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IMPACTS OF PLANT GROWTH REGULATORS AND LIGHT QUALITY ON BANANA (*MUSA SPP*) MICROPROPAGATION

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Abstract

An experiment was carried out in the laboratories of Date palm micropropagation Unit at the College of Agriculture, University of Kufa during the period from August 2017 to June 2018 to study the impact of plant growth regulators and light quality on propagate banana (*Musa* spp.) CV. Dwarf Cavendish (using plant tissue culture technique, BAP (3,4,5,6mg.L^{-1}) and TDZ (0.1, 0.2, 0.3 mg.L^{-1}) alone or incombination with NAA (0.5, 1, 1.5mg.L^{-1}) and light sources, Florescent white and LED light (18 red :2 blue), on shoot multiplication was studied. Shoot cultured on MS with 0.1 TDZ + 1.5 NAA under LED light gave highest values for shoot number and length, chlorophyll and carotin contents (9.25 shoot, 4.12 cm, 50.18 mg/100g FW and 5.070 mg/100g FW) respectivity,but shoot on MS with 0.2 TDZ + 1.5 mg NAA under LED light gave highe shoot fresh and dry weight and shoot containt of carbohydrate which gave 20.28 g,0.6925 g and 2.917 respectivty.

Key words : Banana micropropagation, TDZ, BAP, LED.

Introduction

The banana (*Musa* spp.) which belongs to the Musaceae family is one of the most important fruit production and consumption due to its great nutritional importance. Banana production reached 10704 million tons in 2013 of bananas and 39 million tons of plantain, grown for commercial purposes in 135 countries (FAO, 2016). One of the most important commercial bananas is *M. acuminate*, which carries the genome A and *M. balbisiana*, which carry the genome B, and the commercial varieties of bananas, as well as the hybrid varieties. Among these varieties is Dwarf Cavendish cultivar belonging to the Cavendish group, which is the most widely cultivation for its resistant to Panama disease, as well as adapting better to the cold climate than any other cultivars (Robinson and GalánSaúco 2010). Conventionally, bananas are propagated by suckers but this method may be caused transmission of nematode, fungal and viral diseases to generated plants. In the past two decades, plant tissue culture techniques have been invested in the large quantities of virus-free plantlets production of banana in short time with ease of transport between laboratories and between countries (Bairwa *et al.,* 2015).Various cytokinines, including benzylamino purine (BAP), 2-isopentenyl adenine (2ip) and Kinetin (Kin), were used in micropropagation to increase the number of shoots in bananas. It was observed that the type and its concentration affected the shoot multiplication according to previous studies on bananas. One of Cytokinines, which have been used in banana micropropagation in the last few years, are the derivatives of diphenyl urea, including Thidiazuron (TDZ). (Ngomuo *et al.,* 2014).White Fluorescent Light has long been used as a source of light in tissue culture laboratories at (1000-10000 Lux) which has a wavelength of 350 to 750 nanometers and Its power consumption is high, as well as the heat producing with lighting. Recently, light emitting diodes (LED) another type of light source has been used in the field of plant growth in commercial protected facilities, including glasshouses and growth chambers in tissue culture, which are characterized by low power consumption, high efficiency and long life (50 to 100 thousand hours) with little heat producing and there are white, red, blue, yellow, green and mixture between them(Gupta and Jatothu, 2013). The aim of this research was to test the efficiency of plant growth regulators and light quality on *in vitro* banana shoot multiplication.

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Materials and Methods

The study was carried out in the date palm micropropagation unit at the Faculty of Agriculture / University of Kufa during the period from August 2017 to June 2018 to study the effect of growth regulators (BAP,TDZ, NAA) and light quality on banana (*Musa sp.*) CV. Dwarf Cavendish*in vitro* shoot multiplication. Suckers (6 month old) were brought from the Horticulture office–Ministry of Agriculture-Iraq. According to Mohammed (2015). Their base were washed under running tap water to remove adherent soil. It was then dissected to remove the leaves from the base to the top gradually to reach the shoot tip and then transfer them to the Laminar Air Flow Cabinet for sterilization, by soaking for 20 min in commercial bleach (5.25% NaOCl) diluted to 20% (v/v),this was followed by rinsing for 3 times with autoclaved distilled water (Fig. 1).

Culture initiation

Each shoot tip was cut longitudinally into two pieces(half-shoot tip). Half-shoot tip was cultured on Murashige and Skoog (MS) basal medium (Murashing and Skoog, 1962), with 100 mg Inositol and a mix of vitamins (pyridoxine 0.1 mg, L^{-1}, thymine, 10 mg, L^{-1}, and nicotine, 1 mg.), 30g sucrose and 5 mg.L^{-1} BAP + 0.5 g.L^{-1} activated charcoal(AC). The medium was solidified with 7g agarand the pH of the Medium was adjusted to 5.8 before autoclaving and temperature was maintained at 27 and 16 hrs photo period (1000 lux) for one month.

Shoot multiplication

For multiplication shoot the developed shoot from initiation stage was cultured on MS medium supplemented with 100 mg Inositol and a mix of vitamins (pyridoxine 0.1 mg, L^{-1}, thymine, 10 mg, L^{-1}, and nicotine, 1 mg.), 30g sucrose and plant growth regulators as follow:

A- 4 BAP + 1.5 NAA

B- 5 BAP + 1.5 NAA

C- 0.1 TDZ + 1.5 NAA

D- 0.2 TDZ + 1.5 NAA

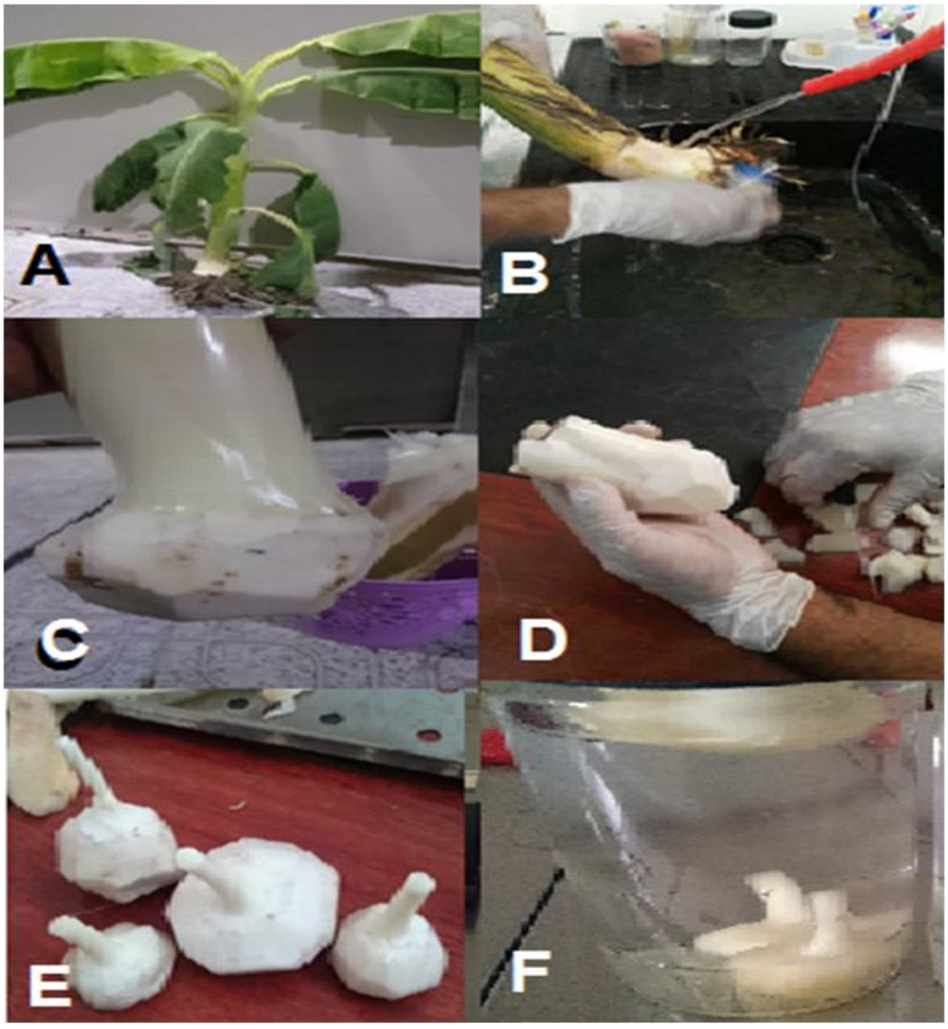

Fig. 1: Shoot tip preparation. A= Sucker B= base were washed under running tap water, C

The cultures incubated in chamber rom under temperature at 27 and 16 hrs photo period (1000 lux) (1000 lux) under two light sources, LED (18 Red : 2 Blue)and Whit fluorescent light for one month.At the end of incubated period the growth parameters was calculated as mean number of shoot, mean shoot length, shoot fresh and dry weight, and shoots content of chlorophyll and carotene and carbohydrate. Chlorophyll and carotenedeterminateaccording to the proposed method byGoodwin (1976) and carbohydrate. according to the proposed method by Herbert *et al.*, (1971) using spectrophotometer spectroscopy at a wavelength of 488 nm.

Experimental design and statistical analysis

The experiment was performed as a factorial experiment using complete randomized design (CRD), with two factor(Plant growth regulators and light quality). The data recorded were analyzed statistically using analysis of variance technique including two-way ANOVA. The averages of the treatments were compared using a Least Significant Differences (L.S.D) at a probability level of 0.05 to test the differences between the averages.

Results and Discussion

Number of shoots

Table 1 show that 0.1 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA was the best plant growth regulators combination for promote shoot multiplication, which gave the highest number of shoots. The results were agreeing with the results of Gubbuk and Pekmezci (2004, 2006), Makara *et al.,* (2010), and Bohra *et al.,* (2016) in which TDZ was stronger than BAP in encouraging the multiplication of Shoots in bananas. TDZ and NAA added to the nutrient medium is important in multiplying the number of shoots by tissue culture because of their positive effect on cell division and size, so they encourage more buds to form and enhance shoots growth. Light quality had a significant effect on the number of shoots. The highest shoot number (6.93 shoot) was under LED than under White fluorescent light. The interaction between plant growth regulators (TDZ and BAP, with consistently the concentration of NAA) and the type of lighting had a significant effect. The highest number of shoots (9.25) was in MS media equipped with 0.1 mg.L^{-1} TDZ and incubated under LED lighting, Which was significantly higher than the rest of the interaction treatments except for the interaction of 0.2 mg.L^{-1} TDZ under the LED light source (Fig. 2). This result may be due to the combined positive effect of both TDZ and LED lights on plant growth.

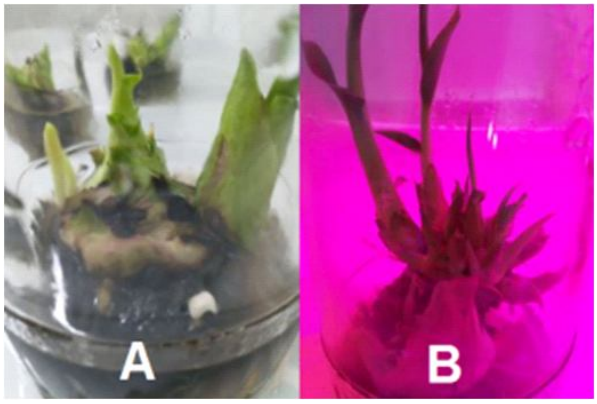

Fig. 2: Number of bud and multiplicated shoots of banana (*Musa spp*.) after 6 weeks of culturing on MS medium. supplemented with 0.1 mg.L^{-1} TDZ + 1 mg.L^{-1} NAA and incubated under White fluorescent light (A) and LED lamps(B).

Shoot Length

Table 2 indicates that there are significant differences in the shoot length The best plant growth regulator combination is 0.1 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA, which gave the highest shoot length)4.87cm), which differed significantly from the rest of the combinations followed by the combination of 0.2 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA and 5 mg.L^{-1} BAP + 1.5 mg.L^{-1} NAA respectively, while the combination 4 mg.L^{-1} BAP+1.5 mg.L^{-1} NAA gave the minimum length of shoots (1.37 cm). These results agree with the results of Youmbi *et al.,* (2006), Roy *et al.,* (2010) and Waman *et al.,* (2016) in that TDZ was stronger than BAP in promoting shoot length in bananas. This may be due to the same reasons given for the interpretation of the effect of TDZ in the number of shoots, as well as the role of cytokinein and NAA interference in the activation of chlorophyll and the absorption of water, causing shoot growth and elongation. As for the effect of the light quality in the length of the shoots there are no significant differences between them.As for the effect of the interaction between different concentrations of TDZ and BAP with a constant concentration of 1.5 mg.L^{-1} NAA and lightb quality the results of the same table showed that the highest rate of shoot length (5.00 cm) was found in cultures grown on MS medium with 0.1 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA and incubated under white fluorescent light, which differed significantly from the other interactions except 0.1 and 0.2 mg.L^{-1} TDZ and LED light source. This result was agreeing with result obtained by Trivedi and Sengar (2017). This result may be due to the combined positive impact of TDZ and LED on the shoots growth.

Shoots Fresh weight (g)

Table 3 shows a significant effect of growth

Table 1: The effect of Plant growth regulators and the Light quality and their interactions on the number of multiplicated shoots for banana (*Musa spp.*)CV.Dwarf Cavendish after 6 weeks of culturing on MS medium.

Plant growth regulators ($mg.L^{-1}$)	Light quality		Mean
	White fluorescent light	LED (18Red:2Blue)	
4 BAP + 1.5 NAA	2.75	4.00	3.37
5 BAP + 1.5 NAA	5.00	5.75	5.37
0.1 TDZ + 1.5 NAA	5.50	9.25	7.37
0.2 TDZ + 1.5 NAA	4.75	8.75	6.75
Mean	4.50	6.93	
LSD (0.05)	Plant growth regulators = 1.84 Light source = 1.30 Interaction = 2.61		

Table 2: The effect of Plant growth regulators and the Light quality and their interactions on the length of shoots for banana (*Musa spp.*)CV.Dwarf Cavendish after 6 weeks of culturing on MS medium.

Plant growth regulators ($mg.L^{-1}$)	Light quality		Mean
	White fluorescent light	LED (18Red:2Blue)	
4 BAP + 1.5 NAA	1.25	1.50	1.37
5 BAP + 1.5 NAA	1.73	1.80	1.76
0.1 TDZ + 1.5 NAA	5.00	4.75	4.87
0.2 TDZ + 1.5 NAA	3.62	4.12	3.87
Mean	2.90	3.04	
LSD (0.05)	Plant growth regulators = 0.67 Light source = NS Interaction = 0.95		

regulators in the MS medium on shoot fresh weight. A combination of 0.2 $mg.L^{-1}$ TDZ + 1.5 $mg.L^{-1}$ is superior for this parameter. The effect of cytokinins in general is its role in activating the absorption of water by the developing shoots and the transfer of nutrients within the shoots and it stimulates the division of cells and increase the number and then increase the carbohydrates accumulation, resulting in the shoot fresh and dry weight increase (Tan *et al.,* 2007 and Dabrowski *et al.,* 2015),This was observed in the results of our study in Tables 5 and 6 for chlorophyll, caroten and carbohydrate table 7. There was a significant effect on the light source in the light weight, with LED lamps significantly surpassing the fluorescent lamps by giving them the highest rate of 13.68 g. This is consistent with Nhut *et al.,* (2003) when exposing banana cultures to a mixture of red and blue LED. As for the effect of the interaction between different concentrations of TDZ and BA and light source, the results of the same table showed that the highest mean of fresh weight (20.28 g) was in cultures grown in a medium with 0.2 $mg.L^{-1}$ TDZ + 1.5 $mg.L^{-1}$ NAA and incubated under LED lamps, which was significantly higher than the rest of the interactions, while the lowest means (2.80 g) was in 4 $mg.L^{-1}$ BAP + 1.5 $mg.L^{-1}$ NAA with white fluorescent lamps. This result may be due to the combined positive impact of TDZ and LED in shoot growth.

Shoots Dry weight (g)

The dry weight of the shoots were also influenced by plant growth regulators when added in combination. The dry weight of the shoots varied under various cytokinin (BA and TDZ) and combinations with NAA(Table 4). With a combination of 0.2 $mg.L^{-1}$ TDZ + 1.5 $mg.L^{-1}$ NAA the highest mean (0.565g) was given, which differed significantly from the others interactions, while the combination 4 $mg.L^{-1}$ BAP + 1.5 $mg.L^{-1}$ NAA give the lowest mean (0.170 g). This is due to the same

Table 3: The effect of Plant growth regulators and the Light quality and their interactions on the fresh weight of shoots (g) for banana (*Musa spp.*)CV.Dwarf Cavendish after 6 weeks of culturing on MS medium.

Plant growth regulators ($mg.L^{-1}$)	Light quality		Mean
	White fluorescent light	LED (18Red:2Blue)	
4 BAP + 1.5 NAA	2.80	4.42	3.61
5 BAP + 1.5 NAA	9.98	11.48	10.73
0.1 TDZ + 1.5 NAA	12.95	18.56	15.75
0.2 TDZ + 1.5 NAA	15.18	20.28	17.73
Mean	10.22	13.68	
LSD (0.05)	Plant growth regulators = 0.96 Light source = 0.68 Interaction = 1.36		

Table 4: The effect of Plant growth regulators and the Light quality and their interactions on the shoots Dry weight (g) of banana (*Musa spp.*)CV.Dwarf Cavendish after 6 weeks of culturing on MS medium.

Plant growth regulators ($mg.L^{-1}$)	Light quality		Mean
	White fluorescent light	LED (18Red:2Blue)	
4 BAP + 1.5 NAA	0.140	0.200	0.170
5 BAP + 1.5 NAA	0.377	0.480	0.428
0.1 TDZ + 1.5 NAA	0.407	0.577	0.492
0.2 TDZ + 1.5 NAA	0.437	0.692	0.565
Mean	0.340	0.487	
LSD (0.05)	Plant growth regulators = 0.049 Light source = 0.0349 Interaction = 0.0698		

reasons as explained for shoot fresh water. The type of lighting lamps had a significant effect on the dry weight, as the source of LED lighting was better than the white fluorescent by giving the highest mean(0.487 g).As for the effect of inter**action** between the plant growth regulator and the type of lighting, the results in same table showed that the highest dry weight was in the cultured in MS with 0.2 mg.L^{-1}TDZ + 1.5 mg.L^{-1}NAA and incubated under the LED lighting source, which differed significantly from the **others** inter**actions**. This result may be due to the combined positive effect of both TDZ and LED.

Chlorophyll content of leaf

The results in Table 5 indicate that the type and concentration of cytokinins in MS has significant effect on leaf content of total chlorophyll, TDZ exceeded BAP. The highest content of chlorophyll (3.49 mg. 100 g^{-1} FW) for 0.1 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA, while the lowest content (4.97 mg.) was with 4 mg.L^{-1} BAP+ 1.5 mg.L^{-1} NAA. The reason is that TDZ works to activate the enzymes involved in the chlorophyll pigment synthesis.The same table shows that the source of light has a significant effect on this parameter with the superiority of the leaves of shoots under the LED lamps, which gave the highest content of chlorophyll (27.85 mg.100 g^{-1} FW) compared to the leaves under the white fluorescent lamps. This result is consistent with Viera *et al.,* (2015) and Trivedi and Sengar (2017) in bananas. As for the interaction between the type and concentration of cytokines with constant concentration of 1.5 mg.L^{-1} NAA and light source, the results of the same table show significant differences between them. With the interaction of 0.1 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA under LED lamps the highest content of chlorophyll was 50.18 mg, 100 g-1 soft weight, while interaction 4 mg.L^{-1} BAP + 1.5 mg.L^{-1} NAA under white fluorescent lamps with a minimum content of 4.47 mg. This result may be due to the combined positive effect of both TDZ and LED in shoot growth and promote the enzymes involved in chlorophyll biosynthesis.

Table 5: The effect of Plant growth regulators and the Light quality and their interactions on leaf chlorophyll content. (mg.100g^{-1}FW) for banana (*Musa spp*.)CV.Dwarf Cavendish after 6 weeks of culturing on MS medium.

Plant growth regulators (mg.L^{-1})	Light quality		Mean
	White fluorescent light	LED (18Red:2Blue)	
4 BAP + 1.5 NAA	4.47	5.48	4.97
5 BAP + 1.5 NAA	10.38	13.08	11.73
0.1 TDZ + 1.5 NAA	24.80	50.18	37.49
0.2 TDZ + 1.5 NAA	19.95	42.68	31.31
Mean	14.90	27.85	
LSD (0.05)	Plant growth regulators = 0.90 Light source = 0.63 Interaction = 1.27		

Table 6: The effect of Plant growth regulators and the Light quality and their interactions on leaf carotene content. (mg.100g^{-1}FW) for banana (*Musa spp*.)CV.Dwarf Cavendish after 6 weeks of culturing on MS medium.

Plant growth regulators (mg.L^{-1})	Light quality		Mean
	White fluorescent light	LED (18Red:2Blue)	
4 BAP + 1.5 NAA	0.362	1.723	
5 BAP + 1.5 NAA	0.598	1.880	
0.1 TDZ + 1.5 NAA	1.663	5.070	
0.2 TDZ + 1.5 NAA	1.588	4.955	
Mean	1.052	3.407	
LSD (0.05)	Plant growth regulators = 0.286 Light source = 0.202 Interaction = 0.404		

Carotene content of leaf

The results of Table (6) show that the concentrations of 0.1 and 0.2 mg.L^{-1} TDZ added to the MS medium with a concentration constant of 1.5 mg.L^{-1} NAA resulted in a significant increase in the content of the shoots of carotene compared with the BAP. The highest mean (3.366 mg.100 g^{-1} FW) was given by added 0.1 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA, which was followed by 0.2 mg.L^{-1} NAA, while 4 mg.L^{-1} BAP + 1.5 mg.L^{-1} NAA gave the lowest content of carotene (1.042 mg.100 g^{-1} FW). This may be due to the effect of TDZ in increasing the activation of enzymes contributing to the carotene biosynthesis in shoot. The results of the same table show that the lighting source has a significant effect on the content of the carotene, as the growing shoots under the LED lamps have been more successful by giving them 3.407 mg.100 g^{-1} FW compared to the growing shoots under the white fluorescent lamps which gave 1.052 mg.100 g^{-1} FW.As for the interaction, added 0.1 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA under LED lamps in the carotene content (5,070 mg.100 g^{-1} FW) was superior to all other interference treatment plants except for the treatment of 0.2 mg.L^{-1} TDZ + 1.5 mg.L^{-1} NAA under LED lamps, while 4 mg.L^{-1}mg.L^{-1} BAP + 1.5 mg.L^{-1} NAA with white fluorescent lamps interference was given. the lowest content of carotene (0.362 mg.100 g^{-1} FW). This result may be due to the combined positive effect of both TDZ and LED for carotene biosynthesis.

Table 7: The effect of Plant growth regulators and the Light quality and their interactions on shoots total carbohydrate (%) for banana (*Musa spp*.)CV.Dwarf Cavendish after 6 weeks of culturing on MS medium.

Plant growth regulators ($mg.L^{-1}$)	Light quality		Mean
	White fluorescent light	LED (18Red:2Blue)	
4 BAP + 1.5 NAA	1.958	2.035	1.996
5 BAP + 1.5 NAA	2.025	2.275	2.150
0.1 TDZ + 1.5 NAA	2.125	2.750	2.437
0.2 TDZ + 1.5 NAA	2.260	2.917	2.588
Mean	2.092	2.494	
LSD (0.05)	Plant growth regulators = 0.118 Light source = 0.085 Interaction = 0.167		

Total carbohydrate content of shoots

From the results of Table (7), there is a significant differences between BAP and TDZ in their effects in total carbohydrate content of the shoots. TDZ at 0.2 $mg.L^{-1}$ plus 1.5 $mg.L^{-1}$ NAA gave the highest carbohydrate content (2.588%), which differed significantly from the others, while 4 $mg.L^{-1}$ BAP + 1.5 $mg.L^{-1}$ NAA gave the lowest percentage of carbohydrates amounted to 1.996%.The lighting type had a significant effect on carbohydrate content, with LED lamps significantly exceeding normal lighting by giving them the highest carbohydrate content. This may be due to the results of our study in Tables 5 and 6 in terms of the effect of LED light on the increase in the pigments content of the multiplying shoots,This has been positively reflected in the increase in the carbohydrate content of the shoots. As for the effect of the interaction between different concentrations of TDZ and BAP with a constant concentration of 1.5 $mg.L^{-1}$ NAA and the type of light, the results of the same table showed that the highest carbohydrate content (2.917%) was in the shoots culture in a medium equipped with 0.2 $mg.L^{-1}$ TDZ + 1.5 $mg.L^{-1}$ NAA and incubated under LED lamps which gave significant differences from the other interactions, except for the 0.1 $mg.L^{-1}$ TDZ + 1.5 $mg.L^{-1}$ NAA and LED lighting. This result may be due to the combined positive effect of both TDZ and LED in shoot growth.

Conclusions and Recommendations

The effect of TDZ was stronger than the BAP effect in shoots multiplication, and the addition of NAA to the multiplication medium provided by BAP or TDZ improved the multiplying of shoots and their lengths. The use of LEDs (red + blue) by 2:18 was more effective than the use of white fluorescent lamps in number of shoots and their phenotypes and their content of plant pigments and carbohydrates. We recommend using BAP at a concentration of 5 $mg.L^{-1}$ or TDZ 0.2 $mg.L^{-1}$ plus 1.5 $mg.L^{-1}$ NAA in nutrient medium of the multiplication with the use of LEDs (red + blue) at 2:18 at this stage

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