Studying safflower hypocotyl cultivation in different concentrations of NacL

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Abstract
Considering the importance and extent of soil and water salinity and the need to produce organic oils in Iran, a research was conducted to determine the strategies and methods of tolerating salt (NaCl) in safflower plant. The use of tissue culture is one of the methods to determine the effect of processes at cellular level to assess salt tolerance; this technique has been used for improving salt tolerance in some crops. The safflower is considered to be a salt semi-tolerant plant. Reviewing the research, there were no study on effect of salinity on tissue culture of safflower and almost all works have focused on whole plant. However, this study aimed to evaluate the effect of NaCl salinity on growth and development of safflower at cellular level and study the effect of salinity on evolutional changes of safflower; this could be important in terms of both fundamental and applied aspects.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 concentration led to increased callus index and better calluseswere formed.

Keywords: Cotyledon of Safflower; NacL, Hypocotyl Culture.

1. Introduction

In ancient times, the safflower was planted in India, Iran, Middle East, East Africa, Turkmenistan, and Russia mainly as a source of coloring in carpet and textile industry. This genus contains 25 species which have been transferred from Spain through North Africa and West Asia to India and many of them are native to Mediterranean region. According to new investigations and considering the close relationship between wild species, the possible homeland of cultivated safflower is a region between eastern Mediterranean and Persian Gulf (Knoles, 1996).

The safflower has long been cultivated. It was primarily cultivated for using orange dye which was obtained from its florets. Later, its oil was extracted and used. The CARTHMEUS is the Latin translation of Arabic word of Qartum; it refers to its pigments' color which had several uses in Arabic countries (Whise, 2000).

In Afghanistan, the safflower oil is mainly used as edible oil and its florets are added to rice, bread, and pickled cucumbers. This plant'shistory in Japan dates back to third century; it was transferred from China to this country. In America, although its cultivation is limited, the type of safflower with high percentage of oleic acid and more stable than other oils is mostly used. This type of safflower which accounts for 20% of cultivated area in this country is used in production of baby food and may be mixed and used with other consumable oils (Smith, 1985). In general, the safflower is used for following cases:

1. As edible oil for patients with heart disease and obesity
2. As a nutritious meal for livestock and poultry
3. As edible vegetables for home use
4. As a natural protection to home
5. As soil reinforce
6. In addition to its use in dye industry, it is also used in pharmaceutical industry and affects the circulatory system.

2. 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:

Salt solution	Amount of salt dissolved in one liter
60 mM	3.506 $g/_{lit}$
120mM	7.01280 $g/_{lit}$
180mM	10.51720 $g/_{lit}$

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.

3. Discussion and Findings

After determining the best hormone concentration, the resistant (LRV) and sensitive (537 - PI) hypocotyls of safflower were cultivated in different salinity treatments including control, Mm60 treatment, Mm120 treatment, and Mm180 treatment. The effects of mentionedNacltreatments on callogenesis of cultured items were studied for 3 months.

Since both sensitive and resistant varieties of safflower were selected for tissue culture, the repetitions of each variety was evaluated in two light and darkness situations. The results are as follows.

In resistant variety, the 5151 - LRV was observed in brightness. During the callogenesis, the control treatment and mM60 had almost the same situation and relatively good calluses were formed. In mM120 treatment, the callogenesis was lower than control treatment. The mM180 had better situation than mM120, but the callogenesis was lower than control treatment. The callus index was observed in highest and lowest rates in control and MM120 treatments, respectively.

Comparing the sensitive and resistant varieties in dark situation, it was observed that the (537) variety has better callogenesis in control treatment than (LRV). In (mM60) and (mM180) treatments, the sensitive variety has better callogenesis than resistant variety. In (mM120) treatment, it was observed that the callogenesis is better in resistant variety than sensitive variety.

It may be concluded that in darkness like brightness, the sensitive variety has bettercallogenesis than resistant variety.

In relation to effect of salinity on callogenesis, it may generally be stated that in both varieties in both dark and light situations, the increased (Nacl) concentration have positive effect on callogenesis; this means that with increasing of salinity, the callus index increased and better calluses formed. However, some repetitions of mentioned varieties showed that the MM120 treatments had severe reduction in callogenesis.

The calluses, callogenesis rate, and callus index of different salinity treatments of (Nacl) are shown in figures (1 to 8), tables (1 to 4), and charts (1 to 4).

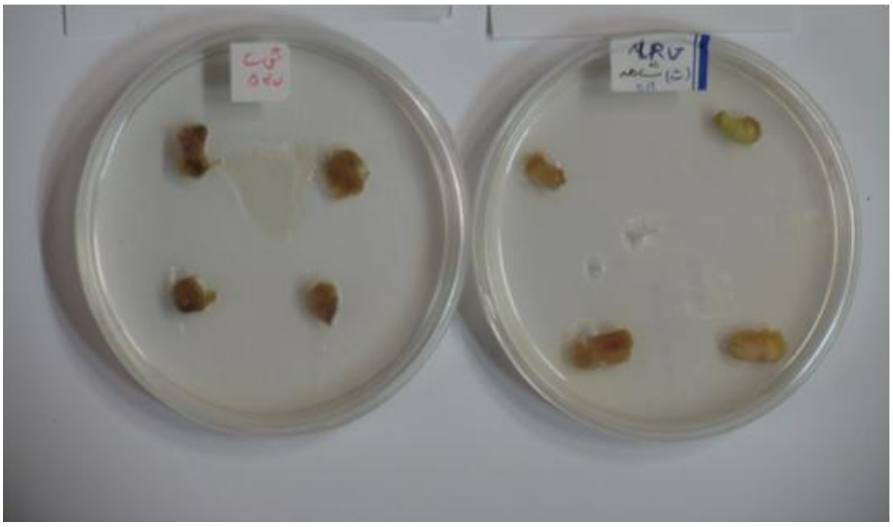

Figure 1: Callogenesis in hypocotyl of safflower at concentration of NaCl= 0mM (control) and in darkness situation

(Right: LRV resistant variety Left: PI – 537 sensitive variety)

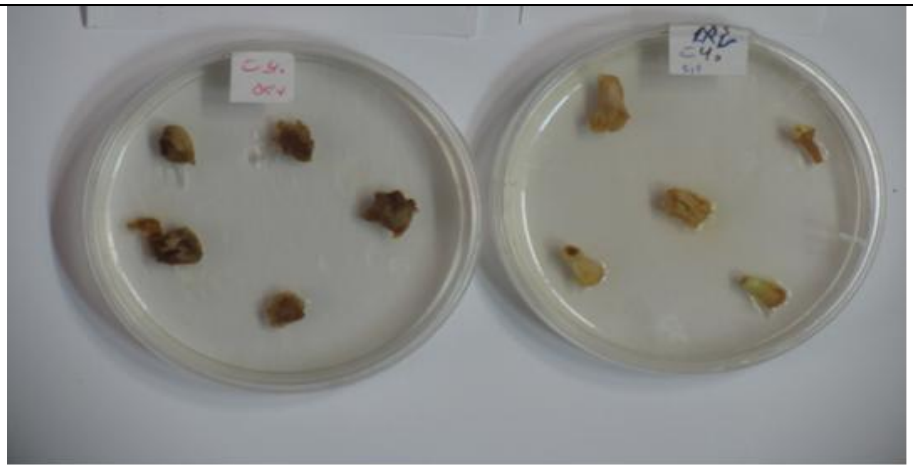

Figure 2: Callogenesis in hypocotyl of safflower at concentration of NaCl = 60mM and in darkness situation

(Right: LRV resistant variety Left: PI – 537 sensitive variety)

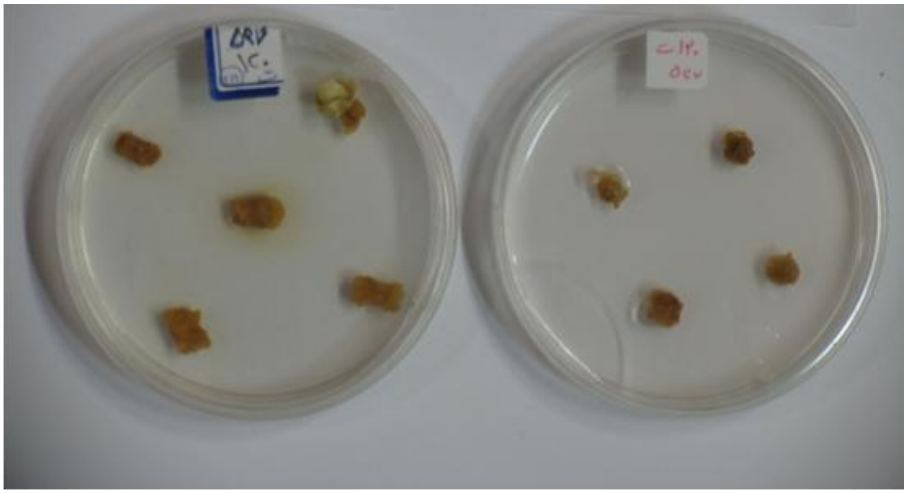

Figure 3: Callogenesis in hypocotyl of safflower at concentration of NaCl = 120mM and in darkness situation

(Left: LRV resistant variety Right: PI – 537 sensitive variety)

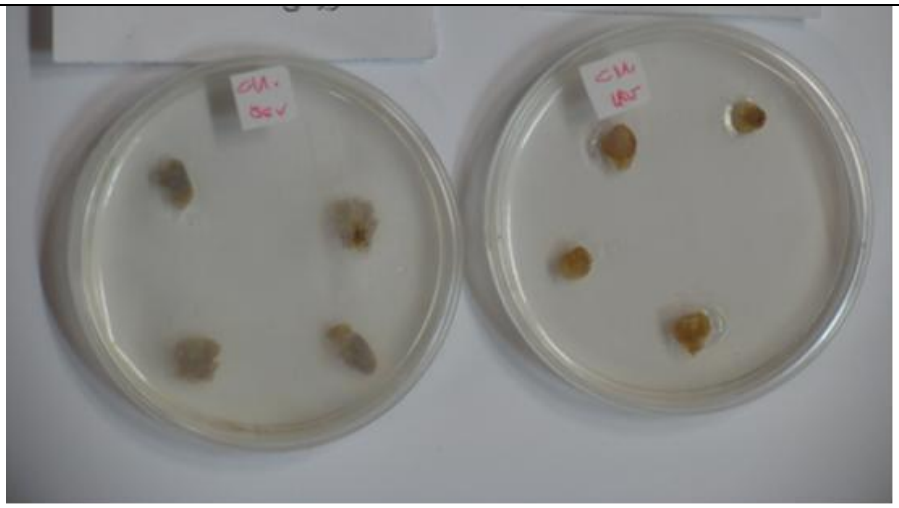

Figure 4: Callogenesis in hypocotyl of safflower at concentration of NaCl = 180mM and in darkness situation

(Right: LRV resistant variety Left: PI – 537 sensitive variety)

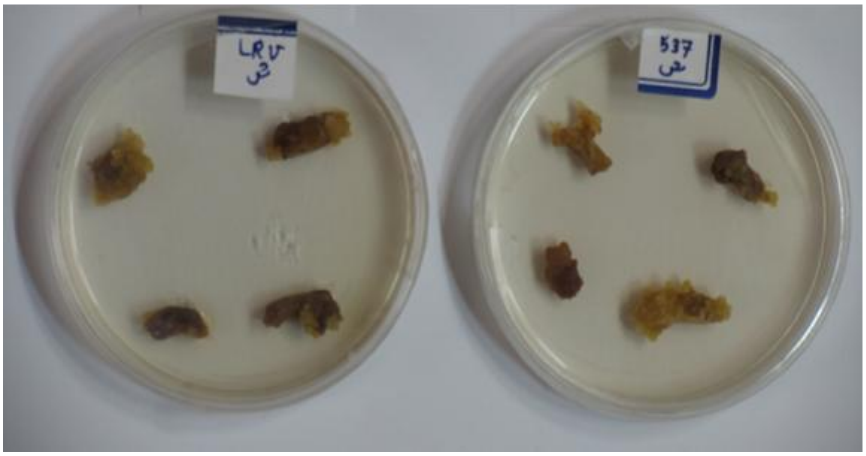

Figure 5: Callogenesis in hypocotyl of safflower at concentration of NaCl= 0mM (control) and in brightness situation

(Left: LRV resistant variety Right: PI – 537 sensitive variety)

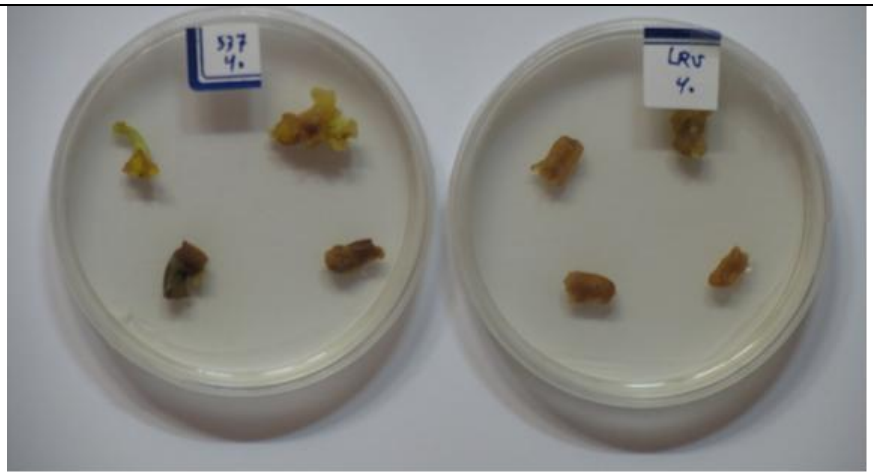

Figure 6: Callogenesis in hypocotyl of safflower at concentration of NaCl = 60mMand in brightness situation

(Right: LRV resistant variety Left: PI – 537 sensitive variety)

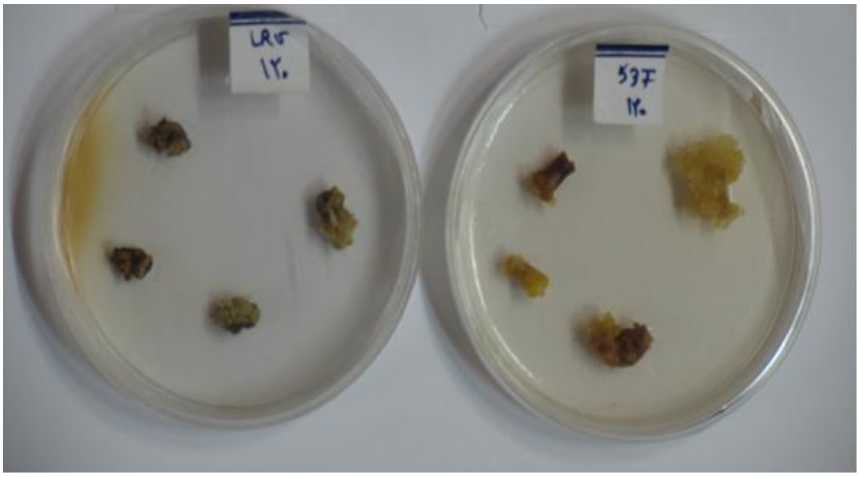

Figure 7: Callogenesis in hypocotyl of safflower at concentration of NaCl = 120mMand in brightness situation

(Left: LRV resistant variety Right: PI – 537 sensitive variety)

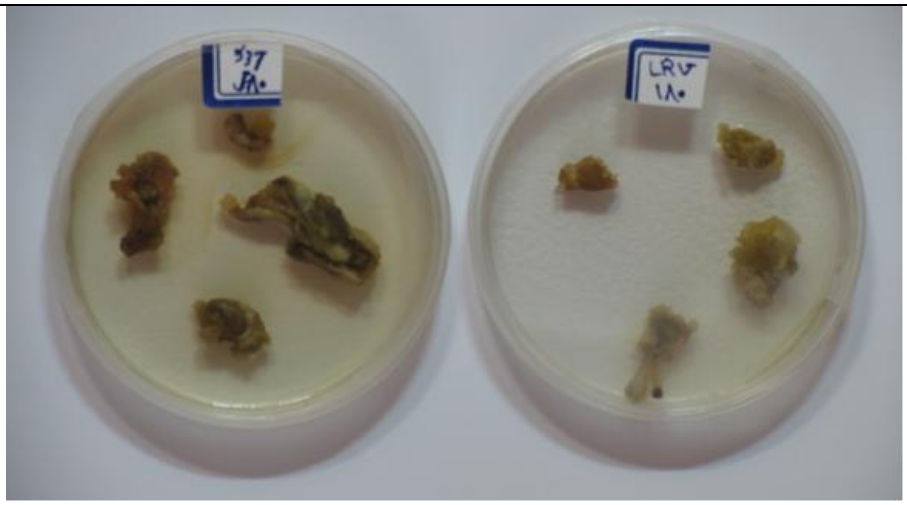

Figure 8: Callogenesis in hypocotyl of safflower at concentration of NaCl = 180mM and in brightness situation

(Right: LRV resistant variety Left: PI – 537 sensitive variety)

Table 1: Callogenesis and callus index of resistant variety of safflower hypocotyl in different concentrations of NaCl in brightness condition

Callus - Brightness –LRV index	
0mM	300
60mM	240
120mM	300
180mM	350

Table 2: Callogenesis and callus index of resistant variety of safflower hypocotyl in different concentrations of NaCl in darkness condition

Callus - Darkness–LRV index	
0mM	150
60mM	150

120mM	200
180mM	350

Table 3: Callogenesis and callus index of sensitive variety of safflower hypocotyl in different concentrations of NaCl in brightness condition

Callus – Brightness-PI537 index	
0mM	300
60mM	250
120mM	350
180mM	400

Table 4: Callogenesis and callus index of sensitive variety of safflower hypocotyl in different concentrations of NaCl in darkness condition

Callus-darkness- PI537 index	
0mM	180
60mM	250
120mM	180
180mM	300

4. Conclusion

Discussion on results of safflower tissue culture and effects of different concentrations (NaCl) on its callogenes is:

The analysis of safflower tissue culture showed that the hypocotyl is the best part for callogenesis. Among different hormonal treatments considering the callogenesis rate, the best situation was for hormone concentration of Kinetin $(\cdot/۲\,\mu m)$ and D-4 and 2 hormone with concentrations of $(۲\mu m)$.

The other studies showed that the cotyledon safflower and $(2,4\text{-}D = 1mgli^{-1}, Kin = 0/1mgli^{-1})$ are the most suitable and important culture point and hormone site for callogenesis.Overall, this study concluded that the increasedcallogenesis in culture situation is affected by used hormones and mass production of undifferentiated tissue(Fahimi, 1996).

Other studies have shown that the callus significantly increases by adding simultaneously the kinetin vaccine. From a total of 486 different compounds of auxin and cytokinin that were tested in various concentrations, the safflower callogenesis was better by B.A and kinetin. The growth regulators of safflower hypocotyl callus included Kinetin and D-2, 4 (Nikam&Shitole, 1999).

In safflower hypocotyl culture, it was concluded that the salinity impacted positively on callogenesis of both sensitive (537-PI) and resistant (5151- LRV) varieties in both light and dark situations; the increased (NaCl) concentration led to increased callus index and formation of better calluses.

The studies which were conducted to determine the interaction between salt (NaCl) and callus growth hormone, it was found that the amount of oil in extraction of calluses which are formed in light is more than those which are formed in darkness. The safflower oil content in extraction of calluses which were formed in different concentrations of NaCLin culture medium decreased in brightness with increasing of salinity; however, the calluses which grown in darkness were not impacted by increasing of salinity (Francois, 1990).

The callus growth in environments with (NaCl) and (CaCl2) was better than environments with (NaCl). Further investigations and comparison with whole plant, it was found that the potassium increased the amount of oil (Hashemi, 1974).

Given that the safflower is one of the varieties which is relatively resistant against salt and drought, it may be concluded that there is correlation between resistance or tolerance of plant at cellular level and whole plant (Fahimi, 2000).

The results of bean tissue culture showed that the callogenesis in environments with (NaCl) reduced the speed of callogenesis and in environments with (NaCl) and salicylic acid, it increased the speed of callogenesis (Kavandi, 2006).

In safflower tissue culture, it was observed that over time, some callus color gets brown; this may be due to polyphenol oxidase activity which oxidizes phenolic compounds and converts them to brown melanin pigments. These compounds prevent from growth of callus. However, there were no logical relationship about effect of increasing or decreasing of amount of (NaCl) on callus color change.

The results of bean callogenesis in medium containing SA and (NaCl) also confirmed this fact (Maddah, 2005). Measuring the fresh weight of safflower callus, it was observed that in both light and dark situations, the increased concentration of (NaCl) reduced the fresh weight of callus compared with control treatment.

The study of beans showed that the fresh weight of calluses which are grown in medium containing (NaCl) dramatically reduced with increasing of salinity. This was confirmed by Citrine and colleagues (1992) in jojoba calluses.

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