Current Trends in Natural Sciences
Vol. 9, Issue 17, pp. 216-221, 2020
https://doi.org/10.47068/ctns.2020.v9i17.027
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

EFFECT OF DIFFERENT TEMPERATURE AND MOISTURE ON DEVELOPMENT OF *IN VITRO* DERIVED BANANA PLANTLETS

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Current Trends in Natural Sciences

Abstract
Seedling development is important in vitro production. Sometimes, lots of in vitro plant materials have died under acclimatization. Humidity and temperature are important factor during acclimatization of banana plantlets. But these factors have varied as banana cultivars. In this study, it is investigated effect of different temperature and moisture on development of in vitro derived banana seedlings belong to two banana cultivars. In present study, it is aimed to determine of effect of different temperatures and moisture on development of in vitro derived banana seedlings. Rooted seedlings of two different banana cultivar which grown in Turkey which about 5-6 cm height were transferred to peat: petlite media and they were grown under 22, 24, 26 ^{0}C temperature and 70%, 80% and 90% humidity. As obtained results, Nefir Deniz cultivar had highest plant height under 80% humidty+26 ^{0}C (8,26 cm) and lowest plant height was under 90% humidty+22 ^{0}C (6.24 cm). Nefir AZ banana cultivar had highest plant height under 70% humidty+22 ^{0}C (9.53 cm) and lowest plant height was under 80% humidty+24 ^{0}C (6.12 cm). Obtained results show that different humidity and temperature conditions should applied for different banana cultivar seedling acclimatization.

Keywords: banana, in vitro seedling acclimatization.

1. INTRODUCTION

Seedling development is important in vitro production. Sometimes, lots of in vitro plant materials have died under acclimatization. Humidity and air temperature is mentioned as a key factor in controlling during acclimatization of banana plantlets, prior to in vivo transplantation. On a commercial scale, banana plantlets have been produced by micropropagation through plant tissue culture, which is successfully implemented. Normally, the environments in in vivo are quite different when compared to in vitro conditions, in terms of relative humidity (RH), constant temperature, air ventilation, nutrient levels, etc (Kozai et al., 1997; Chen, 2004; Hazarika, 2006). In vitro acclimatization, or hardening, is one of the main processes in the production of healthy plantlets before their transplantation to in vivo (Pospíšilová et al., 1999a). But these factors have varied as banana cultivars. There are many techniques for controlling the RH in the culture vessel of plant tissue culture, such as, saturated salt addition to the culture chamber and increasing the air ventilation rate (Cui et al., 2000; Cha-um et al., 2003; Shim et al., 2003). Acclimatized plantlet adaptation is an important mechanism in the transplanting process of plant micropropagation, relating to survival percentage, growth and development (van Huylenbroeck et al., 1998; van Huylenbroeck et al., 2000; Kadleček et al., 2001; Fila et al., 2006). Healthy, acclimatized plantlets

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have been identified using physiological characteristics including chlorophyll content, chlorophyll a fluorescence parameters, CO2 assimilation, net photosynthetic rate (Pn), stomatal conductance (gs) and transpiration rate (E), which have been demonstrated in many plants such as orchids (Jeon et al., 2005), Calathea louisae (van Huylenbroeck et al., 2000), tobacco (Pospíšilová et al., 1999b; Kadleček et al., 2001), *Spathiphyllum floribundum* (van Huylenbroeck et al., 1998), strawberry (Borkowska, 2001), grapevine (Carvalho and Amâncio, 2002a; Fila et al., 2006) and chestnut (Carvalho and Amâncio, 2002b). Banana plantlet (Musa sp.) acclimatization can be divided into two phases. In the first, in vitro plantlets are transferred to controlled environments (greenhouse or box shade, under the conditions of 20°C to 28°C, 80 to 90% RH, and 70% shade cloth) for a three to six-week period. In the second phase, plantlets are shifted to trays, pots or bags, under 50% shade, in a temperature range from 18°C to 34ºC, and a relative humidity higher than 75%, for a gradual hardening (Souza et al., 1997; Hoffmann, 2002). When plantlets reach 25–30 cm height they are considered acclimated and become available to the market (Silva et al., 1999). In this study, it si investigated effect of dıfferent temperature and moisture on development of ın vıtro derıved banana seedlings belong to two banana cultivars.

2. MATERIALS AND METHODS

Two different banana cultivars (Nefir Deniz and Nefir AZ which improved at banana breeding program as new banana cultivar in Turkey as plant material.

Thirty-six in vitro derived banana seedlings were used for each application with three replications (12x3=36 seedlings). Rooted seedlings which about 5-6 cm height were transferred to peat: petlite media and they were grown under 22^0C, 24^0C, $26\ ^0$C temperature and 70%, 80% and 90% humidity. Plant diameter (cm) and plant height (cm) measured weekly. Obtained results were analyzed using EXEL program. Also, data analyzed using JUMP statistic program.

3. RESULTS AND DISCUSSIONS

The results are given in Table 1, Table 2. According to the findings obtained, Plant height and plant diameter measured in Nefir Deniz Banana cultivar for 4 weeks and in the last week, scoring was done for the visual scale. Graph created with the findings obtained in the 4th week and In Nefir Deniz banana cultivar, plant height is obtained in the 4th week at the lowest 90% humidity + 22 ^{0}C (6,24 cm) temperature and humidity application, the highest was obtained from 80% Humidity + 26 ^{0}C (8.26 cm) temperature application.

On the other hand, plant height and plant diameter were measured for 4 weeks in Nefir AZ Banana variety and scoring was done for visual scale in the last week. Graph was created with the findings obtained in the 4th week (Table 2) and In Nefir AZ banana cultivar, plant height is obtained in the 4th week at the lowest 80% humidity + 24 ^{0}C (6.12 cm) temperature and humidity application, while the highest is obtained from 70% Humidity + 22 ^{0}C (9.53 cm) temperature application. The application of 80% Moisture + 26 ^{0}C, which is the most successful application in the Nefir Deniz banana variety, also gave a successful result in the Nefir AZ banana variety compared to other applications. In addition, the data obtained from the study were subjected to variance analysis using JUMP statistical software. The differences were found significant in terms of the characteristics examined between cultivars and applications. Statistically significant characters at $p < 0.05$ level were listed according to the Minimum Significant Difference (OEF) method, and the level of difference and significance was revealed (Table 3, Table 4).

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Table 1. Data obtained from the effect of Nefir Deniz banana variety on seedling growth under 3 different temperature (22 ^{0}C, 24^{0}C, 26 ^{0}C) and 3 different humidity (70%, 80%, 90%) conditions

Cultrivar	1. week		2. week		3. week		4. week		
Nefir Deniz	Plant height(cm)	Plant diamater(cm)	Plant height(cm)	Plant diamater(cm)	Plant height(cm)	Plant diamater(cm)	Plant height(cm)	Plant diamater(cm)	Scale(1-5)
90%+22 ^{0}C	4.00	0.31	4.60	0.35	5.44	0.39	6.24	0.44	3
90%+24 ^{0}C	7.54	0.36	6.98	0.41	6.26	0.42	6.58	0.46	3
90%+26 ^{0}C	5.74	0.37	6.24	0.39	6.34	0.45	6.94	0.48	4
80%+22 ^{0}C	5.72	0.31	6.16	0.44	6.58	0.49	7.32	0.59	4
80%+24 ^{0}C	6.78	0.42	7.22	0.46	7.44	0.48	7.54	0.57	4
80%+26 ^{0}C	5.76	0.51	6.54	0.57	7.32	0.60	8.26	0.63	5
70%+22 ^{0}C	4.90	0.35	5.44	0.45	5.80	0.50	6.42	0.51	3
70%+24 ^{0}C	4.63	0.30	5.78	0.41	6.23	0.44	7.08	0.49	4
70%+26 ^{0}C	6.32	0.31	5.90	0.42	5.98	0.45	6.76	0.49	4
Max	7.54	0.51	7.22	0.57	7.44	0.60	8.26	0.63	5.00
Min.	4.00	0.30	4.60	0.35	5.44	0.39	6.24	0.44	3.00

Table 2. Data obtained from the effect of Nefir AZ banana variety on seedling growth under 3 different temperature (22 ^{0}C, 24^{0}C, 26 ^{0}C) and 3 different humidity (70%, 80%, 90%) conditions

Cultivar	1. week		2. week		3. week		4. week		
Nefir AZ	Plant height(cm)	Plant diamater(cm)	Plant height(cm)	Plant diamater(cm)	Plant height(cm)	Plant diamater(cm)	Plant height(cm)	Plant diamater(cm)	Scale(1-5)
90%+22 ^{0}C	4.78	0.32	5.64	0.36	6.10	0.42	6.68	0.49	4
90%+24 ^{0}C	5.08	0.37	6.76	0.42	8.34	0.44	9.44	0.52	5
90%+26 ^{0}C	4.68	0.33	5.84	0.41	6.90	0.46	7.92	0.52	4
80%+22 ^{0}C	4.72	0.29	5.62	0.32	5.80	0.39	7.64	0.43	4
80%+24 ^{0}C	4.28	0.32	5.08	0.42	5.46	0.43	6.12	0.46	3
80%+26 ^{0}C	6.14	0.32	7.14	0.40	7.68	0.48	8.98	0.52	5
70%+22 ^{0}C	7.10	0.42	7.93	0.47	9.07	0.50	9.53	0.59	5
70%+24 ^{0}C	5.96	0.29	6.60	0.43	6.96	0.46	7.50	0.50	4
70%+26 ^{0}C	4.40	0.27	5.14	0.36	5.86	0.43	6.64	0.52	4
Max.	7.10	0.42	7.93	0.47	9.07	0.50	9.53	0.59	5.00
Min.	4.28	0.27	5.08	0.32	5.46	0.39	6.12	0.43	3.00

Table 3. Statistical analysis table of the effects of different applications on seedling growth (Plant height) in Nefir Deniz and Nefir AZ banana varieties

Plant height(cm)						
Cultivar	Humidty	Air temperature			Mean. (Humidty)	Mean (Cultivar)
		22 ^{0}C	24 ^{0}C	26 ^{0}C		
Nefir Deniz	90%	6.680 cd	9.440 a	7.920 a-d	8.013	7.827
	80%	7.64 a-d	6.120 d	8.980 ab	7.580	
	70%	9.520 a	7.50 a-d	6.640 cd	7.887	
	Mean	*7.947*	*7.687*	*7.847*		
Nefir AZ	90%	6.240 cd	6.940 b-d	6.580 cd	6.587	7.002
	80%	8.260 a-c	7.540 a-d	7.320 b-d	7.707	
	70%	6.760 cd	6.960 b-d	6.420 cd	6.713	
	Mean	*7.087*	*7.147*	*6.773*		

According to the results of statistical analysis, Variety, Temperature * Humidity and Humidity * Variety * Temperature applications were found important in terms of plant height (Table 5).

Patel et al. (2015) reported that even though there is not much difference in the survival rate of plants emerging from tissue culture of different banana varieties in Coco peat environment, it varies according to cultivars (Grand Naine (87.5%), Mahalaxmi (85.6%), Shrimanti (81.9%), and Basarai (83.7%). Scaranari et al. (2009) investigated the effect of 3 different shades of red (70%, 50%, 30%) and 1 shade of black (50%) on the development of banana seedlings belonging to the Grand nain cultivar and the most successful result was black (50%) and red. They obtained from 70% application. The objective of this study was to evaluate the development of pre-acclimatized banana

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plantlets cv. Pioneira (*Musa* sp., Group AAAB) obtained from tissue culture. Seedlings were multiplied in five subcultures (30 days each), on MS medium supplemented with 5 mg.L-1 BA (benzyl aminopurine), solidified with agar gel, pH 5.8, under 20 hours photoperiod, 2,000 lux of light intensity and temperature of 25±2°C. The in vitro rooting was performed on MS medium. Plantlets were pre-acclimatized for 30 days in a greenhouse, under controlled conditions of temperature and irrigation. These plantlets, 4.8±1.5 cm high, were then transferred to plastic bags containing organic substrate. Growth and development of the plantlets were evaluated under several conditions: greenhouse, humid chamber in greenhouse, screen house, humid chamber in screen house, under tree canopy, humid chamber under tree canopy and in-field conditions. A completely randomized design was used with five replications, each replicate containing 10 plantlets. Evaluations of plantlet development were performed at 60 and 120 days after transplanting. All treatments showed 100% plantlet growth, except for the direct field transplanting (39.7%). The plantlet development was similar in greenhouse, screen house, and under tree canopy conditions. The proposed system shows feasibility for use in long-distance distribution of micro propagated plantlets (Silva et al. 1997).

Table 4. Statistical analysis of the effects of different applications on seedling growth (Plant diameter) in Nefir Deniz and Nefir AZ banana varieties

Plant diameter(cm)						
Cultivar	Humiditiy	Air Temperature			Mean. (Humiditiy)	Mean (Cultivar)
		22 °C	24 °C	26 °C		
Nefir Deniz	90%	0.490	0.520	0.520	0.510 bc	0.506
	80%	0.430	0.460	0.520	0.470 bc	
	70%	0.590	0.500	0.520	0.537 ab	
	Mean	*0.503*	*0.493*	*0.520*		
Nefir AZ	90%	0.440	0.480	0.460	0.460 c	0.520
	80%	0.630	0.570	0.590	0.597 a	
	70%	0.490	0.510	0.510	0.503 bc	
	Mean	*0.520*	*0.520*	*0.520*		

Table 5. F-values of some growth characteristics of banana cultivars

F Value				
	Degree of freedom	Plant height	Plant diameter	
Cultivar	1	5.793*	0.507	
Humidity	2	0.447	2.018	
Temperature	2	0.121	0.147	
Humidty*Cultivar	2	1.973	7.703*	
Temperature*Cultivar	2	0.205	0.147	
Temperature*Humidty	4	3.553*	0.498	
Humidty*Cultivar*Temperature	4	2.518*	1.001	

In present study, three different humidity and temperature were applied at two different banana cultivars and differences were obtained between seedling development of banana cultivars with regard to temperature and humidity. Also it was determined differences among applications as statistically. When differences between banana cultivars, the best application banana cultivar seedlings was 80 % humidity +26 ^{0}C temperature for Nefir Deniz and 70% humidty+24 ^{0}C temperature for Nefir AZ. As our observation at open field and greenhouse conditions, Nefir AZ

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banana cultivar was more tolerant than Nefir Deniz banana cultivar with regard to chilling. Therefore, the reaction to the humidity and temperature difference between the two banana varieties may therefore have occurred. As a result of present study, a protocol was established for seedling development at appropriate temperature and humidity after tissue culture for both types of bananas.

4. CONCLUSIONS

The most suitable temperature and humidity protocol for the development of seedlings extracted from the tissue culture of Nefir Deniz banana variety is the application of 80% Humidity + 26 ^{0}C temperature. And also, The most suitable temperature and humidity protocol for the development of seedlings extracted from the tissue culture of Nefir Deniz banana variety is 70% Humidity + 22 ^{0}C temperature application.

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