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PINEAPPLE MICROPROPAGATION (*ANANASCOMOSUSL.* SMOOTH CAYENNE) AND PLANT GROWTH FEATURES IN THE PROCESS OF ADAPTATION IN HYDROPONICS

Pineapple microplants adaptation in hydroponics

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Abstract

The present research aimed at optimizing the propagation of pineapple (*Ananascomosus L.*) variety 'Smooth Cayenne' in vitro with the development of a hydroponic adaptation system of microplants. At the stages of proliferation and rhizogenesis, the previously known optimal concentrations of cytokinins and auxins were used. The material was washed in running tap water for one hour, and then sterilized with silver nitrate ($AgNO_3$) at 1% concentration for 10 minutes; the buds survival rate afterwards was 67%. For proliferation, the concentration of 6-benzylaminopurine (6-BAP) was 1.0 mg/L, with an average of 24.21 shoots per explant over three passages. In the variants with 6-BAP at 0.5 mg/L and 2-isopentenyladenine (2-iP) at 1.0 mg/L, 60.8% and 46.8% of the plants had a height of more than 3 cm, respectively; 100% of the shoots rooted in all studied variants. Adaptation of microplants in hydroponics ensured the survival of 100% of plants in different growth groups: 1) small (4.0-5.9 cm), 2) medium (6.0-7.9 cm), 3) large (8.0 cm and more). Within one month, indicators of shoot length, root length and plant mass gradually increased by 10-20%, 50-90% and 186-232%, respectively.

Keywords: in vitro, pineapple micropropagation, *Ananascomosus L.*, microplant, cytokinin, auxin, adaptation, hydroponics, fruit quality

Author contributions statement. The author directly initiated and maintained sterile pineapple culture, carried out experiments to study the characteristics of propagation and rooting under the influence of various growth regulators and developed a technique for adapting pineapple microplants under hydroponics conditions.

Introduction

Pineapple has a long history as the culture that defines the economy of several tropical countries (Zepeda and Sagawa, 1981). Traditionally, pineapple is propagated vegetatively, by side shoots or basal rosettes. This technology is well known yet very laborious and insufficient, since it involves using material with a high degree of heterogeneity, of root system and of the aerial part development, which affects the survival of plants in field conditions. These problems can be successfully solved by clonal micropropagation which involves the mass vegetative propagation of the material in sterile conditions. This method is especially useful for rapidly increasing the amount of planting material of modern pineapple varieties the selection of which is actively carried out for a number of indicators, including the quality of fruits and the complex resistance to dangerous pathogens (Li et al, 2011; Moreira et al, 2016). There is extensive information on the successful propagation of pineapple using the in vitro method (Firooozabady and Gutterson, 2003; Be and Debergh, 2006; Danso et al., 2008; Farahani, 2014). The publications highlight certain contradictions and disagreements in the approaches to such propagation, but in most cases the authors speak of very high rates – 30,000-100,000 shoots from one bud in 6-9 months (Fitchet, 1990; Kiss et al., 1995; Dal-Vesco et al., 2001; Soneji and Mhatre, 2002; Almeida et al., 2002; Bhatia and Ashwath, 2002; Sripaoraya et al., 2003; Dutta et al., 2013; Hamad et al., 2013). Typically, the apical buds of several plants or the axillary buds of a single plant are used to initiate a pineapple culture (Sonejijr and Mhatre, 2002; Amin et al., 2005;

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Ibrahim et al., 2013). Further propagation success depends on the type of cytokinins used – 6-benzylaminopurine (6-BAP), 6-furfurylaminopurine (kinetin), 2-isopentenyladenine (2-iP) in 0.25-5.0 mg /L concentrations; these are the basis for growth regulation at the proliferation stage (Liu et al., 1989; Khoa, 2004; Mhatre, 2007). At the rhizogenesis stage, auxins such as β-indole acetic acid (IAA), β-Indolebutyric acid(IBA) and α-naphthalene acid (NAA) are used mainly in concentrations 0.5-3.0 mg/L, separately or as combination with low concentrations of cytokinins in the nutrient medium (Pierik et al., 1984; Zuraida et al., 2011).

It is generally agreed that special attention should be paid to the adaptation of microplants to non-sterile conditions, since this determines the effectiveness of the whole process. To date, quite a lot of adaptation systems are developed, making it possible to modernize and reduce the cost of propagation by combining some processes, but no final solution exists (Escalona et al., 1999; Tavares et al., 2008). In addition, a comparative assessment of plants obtained in vitro with traditionally grown plants was carried out under field conditions (Sopie et al., 2011; Villalobos-Olivera et al, 2019).

The purpose of this work was to study the possibilities and features of the adaptation of pineapple microplants to non-sterile conditions of the hydroponic system, determining their viability and suitability for planting in natural conditions.

Materials and methods

Initiation of tissue culture. First, the apical part of the mother plant was removed along with the crown fragment, and the remaining leaves were shortened to 5-6 cm. 1 ml of solution containing 5 mg of 6-BAP was applied to the cut surface of the stem, after which the pot with the plant was placed in the dark at the temperature of + 26 °C and the humidity of 60% for one month. Afterwards, the leaves of the mother plant were removed at the base, and the most developed axillary buds were cut out with adjacent tissues. This material was first washed in running tap water for one hour, then sterilized with 1% silver nitrate ($AgNO_3$) for 10 minutes; then the material was washed three times with sterile distilled water (standard procedure). Isolation of meristematic zones from the buds and their successive planting on MS (Murashige& Skoog, 1962) nutrient medium (containing 0.25 mg/L of 6-BAP as a growth regulator) was carried out in a laminar flow cabinet. Planted explants were placed in a bright room with the following parameters: temperature + 26 °C, illumination 5000 lux, photoperiod 16 hours. The final record of surviving, dead and infected explants was carried out in a month.

Proliferation, rooting and adaptation. Sterile shoots were also cultured on MS medium. At the stage of proliferation, the research options were as follows: 6-BAP – 0.5 mg/L and 1.0 mg/L, 2-iP – 1.0 mg/L and 2.0 mg/L. For propagation, 200×21 mm tubes were used as culture vessels. Cultures were incubated under the same conditions as at the initiation stage. The development of shoots was monitored every two weeks. The number of shoots was counted after six weeks of cultivation; the height of the shoot was set at the longest leaf.

To induce root formation, pruned seedlings were transferred to MS solid nutrient medium containing different concentrations of IAA or NAA (1.0 or 2.0 mg/L). In 100-ml flasks with 30 ml of nutrient medium six plants were placed; incubation was performed as described above. The percentage, number and length of the roots were recorded six weeks after planting, the length of the root system being set at the longest root. Before taking into account the parameters of rooted plants, they were ranked by three weight groups: 1) 0.1-0.30 g, 2) 0.31-0.90 g, 3) 0.91 g or more. The hydroponic system developed by the author and well-proven earlier with strawberry microplants was used to adapt the rooted pineapple shoots. Plastic pallets (0.5 x 0.3 x 0.04 m; four-liters capacity) were used; on the surface of the pallets pieces of light synthetic non-woven material equivalent in area to the pallets were pre-fixed and perforated with planting holes in accordance with the selected growing pattern (6.5 x 6.5 cm).

Microplants were removed from the flasks and prepared for planting – the agar nutrient medium was washed off the root system by a stream of running water. Planting was carried out directly at the site of subsequent adaptation. Before planting on pallets, three growth groups of 39 plants each were formed: 1) small (4.0-5.9 cm), 2) medium (6.0-7.9 cm), 3) large (8.0 cm and more) to study the effectiveness of adaptation. Using tweezers, the roots of microplants were placed into the planting holes so that the seedlings remained on the surface of the non-woven (synthetic) material, after which the capacity of the pallet was filled with a solution of mineral salts. The mineral part of the MS medium was used as a nutrient solution. To preserve the viability of the stem part of the plant, the trays were covered with polyethylene. The circulation of the solution in the pallets was provided automatically every two hours during the day for three weeks. After that, the caps were removed, and the plants continued to be in hydroponics for another week. After a month in total, the plants were transplanted to non-sterile soil substrate (peat) under normal conditions without any additional operations.

The experiments were organized in accordance with generally accepted methodological requirements. At the propagation stages, the repetition was tenfold (tubes), and rooting was fivefold (flasks). Each microshoot was placed in one tube, six microshoots were planted in one flask. The significance of differences in the experiments was evaluated using the LSD test at the significance level of 5% (Steel and Torri, 1980).

Results and discussion

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Initially, the author of the present study experienced a certain shortage of plant material. The earlier described technique made it possible to obtain 9 buds kidneys suitable for further work. However, since they did not have contact with the environment, after rinsing with running water and surface sterilization with $AgNO_3$ (1%), they showed a good survival rate. One month after planting, 6 out of 9 buds (67%) were free of pathogens and were used in the next breeding stage. By the end of the 1st month, 1-4 rudiments of axillary buds developed in 4 of them. In addition, to increase the amount of starting material, within two months the author divided the conglomerates into parts and transplanted the explants onto the medium of the same composition that was used for culture introduction; this made it possible to carry out further experiments with a sufficient number of microshoots. In variants of experiments on the proliferation of sterile cultures, cytokinins were included in concentrations that showed the best efficacy in the studies by a number of scientists (Fitchet, 1990; Almeida et al., 2002; Ibrahim et al., 2013). Table 1 shows the results of in vitro proliferation of pineapple under the influence of two cytokinins with different concentrations. It was found that the growth regulators used successfully contributed to the development of axillary meristems and the growth of lateral shoots. Of the studied variants, the 1.0 mg/L concentration of 6-BAP turned out the most effective, at which 24.21 shoots per explant were obtained on average for three passages. The significance of the excess of this indicator over the variants with 0.5 mg/L 6-BAP, with 1.0 mg/L 2-iP and over the control variant was mathematically confirmed. In the control variant (without growth regulators), spontaneous formation of shoots was also recorded; nevertheless, it was insignificant.

The fluctuations in the average length of the shoots (the second important indicator the author recorded) were noted within the limits of experimental error, with the exception of control. They were recorded in variants within 2.34-2.91 cm after 6 weeks of observation. Scientific literature provides essential information on the fact that more productive options are possible when other concentrations and regulators are used (Danso et al., 2008, Atawia et al., 2016). However, the author of the present research was more interested in the qualitative component of the material obtained during propagation. Larger plants are always more convenient for practical use, since they are more technological, more viable and less demanding. Analyzing the results for this indicator makes it possible to distinguish options with 6-BAP (0.5 mg/L and 2-iP (1.0 mg/L) where 60.8% and 46.8% of the plants had a height of more than 3 cm (Fig. 1); this is consistent with the results obtained by other researchers (Farahani, 2014; Atawia et al., 2016).

Moreover, the research revealed the pineapple is a very viable in vitro culture and, unlike many other plants, allows for long (more than 3 months) cultivation without transplantation to fresh medium. This testifies in favor of the fact that increase in cultivation duration at low concentrations of hormones will allow obtaining higher quality shoots.

Table 1. Effect of 6-BAP and 2-iP concentrations on some growth characteristics of *Ananascomosus*in vitro culture*

Cytokinintreatment (mg L^{-1})	Total shoots number (units)	Shoots number in shoot length ranges			Shoot length (cm)
		< 2cm	2-3cm	> 3cm	
Control 0.0	3.51	1.92	1.32	0.27	1.51
6-BAP 0.5	16.33	2.01	4.39	9.93	2.91
6-BAP 1.0	24.21	2.51	16.24	5.46	2.34
2-iP 1.0	14.63	1. 33	6.45	6.85	2.82
2-iP 2.0	18.81	3.39	8.88	6.54	2.57
Mean	15.50	1.90	7.46	6.14	2.43
LSD 5%	6.12	0.76	3.03	2.47	0.45

*- averagefor 3 subcultures

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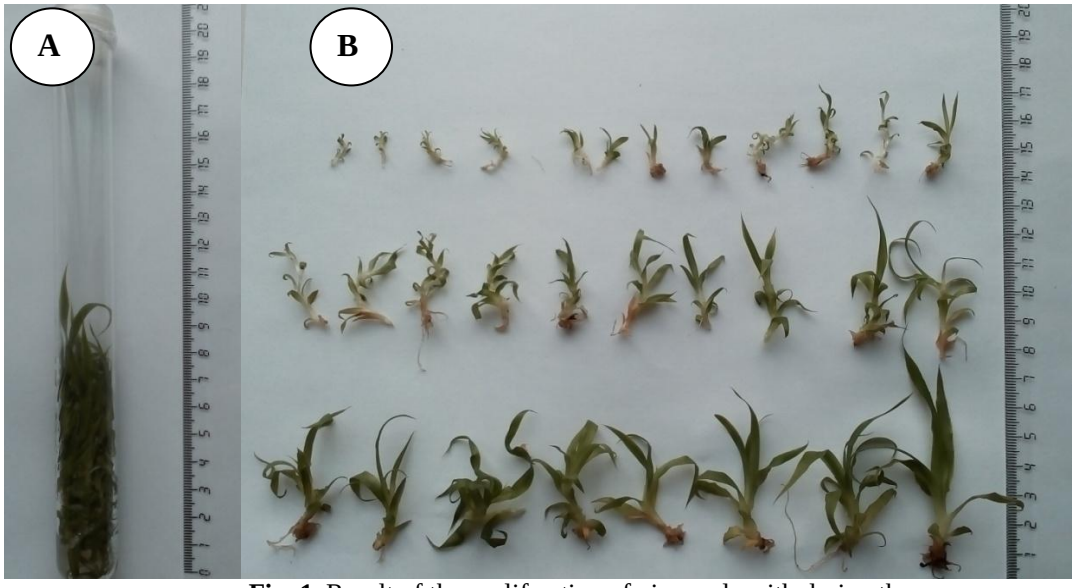

Fig. 1. Result of the proliferation of pineapple with during the passage:
A) Test-tube with conglomerate shoots before division, B) shoots after separation

Rooting of pineapple shoots revealed a good rhizogenic ability of the culture. High percentage of spontaneously rooted microshoots was observed even in the control variant (on the medium without auxins); however, in this case rooting began a few weeks later, and there were fewer roots, which significantly restrained the growth of the stem. The ranking of rooted plants by mass after six weeks of rooting allowed revealing certain patterns of their development against the background of various auxins and their concentrations (Table 2). The tendency is that an increase in the auxin concentration in the nutrient medium leads to a decrease in the proportion of plants weighing more than 0.9 g, especially in the variant with 1.0 mg/L of NAA. At the same time, this group had the best indicators of the number of roots and their length among all the studied variants.

The next focus of the study was the pineapple plants development dynamics in the process of adaptation. Table 2 shows that differences in the length of the shoots among the weight groups in all the variants were evident already after the rooting stage, and between the third and first groups, they were even more significant (excluding the control group). Obviously, this effect is a known result of correlations between the root system and the stem; that is, a more actively developing root system stimulated a correspondingly stronger development of the stem, which affected the weight and height of the plants. Fig. 2 shows the dynamics of changes in the growth rate of pineapple microplants during the adaptation period under hydroponic conditions. Within one month, indicators of shoots length, of roots length and of plants weight went significantly upwards – by 10-20%, 50-90% and 186-232%, respectively.

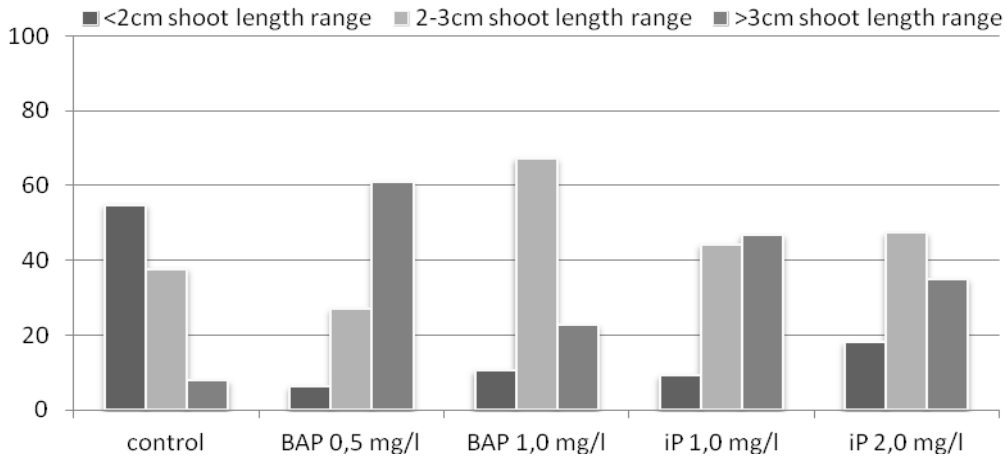

Fig. 2. The influence of cytokinin type and its concentration on the qualitative content of shoots in height (%)

At the same time, the greatest development progress was noted in the first group. By the final indicators of the root system length and the plant weight, they surpassed the starting indicators of the second group and were almost equal to the plants of the third group of growth.

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Table 2. Effect of auxin type and its concentrations on rooting pineapple plants *in vitro**
***-** accounted before planting to adaptation

Auxin treatment (mgL^{-1})	Total rooting (%)	Weight ranking group (g)	Plant weight (g)	Weight content (%)	Root number	Root length (cm)	Shoot length(cm)
Control (without auxin)	96	1 (0.1-0.30) 2 (0.31- 0.90) 3 (0.91g and >)	0.15 0.27 0.63	21.1 45.5 33.4	2.14 2.33 2.81	3.54 3.59 4.12	4.23 4.87 5.02
IAA 0.5	100	1 (0.1-0.30) 2 (0.31- 0.90) 3 (0.91g and >)	0.23 0. 54 1.31	24.8 49.6 25.6	3.59 3.86 5.07	3.52 5.08 6.88	5.23 7.02 9.27
IAA1.0	100	1 (0.1-0.30) 2 (0.31- 0.90) 3 (0.91g and >)	0.25 0.57 1.19	28.7 48.8 22.5	3.77 4.41 5.15	3.49 4.94 6.51	4.29 6.46 8.77
NAA 0.5	100	1 (0.1-0.30) 2 (0.31- 0.90) 3 (0.91g and >)	0.31 0.59 1.11	26.2 51.7 22.1	4.95 5.36 5.38	3.52 4.84 5.42	5.03 6.42 8.29
NAA 1.0	100	1 (0.1-0.30) 2 (0.31- 0.90) 3 (0.91g and >)	0.29 0.65 1.08	28.1 61.2 10.7	5.33 5.82 6.15	3.39 3.92 4.51	4.83 6.92 7.97
LSD 5%					1.33	1.19	2.04

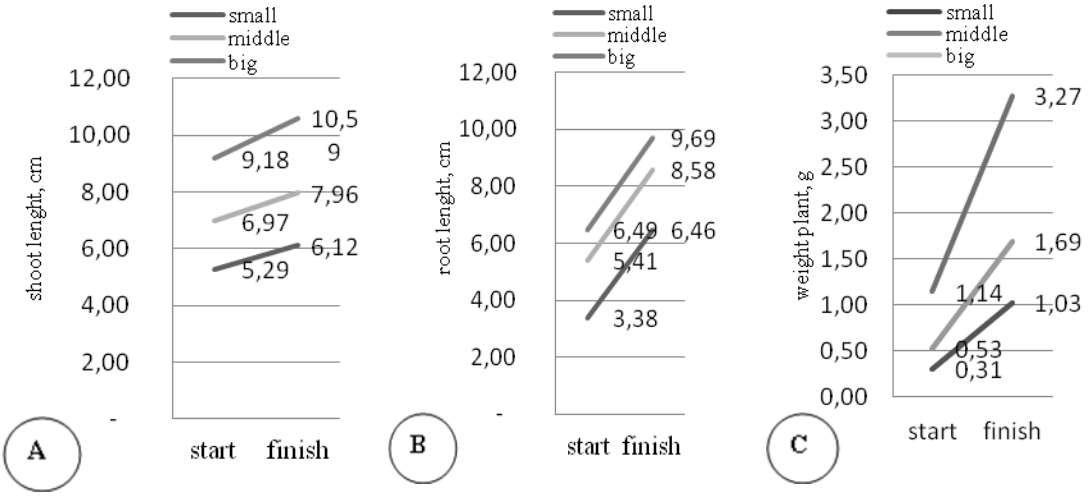

Fig. 3. Dynamics of changes in various parameters of pineapple plant development in the period of adaptation to the different growth groups: (A) - shoots length before and after adaptation; (B) - roots length before and after adaptation; (C) – plants weight before and after adaptation

This suggests that even relatively small plants can be successfully used to obtain pineapple planting material after adaptation in hydroponics; these plants reach acceptable sizes and are ready for planting in greenhouse conditions. Adaptation of pineapple microplants with sterile roots in hydroponic conditions showed 100% survival efficiency in all the three study groups. The plants were kept in a humid chamber for three weeks. Finally, the adaptation was completed within one more week, when the plastic coating was removed completely, which made it possible to replant the plants without any additional measures in non-sterile peat. Figures 4 and 5 show the completion of adaptation.

The use of hydroponics made it possible to eliminate a number of problems associated with the adaptation of plants obtained in vitro. Firstly, there was no need for special preparation of the soil substrate, which, as a rule, implies its autoclaving.

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Fig. 4. Pineapple plants after removing the film coating three weeks after planting

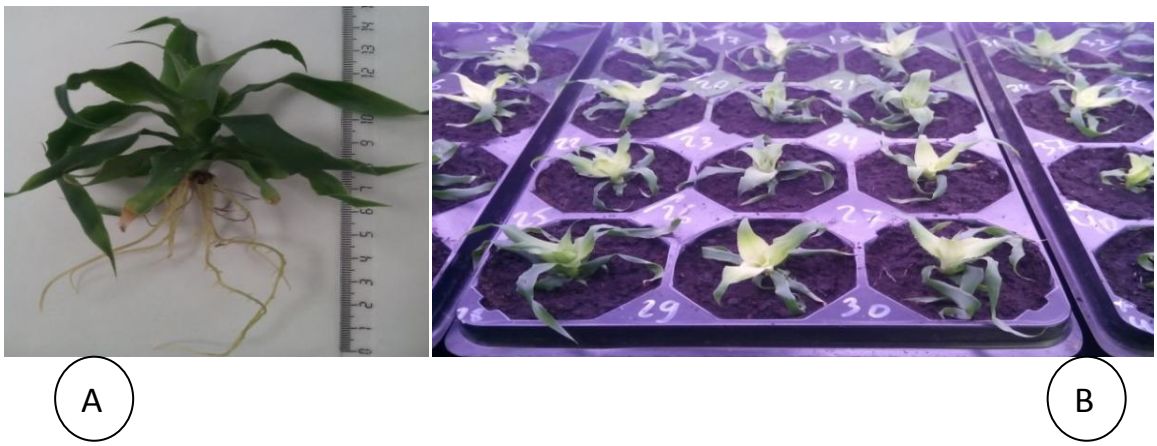

Fig. 5. Pineapple plants after adaptation for one month in hydroponics:
(A) A general view of the seedling; (B) Planting in a non-sterile substrate under normal conditions

Second, the substrate was not required at all, since an aqueous solution of mineral salts was used, which, unlike organic substrates, is little affected by bacterial and fungal contamination and by the spread of insects. Aeration of the root system, which is necessary for its vital activity, was maintained due to the circulation of the solution; under the cover, the leaves received good development and sufficient adaptation to lower air humidity for three weeks. The author believes the laws of pineapple microshoots development when adapting to non-sterile conditions in hydroponics, established during these experiments, can be successfully taken into account in the practice of mass cultivation of planting material in vitro. Moreover, the experience can be transferred to other cultures with a similar type of stem, for example, the banana.

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