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# EFFECT OF MAGNESIUM SULPHATE AND LIGHT DURATION ON MULTIPLICATION RATE OF BANANA (*Musa sp*.) IN VITRO CULTURE.

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## ABSTRACT

This investigation was conducted in the tissue culture laboratory vegetable department, Faculty of Agriculture, Assiut University, to study the effect of Magnesium Sulphate ($MgSO_4$) and light duration in-vitro multiplication rate of five subculture on banana. Magnesium Sulphate ($MgSO_4$) was add at 1, 2.5, 5 and 10 ppm and light duration treatment applied at (darkness for 4 weeks, natural light for 4 weeks, 16 hours light for 4 weeks and 2 weeks at darkness followed by 2 weeks at 16 hours light). Results indicated that light duration treatment are more significantly effect on multiplication rate, chlorophyll a, chlorophyll b and carotene contents. Darkness for 2 weeks followed by light 16 hours for 2 weeks recorded the highest rate of multiplication rate. Meanwhile, Magnesium Sulphate and their interactions with light duration treatments are of non-significant effect on multiplication rate. The best results was regard to multiplication and improvement of plant morphogenesis at final stage of development in vitro of Grand Naine Banana when explants incubated at dark for a period 15 days followed by 16 hours light for 15 days.

**Keywords:** *light duration; multiplication rate; darkness; natural light; Magnesium Sulphate.*

## INTRODUCTION

Banana (*Musa spp.)* is one of the most important fruit crops cultivated in tropical and subtropical regions of the world. It is a staple food and export commodity. It contributes to the food security of millions of people in the developing world. It is grown in humid tropical region and constitutes the 4 $^{th}$ largest fruit crop of the world and as well as in Egypt. Banana production occupies an important share in the total fruit crops of Egypt. The total area of banana in the world attained about 4,923,548 ha (about 11,722,819 fed.) with an annual production at about 97.378.272 tons. In Egypt, the total cultivated area of banana in 2009 was about 2700 ha(about 6480 fed.) and produced about 1,100,000 tons (FAO, 2009).

Tissue culture systems are aseptic and easily kept free from fungi, bacteria, viruses, and insects parasites. An important use of tissue culture at present is the culturing of meristems so as to obtain virus free plants, and the subsequent storage of such plants as pathogenic free stocks by plant breeders. Micro propagation or in vitro techniques were established for fast multiplication of bananas (Vuylsteke D, 1989). Commercial production of Micropropagated bananas is now common in many countries and it is estimated that 25 million plants are produced worldwide each year. Micropropagation of banana is highly efficient, allowing a large turnover of plants in a very short period of time with in very little space (Arias, O. 1992), Arvanitoyannis, I.S, A *et al* 2007).The basic MS nutrients (Murashige T, Skoog F ,1962) are the most widely used media.

Magnesium, besides being the central atom of the chlorophyll molecule, representing approximately 10 % of the total foliar magnesium (Hopkins, 1995), is also a cofactor of most enzymes acting on phosphorylated substrates, making it very important in energy metabolism. Enzymatic reactions acting on the carboxylic group, nucleotide transfer, some dehydrogenases, mutates and lyses are also processes stimulated by Magnesium (Mengel&Kirkby, 1987).

Banana explants are occasionally cultured on medium supplemented with Magnesium Sulphate (MgSo4) to increase the number of shoots during multiplication (Dhanalakshmi. s and Stephan. R, 2014).

Light source is one of the important factors controlling plant growth and development (Nhut, D.T., *et al* , 2005). Light is an important stimulus for plant development. It is also widely known that spectral quality is a key factor in plant morphogenesis (Okamoto *et al*, 1997). Conventional fluorescent lamps, which have a wide range of wavelengths from 350 to 750 nm, are the most commonly used light source in plant tissue culture (Economou & Read, 1987)

Dark conditions enhanced higher regeneration shoot than light condition. The optimal collaboration of a dark incubation period together with growth regulator hormone increased regeneration shoot. Although light may be essential for plant development, darkness is also beneficial for plant morphogenesis, mainly at itsinitial stage of development in vitro ( Nisyawati & Kariyana. k , 2013 ).

Dark conditions enhanced higher proliferation rates than light conditions in some cultivars suggesting that banana in vitro proliferation is a photomorphogenically responsive process that is enhanced under dark conditions ( Makara, A. M. *et al*, 2010 ).

## MATERILS AND METHODS

The work of this study was carried out during 2014 season in vitro multiplication rate of five subculture on Grand Naine Bananas in Tissues Culture Laboratory faculty of Agriculture Assiut university.

**Source of banana plants :**

Tissue samples used in the experiment are imported from tissue culture AL- Nawa laboratory,

Jordon. cut from mother plants of banana (*Musa spp*; cv: Grand – Nain Cavendish. subgroup, AAA) the banana tissue were immediately transferred to the laboratory.

**Culture media preparation**

The stock salt solutions of medium were prepared with distilled water ( Murashige and Skoog, 1962 ). MS basal medium was used with some additions. The medium consisted of half-strength MS basal media supplemented with growth regulator hormone BAP 3 ppm $1^{-1}$ , stab a vitamins, sucrose 30 g $^{-1}$, agar 3.5 $g^{-1}$, jel 1 g $^{-1}$ to solidify the medium .The pH of the medium was adjusted to 5.7 $\pm$ 0.1 before sterilization using 0.1 or 1M of potassium hydroxide. Fifty $ml^3$ of the medium was poured into 500 $ml^3$ glass jars. All media were autoclaved under 1.5 $IP/b^2$ at 121 C for 20 min. and then kept overnight at room temperature before culture.

**Plant Micro propagation :**

The explants were subculture on half- strength MS medium. Contamination did not exceed 4-5%. The explants were incubated in a growth chamber for three weeks at 27 C $\pm$ 2 under 16 hours of cool white fluorescent light (21 umol/ $s^{-1}$ / $m^{-2}$ )and 8 hours of darkness. All explants every three weeks were transferred to a fresh medium for micropopagation till subculture number 5 increased number of explants. Every explants in subculture fifth of the experiment was ready environment.

**The experiment treatments :**

**The experimental included the following two factors:-**

1-the first factor (A)consisted of Five concentration of Magnesium Sulphate ($MgsO_4$):-

$A_1$ . Control ( without Magnesium Sulphate).
$A_2$ . Magnesium Sulphate at 1 ppm.
$A_3$ . Magnesium Sulphate at 2.5 ppm.
$A_4$ . Magnesium Sulphate at 5 ppm.
$A_5$ . Magnesium Sulphate at 10 ppm.

2- The second factor (B) included the four levels of lighting and darkness on explants incubated at:-

$B_1$ . Darkness for 4 weeks.
$B_2$ . Natural light for 4 weeks.
$B_3$ . 16 hours light for 4 weeks.
$B_4$ . Darkness for 2 weeks followed by light 16 hours for 2 weeks.

All culture condition have room temperature 27± 2o C and lighting using Phillips lamps of 20 watt which were placed in 20 cm above bottles. Total sample of each treatment was 3 replicates.

**Experimental design**

The experiment was set in completely randomized block design in split plot arrangement witch three explants replicates of each treatment, Five concentrate ion of Magnesium Sulphate treatments occupied the main plots and four levels of lighting and darkness occupied the subplots.

**Studied measurement**

At the end of the experiment (after 4 weeks), data were collected and included the following investigations :

1) Number of shoots produced by explants.
2) Shoots height ( cm ).
3) Number of leaves.
4) Determination of photosynthetic pigments.

The photosynthetic pigments were extracted from a definite fresh leaf sample in 5 ml of 95% ethyl alcohol in a test tube at 60 c, until colorless. Then the total volume completed into 10 ml with 95% ethyl alcohol and absorbance readings were determined with a spectrophotometer (unico UV 2100 spectrophotometer.) chlorophylls and carotenoids concentrations were calculated as mg/g FW at 663,644 and 452 nm using the following equations (Lichtenthaler H. K. 1987)

Chl. *a* = ( $13.36*A_{663}$ ) – ( $5.19*A_{644}$)
Chl. *b* = ( 27.49* A 644) – ( 8.12*A 663)
Carotene = { ( 1000*A452) – (2.13* Chl. *a* ) – ( 9.76* Chl. b)} /209.

**Statistical analysis:-**

Statistical analysis was dome according to mead *et al* (1993) using L.S.D test to differentiate various treatments means.

## RESULTS AND DISCUSSION

The mean multiplication rate of banana formation are shown in Table (1). Light duration treatments are of more significant effect on multiplication rate; darkness for 2 weeks followed by light 16 hours for 2 week(treatment B4)recorded the highest rate of multiplication(13.39).according to (Nisayawati and Kariyana, 2013) of banana. Meanwhile, natural light for 4 weeks(treatment B2) recorded the lowest values of multiplication rate(10.06). according to (Kodym *et al*, 2001) of banana. On the other hand, Magnesium levels and interaction between Light duration and Magnesium treatments are of non-significant effect on multiplication rate.

**Table (1): Effect of light duration and Magnesium Sulphate (MgSO4) on multiplication rate of Grand Nain Banana in vitro culture in 2014 season .**

| Light duration MgSO4(conc.) | B1 | B2 | B3 | B4 | Mean(A) |
|---|---|---|---|---|---|
| A1 | 11.78 | 10.17 | 10.43 | 12.40 | 11.19 |
| A2 | 12.17 | 8.44 | 9.44 | 12.11 | 10.54 |
| A3 | 12.39 | 10.47 | 10.77 | 13.44 | 11.77 |
| A4 | 13.11 | 10.81 | 11.35 | 14.00 | 12.32 |
| A5 | 15.72 | 10.43 | 10.22 | 15.00 | 12.84 |
| Mean(B) | 13.03 | 10.06 | 10.45 | 13.39 | --------- |
| LSD at 5% | A (n.s) | | B (2.28) | | A*B (n.s) |

**Table(2): Effect of light duration and Magnesium Sulphate (MgSO4)on shoots height (cm) of Grand Nain Banana in vitro culture in 2014 season .**

| Light duration / MgSO4(conc.) | B1 | B2 | B3 | B4 | Mean(A) |
|---|---|---|---|---|---|
| A1 | 4.67 | 2.94 | 3.14 | 4.15 | 3.73 |
| A2 | 6.39 | 3.67 | 3.44 | 5.44 | 4.74 |
| A3 | 5.72 | 3.62 | 4.22 | 3.83 | 4.35 |
| A4 | 6.27 | 4.17 | 5.00 | 4.83 | 5.07 |
| A5 | 5.55 | 4.42 | 4.44 | 4.17 | 4.65 |
| Mean(B) | 5.72 | 3.76 | 4.05 | 4.49 | --------- |
| LSD at 5% | A | | B | | A*B |
| | (n.s) | | (0.78) | | (n.s) |

**Table (3):Effect of light duration and Magnesium Sulphate (MgSO4) on number of leaves of Grand Nain Banana in vitro culture in 2014 season.**

| Light duration / MgSO4(conc.) | B1 | B2 | B3 | B4 | Mean(A) |
|---|---|---|---|---|---|
| A1 | 2.22 | 2.52 | 2.62 | 2.71 | 2.52 |
| A2 | 2.39 | 2.78 | 3.33 | 2.89 | 2.85 |
| A3 | 2.31 | 2.53 | 2.67 | 2.77 | 2.57 |
| A4 | 2.00 | 2.58 | 2.67 | 3.22 | 2.62 |
| A5 | 2.00 | 2.83 | 3.00 | 2.67 | 2.63 |
| Mean(B) | 2.18 | 2.65 | 2.86 | 2.85 | --------- |
| LSD at 5% | A | | B | | A*B |
| | (n.s) | | (0.31) | | (n.s) |

The mean shoots height (cm) of banana formation are shown in Table (2). Light duration treatments are more significantly effect on shoots height; darkness for 4 weeks followed by light 16 hours for 4week (treatment B1) recorded the highest rate of shoots height (5.72 cm). according to (Cybularz-Urban *et al.* 2007) of Cattiea Hybrid. Meanwhile, natural light for 4 weeks(treatment B2) recorded the lowest values of shoots height (3.76 cm). On the other hand, Magnesium levels and interaction between Light duration and Magnesium treatments are of non-significant effect on shoots height.

The mean Number of leaves of banana formation are shown in Table (3). Light duration treatments are more significantly effect on Number of leaves; light 16 hours for 4 week (treatment B3) recorded the highest rate of Number of leaves (2.86). according to (Nhut *et al*, 2005) of Spathiphyllum. Mean while, Darkness for 4 weeks (treatment B1) recorded the lowest values of Number of leaves (2.18). On the other hand, Magnesium levels and interaction between Light duration and Magnesium treatments are non-significant effect on Number of leaves.

The mean chlorophyll *a* of banana formation are shown in Table (4). Lightduration treatments are more significantly effect on chlorophyll *a*; darkness for 2 weeks followed by light 16 hours for 2 week (treatment B4) recorded the highest rate of chlorophyll *a* (0.69mg/g). Meanwhile, Darkness for 4 weeks (treatment B1) recorded the lowest values of chlorophyll *a* (0.11mg/g). Magnesium levels treatments are more significantly effective on chlorophyll *a* ; Magnesium (treatment A4 ) recorded the highest rate of chlorophyll *a* (0.54 mg/g). WYDRZYNSKI *et al* (1975). mean while, Magnesium (treatment A1) recorded the lowest values of chlorophyll *a* (0.31 mg/g) . On the other hand, Magnesium levels and interaction between Light duration were of non-significant effect on chlorophyll *a*.

**Table (4): Effect of light duration and Magnesium Sulphate (MgSO4) on chlorophyll *a* of Grand Nain Banana in vitro culture in 2014 season.**

| Light duration / MgSO4(conc.) | B1 | B2 | B3 | B4 | Mean(A) |
|---|---|---|---|---|---|
| A1 | 0.05 | 0.33 | 0.42 | 0.45 | 0.31 |
| A2 | 0.08 | 0.61 | 0.49 | 0.77 | 0.49 |
| A3 | 0.13 | 0.68 | 0.57 | 0.75 | 0.53 |
| A4 | 0.14 | 0.64 | 0.57 | 0.81 | 0.54 |
| A5 | 0.13 | 0.65 | 0.59 | 0.66 | 0.51 |
| Mean(B) | 0.11 | 0.58 | 0.53 | 0.69 | --------- |
| LSD at 5% | A | | B | | A*B |
| | (0.07) | | (0.08) | | (n.s) |

**Table (5): Effect of light duration and Magnesium Sulphate (MgSO4) on chlorophyll *b* of Grand Nain Banana in vitro culture in 2014 season.**

| Light duration MgSO4(conc.) | B1 | B2 | B3 | B4 | Mean(A) |
|---|---|---|---|---|---|
| A1 | 0.06 | 0.12 | 0.15 | 0.16 | 0.12 |
| A2 | 0.16 | 0.29 | 0.28 | 0.25 | 0.24 |
| A3 | 0.18 | 0.34 | 0.33 | 0.34 | 0.30 |
| A4 | 0.14 | 0.31 | 0.29 | 0.39 | 0.28 |
| A5 | 0.15 | 0.34 | 0.33 | 0.33 | 0.29 |
| Mean(B) | 0.14 | 0.28 | 0.27 | 0.29 | --------- |
| LSD at 5% | A | | B | | A*B |
| | (0.07) | | (0.04) | | (n.s) |

The mean chlorophyll *b* of banana formation are shown in Table (5). Light duration treatments are more significantly effective on chlorophyll *b*; darkness for 2 weeks followed by light 16 hours for 2 weeks (treatment B4) recorded the highest rate of chlorophyll *b* (0.29 mg/g). Meanwhile, Darkness for 4 weeks (treatment B1) recorded the lowest values of chlorophyll *b* (0.14 mg/g). Magnesium levels treatment are more significantly effective on chlorophyll *b* ; Magnesium (treatment A3 ) recorded the highest rate of chlorophyll *b* (0.30 mg/g). Mean while, Magnesium (treatment A1) recorded the lowest values of chlorophyll *b* (0.12 mg/g) . On the other hand, Magnesium levels and interaction between Light duration were of non-significant effect on chlorophyll *b*.

The mean carotene content of banana formation are shown in Table (6). Light duration treatments are more significantly effective on carotene. Darkness for 2 weeks followed by light 16 hours for 2 week (treatment B4) recorded the highest rate of carotene (0.36 mg/g). Meanwhile, Darkness for 4 weeks (treatment B1) recorded the lowest value of carotene (0.09 mg/g). Magnesium levels treatment are more significantly effect on carotene; magnesium (treatment A3) recorded the highest rate carotene (0.30 mg/g). mean while, Magnesium (treatment A1) recorded the lowest value of carotene (0.17 mg/g) . On the other hand, Magnesium levels and interaction between Light duration were of non-significant effect on carotene.

**Table (6): Effect of light duration and Magnesium Sulphate (MgSO4) on carotene of Grand Nain Banana in vitro culture in 2014 season.**

| Light duration MgSO4(conc.) | B1 | B2 | B3 | B4 | Mean(A) |
|---|---|---|---|---|---|
| A1 | 0.06 | 0.18 | 0.21 | 0.23 | 0.17 |
| A2 | 0.09 | 0.32 | 0.27 | 0.42 | 0.28 |
| A3 | 0.11 | 0.37 | 0.32 | 0.39 | 0.30 |
| A4 | 0.09 | 0.34 | 0.29 | 0.42 | 0.29 |
| A5 | 0.09 | 0.34 | 0.31 | 0.34 | 0.27 |
| Mean(B) | 0.09 | 0.31 | 0.28 | 0.36 | --------- |
| LSD at 5% | A | | B | | A*B |
| | (0.04) | | (0.04) | | (n.s) |

## CONCLUSION

The research described an efficient protocol for shoot regeneration of banana cultivar in vitro culture. Higher number of shoots were produced on media with addition of Magnesium Sulphate. Dark conditions enhanced higher multiplication rate than light condition. The optimal collaboration of a dark incubation for a period of 15day followed by light 16 hours for 15 day on increased multiplication rate, and improved plant morphogenesis at Final stages of development in vitro.

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## تأثير كبريتات المغنيسيوم والإضاءة المختلفة علي معدل تضاعف نباتات الموز في المعمل.

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****قسم الخضر- كلية الزراعة جامعة أسيوط مصر**

أجريت هذه التجربة في مختبر زراعة الأنسجة بقسم الخضر, كلية الزراعة, جامعة أسيوط, لدراسة تأثير كبريتات المغنيسيوم ($MgSO_4$) والإضاءة المختلفة علي معدل التضاعف معمليا في النقلة الخامسة لنبات الموز, وكانت إضافات كبريتات المغنيسيوم بتركيزات (1 و 2,5 و 5 و 10 جزء في المليون), ومعاملات الإضاءة المختلفة (الظلام لمدة٤ أسابيع, الإضاءة الطبيعية لمدة ٤ أسابيع, الإضاءة ١٦ ساعة لمدة ٤ أسابيع , الظلام لمدة ٢أسبوع يليها الإضاءة ١٦ ساعة لمدة ٢ أسبوع). وأشارت النتائج أن للإضاءة المختلفة تأثير كبير علي معدل التضاعف والكلوروفيل ( أ,ب) و الكاروتين , وسجلت معاملة الظلام لمدة ٢ أسبوع يليها الإضاءة ١٦ ساعة لمدة ٢ أسبوع أعلي نتائج علي التضاعف. ومن ناحية أخري , فإن تأثير كبريتات المغنيسيوم وتفاعلاتها مع الإضاءة المختلفة غير ملحوظ علي التضاعف. وتعتبر أفضل النتائج التي تم ملاحظتها علي التضاعف وتحسين الصفات المرفولوجيه في نهاية النقلة الخامسة لزراعة الأنسجة لنباتات الموز الجراندنان عند تعريضها إلى الإظلام لمدة ٢ أسبوع يليها الإضاءة لمدة ١٦ ساعة لمدة ٢ أسبوع.