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Induction high amount pigment of Sunflower callus in vitro by salt stress

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Abstract: In this experiment, production callus from flower petals of safflower cultured before flowering after surface sterilization and then inoculated on MS media with different concentrations of growth regulators Kn and 2,4-D (0.0, 1.0, 2.0)(0.0, 0.2, 0.5)mg respectively, and then the callus composed of the best concentration of growth regulators was transferred to different concentrations of salinity NaCl (0.0, 50, 100, 150, 200) mM. .The results showed that the highest concentration of callus production of safflower was at concentration of Kn and 2,4-D were (2.0, 0.2) mg respectively, also the results showed that the concentration of red and yellow safflower was increased by increasing salinity concentration with the best concentration at 150 mM 1.53 at red pigment and 1.92 at yellow pigment. Because the concentrations of pigments in the concentration 150 were higher than the normal rates in the petals of safflower, so possibility used as food flavors for desserts, desserts, and ice cream.

Keyword: safflower, invitro, pigment

Introduction

Safflower(*Carthamus tinctorius* L.) belongs to oil crops, there are many varieties of safflower according to the presence and density of thorns, leaves, floral cup, floral color, fluff or lack of floral (Taha and Matthaus, 2018). A three-purpose crop uses oil for cooking as used in the treatment and removed rancid fat, one of the most difficult areas to dwindle(Machewad et al 2013) The Gain is yield derived from the age of seeds (after separated oil) in animal nutrition and can be provided to all types of poultry and other good value food because it contains up to 25% crude protein in addition to carbohydrates as an energy source, Its seeds are for feeding birds or for animal feed, where it enters concentrated feed mixes (Toshiro et al, 1997) -Petals: Floral petals are used as natural melons for some foods, sweets and ice cream as they were used as pigments for clothes due to the presence of chromatin dye and high prices (Kadhim, 2017).

Despite the importance of the crop and the special dyes, there are problems facing traditional agriculture, including that it is

grown in one season in Iraq and the difficulty of harvesting because of thorns in the varieties of chocolate, in addition to the cost of agriculture, production and harvesting because it needs special attention (Joachim et al, 1996).Quality control of Chinese herbal medicine (CHM) This organization has developed special standards to evaluate the quality of the weeds including color, shape, hardness, and size. In all of its studies, it proved that the natural products in most products are below the required level(Zhao et al 2010).Therefore, it is required to find a way to increase the natural product in a quantity exceeding the yield in the natural state. It is very difficult to produce the active material in the natural state artificially, except in the case of the use of plant tissue culture technique by inoculating explants of the active substance only of the active ingredient only without the need for traditional cultivation, without the restriction of season And unlimited (Claudia et al 2012).In addition, it is possible to increase the secondary metabolism by using tissue culture technique by increasing the stress on the callus induced, the available stress and the low salinity stress (Daneshmand et al. 2010). which has proved it is increasing the metabolism in many plants (Akula and Gokare 2011) so we seek in this research to stimulate the production of callus from the areas where it is concentrated The active metabolism in petals (Ghorbani et al, 2015)Callus was exposure to different levels of salt stress to study the effect of stress on the active metabolism yellow and red pigments in the callus tissue produced

Material and methods:

Experiments were carried out in tissue culture laboratories, Department of Plant Production at the Technical College of Musib – Furat University. The seeds were collected from the crop laboratory of the University of Al Qasim Green. Seed implantation in the fields belonging to the technical collage Musaib to follow the growth of plants and harvest them at appropriate stage for inoculation in plant tissue culture laboratory.

After the arrival of the stage of the flower before blooming plants are taken to the laboratory are separated non-open flowers then placed in the Baker, add the sterile substances alcohol at a concentration of 90% for five seconds and add hypochlorite at 20% concentration of the solution of the minor chlorine percentage of 6% for 20 minutes inoculation of flower petals on the MS medium contains different concentrations of growth regulators Kn and 2,4-D(0.0, 1.0, 2.0)(0.0, 0.2, 0.5) mg respectively. The best concentration of callus production was determined after 46 days of inoculating by calculating the soft weight of the callus. Callus was grown on the best combination of the food medium and growth organizations and on different concentrations of salinity (0.0, 50, 100, 150, 200) mM of NaCl to test the extent of the material of colors in the callus after

exposure to salt stress. Determination of pigment content: Flow method for extraction of yellow color from safflower petals callus. Because of yellow safflower pigment soluble in water so following Fatahi (2008) method followed the same old way with some modifications. Took same amount of callus and dried in oven with treatment after that immersion in distilled water in a liquor percentage of 0.01 at 40C for 2 hr by using shaking bath repeat it two time and add first one with second to filtration them together concentrated after that with a vacuum evaporator and freeze them to dry at -40 c to obtain colorant powder. The color absorption properties of colored solutions showed yellow shadows pigments (water extraction)in the visible range between 400-420 nm, and red color carthamin from 385 -500 nm. After washing the callus induced from the petals of safflower leaves of the medium is placed in a solution of sodium carbonate at 1% concentration and at a temperature between 20 – 25C^{o} is cut and disassembled every 30 minutes. Repeat the process twice and then filter the mixture with a nylon strainer 200 mesh The mixture was placed in the centrifuge 2000 for 10 minutes. Place the appropriate 1% citric acid in a transparent liquid and then leave the solution for 30 minutes on consistency to separate sediment.

Results and Discussion:

From the first table we observe the effect of growth regulators Kn and 2, 4-D on the weight of callus, where no comparison with control treatment not showed any amount of callus, especially with concentrations of kn at 0.0 mg while the callus was produced in concentrations (1, 2) mg of Kn with 0.0 mg of 2,4-D, best concentration production callus was in Kn and 2,4-D (2.0 and 0.2) mg which differed significantly from the rest of the concentrations. From the above it was found that the lack of production of Callus without adding growth regulators is evidence of the effect of growth regulators in stimulating the growth and production of Callus, but on the production of callus in the control treatment of Kn shows the balance in between growth regulators additive Cytokinin (Kn) with internal growth regulators (Auxins) although the quantity was few From the importance of balance between the internal growth regulators with the external added several concentrations of growth, regulators were added to reach the ideal concentration.

The increase in growth regulators from the appropriate balance leads to a negative effect on the stimulation and production of callus because of its toxic effect, which leads to an inhibition of growth

Table 1: effect of different growth regulators on callus induction from safflower petals after 46 days.

Kn / 2,4-D	0.0	0.2	0.5
0.0	0.0	0.0	0.0
1.0	0.47	1.32	0.54
2.0	0.62	2.11	0.77
L.S.D. 2,4-D* Kn=0.74			

Table 2: effect of different concentration of NaCl on safflower callus color yellow and red pigment.

NaCl concentrations mM	Red Pigment	Yellow Pigment
0.0	0.42	0.49
50	0.73	1.17
100	1.27	1.32
150	1.53	1.92
200	0.83	1.42
LSD	0.28	0.33

From the second table shows that the two dyes yellow and red in safflower callus had to color even without exposure to saline stress as notes that the concentration of dye began increasing with increasing concentration of salt NaCl has been significantly different from the first addition 50mM of NaCl, and also shows same table that the increase in the amount of dye continued to increase salt concentration. up to 150 mM concentration where the amount of pigment decreased at a concentration of 200 mM from the highest concentration 150mM.

The increase of the dye significantly by increasing the concentration of salt stress in media is indicative of the effect of salinity by increasing the active ingredient in the plant tissue because the stress increases the concentration of secondary metabolism in the

plant tissues in many plants (Zahra et al 2015, Gao et al. 2005).

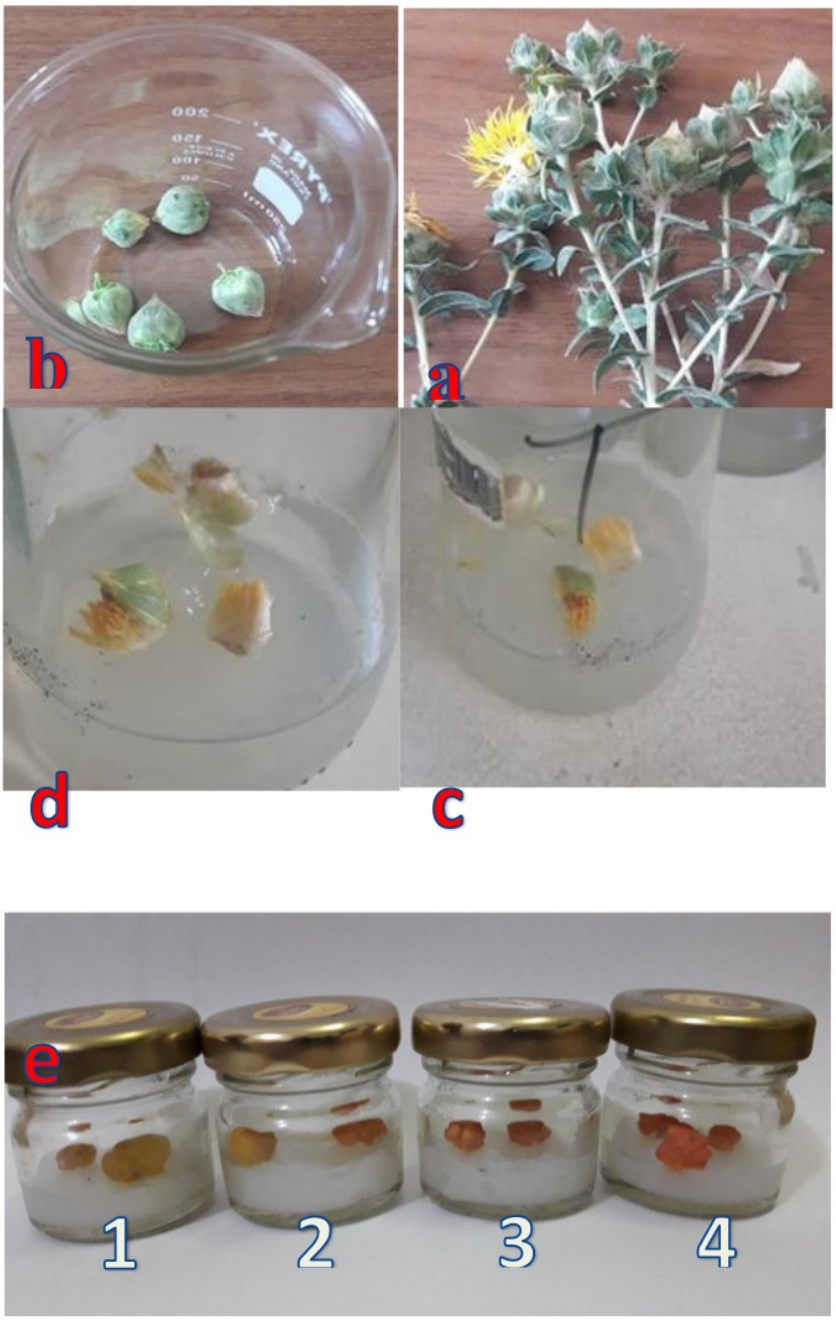

Fig 1) callus step induction and stress test :

a- Parts of the plant with flowers in different stage of bloom.

b- no open flowers in class for sterilization.

c, d- petals inoculation on MS medium with different concentrations of growth regulators.

e- callus (1, 2, 3, 4) on different concentrations of salinity (0.0, 50, 100, 150, 200) mM of NaCl.

The(fig, 1) shows that the part used to produce callus is the petals of flower and it was found that the period of taking is before the opening flowers(fig, 1-a,b) it closed to facilitate sterilization and keep them free of pathogens(fig, 1 c,d) , the fig also showing increased the amount of pigment by increasing salt concentration(fig, 1-e).

From the above(fig, 1) we saw that flowers were inoculated before blooming because of the difficulty of sterilization after blooming and because the tissues are good at this stage can be procreated and producing callus in addition to the disposal of internal contaminants.

As shown in fig 1, the increase in color is increased by increasing the concentration of salinity in the center of the plant from the first concentration and then increase with salinity (Claudia et al. 2012).

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