0213 *	0.0798	0.8015
Incubation*Hormone*Media	10	0.3307	0.0096 **	0.0277 *	0.9418
Error	72				
Total	108				

Table (3). Effect of different media types, hormone treatments and incubation periods on the shoot formation of Smooth cayenne pineapple

Hormone treatments (mg /L)	**Incubation** (Days)	**Media** CH	DP	LS	MS	MT	N
			Shoots/explant				
BAP (2.25)	30	4 efg	2 g	4 efg	3 fg	7 bcde	5 defg
	60	10 abcd	8 abcde	5 defg	9 abcd	9 abcd	7 bcde
	75	11 abc	11 abc	10 abcd	10 abcd	9 abcd	12 ab
BAP (3.25) IAA (1.75)	30	5 defg	4 efg	7 bcde	3 fg	3 fg	4 efg
	60	9 abcd	8 abcde	7 bcde	5 defg	7 bcde	6 cdef
	75	14 a	9 abcd	9 abcd	13 ab	8 abcde	7 bcde