Effect of light Intensity and Temperature on in vitro Somatic Embryos Germination Stage in Date Palm

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KEYWORDS

Embryos germination, light intensity, Phoenix dactylifera L., Physical factors, Somatic embryogenesis, Temperature.

ABSTRACT

The aim of this study is to detect the extent of physical factors (light intensity and temperature) effect on proliferation and germination of date palm somatic embryos i*n vitro*. The treatments of this study were carried out by using two degrees of temperature (18±2ºC & 28±2ºC) with four levels of light intensity (0, 20, 40 and 60 µmol m^{-2} s^{-1}). The obtained results revealed the following: The results showed that there are significant differences between the eight treatments of physical factors under study as the results monitored that the highest average for No. of somatic embryos germination was for the explants cultured at light intensity of 40 µmol m^{-2} s^{-1} and under low temperature of 18±2ºC, whereas the highest average for germination lengths was for the explants cultured at light intensity of 40 µmol m^{-2} s^{-1} and high temperature of 28±2ºC all over the study. While, the lowest values were for the explants cultured at total darkness.

تأثير شدة الإضاءة ودرجة الحرارة على مرحلة إنبات الأجنة الجسدية في نخيل التمر معملياً

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الكلمات الدالة

نبات الأجنة الجسدية، شدة الإضاءة، نخيل التمر، الظروف الفيزيائية، الأجنة الجسدية، درجة الحرارة.

المُستلخص

إن الهدف من هذه الدراسة هو التعرف على مدى تأثير العوامل الفيزيائية (شدة الإضاءة ودرجة الحرارة) على تكشف وإنبات الأجنة الجسدية لنخيل التمر معملياً. وقد نفذت هذه الدراسة باستخدام أثنين من درجات الحرارة (18±2ºC & 28±2ºC) مع أربعة مستويات من شدة الإضاءة (0, 20, 40 and 60 µmol m^{-2} s^{-1}). وقد كشفت النتائج المتحصل عليها ما يلي: أظهرت النتائج أن هناك فروقاً معنوية بين الثمانية معاملات للعوامل الفيزيائية قيد الدراسة، كما رصدت النتائج أن أعلى معدل لعدد إنبات الأجنة الجسدية كان من الأجزاء النباتية المنزرعة في شدة إضاءة (40 µmol m^{-2} s^{-1}) وتحت درجة الحرارة المنخفضة (18±2ºC). في حين كان أعلى متوسط لاستطالة الأجنة المنبتة مع الأجزاء النباتية المنزرعة تحت نفس شدة إضاءة (40 µmol m^{-2} s^{-1}) ولكن مع درجة الحرارة المرتفعة (28±2ºC). بينما كانت أدنى قيمة بشكل عام مع الأجزاء النباتية المنزرعة في إظلام تام.

Introduction

The somatic embryogenesis is one of *in vitro* date palm (*Phoenix dactylifera* L.) micropropagation techniques that tissue culture laboratories greatly counted on in addition to organogenesis. Despite the high yield of embryogenesis it takes a long period of time because of time needed for going through callus formation stage in addition to the probability of mutations occurrence or occurrence of somatic differences than the origin on the explant at any stage of the different growth stages. On contrary to the direct method (organogenesis) that gives true to type plants in a short period of time but with lower yield than embryogenesis **(Al-Sakran and Muneer, 2006), (Belal, *et al*., 2008a), (Shehata, 2008), (Al-Khayri, 2010), (Abul-Soad and Mahdi, 2010), and (Fki, *et al*., 2011)**.

Date palm is unisexual dicotyledonous plant thus it needs pollination and fertilization to from sexual zygote **(Lunde, 1978)**. While, the *in vitro* somatic zygote is formed as a result of fission and fusion of undifferentiated somatic parenchymal cells (callus) to form the somatic zygote which forms 100 % true to type plants that only acquire the characters of the mother plant on contrary to the sexual zygote that acquire the characters of both parents besides the Fifty/Fifty probability of producing males and females **(Razdan, 2003), (Fehér, 2005) and (Belal, *et al*., 2008b)**.

Somatic embryogenesis and embryos germination are highly important i*n vitro* growth and development stages of date palm through embryogenesis that extremely determine the number of the *in vitro* resultants plants ready for later acclimatization stage inside greenhouse **(Zimmerman, 1993) and (Ramos, *et al*., 2012)**.

Generally, any embryo (somatic or sexual) passes through different main must five growth stages in its way to germination **(Tisserat, *et al*., 1979), (Singh, 1978), and (Torres, 1989)** starting with being a cell at which primary fission occurs to form complicated undifferentiated cells (callus) passing through globular embryo then heart embryo then torpedo embryo with its bipolar shape at which the embryo becomes cotyledonous then one pole goes up towards light (phototropism) to form shoots and the other goes downwards (gravitropism) to form roots till it reaches the final stage of growth by being a mature plant **(Kyte, 1987) and (Razdan, 2003)**. Any embryo must passes through these main five stages, either somatic or sexual embryos. Regarding to the importance of embryogenesis stage for date palm many research studies were carried out on this important stage but most of them concentrated on studying the chemical factors like **(Barrett, *et al*., 1997), (Khlifi and Tremblay, 1995), (Al-Khayri, 2001), (Alkhateeb, 2008), (Sghaier, *et al*., 2009), (Al-Khayri, 2011), and (Boufis, *et al*., 2014)**, only few studies showed interest about studying the physical factors (environmental) like **(Veramendi and Navarro, 1996), (Shehata, 2008), and (El-Ashry, *et al*., 2013)**.

Subsequently, the concept of this study was to detect the extent of effect of physical factors represented by two degrees of temperature and four levels of light intensity on *in vitro* date palm somatic embryos germination because of the important role and the noteworthy effect of these factors for all (whether superior or inferior) human beings in general on completion of most biological processes particularly in plant cells and tissues in addition to the high importance of this growth stage. This study will determine the extent of effect of those physical factors on growth and germination of the somatic embryos of date palm.

1. MATERIALS AND METHODS

This work was carried out in the Tissue Culture Lab. of Plant Production, College of Environmental Agricultural Sciences, North Sinai, El-Arish University, Egypt.

1.1. Somatic embryogenesis materials

Somatic embryos obtained from culturing explant cut from 5 years old date palm Samany *cv*., were used in this study these resultantant somatic embryos (Table 2), were formed from callus resulted from culturing the explant on the callus induction modified medium (DP_1), for six months followed by reculturing on the embryogenic callus modified medium (DP_2), for three months. Then transferred on the embryogenesis modified medium

(DP_3) for seven months with the aim of formation of the somatic embryos from the cultured callus (Table 1). The pH level was adjusted at 5.7±0.1 before adding gerlite and autoclaving the medium at 1.05 $Kg.cm^{-2}$ equivalent to 121ºC for 20 min. The nutrient media were dispensed into medial jars 50 ml. for seven subcultures to obtain germination of somatic embryos (Table 2).

Table 1. Nutrient medium composition for in vitro germination of somatic embryos stage of date palm.

Modified media Salts Strength	**Composition medium (mg l^{-1})**			
	Organic Constituent›s	**Growth regulators**	**Complex addenda**	
1. Callus induction (DP_1)	**Full MS** *	100 Myo-Inositol + 80 Adenine Sulfate + 170 $NaH_2PO_4.2H_2O$ + 2 Thiamine-HCl + 2 Glutamine + 2 Ca-Pantothenate + 2 Biotin	10 NAA + 10 IAA + 30 2,4-D	30000 Suc. + 2000 Gelrite + 3000 Activated charcoal (AC)
2. Embryogenic callus (DP_2)			Free hormones	
3. Embryogenesis (DP_3)			2.5 NAA + 3 BAP + 2 Kin	
4. Embryo germination (DP_3)		The same hormones		

* MS medium **(Murashige and Skoog, 1962)**.

1.2. Physical factors (conditions)

Eight treatments were used in this study, as embryos were cultured at growth room for seven months under two degrees of temperature (low: 18±2 ºC and high: 28±2 ºC), and four levels of light intensities (0, 20, 40 and 60 µmol m^{-2} s^{-1} ≈ 0, 1000, 2000, 3000 Lux, respectively), at photoperiod of 16/8 hrs of light / dark cycles daily. Cool white fluorescence lamps of 36 watt were put at distance of 45 cm from jars.

Noted: µmol. Photons m^{-2} s^{-1} = Lux X 0.0165 **(Hershey, 1991)**,

1.3. Statistical analysis

Data was statistically analyzed by analysis of variance (ANOVA) for the completely randomized design according to **(Snedecor and Cochran, 1990)**. The treatment means were compared using Least Significant Difference (LSD) at 5 % level of probability. Mean separations were done by using a Mstat computer program v.4 **(Duncan, 1955)**. Data were recorded for every treatment at the end of the seven subcultures 1, 2, 3, 4, 5, 6 and 7 mon. from initial culture respectively). Each treatment had five replicates and each replicate = 5 jars and each jar contains 2-3 embryonic callus. The data were presented as the average per embryoids as following: embryo germination number and lengths (cm).

2. Results

The objective of this study is to test the effect of physical conditions (two degrees of temperature and four levels of light intensity) on growth and development of embryos germination (*Phoenix dactylifera* L. *cv*. Samany) *in vitro*.

2.1 Effect of physical conditions on proliferation and formation of somatic embryos

Data present in (Table 2) showing that the interaction effect of temperature and light intensity on proliferation and formation of somatic embryos of date palm Samany *cv*. *in vitro*. Revealed that the highest rate of embryogenic callus (5.00a and 4.00b, respectively) was at 18±2 ºC followed by 28±2 ºC with darkness (0.0 µmol m^{-2} s^{-1}). While, the worst rate (1.00e and 2.00d) was at 28±2 ºC & 18±2 ºC, respectively with high light intensity of (60.0 µmol m^{-2} s^{-1}) during somatic embryogenesis stage.

On the other hand, concerning the interaction effect of physical conditions on No. of embryos: The results in the same table, show the interaction effect of eight different treatments between temperatures and light intensities on number of embryos during seven months of cultures of Samany *cv*. The data reflected that the highest mean (36.00a) was achieved at 18±2 ºC in darkness

followed by 28±2 ºC under the same light intensity (0.0 µmol m^{-2} s^{-1}). Whereas, the lowest mean of number of embryos (16.80b) was noticed at 28±2 ºC with light intensity (60.0 µmol m^{-2} s^{-1}) during the same stage. **(Veramendi and Navarro, 1996), (Shehata, 2008), and (Tongtape and Te-chato, 2010)** obtained similar results.

Table 2: Average of embryos number formation during somatic embryogenesis stage of Samany *cv. in vitro.*

Temperature (ºC)	18±2				28±2			
Light intensity (µmol m^{-2} s^{-1})	**0.0**	**20.0**	**40.0**	**60.0**	**0.0**	**20.0**	**40.0**	**60.0**
Embryogenic callus	5.00a	3.00c	3.00c	2.00d	4.00b	3.00c	2.00d	1.00e
Embryos number	36.00a	28.60bc	25.40bcd	19.60de	31.00ab	23.20cd	19.60de	16.80e

* P-values less than 0.05 are significant.

2.2 Effect of physical conditions on numbers and lengths of embryos germination

Table (3) shows the specific effect of temperature on embryos germination of date palm Samany *cv*. *in vitro*. The results declared that low temperature (18±2 ºC) was better than the high one for germinated embryos number (16.69a), conversely the high temperature (28±2 ºC) gave the highest average for shoot length of the germinated embryos (2.78a), than the lower one.

The results also showed the specific effect of light intensity on this stage and the results declared that the best light intensity for embryos germination no. (18.84a) was 40.0, then 20.0, then 60.0 µmol m^{-2} s^{-1}, respectively. The moderate light intensity (40.0 µmol m^{-2} s^{-1}) gave the highest means for the germinated embryos length (3.09a cm) then 60.0 then 20.0 µmol m^{-2} s^{-1} (2.92a & 2.58ab, respectively). While, embryos cultured at total darkness gave the lowest means in general for both embryos number and shoot length (8.33c and 1.54b, respectively).

These results generally matches with the nature as the internal processes like fission growth and development of cells and tissue as well as growth and development of embryos require low temperature and low light intensity almost zero **(Shehata, 2008)**. While, external processes like embryos germinating, shooting, rooting, flowering and fruiting require high temperature and high light intensity for completion of these processes **(Belal, *et al*., 2008b)**.

On another hand, Table (4) shows the interaction effect between physical factors under study (temperature and light intensity) on germination number of somatic embryos *in vitro* and on the germinated embryos length (cm), the results revealed that:

The highest mean for temperature effect on embryos number (15.69a) was achieved at the low temperature (18±2ºC). While, the highest averages concerning the germinated embryos length (2.82a) was at the high temperature (28±2 ºC).

Concerning light intensity, the highest mean for light intensity effect on germinated embryos no. (18.97a) was achieved at (40.0 µmol m^{-2} s^{-1}), followed by (20.0 µmol m^{-2} s^{-1}) that achieved (16.97ab) and their lowest average was for the embryos cultured at total darkness (9.04c). While, the highest mean of germinated embryos length (3.07a cm) were achieved at light intensity of 40 then 60 followed by 20 µmol m^{-2} s^{-1} respectively, eventually the lowest averages for germinated embryos length (shoot length-cm) were for those cultured at total darkness (1.61b).

Thus, it can be concluded that the best result for the interaction effect between the physical factors on somatic embryos germination No. was at the low temperature (18±2 ºC) and light intensity of (40, 20 µmol m^{-2} s^{-1}, respectively). Whereas, the best result for the interaction effects on shoot length was at the high temperature (28±2 ºC) and at the high light intensity of 40, 60, then 20 µmol m^{-2} s^{-1}, respectively. While, the lowest results of physical factors interaction effect on parameters of the study were with the embryos cultured at total darkness in general either at the high temperature concerning the germinated embryos number or at the low temperature concerning germinated embryos length.

3. Discussion

The obtained results revealed that somatic embryos proliferation and germination was better at the high temperature (28±2 ºC) with moderate light intensity of (40.0 µmol m^{-2} s^{-1}) as there were high significant differences than the other seven treatments. On another side, the lowest somatic embryos germination rate occurred with those cultured at low temperature (18±2 ºC) with the different light intensity levels, as well as with those cultured at total darkness with the different temperature degrees (18±2 ºC & 28±2 ºC).

The results also declared that somatic embryos cultured at total darkness gave a convenient percentage of germination but with weak and short shoots, this is may be attributed to the low chlorophyll content (SPAD) in the produced shoots, as they were white then lately, turned into green after subjection to light, and grown naturally, which reflects the important role of light which probably affected the metabolic rate greatly that led to weakening their growth greatly because of lowering the metabolic rates specially photosynthesis rate **(Friedrich, 1998), and (Kornberg, 2000)**.

On the contrary, this is may be attributed to that the catabolism rate was higher than the photosynthesis rate due to elevation of the temperature and the light intensity over the normal levels which subsequently led to lowering photosynthesis rate of the somatic embryos that negatively affect their germination rate greatly. That is what has been clearly revealed by the study parameters represented in the germination embryos number and their weak growth obviously **(Hothersall and Ahmed, 2013)**.

Plants differ from other Organisms by being autotrophic through photosynthesis process characteristic for plants only. Thus, light and temperature play a very important and effective role in completion of this highly important process **(Friedrich, 1998)**. Which, simple substances turns into more complex ones like carbohydrates, lipids and proteins **(Kornberg, 2000). (Veramendi and Navarro, 1996), (Shehata, 2008), and (El-Ashry, *et al*., 2013)** also reported the important role of both light intensity and temperature in formation and growth of date palm somatic embryos *in vitro*.

As either plant cells or tissues or organs can't carry out metabolism in absence of light source especially *in vitro*, as they suffer from high humidity ratio and low gas exchange rate inside the jars even if they were supplied with variant sources and high concentrations of some vitamins **(Al-Khayri, 2001)**, or carbohydrates **(Alkhateeb, 2008) or hormones (Al-Khayri, 2003), (Sghaier, *et al*., 2009), and (Boufis, *et al*., 2014)** as they can't compensate for light that is a very important basic factor for completing metabolism process at any stage of the different growth stages for any plant **(Veramendi and Navarro, 1996), (Belal, *et al*., 2008a&b), (Shehata, 2008), and (El-Ashry, *et al*., 2013)**.

Table 3. Specific effect of temperature and light intensity on embryo germination stage of Samany *cv. in vitro.*

Param.	Factors	Treatments	Culture period (Month)							Mean
			1 mon	2 mon	3 mon	4 mon	5 mon	6mon	7 mon	
a. No. of somatic embryos germination	Temperature (ºC)	18±۲	4.55a	7.75a	12.05a	15.25a	20.75a	25.50a	30.95a	**16.69a**
		28±۲	3.50b	5.30b	8.45b	12.65b	19.55b	23.20b	28.00b	**14.38b**
	Temperature Mean		**4.03**	**6.53**	**10.25**	**13.95**	**20.15**	**24.35**	**29.48**	
	Light intensity (µmol m^{-2} s^{-1})	**0.0**	1.70c	3.50b	4.30c	7.60b	9.50c	13.10c	18.60c	**8.33c**
		20.0	4.40ab	7.10a	13.50a	16.10a	22.40ab	27.80ab	31.40ab	**17.53ab**
		40.0	6.10a	9.20a	13.40a	14.40a	25.10a	30.20a	33.50a	**18.84a**
		60.0	3.90b	6.30ab	10.20b	11.30ab	19.60b	25.70b	30.00b	**15.29b**
	Light intensity Mean		**4.03**	**6.53**	**10.35**	**12.35**	**19.15**	**24.20**	**28.38**	

b. Germination lengths (cm)	**Temperature** (ºC)	**18±۲**	1.78a	2.18a	2.20b	2.30b	2.53b	2.53b	2.75b	**2.32b**
		28±۲	1.63b	2.01b	2.93a	3.21a	2.75a	3.43a	3.51a	**2.78a**
	Temperature Mean		**1.71**	**2.10**	**2.57**	**2.53**	**2.87**	**2.98**	**3.13**	
	Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	**0.0**	1.00b	1.25b	1.45b	1.55b	1.60b	1.70c	2.25b	**1.54c**
		20.0	1.40ab	2.23a	2.63ab	2.75a	2.83ab	2.85b	3.40ab	**2.58b**
		40.0	2.15a	2.50a	3.03a	3.28a	3.35a	3.55a	3.80a	**3.09a**
		60.0	2.00a	2.25a	2.88a	3.15a	3.20a	3.33ab	3.65a	**2.92a**
	Light intensity Mean		**1.64**	**2.06**	**2.50**	**2.68**	**2.75**	**2.86**	**3.28**	

* P-values less than 0.05 are significant.

Table 4. Interaction effect of temperature and light intensity on embryo germination stage of Samany *cv. in vitro.*

Parameters	**Temperature (ºC)**	**Time (Month)**	**Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)**				**Time average**	**Temperature average**
			0.0	**20.0**	**40.0**	**60.0**		
a. No. of somatic embryos germination	**18±2**	**First**	1.60d	5.00b	7.40a	4.20b	**4.55**	**15.69a**
		Second	4.80cd	8.00b	11.40a	6.80bc	**7.75**	
		Third	6.00c	15.80a	16.80a	10.40b	**12.25**	
		Fourth	3.60c	16.00ab	17.80a	10.80 b	**12.05**	
		Fifth	5.60d	21.40b	30.40a	17.60bc	**18.75**	
		Sixth	9.60d	30.60ab	35.80a	26.00b	**25.50**	
		Seventh	14.40c	28.80b	40.40a	32.40b	**29.00**	
	28±2	**First**	1.80cd	3.80b	4.80b	3.60bc	**3.50**	**14.44b**
		Second	2.20d	6.20bc	7.00bc	5.80bc	**5.30**	
		Third	2.60d	11.20b	10.00b	10.00b	**8.45**	
		Fourth	11.60b	14.40ab	12.80ab	11.80b	**12.65**	
		Fifth	13.40c	23.40b	19.80b	21.60b	**19.55**	
		Sixth	16.60c	26.20b	24.60b	25.40b	**23.20**	
		Seventh	32.80b	26.80b	26.60b	27.60b	**28.45**	
Light intensity average			**9.04c**	**16.97ab**	**18.97a**	**15.29b**		
b. Germination lengths (cm)	**18±2**	**First**	0.80c	1.20bc	1.70abc	1.90ab	**1.40**	**2.29b**
		Second	1.60a	2.40a	2.80a	2.10a	**2.23**	
		Third	1.30c	2.70ab	3.10a	2.40ab	**2.38**	
		Fourth	1.10c	2.50b	3.00ab	2.50b	**2.28**	
		Fifth	1.30c	2.70ab	2.90ab	2.60ab	**2.38**	
		Sixth	1.00e	2.50cd	3.20bc	3.00c	**2.43**	
		Seventh	2.10c	3.10abc	3.20abc	3.40ab	**2.95**	
	28±2	**First**	1.20bc	1.60abc	2.10a	2.10a	**1.88**	**2.82a**
		Second	1.80a	2.05a	2.60a	2.10a	**2.01**	
		Third	1.60bc	2.55ab	3.05ab	3.40a	**2.75**	
		Fourth	2.00bc	3.00ab	3.45a	3.65a	**2.93**	
		Fifth	2.10bc	2.95ab	3.90a	3.90a	**3.21**	
		Sixth	2.20d	3.20bc	3.90a	4.05a	**3.34**	
		Seventh	2.40c	3.70ab	4.10a	4.20a	**3.60**	
Light intensity average			**1.61c**	**2.58b**	**3.07a**	**2.95a**		

* P-values less than 0.05 are significant.

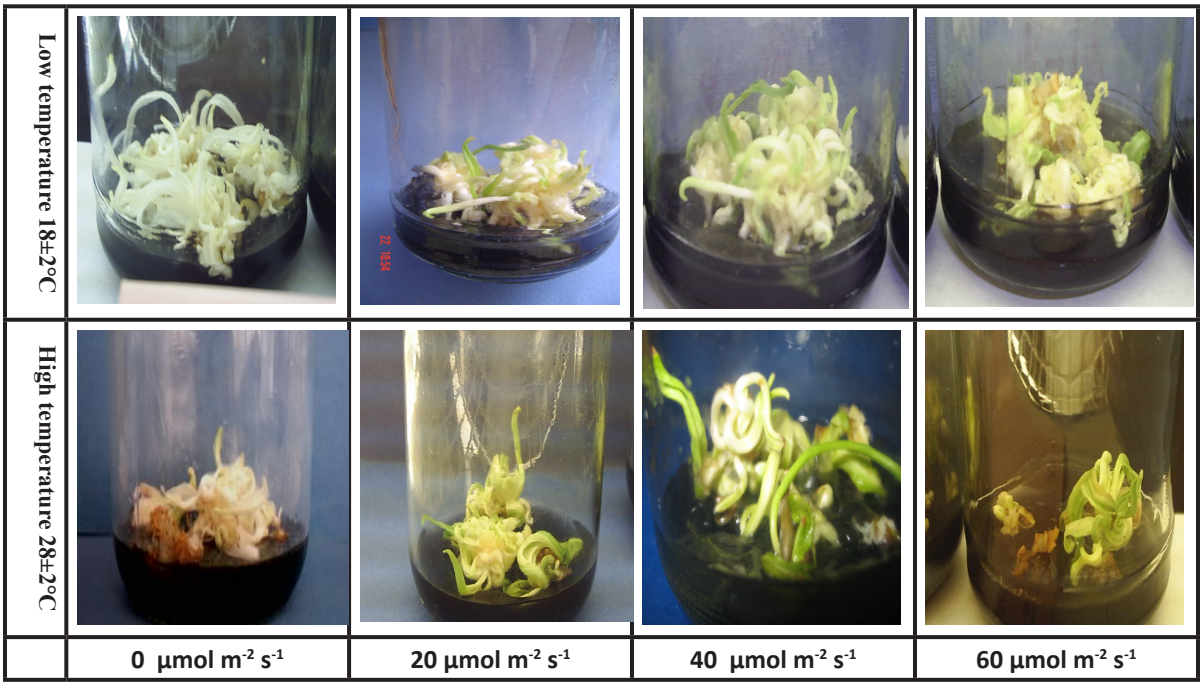

Figure 1. Show the interaction effect of different two degrees from temperature and four levels from light intensities on embryo germination stage in Samany *cv. in vitro.*

Conclusion (Recommendations)

According to this study and to the ex-studies investigating the effect of physical factors (represented by temperature and light intensity) on different growth and development stages of date palm *in vitro*, we can summarize the most important results as the following:

The optimum temperature for all date palm growth stages *in vitro* is (25±3 ºC) except for both proliferation and production of somatic embryogenesis and somatic embryos germination stages, that requires low temperature about (18±2 ºC), and lower temperature than 18 ºC results in lowering fission and growth rate of the explant in general. While, higher temperature then 28 ºC results in adverse effects for all growth stages.

Concerning light intensity, it was found that the optimum light intensity for all different growth stages is (20, 40 µmol m^{-2} s^{-1} ≈ 1000, 2000 lux) except for callus formation and somatic embryogenesis stages that absolutely don't need any light source (i.e cultured at total darkness). The higher light intensity than (40 µmol m^{-2} s^{-1}) adversely affects the results despite that rooting stage require high light intensity (i.e more than 60 µmol m^{-2} s^{-1} ≈ 3000 lux).

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