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Full Length Research Paper

Growth, flowering and fruiting *in vitro* pineapple (*Ananas comosus* L.) in greenhouse conditions

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The experiment was carried out in a greenhouse, located in Tehran city, Iran. The objectives of this study was to evaluate the effects of fertilizer and acidic soil on the foliar and radicular growth of micropropagated plantlets of the pineapple cv. Merr (*Ananas comosus* L.). We evaluated the growth of that genotype in five different ages of acclimatizing: 1, 2, 3, 4, 5 and 6 months in greenhouse. The hardening of plantlets increased length of shoot, leaf length and leaf number and slip production, accelerated flowering and fruit maturity, caused uniform flowering and fruit ripening, and had no effect on sucker development. When hardening plantlets were at least 60 to 70 cm tall and 10 to 12 months old, an inflorescence bud was observed to form in the center of the leaves. Flowers (light red in color) opened row by row over a period of about two weeks. When fruits were about six months old, about four months after flowering has occurred, these changes were observed. The color of the shell changed from green to rich gold. When the fruit was golden half way up, it could be picked and eaten. The color change of the shell occurred first at the bottom of the fruit and moved upwards. During this change, the fruit became sweeter and the color of the flesh changed from white to yellow.

Key words: Pineapple, *Ananas comosus* L., flowering, fruiting, growth.

INTRODUCTION

Pineapple (*Ananas comosus* L.) is one of the most economically important tropical fruits (Duval et al., 2001). In terms of worldwide production, it is currently the third most important tropical fruit after bananas and mangoes (FAO, 2008). This bromeliad is routinely propagated vegetatively by means of lateral shoots, basal suckers or crowns. Pineapple micro propagation can be considered to be easy, but the multiplication rate is low and it would take eight years to obtain enough propagules from one mother plant (Almeida et al., 2002). In conventional breeding, clonal selection is tedious and requires several generations of back crossing in order to develop pineapple varieties with desired traits. Being a vegetatively propagated plant, conventional hybridization techniques for the generation of better pineapple varieties are cumbersome and time consuming (Mhatre, 2007).

Hence, the need to improve the multiplication rates of selected elite genotypes led to the development of tissue culture techniques for the *A. comosus* (L. Merr) pineapple (Almeida et al., 2002). *In vitro* micropropagation of pineapple plantlets has many advantages over conventional methods of vegetative propagation. For instance, this technique allows an efficient and rapid increase of selected elite pineapple varieties. Many authors have reported successful production of pineapple via micropropagation system during the last few years (Firooozabady and Gutterson, 2003; Be and Debergh, 2006; Danso et al., 2008).

The flowering is an essential component of pineapple (*A. comosus* var. comosus) (Hepton, 2003) cultivation, especially for those cultivars intended for fresh consumption. Natural flowering out of season can cause serious scheduling problems for growers. Induction of reproductive development in pineapple under natural conditions is favoured by shortened day-length and cool night temperatures (Van Overbeek and Cruzado, 1948;

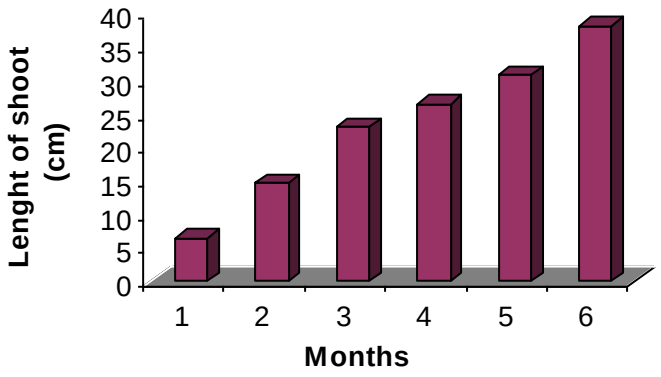

Figure 1. Mean length of pineapple shoot during acclimatization period.

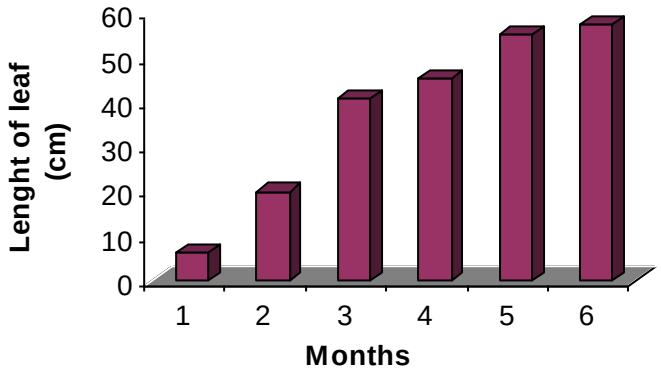

Figure 2. Mean length of pineapple leaves during acclimatization period.

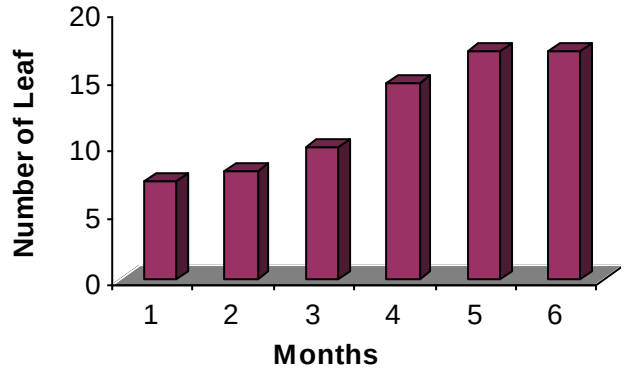

Figure 3. Mean number of pineapple leaves during acclimatization period.

Gowing, 1961; Friend and Lydon, 1979; Friend, 1981); however, other stresses can also induce flowering (Bartholomew et al., 2003). In some regions, natural induction begins in late November as a result of sudden drops in temperature that coincide with passing cold fronts (Wang et al., 2007).

Hence, the present study was conducted with the following objectives: 1) measuring growth foliar of i*n vitro* plantlets pineapple during acclimatization, 2) flowering of pineapple cv. Merr and 3) fruiting in greenhouse, exposing them to natural conditions of weather.

MATERIALS AND METHODS

The pineapple buds (cv. Merr) were collected from field-grown plants and cultured following the protocol described by Daquinta and Benegas (1997). Explants were placed in conventional plant containers for micropropagation (300 ml) where culture medium fed five explants. The culture medium included Murashige and Skoog salts (1962), 30 gl^{-1} sucrose, 4.4 µM 6- benzyladenine and 5.3 µM naphthalene acetic acid. Shoots were transferred to the multiplication culture medium (as described above except: 9.3 µM 6-benziladenine and 1.6 µM naphthalene acetic acid). They were sub cultured at 42 days intervals.

The *in vitro* plantlets were obtained and later hardened in *ex vitro* for 6 months (Yanez et al., 2004).For *ex vitro* hardening, plantlets were placed in pots containing 82 cm^3 of a mixture pit moss + perlite (2:1) as acidic soil. During hardening of *in vitro* plantlets, they were transferred to the greenhouse environment (photon flux density of 458 $\mu molm^{-2}s^{-1}$ and humidity 70% to 80%). The most important pineapple phenotypic traits were recorded during 18 months (acclimatization, flowering and fruiting phases). The agricultural evaluations were made in greenhouse conditions.

The fertilizer used was adapted from Moreira (2001) and recommended by Malavolta (1980), using doses of NPK fertilizer; 0.3 g N, P 0.2 g and 0.15 g K per kg of substrate each month from 30 days after transplanting.

The Statistical Package for Social Sciences (Version 8.0 for Windows, SPSS Inc.) was used to perform one-way ANOVA and LSD tests ($P \leq 0.05$) were done.

RESULTS AND DISCUSSION

Direct regeneration of shoots and shoot buds (with no callus) was obtained with MS media containing different BAP/IAA 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.

It was possible to observe differences in pineapple plantlets during the acclimatization period due to acidic soil and fertilizers. The advantages of fertilizer use on plant growth and flowering were quantified by statistical experimental design. In fact, the length of shoot increased to 38.15 cm in the sixth month compared to the first month; a relative increment of 95% (Figure 1). Number and length of leaves increased during 6 months acclimatization; in the first month, mean number and length of leaves were 7.3 and 6.05 cm, respectively, and in the sixth month, they were 17.1 and 57.35 cm (Figures 2 and 3).

The plantlets produced a low branch during acclimatization and branching was very week, also no branch was observed in hardening time.

Table 1a. Representative mean difference test (LSD) for morphological characters among 1, 2, 3, 4, 5 and 6 months acclimatization.

Length of shoot (I)	Length of shoot (J)	Mean difference (I-J)	Significance
LS1	LS2	(*)-8.2000	0
	LS3	(*)-16.7000	0
	LS4	(*)-20.1000	0
	LS5	(*)-24.4500	0
	LS6	(*)-31.8000	0
LS2	LS1	(*)8.2000	0
	LS3	(*)-8.5000	0
	LS4	(*)-11.9000	0
	LS5	(*)-16.2500	0
	LS6	(*)-23.6000	0
LS3	LS1	(*)16.7000	0
	LS2	(*)8.5000	0
	LS4	(*)-3.4000	0
	LS5	(*)-7.7500	0
	LS6	(*)-15.1000	0
LS4	LS1	(*)20.1000	0
	LS2	(*)11.9000	0
	LS3	(*)3.4000	0
	LS5	(*)-4.3500	0
	LS6	(*)-11.7000	0
LS5	LS1	(*)24.4500	0
	LS2	(*)16.2500	0
	LS3	(*)7.7500	0
	LS4	(*)4.3500	0
	LS6	(*)-7.3500	0
LS6	LS1	(*)31.8000	0
	LS2	(*)23.6000	0
	LS3	(*)15.1000	0
	LS4	(*)11.7000	0
	LS5	(*)7.3500	0

LS, Length of shoot. The mean differences are significant at the 0.05 level.

The 0.3 g N enhanced all growth parameters. Plantlets performed almost similar in medium and large size pots without any significant difference in all measured parameters. Irrigation with fertilizer water increased length of shoot, leaf number and length of leaf (Table 1a, b, c). However, the water quality did not influence branching. Under field conditions, Mohamed (1983) recognized the height need of this crop for mineral nitrogenous nutrition.

The native habitat of pineapple is the equatorial forests of Brazil, and the major production areas throughout the world are either equatorial or subequatorial with cloudy rainy skies (Samson, 1989). Clouds and forests permit partial exposure to sunlight. Mounting clear plastic (in greenhouse) with netting would result in light cuts and this might explain the gains in number of leaves. The influence of light intensity was not studied in previous post-weaning investigations on pineapple, but Folliot and Marchall (1991) reported enhanced *ex vitro* growth attributes with lengthy photoperiods.

The presence of growth or nutrient accumulation after the 180-day acclimatization period suggested that the

Table 1b. Representative mean difference test (LSD) for morphological characters among 1, 2, 3, 4, 5 and 6 months acclimatization.

Number of leaf (I)	Number of leaf (J)	Mean difference (I-J)	Significance
NL1	NL3	(*)-2.5500	0.001
	NL4	(*)-7.3000	0
	NL5	(*)-9.8000	0
	NL6	(*)-9.8000	0
NL2	NL3	(*)-1.8500	0.013
	NL4	(*)-6.6000	0
	NL5	(*)-9.1000	0
	NL6	(*)-9.1000	0
NL3	NL1	(*)2.5500	0.001
	NL2	(*)1.8500	0.013
	NL4	(*)-4.7500	0
	NL5	(*)-7.2500	0
	NL6	(*)-7.2500	0
NL4	NL1	(*)7.3000	0
	NL2	(*)6.6000	0
	NL3	(*)4.7500	0
	NL5	(*)-2.5000	0.001
	NL6	(*)-2.5000	0.001
NL5	NL1	(*)9.8000	0
	NL2	(*)9.1000	0
	NL3	(*)7.2500	0
	NL4	(*)2.5000	0.001
NL6	NL1	(*)9.8000	0
	NL2	(*)9.1000	0
	NL3	(*)7.2500	0
	NL4	(*2.5000	0.001

NL, Number of leaves. The mean differences are significant at the 0.05 level.

response to foliar acidic soil application to promote growth of pineapple plantlets might been a time-related process, since Baldotto et al. (2009) observed differences only after 150 days of acclimatization. The period of pineapple acclimatization is critical due to low growth rate of roots and shoots. In this phase, structural and physiological adjustments of plantlets to *ex vitro* conditions are crucial for the success in subsequent phases (flowering and fruiting) (Barboza et al., 2006).

Inflorescence induction in pineapple generates an apical reproductive structure. In the typical plant with determinate flowering, the developing fruits are supported exclusively by the pre-existing subtending leaves.

Flower development in greenhouse typically occurs in late December or January when the days are short (about 10.5 h) and the nights are cool (about 13 to 18°C).

When hardening of plantlets were at least 40 to 50 cm tall and 8 to 10 months old, an inflorescence bud was observed to form in the center of the leaves (Figure 4a). At that time, the stem elongated and enlarged near the apex and puts forth an inflorescence of small purple or red flowers (Figure 4b). The developing fruit did not show until about two months later when a bright red cone emerged. Generally, the first flowers open 50 or so days after flower induction and flowering continues for 20 to 40 days. Usually, one to ten flowers open daily beginning around midnight and close the following evening.

Later, flowers opened row by row over a period of about two weeks, starting from the bottom. The petals of the last flower had dried, when the fruit begun to develop (Figure 4c). However, in pineapple, the inflorescence and developing fruit were augmented with new photosynthetic

Table 1c. Representative mean difference test (LSD) for morphological characters among 1, 2, 3, 4, 5 and 6 months acclimatization.

Length of leaf (I)	Length of leaf (J)	Mean difference (I-J)	Significance
LL1	LL2	(*)-13.8500	0
	LL3	(*)-34.6500	0
	LL4	(*)-39.2500	0
	LL5	(*)-49.0000	0
	LL6	(*)-51.3000	0
LL2	LL1	(*)13.8500	0
	LL3	(*)-20.8000	0
	LL5	(*)-35.1500	0
	LL6	(*)-37.4500	0
LL3	LL1	(*)34.6500	0
	LL2	(*)20.8000	0
	LL5	(*)-14.3500	0
	LL6	(*)-16.6500	0
LL4	LL1	(*)39.2500	0
	LL2	(*)25.4000	0
	LL5	(*)-9.7500	0.003
	LL6	(*)-12.0500	0
LL5	LL1	(*)49.0000	0
	LL2	(*)35.1500	0
	LL3	(*)14.3500	0
	LL4	(*)9.7500	0.003
LL6	LL1	(*)51.3000	0
	LL2	(*)37.4500	0
	LL3	(*)16.6500	0
	LL4	(*)12.0500	0

LL, Length of leaves. The mean differences are significant at the 0.05 level.

leaves in developing crowns and sometimes augmented with leaves of slips (Bartholomew and Malezieux, 1994; Marler, 2011). The sink activity due to the presence of fruit generally increased net photosynthesis of leaves near the fruit (DeJong, 1986; Lenz, 1979; Mondal et al., 1978; Schaffer et al., 1986, 1987). When fruit was about six months old, about four months after flowering has occurred, changes begun to be observed. The color of the shell changed from green to rich gold. The color change of the shell occurred first at the bottom of the fruit and moved upwards (Figure 4d).

During this change, the fruit became sweeter and the color of the flesh changed from white to yellow. When the fruit was golden half way up, it could be picked and eaten. Ripe fruit had a yellow peel and pleasant aroma. The pulp was yellow to golden yellow, sweet and juicy (Figure 4e).

According to Pinto du Cunha (2005), flowering is a unique and integrated process, of very complex nature and multifactorial control, that has been studied extensively, from ecophysiology to biophysics aspects (Bernier et al., 1981a, b; Bernier et al., 1993; Kinet et al., 1981; Kinet, 1993). Most of the plants react to environmental signals regulating the transition into flowering, since all individuals of a given species have to bloom synchronously for the success of crossings and, also because they should complete sexual reproduction under favorable external conditions (Bernier et al., 1993).

Basically, the initiation of pineapple flowering depends on the physiological state and nutritional reserve of the plant, day length and temperature (Bartholomew and Malezieux, 1994). According to these authors, a minimum difference between day and night temperatures is necessary to elicit natural flowering, in addition to the

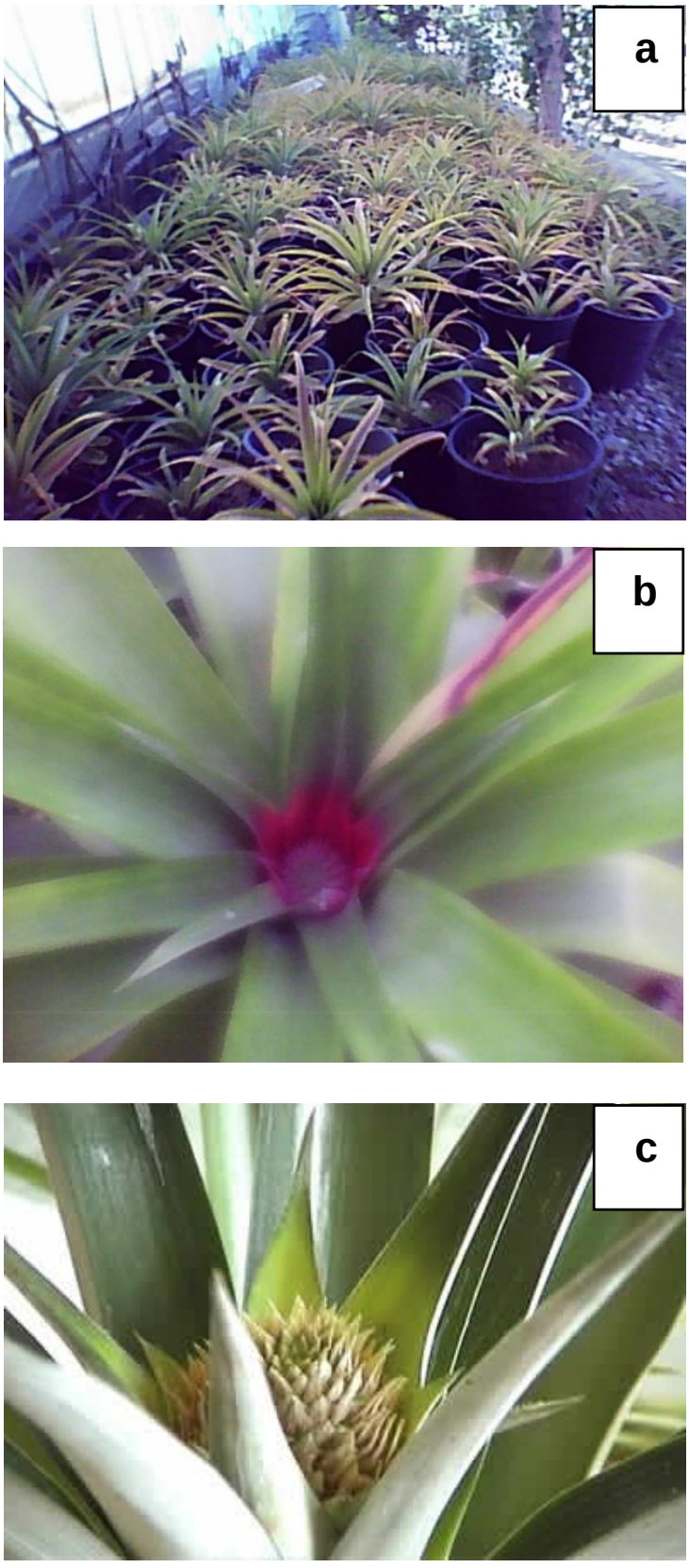

Figure 4. Flower and fruit of formation and development phases a) pineapple plants after acclimatization b) flower of formation c) growth flower d) formation fruit e) ripen fruit.

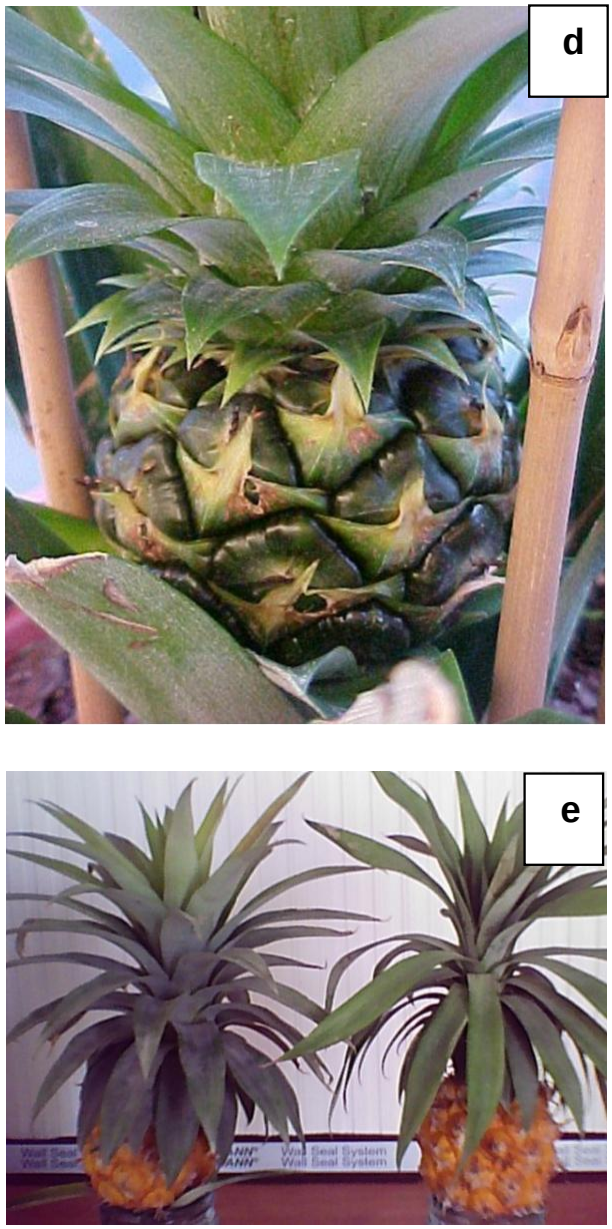

Figure 4. Continued.

effect of short days.

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