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Studying free proline content of calluses which are created in safflower hypocotyl in different concentrations of NacL

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ABSTRACT

Investigating the effect of salinity on safflower hypocotyl callus, it was shown that the salinity positively impacted on callogenesis of both sensitive and resistant types in both light and darkness conditions; the increased NaCl concentrationled to increased callus index and better calluseswere formed. Measuring free proline content in callus of safflower, it was indicated that the increased salt concentration in culture medium led to increased accumulated proline in sensitive varieties and decreased proline content in resistant varieties which are under salt stress. The polyphenol oxidase enzyme activity increased largely with increasing of salinity; this increase was observed in both resistant and sensitive varieties of safflower and there was no significant difference between varieties in light and darkness situations. Comparing sensitive and resistant varieties, it was shown that more enzymes were produced in sensitive variety by stress. This study investigated the effect of different NaCl (180, 120, 60, and zero mM) treatments oncallogenesisrate, qualitative and quantitative measurements of polyphenol oxidase enzyme activity, and proline content in calluses of safflower hypocotyl in MS medium. The hormonal ratio of $^{mg}/_{li}$ =0.2Kin و$^{mg}/_{li}$2 =2, 4 – D was used to study the effect of salinity on callogenesis of hypocotyl of resistant and sensitive varieties of safflower in MS medium. The findings showed that the different hormonal treatments (in terms of concentration) have different effects on growth and appearance of calluses.

Keyword:
Free ProlineSallus, Cotyledon of Safflower; NacL

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INTRODUCTION

The safflower should be introduced as anonymous familiar.Despite the long history of this plant in Iran and considering the potential capacity of this oilseed, Iran has not paid much attention to oil seed production in recent decades; according to formalstatistics,the production of safflower in Iran has decreased from seven thousand to six thousand tons during 1975 - 1978.This plant wascultured in Iran since 1967 and continued until 1974. In 1974, the crop acreage and average yield per hectare was 382 hectares.After this year, due to technical problems of this plant during its life, it was less considered. For this reason, its culture was significantly reduced and there is no formal statistics (Karimi, 1996).

In connection of using safflower in Iran, its flowers were used to color food and its grains were used for feeding poultry and oil extraction. There have been conducted many experiments on different varieties of safflower and it was shown that firstly, the cultivation and development of new varieties of safflower with high oil content may compensate partly the shortage of organic oils and secondly, the resulting proteins may be widely used for animal feeds. As was mentioned, this plant has no place in Iran, but considering the high tolerance of this plant against salinity and its frequency, the cultivation of it is considered to be a necessity(Foroozan, 1989).

Since this plant is resistant to high temperatures, if the pests and diseases issues will be solved in tropical regions, it will be suitable oil seed for those areas. The safflower cultivation and growth period is 150 days; though it varies depending on growing season and varieties.In total, the safflower is a tropical vegetable which will not be destroyed in case of drought; it adapts itself to current situation,shortens its growth period, and has less product in such circumstances. The early planting of safflower will led to higher branches and enhance product performance.There are different varieties of safflower in Iranincluding:

1) LRV – 5151
2) gilla
3) Cyprus - bregon
4) Isfahan - Local
5) PI - 537
6) PI - 596 7- PI - 598.

Methodology

According to Agricultural Research Center of Zanjan, 7 varieties of safflower were identified:

1. Gilla
2. Cyprus bregon
3. PI – 250537
4. PI – 250536
5. PI – 537598
6. LRV – 5151
7. Isfahan Local.

Since there were no information on above mentioned varieties resistance level to salinity stress and the sensitive and resistant types were needed to be compared in experiment, the germination was tested at first stage to assess the varieties resistance to salinity stress.The experiment was conducted in two stages with randomized complete block design as factorial with four replications. The factors included different levels of sodium chloride (60, 120, and 180 mM sodium chloride treatments and a zero level of salt as control) and mentioned types.Since the study aimed to evaluate the effect of salinity on growth of safflower, the effects of different salt concentrations (60, 20, and 180 mM) on germination of safflower seeds were first examined. Then, determining the resistant and sensitive varieties, the effect of salinity on safflower tissue culture was studied.In this regard, the germination was tested in two following ways:

Germination in petri dishes:

In this experiment, a total of 84 petri dishes were needed to test the germination of seven varieties of safflower seed in four different levels of salinity for three times.

1. After preparation and sterilization of containers, the industrial ethanol and filter paper were used to autoclave the petri and then, the petri was located inside them. The seeds were put in a liquid disinfectant (sodium hypochlorite 5%) for 5 to 7 minutes and then they were washed 3 times with distilled water.
2. Inside each petri dish, a filter paper was placed and then, 50 sterilized seeds were put on it. Again, another filter paper was put on seeds and petri door was then laid on them.
3. The procedure for preparation of saline solutions: first, 44 / 58 g of pure NaClwas solved in1000 mM distilled water and then, the following salt concentrations were prepared:

محلول نمكي	مقدار نمك حل شده در يك ليتر
mM 60	$\frac{g}{lit}$ 3.506
mM120	$\frac{g}{lit}$ 7.0128
mM180	$\frac{g}{lit}$ 10.51720

4. The above-mentioned saline solutions were added to 63 petri dishes. It should be noted that the distilled water was added to 21 dishes as control varieties.
5. Then, the dishes were transferred into Incubator at temperature of 25 C.After 24 hours, the number of germinated seeds were counted and this was repeated daily for 14 days (ISTA, 1985). During this period, despite sanitation, there was seen some mold and fungal contamination in some samples. In the case of low contamination, the contamination was removed and in the case of high contamination, the treatment was repeated.

Germination by rolled filter paper method:

In this method, the washing and sterilization of seeds are similar to previous method. In this method, the sterilized seeds were placed in middle of filter papers (size of 25*25 cm). This aimed to determine the growth rate of embryonic hypocotyl in germinated seeds.This method procedure is as follows:

1. The seeds were sterilized similar to previous method.
2. The filter papers were prepared in square shape in 25*25 size.
3. Ten seeds were placed in a line in middle of filter paper.

4. The second filter paper was put on first paper.
5. The prepared salt and control solutions were poured into beaker dishes.
6. The filter papers which contained seeds were rolled and put into the containers of solutions.
7. The dishes were covered by nylon to prevent evaporation of water.
8. The germination of samples were studied for 14 days. During this procedure, the germinated seeds were located at other environment.
9. During the investigation of germination, the seedling roots, seedlings stems, and other roots were also measured.
10. At the end of fourteenth day, the seedling roots and seedlings stems were separated and weighted separately to calculate the pure weight of seedlings roots and stems.

At this stage, the TATC MSand SAS software were used to analyze the germination data and cluster, respectively. The traits with statistically significant difference were compared with Duncan test at 5% probability level. The charts were plotted using Exell software. In this way, the resistant and sensitive varieties were identified to be used later for tissue culture.

Discussion and findings

Measuring proline content in different (NaCl) concentrations showed that in most varieties of safflower, the increased NaCl concentration led to decreased proline content compared to control group. In treatments of resistant variety (LRV), the decrease continued to 120mM in samples which were cultured in light. In 180 mM concentration,a sudden increase happened in proline.

In varieties in darkness (LRV), the decreased proline was clearly seen with increase of NaCl.

In varieties in brightness, the sensitive (537 - PI) was observed. The increased salt concentration decreased proline to extent of 120mM; in 180 mM concentration, a sharp increase was observed.

Table 1: Free proline content index of resistant variety of safflower hypocotyl in different concentrations of NaCl in brightness condition

NaCl (mM) concentration	Proline
0	11.219
60	5.214
120	3.464
180	9.866

Table 2: Free proline content index of resistant variety of safflower hypocotyl LRV in different concentrations of NaCl in darkness condition

NaCl (mM) concentration	Proline
0	9.866
60	0.723
120	0.371
180	0.32

Table 3: Free proline content index of sensitive variety of safflower hypocotyl PI – 537 in different concentrations of NaCl in brightness condition

NaCl (mM) concentration	Proline
0	13.583
60	11.285
120	2.928
180	12.631

Table 4: Free proline content index of sensitive variety of safflower hypocotyl PI – 537 in different concentrations of NaCl in darkness condition

NaCl (mM) concentration	Proline
0	5.333
60	4.021
120	5
180	7.303

Table 5: Correlation of traits in proline experiment (ANOVA test)
Data file: &k0S&k2GPROLIN&k0S
Title:
Function: FACTOR
Experiment Model Number 1:
Two Factor Completely Randomized Design
Data case no. 1 to 48.
Factorial ANOVA for the factors:
Replication (Var 1: r) with values from 1 to 3
Factor A (Var 2: tre) with values from 1 to 4
Factor B (Var 3: var) with values from 1 to 4
Variable 5: prolin
Grand Mean = 6.411 Grand Sum = 307.704 Total Count = 48

T A B L E O F M E A N S

1	2	3	5	Total
*	1	*	10.000	120.003
*	2	*	5.311	63.729
*	3	*	4.402	52.818
*	4	*	5.929	71.154
*	*	1	7.301	87.612
*	*	2	2.820	33.840
*	*	3	10.107	121.281
*	*	4	5.414	64.971
*	1	1	11.219	33.657
*	1	2	9.866	29.598
*	1	3	13.583	40.749
*	1	4	5.333	15.999
*	2	1	5.214	15.642
*	2	2	0.723	2.169
*	2	3	11.285	33.855
*	2	4	4.021	12.063
*	3	1	9.307	27.921
*	3	2	0.371	1.113
*	3	3	2.928	8.784
*	3	4	5.000	15.000
*	4	1	3.464	10.392
*	4	2	0.320	0.960
*	4	3	12.631	37.893
*	4	4	7.303	21.909

A N A L Y S I S O F V A R I A N C E T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	3	220.358	73.453	356.9106	0.0000
4	Factor B	3	340.073	113.358	550.8111	0.0000
6	AB	9	325.071	36.119	175.5037	0.0000
-7	Error	32	6.586	0.206		
	Total	47	892.088			

Coefficient of Variation: 7.08%

s_ for means group 2: 0.1310 Number of Observations: 12

y s_ for means group 4: 0.1310 Number of Observations: 12

y s_ for means group 6: 0.2619 Number of Observations: 3

Conclusion

The proline may be considered as a source of organic nitrogen for plants in critical condition. As a component of Osmoprotectant, the proline plays an important role in iosmotic balance and development of active forms of oxygen (ROS).It has been shown that the osmotic materials are not barriers to natural biochemical reactions and act as Osmoprotectant during osmotic stress (Kumar&Parida, 2005).

The proline accumulates in plants in response to biotic stresses such as drought, salinity, and low temperature.This accumulation is a characteristic of many plants and is the answer to many types of stresses (spinal and Palg, 1981).The results of measuring values of free proline in calluses of safflower hypocotyl indicate that the proline content differed in resistant and sensitive varieties in presence of salt (NaCl); increasing of salt concentration in culture medium leads to increase of accumulated proline in sensitive variety (537-PI) and decrease of proline content in resistant variety (5151-LRV).

The studies of beans calluses indicate that the proline in high concentrations of (NaCl) increases proline. Also at different concentrations of (NaCl), the increase of SA led to 100 micro-molar proline increase firstly and then, it was reduced (kavandi, 2006).

The drought stress in wheat increases on average the proline in leaves. The accumulation of proline observed in case of water stress in all varietiesshowed that the proline accumulation rate increases under stress conditions. Also, there was no significant difference between two sensitive and resistant groups in terms of proline concentration.

Singh et al. (1972 and 1973) found that the barley cultivars showed different amounts of proline in same water potential. The researchers suggested that the capacity of proline accumulation may be used as drought resistance in wheat breeding programs.

The study of tomato hypocotyl callus considering the adding of NaCl in culture showed that their amount decreased and minimized at 150 mM concentration. The increase of proline increased callus formation at high levels of NaCl.The increase of proline at a time when there was no NaCl in medium (control) also led to growth and development of hypocotyl callus (A.E.EL-ENANY, 1997).

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