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MICROPROPAGATION AND GROWTH OF *IN VITRO* PINEAPPLE (*ANANAS COMOSUS* L. MERR) IN IRAN

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Abstract

Although, several pineapple micropropagation protocols have already been published, significant improvement could be achieved, if the stages of *in vitro* culture were better defined. Our work concerned several experiments aiming at the mass production of high quality plantlets. Tissue culture experiments were therefore conducted to develop rapid multiplication procedures for *Ananas comosus* L. (Merr). Terminal buds from suckers were treated with 0.025% (w/v) mercuric chloride for 2 minutes and placed in different media. Explants were transferred to MS medium supplemented with NAA (2 mgl^{-1}) and BA (0, 1, 2, 3, 4, 5, 6, 7 mgl^{-1}) and kept for 2-4 months under 16/8 h photoperiod (40 $\mu mol\ m^{-2}\ s^{-1}$) and 25 ± 2ºC. Results showed that higher multiplication rates for *Ananas comosus* L. were obtained with BA concentrations of 5 mgl^{-1} at 3 months. The *in vitro* proliferated shoots produced roots with maximum frequency (84%) on MS medium without growth regulator at 6 weeks intervals. Using the protocol described in this work, it is possible to obtain 1 million rooted plantlets after 12 months from a single bud, with a 45 day subculture interval.

Key words : *Ananas comusus* L., micropropagation, rooting, pineapple, regeneration.

Introduction

Pineapple is a member of the Bromeliaceae or Bromeliad family, pineapple is cultivated for fruit, used fresh, canned, frozen, or made into juices, syrups or candied (Firoozabady *et al.*, 2003). Today, the pineapple is found in almost all the tropical and subtropical areas of the world and has become one of the leading tropical fruits in international commerce. Major areas of commercial cultivation are found between 30°N and S latitudes, with some areas considered marginal for various reasons (Firoozabady *et al.*, 2004). Despite the xerophytic characteristics of pineapple, growth is adversely affected by prolonged dry periods.

Ananas comosus L. Merr is a woody plant from south America, used as a fruit crop. The agronomic interest of this species and the difficulties in propagating it by conventional methods, led us to test different tissue culture techniques. Crown tips cv. Giants Kew, khulna (Rahman *et al.*, 2001) and cv. Queen (Mandal *et al.*, 2002) explants were prepared from the mature fruits and cultured on MS medium. Dolgov *et al.* (1998) used leaf as explants, then regenerated from callus. Moraes *et al.* (2010) used axillary buds as explants of pineapple cv. Emepa 1. Some of researcher used liquid medium for micropropagation (Dal Vesco *et al*., 2001 and Omokolo *et al*., 2001).

The most important pineapple varieties cultivated in some areas, which are highly susceptible to fusarium wilt (*Fusarium subglutinans* WR), the most serious disease of this crop in some area, causing considerable production losses (Cunha *et al.*, 1994). Besides fusarium wilt, other problems affect the commercial production of pineapple, such as the lack of high quality propagules, low rate of multiplication of plants by conventional methods and the lack of matrix plants have been limiting for pineapple culture (Ruggiero *et al.*, 1992). The need to solve these problems, producing better and clean propagules, improving the rate of plant multiplication and a faster multiplication of elite genotypes, led to the development of tissue culture techniques for the pineapple (Almedia, 1994). Bregonci *et al.* (2008) had as objective to evaluate the foliar and radicular growth of micropropagated plantlets of the pineapple cv. Gold [*Ananas comosus* (L.) Merrill]. Plant regeneration by somatic embryogenesis and organogenesis used for commercial pineapple production that as one way could been solve

Table 1 : Effect of different concentrations of BAP and 2 mgl^{-1} NAA (Treatments) on length of shoot, number of shoot and percentage of rooting from explant culture of *Ananas comosus* (This is grouping according to Duncan's test).

Treatment ($BAPmgl^{-1}$)	Length of shoot (cm)	Number of shoot	Percentage of rooting
0	4.50 b	4.00 ab	78.20 cd
1	2.64 ab	5.00 b	62.40 bc
2	2.32 a	5.80 b	39.90 a
3	2.99 ab	1.80 a	50.60 ab
4	3.66 abc	4.80 b	54.80 ab
5	4.94 c	9.40 c	84.00 d
6	2.22 a	5.40 b	58.00 ab
7	2.80 ab	4.60 ab	59.80 b

problem of production (Sripatoraya *et al.*, 2003). The present work reports production of pineapple plants by organogenesis and shoot proliferation.

Materials and Methods

Shoots of *Ananas comosus* L. Merr was obtained from the Chabahar Agriculture Incorporation. The shoot of segments each with the primordia of two axillary buds, were excised aseptically from the cultured shoots and used for experiments in media culture. Explants were sterilized by immersion in a solution mercuric chloride 0.025% (w/v) under continuous stirring for 2 minutes and then thoroughly rinsed with sterilized water. The axillary buds were placed on MS media (Murashige and Skoog, 1962) supplemented with NAA (2 mgl^{-1}), various amounts of BA (0, 1, 2, 3, 4, 5, 6, 7 mgl^{-1}), sucrose (30 gl^{-1}) and solidified with agar (7 gl^{-1}). The pH was adjusted to 5.7 before agar addition and autoclaving. The plantlets were produced during experiments using for 3 subsequent *in vitro* subcultures (each subculture tested 6 weeks), thus the plantlets were cultures on MS medium without hormones. The shoots were cultured under photoperiod 16 H from white Fluorcent lamps (40 $\mu molm^{-2}\ s^{-1}$) at 25°C±2°C. The number of shoot, length of shoot and percentage of rooting of propagated shoots were recovered after 6 weeks of culture. Shoots that had been cultured on rooting media (MS and free hormone) for 6 weeks then, roots were been measured.

The experiments were repeated for 3 times for each treatment used and morphological data were analyzed by analysis of variance test (ANOVA) followed by least significant difference test (LSD).

Results and Discussion

In tissue cultures containing cytokinin named BA (Banzyl Adenine) explants growth and shoots regenerate (figs. 1a, 1b, 1c). The regenerated plants of the latter sub-cultures showed significant increase ($p<0.05$) in morphological characters like length of shoots, number of shoot, and percentage of rooting (table 1). The analysis of the data showed significant effects for all parameters type of explant and BAP concentration.

Number of shoot and length of shoot

Direct regeneration of shoots and shoot buds (with no callus) was obtained with MS media containing different BA/NAA combinations with Merr variety being the optimal medium . The frequency of shoot regeneration (number of explants regenerating shoots) varied based on the tip shoot multiplication media. For example, when MS medium with BA (5 mgl^{-1}) was used for shoot culture, the average frequency was 9.4 (range 4-9.4). The propagation of shoots was examined with NAA and difference concentrations of BA (table 1). The maximum number and greatest propagated shoots were obtained with MS medium that contained 2 mgl^{-1} NAA and 5 mgl^{-1} BA. In order to determine the optimum concentration of BA, we also tested lower concentrations of BA 0 mgl^{-1} to 7 mgl^{-1}, and observed mean of 3 and 25 propagated shoot per dish, respectively (fig. 2a). Mean length of shoot were significant in 0, 2 and 5 mgl^{-1} BA concentrations.

The shoot apices behaved differently on different culture media. On media containing NAA (2 mgl^{-1}) and BA (5, 6, 7 mgl^{-1}), the apex developed into a green shoot system within 6 weeks. The shoot apices developed many buds in the presence of BA (5 mgl^{-1}), but bud proliferation was enhanced when NAA (2 mgl^{-1}) was combined with BA (6, 7 mgl^{-1}) (fig. 2b). Bud proliferation was low in NAA (2 mgl^{-1}) with BA (0. 2, 3, 4 mgl^{-1}), but number of shoot were significant in range 1-6 mgl^{-1} BA concentrations with 2 mgl^{-1} NAA (table 2).

Hamad *et al.* (2010) used BA and NAA with different concentration and they researched proliferation capacity and shoot formations pattern of pineapple (*Anaans comusus* L. Merr).

The effect of BA levels on the micropropagation of pineapple has been reported (Pescador and Koller, 1990; Kiss *et al.*, 1995; Almeida *et al.*, 1997; Guerra *et al.*, 1999). According to Albuquerque *et al.* (2000), the use of BA in MS medium was essential for the regeneration of plants from shoot apices of pineapple, aiming at plants free of Fusarium. Paiva *et al.* (1999) obtained the best results in the shoot induction of pineapple, Skay, with either 1 mgl^{-1} BAP or 0.1 mgl^{-1} TDZ.

Barbosa and Caldas (2001) working with etiolated

Fig. 1 : Micropropagation of pineapple , cv. Merr. **a)** segment used as explant source after removal of the primordia to expose the axillary buds , **b)** shoot multiplication, **c)** length of shoot.

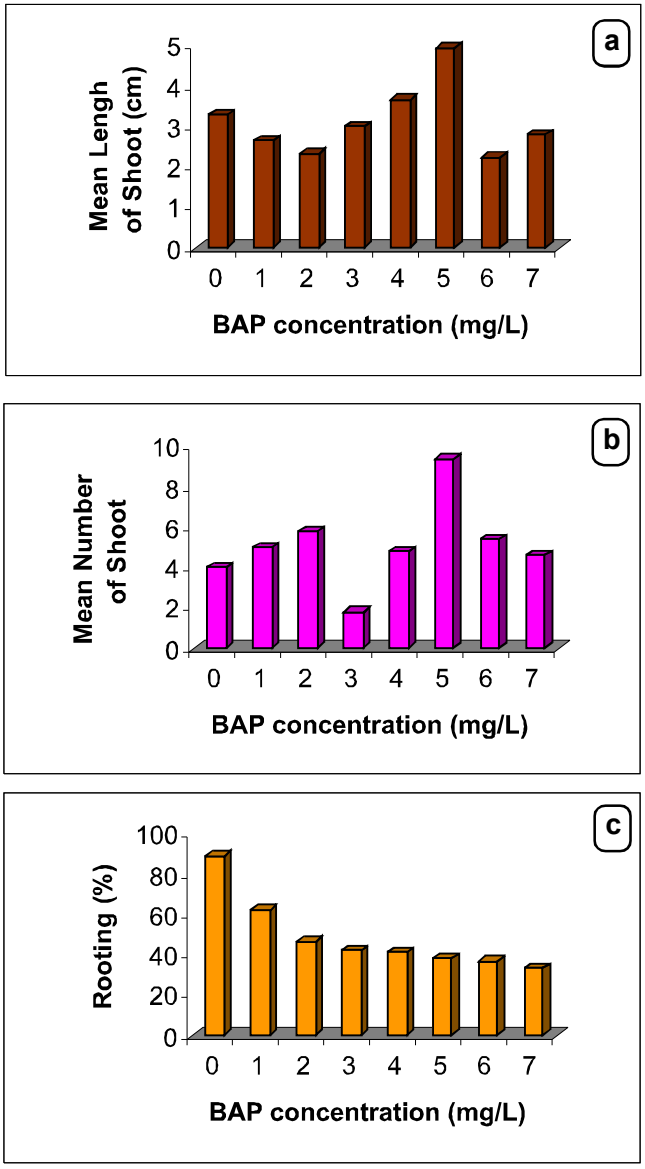

Fig. 2 : a) Mean length of shoot., b) mean number of shoot; c) mean percentage of rooting, under different concentration of hormone (BAP).

segments for micropropagation of the pineapple hybrid PE X CS-52, observed that BAP promoted the highest number of plants per shoot and per nodal segment, when compared with KIN., or a combination of BA and NAA Grattaglia and Machado (1998) cited BAP as the best cytokinin for the multiplication of aerial plant parts and for the induction of adventitious shoots.

Percentage of rooting

The shoot regenerated transferred to MS medium free hormones, but purposely applied chemical auxin named NAA (Naphtol Acetic Acid) and BA with different concentrations in media. Auxin was remarkable that effect on rooting. According to Kiss *et al*. (1995) the

developing plantlets were 3 to 5 cm high within 20 to 25 days, they then rooted on a growth regulator- free MS medium. The largest mean of rooting was 84% after 6 weeks in new medium (fig. 2c), but it decreased in MS media culture with different concentration BAP. Percentage of rooting were significant in 2, 5 and 7 mgl^{-1} BA concentrations (table 1).

Conclusion

It can be calculated a production of several plants after short time, starting from only one plant with an average of ten slips and twelve axillary buds each.

This comparison demonstrates the advantage of micropropagation of pineapple over the conventional propagation method. The micropropagation not only provides higher rates of multiplication, but also the time and area needs is much smaller when compared to in vivo vegetative propagation methods. The plants obtained in this experiments were rooted *in vitro* and can transferred to the greenhouse for adaptation.

Almeida *et al*. (2002) studying the influence of BA on in vitro proliferation of pineapple.

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References

Albuquerqe, C. C., T. R. Camara, M. Menezes, L. Willadino, I. Muener and C. Ulisses (2000). Cultivo *in vitro* de apices caulinares de abacaxizeiro para limpeza clonal em relacao a fusariose, *Scientia Agricola. Piracicaba,* **57** : 363-366.

Almeida, W. A. B. de (1994). Efeito da benzilaminoopurina nas diferentes fases da propagcao *in vitro* do abacaxizeiro (*Ananas comosus* (L.) Merr.). 83p. Dissertacao9Mestrado)-universidade Federal da Bahia, Escola de Agronomia Cruz das Almas.

Almeida, W. A. B. de., A. P. de. Matos and A. S. Souza (1997). Effects of benzylaminopurine (BAP) on *in vitro* proliferation of pineapple (*Ananas comosus* (L.) Merr). *Acta Horticulturae,* **425** : 242-245.

Almeida, W. A. B., G. S. Santa, P. M. Rodrigez and M. P. de. C. Costa (2002). Optimization of protocol for the micropropagation of pineapple. *Rev. Bras. Frutic.,* **24** : On line version.

Barbosa, S. B. S. C. and L. S. Caldas (2001). Estiolamento e regeneracao na multiplicacao *in vitro* do abacaxizeiro hibrido PE X SC-52. *Pesquisa Agropecuaria Brasileira*, **36** : 417-423.

Bregonci, I. D. S., E. F. D. Reis, G. D. D. Almeida, V. J. Brumand and M. Zucoloto (2008). Evaluation of the foliar and radicular growth of the micropropagated plantlets of the pineapple cv. *Gold in acclimatization. Idesia,* **26** : 87-96.

Cunha, G. A. P. da., A. P. de. Matos, J. R. Cabral, L. F. da. S. Souza, N. F. Sanches and D. H. R. C. Reinhardt (1994). Aspectos tecnicos da produca. Brasilia, DF : Embrapa/ SPI, 41. On line version.

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

Dolgov, S. V. and A. P. Shushkova Firsov (1998). Pineapple (*Ananas comosus* L.) regeneration from leaf explants. *Acta Horticulture*, **461** : 439-444.

Drew, R. A. (1980). Pineapple tissue culture unequalled for rapid multiplication. *Queensland Agricultural Journal,* **106** : 447-451.

Grattaglia, D. and M. A. Machado (1998). Micropropagacao in: Torrea AC, Caldas LS, Buso JA, *Cultura de tecidos e transformacap genetica de plantas,* **1** : 184-250.

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

Firoozabady, E. and Y. Moy (2004). Regeneration of pineapple plants via somatic embryogenesis and organogenesis. *In Vitro Cell Dev. Biol. Plant,* **40** : 67-74.

Guerra, M. P., L. L.dal. Vesco, R. Pescador, A. R. Schuelter and R. O. Nodari (1999). Estabelecimento de um protocolo regenerativo para micropropagacao do abacaxizeriro. *Pesquisa Agropecuaria Brasileira*, **34** : 1557-1563.

Hamad, A. M. and R. M. Taha (2009). Effects of sequentional subcultures on *in vitro* proliferation capacity and shoot formation pattern of pineapple (*Ananas comusus* L. Merr) over different incubation periods. *Scientia Horticulturae*, **117** : 329-334.

Kiss, E., J. Kiss, G. Gyulaai and L. E. Heszky (1995). A novel method for rapid micropropagation of pineapple. *Hort. Science*, **30** : 127-129.

Mandal, A. B., M. Aparna and R. Elanchezhian (2002). *In vitro* Micropropagation of *Ananas comosus* L. (Merr.) var. Queen . *Jour. Appl. Hort.,* **4** :107-112 .

Moraes, A. M. D., F. D. A. C. Almeida, R. D. L. A. Bruno, J. C. Filho, S. T. Nunes andJ. P. Gomes (2010). Micropropagation of pineapple, cv. Emepa, *Revista Brasileira de Engenharia Agrícola e Ambiental*, **14** : 1807-1929.

Murashig, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant,* **15** : 473-497.

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

Paiva, P. D. de. O., M. D. B. Mayer, M. I. Kawamura, M. Pasqual and R. Paiva (1999). Efeito de BAP, tidazuron e sulfato de adanina na propagacao *in vitro* de abacaxi, **46** : 231-237.

Pescador, R. and O. C. Koller (1990). propagacao *in vitro* do abacaxizeiro (*Ananas comosus* (L.) Merril) cv. Perola. *Revista Brasileira de Fruticultura,* **14** : 1-4.

Rahman, K. W., M. N. Amin and M. A. K. Azad (2001). *In vitro* clonal propagation of pineapple, *Ananas comosus* (L.) Merr. *Plant Tissue Cult*., **11** : 47-53.

Rugigiero, R. and O. C. Koller (1992). Propagacao *in vitro* do abacaxizeiro (*Ananas comsus* (L.) Merril) cv. Perola. *Revista Brasilleira de Fruticultura,* **14** : 1-4.

Sripatoraya, S., R. Marchant, B. Power and M. R. Davey (2003). Plant regeneration by somatic embryogenesis and organogenesis in commericial pineapple (*Ananas comosus* L.). *In Vitro Cellular and Developmental Biology Plant,* **39** : 450-454.