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Evaluation of Hormone Treatment on Callus Length and Weight in Summer Safflower Cultivars

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Abstract:

With respect to country need to edible oils, it is more important to extend the cultivation of oilseeds. Although, safflower is the native plant of Iran and it is resistant to drought and salty stresses, but a few studies have been done on it. Today, improvement and manipulating of some of undesirable traits of safflower is possible by genetic engineering tools. During the manipulating process, important stages of tissue culture specially, regeneration phase of deformed plant has high worth. In order to evaluate the effect of explants, type of hormones and hormone levels on callus length and weight in summer safflower cultivars, an experiment was carried out in factorial based on randomized complete block design with 3 replications and four factors (safflower cultivars, explants, type of hormones and hormone levels) in 2011. Results showed that safflower cultivars (Iraqi 222 and 34069) and explants (hypocotyle and cotyledon) had a significant effect on the callus length. Based on the results, the effect of hormones and hormone levels were significant on callus weight. 34069 and hypocotyle were known as a premier compounds due to maximum length of callus and Iraqi 222 at both explants were known as low yield compound due to the shortest callus length. Also it was revealed maximum callus weight was related to hormone levels (2 mg per liter) and Zeatin hormone.

Keywords: Safflower, Hormone Levels, Callus Length and Weight.

1.0 Introduction:

Safflower (*Carthamus tinctorious* L.) is a plant of Asteracea family that favorite and specific traits such as medical, industrial and food usages of its petals, high quality of oil, broad adaptation to low winter and high summer temperatures and short growing season in summer cultivation have made it valuable plant (Ahmadi,et al.1996). It is more important to extend the cultivation of oilseeds because of supplying the main part of oil from overseas and increasing of population. Safflower is one of the oilseeds that have relatively resistance to drought and salty conditions and it can be cultivated in arid and semi arid region (Anwar,et al 1993). Although, safflower is the native plant of Iran and the wild type of it can be found in Iran But it has been ignored and a few studies have been done on it (Ahmadi,et al.1996and Omidi Tabrizi, et al.2000). The average of seed yield of safflower is about 500 kg per hectare in Iran that it is lower than the world average (795 kg per hectare) (Tejovathi,et al. 1990). It has potential of yield about 4 tons per hectare, so that in some experiments it was harvested more than 4.5 tons per hectare. However, the yield of 2 tons per hectare is considered as good yield (Omidi Tabrizi, et al.2000). Safflower's oil is one of the best edible oils due to having about 90 percent of unsaturated fatty acids. There is approximately 270 mg-tocopherols per each kg of oil that this substance causes oil stability at high temperatures (Pasban Slame,2004). The genetic manipulating in safflower cultivars by genetic engineering and achievement to favorite traits is the basic goals. It needs to tissue culture because of this manipulating, and therefore, the achievement to safflower regeneration rate and condition helps more to reach breeding goals. Studies show that the regeneration is possible at concentrations of 0.1 Naphthalene Acetic Acid (NAA) and 2, 2 BAP, but it differs depending on genotype and light conditions (Anwar,et al 1993 and Chatterji, et al 1940). Physiological studies show that giberlic acid is necessary for making pollen (Potter,et al.1940) and absisic acid plays main role in formation of safflower

flower (Baydar,2001). It is widely considered that morphogenesis is strongly affected by genetic and exogenous Factors(Bhaskaran, et al 1990). The main goal of this experiment is selection of type of hormones and hormone levels of callusing and also the evaluation of the effect of explants and safflower cultivars on callus length and weight.

2.0 Materials and Methods:

An experiment was carried out in factorial based on randomized complete block design with 3 replications in 2011. Factor A was including two safflower cultivars named: Iragi 222 and 34069, factor B was including two explants (hypocotyle and cotyledon), factor C was including three kinds of hormone named: Kianitin, zeatin and BAP, and factor D was including four levels of hormone concentrations (0.5, 1, 1.5 and 2 mg per liter). Hypocoloride sodium (2%) was used for disinfection of seeds for two minutes and then the seeds were washed in alcohol 70% for 12 minutes. It was done on vortex to sterile the seeds. Finally the seeds were washed by distilled water to remove remains. It was carried out for both cultivars. The sterilized seeds were cultured on MS medium and were placed in darkness for two days until the seeds have germinated and were etuleged. Then the seeds were placed in light for 6 days of 2000 lux. At last hypocotyl and cotyledon were transferred to medium containing 0.5, 1, 1.5 and 2 mg per liter of BAP, Zeatin and Kainitin hormones. Rate of consumption sucrose was 3% of volumetric weight.

2.1 Growth Chamber Conditions:

1- Lightning: 12 hours with 2500 lux at 25 ° C
2- Darkness: 8 hours at 2 ° C.

2.2 Evaluated Traits:

1- Callus length (were measured after 63 days of culturing)
2- Callus weight (were measured after 72 days of culturing).After ensuring of assumption establish of variance analysis, data were analyzed and averages were compared with Duncan test at 5% probability level. Also SPSS and MSTATC software were used for data statistical analysis.

3.0 Results and Discussion:

3.1.Variance Analysis of Studied Traits:

The results of variance analysis of studied traits have been inserted in table 1. It shows that there was significant difference among safflower cultivars with respect to callus length. Therefore it can be used for callus length in selection programs. The effect of explants (hypocotyle and cotyledon) was significant on the callus length at 1% probability level (table 1). Not significant of callus length under hormone types and levels showed high stability of callus length and lack of difference among safflower cultivars due to the hormone types and levels response. The cultivar × explants interaction was significant for callus length at 5% probability level that It means the dissimilar reaction of two safflower cultivars at explants. Also the cultivar × explants × hormones type triple interaction was significant at 5% probability level. Not significant of other factors and their interaction in regard to callus length showed the similar effect of this factors on callus length.

Table1: variance analysis of studied factors on callus length and weight.

SOURCE OF VARIANT	Means of square	
	df	cl cw
R	2	0.611	0.001
C	1	114.847**	0.004
EX	1	20.4*	0.006
HT	2	2.114	0.441**
HL	3	1.183	0.605**
C.EX	1	8.663*	0.0001
C.HK	2	3.948	0.001
C.HL	3	1.045	0.003
EX.HK	2	0.686	0.009
EX.HL	3	3.564	0.003
HK.HL	6	1.191	0.254**
C.EX.HK	2	5.760*	0.007
C.EX.HL	3	0.418	0.006
C.HK.HL	6	2.194	0.008
EX.HK.HL	6	0.963	0.003
C.EX.HK.HL	6	0.671	0.004
E	94	1.830	0.005
CV(%)		%36.88	%16.31

* and ** , significant in 5% and 1% , respectively
R:Replication; C: Cultivar; EX: Explant; HK: Hormone levels; HL: Hormone levels; E: Error
CV: Coefficient of variation; CL: Callus length; CW: Callus weight

The results of variance analysis revealed that the effect of type of hormones and hormone levels were significant on callus weight at 1% probability level. Also the type of hormones had different reaction related to different levels of applied hormones. Having the significant interaction between these two factors for the callus weight showed the dissimilar reaction of hormone levels × type of hormones with respect to callus weight. Not significant of callus weight under the effect of other factors and their interaction showed the similar effect of factors on callus weight.

Table2- Means comparing of trait related to hormone levels.

CW(gr)	*CL(cm^2)*	**HL(mg/li)**
0.5	3.62a	0.35c
1	3.72a	0.33c
1.5	3.45a	0.45b
2	3.88a	0.62a

Different letters in each column indicate a significant level of 5% is likely.

Table3- Means comparing of callus weight related to hormone type.

CW(gr)	**HT**
0.33b	BAP
	Zeatin
0.48a	kinitine
0.50a	

Different letters in each column indicate a significant level of 5% is likely.

Table4- Means comparing of explants and cultivar on callus length.

CL(cm2)	*EX*	**c**
2.906c	Hipocotil	Iraqi 222
2.643c	Cotyledon	34069
5.182a	Hipocotil	
3.939b	Cotyledon	

Different letters in each column indicate a significant level of 5% is likely.

3.2 Mean Comparing:

The results of mean comparing have been inserted in tables 2, 3, 4 and 5. Based on table 2 all the hormone levels had the similar effect at increase of callus length. The highest and shortest callus length was belonging to the hormone levels of 2 mg per liter and 0.5 and 1 mg per liter respectively. The results of mean comparing of callus weight related to the type of hormones showed that the highest callus weight was belonging to kinetin and zeatin (Table 3).The maximum callus length was belonging to 34069 cultivar and explant of hypocotyle and the minimum was belonging to Iraqi 222 cultivar at both explants (table 4). The results in table 5 showed that the highest callus weight was associated with the hormone levels of 2 mg per liter and Zeatin. However BAP had the lowest callus weight at all hormone levels.

Table5: Means comparing of hormone type and hormone levels on callus weight.

CW(gr)	*HL(mg/li)*	**HT**
0.317d	0.5	
0.315d	1	
0.317d	1.5	BAP
0.358cd	2	
0.338cd	0.5	
0.320d	1	Zeatin
0.380cd	1.5	
0.880a	2	
	0.5	
0.395c	1	Kaintin
0.367cd	1.5	
0.642b	2	
0.610b		

Different letters in each column indicate a significant level of 5% is likely.

4.0 Conclusion:

According to the above it can be concluded 34069 cultivar and hypocotyle were known as premier compound because of highest callus length. Also, Iraqi 222 cultivar at both explants was known as low yield compounds because of lowest callus length. On the other hand it was revealed that the callus weight was related to hormonal levels (2 mg per liter) and Zeatin. Overall the results showed that using the high hormone levels causes noticeable increase at callus weight, but it is not affected on callus length.

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