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

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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 °C temperature and 70%, 80% and 90% humidity. As obtained results, Nefir Deniz cultivar had highest plant height under 80% humidity+26 °C (8.26 cm) and lowest plant height was under 90% humidity+22 °C (6.24 cm). Nefir AZ banana cultivar had highest plant height under 70% humidity+22 °C (9.53 cm) and lowest plant height was under 80% humidity+24 °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 (Pospšilová 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 Huylkenbroeck et al., 2000), tobacco (Pospíšilová et al., 1999b; Kadleček et al., 2001), Spathiphyllum floribundum (van Huylkenbroeck 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 34°C, 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 is investigated effect of different temperature and moisture on development of in vitro derived 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°C, 24°C, 26°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°C (6.24 cm) temperature and humidity application, the highest was obtained from 80% Humidity + 26°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°C (6.12 cm) temperature and humidity application, while the highest is obtained from 70% Humidity + 22°C (9.53 cm) temperature application. The application of 80% Moisture + 26°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).
Table 1. Data obtained from the effect of Nefir Deniz banana variety on seedling growth under 3 different temperature (22°C, 24°C, 26°C) and 3 different humidity (70%, 80%, 90%) conditions

| Cultivar | 1. week | 2. week | 3. week | 4. week | Scale (L-B) |
|----------|---------|---------|---------|---------|-------------|
|          | Plant height(cm) | Plant diameter(cm) | Plant height(cm) | Plant diameter(cm) | Plant height(cm) | Plant diameter(cm) | Plant height(cm) | Plant diameter(cm) | Scale (L-B) |
| Nefer Deniz 22°C | 4.50 | 0.31 | 6.60 | 0.41 | 6.25 | 0.42 | 6.35 | 0.43 | 3 |
| 24°C | 7.54 | 0.36 | 6.36 | 0.41 | 6.25 | 0.42 | 6.35 | 0.43 | 3 |
| 26°C | 5.74 | 0.27 | 5.24 | 0.38 | 5.94 | 0.45 | 6.09 | 0.48 | 4 |

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

| Cultivar | 1. week | 2. week | 3. week | 4. week | Scale (L-B) |
|----------|---------|---------|---------|---------|-------------|
|          | Plant height(cm) | Plant diameter(cm) | Plant height(cm) | Plant diameter(cm) | Plant height(cm) | Plant diameter(cm) | Plant height(cm) | Plant diameter(cm) | Scale (L-B) |
| Nefer AZ 22°C | 4.50 | 0.31 | 6.60 | 0.41 | 6.25 | 0.42 | 6.35 | 0.43 | 4 |
| 24°C | 7.54 | 0.36 | 6.36 | 0.41 | 6.25 | 0.42 | 6.35 | 0.43 | 4 |
| 26°C | 5.74 | 0.27 | 5.24 | 0.38 | 5.94 | 0.45 | 6.09 | 0.48 | 4 |

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

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...
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 °C temperature for Nefir Deniz and 70% humidity+24 °C temperature for Nefir AZ. As our observation at open field and greenhouse conditions, Nefir AZ

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

| Cultivar       | Humidity | Air Temperature | Mean (Humidity) | Mean (Cultivar) |
|----------------|----------|-----------------|-----------------|-----------------|
| Nefir Deniz    | 90%      | 22 °C           | 0.820           | 0.510 bc        |
|                | 90%      | 24 °C           | 0.520           | 0.470 bc        |
|                | 70%      | 26 °C           | 0.520           | 0.537 ab        |
|                | Mean     | 0.503           | 0.493           | 0.520           |
| Nefir AZ       | 90%      | 22 °C           | 0.820           | 0.480 c         |
|                | 90%      | 24 °C           | 0.520           | 0.597 a         |
|                | 70%      | 26 °C           | 0.520           | 0.503 bc        |
|                | Mean     | 0.520           | 0.520           | 0.520           |

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

|                | Degree of freedom | Plant height | Plant diameter |
|----------------|-------------------|--------------|----------------|
| Cultivar       | 1                 | 6.793*       | 0.507          |
| Humidity       | 2                 | 0.447        | 2.018          |
| Temperature    | 2                 | 0.121        | 0.147          |
| Humidity*Cultivar | 2              | 1.973        | 7.703*         |
| Temperature*Cultivar | 2         | 0.265        | 0.417          |
| Temperature*Humidity | 4            | 3.953*       | 0.489          |
| Humidity*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 °C temperature for Nefir Deniz and 70% humidity+24 °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 °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 °C temperature application.

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