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CALLUS INDUCTION AND PLANT REGENERATION FROM *BRYOPHYLLUM* LEAVES AND SALT STRESS EFFECT ON CALLUS CONTENT OF BRYOPHILIN A AND BRYOPHILIN C.

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Abstract

In this investigation, Callus obtained from *Bryophyllum* leaves on MS media with different concentrations of 2, 4-D (1,2,3) mgl^{-1} and containing sucrose and vitamins. The callus re-differentiation on the same medium with modifying growth regulators Kn (1.0, 0.5) mgl^{-1} and IAA (0.5, 0.1) mgl^{-1}, the effect of salt stress on the callus cells level and at different salinity levels (10%, 20%, 30%, 40%) was studied for 28 days. Results showed that, the best concentration for callus production was in the concentration of 2 mgl^{-1} 2,4-D and regeneration of the callus to, plantlets with Kn 1.0 mgl^{-1} and IAA 0.1 mgl^{-1}, with MS media. The results showed that *Bryophyllum* callus was able to tolerance to salt stress up to 30% concentration of NaCl. Although wet and dry weight were affected by salt stress from the first concentration 10% and the higher contain of bryophilin A at 30% was 4.18µg/g while higher result in bryophilin C at 20% was 2.12 µg/g.

Keywords: Induction callus, regeneration plants, *Bryophyllum* and salt tolerance bryophilin.

Introduction

Medicinal plants have been used for treatment or prevent the diseases since before recorded history. Bryophyllum is important medicinal plant of the family Crassulaceae, it's a genus of 125 species of tropical region (Kulka, 2006), *Bryophyllum* has fatty acids, monoarylphenolics, sugars, alcohols, sugar alcohols, sugar acids, carboxylic acids, dicarboxylic acids, tricarboxylic acids, vitamins, alkanes and alkenes (Faboro *et al.* 2016), its useful to treating many diseases renal calculi, hypertension, asthma, cold, bleeding disorders anti-Diabetic and wound recover (Nagaratna and Hegde, 2015) and extracts of leaves anti-cancer (lung cancer) because its contain alkaloids, tannins, flavonoids, saponins, steroids, phenols, terpenoids and anthroquinones (Sharma *et al.,* 2015), (Panduran *et al.,* 2015) and (Nawli, *et al.,* 2012). Succulent plant short thick beautiful plant that do very well indoors and outdoors blooming flowers come pink, (Skoog and Miller, 1957) used from old time folk medicine in India, China and Philippines to treatment many health problems like gastric ulcer, painful, pulmonary infection skin care, inflammation, rheumatism and enter in many medicines and most of the natural sources of drugs extract like anti-cancer activity, anti-diabetic activity, antifungal activity, anti-leishmanial activity, anti-microbial activity and ant-proliferative activity (Sharma *et al.,* 2015, Prioku and Igbe, 2017 and Faboro *et al.,* 2016). These plants contain many macro elements Mg, Ca, K, Na, Fe, Co, Zn, steroidal and cardienolide, amino acids, carbohydrates and proteins. These plants have many of sugars and various enzymes. (Sharma, 2008, Kumar, 2015 and Dodds and Robert 1995 and 1997). The importance of plant comes to attention, this plant is widely adaptive to environmental conditions in many countries in addition to the use of plant in the gardens and it is located as wide on the hills and despite the fact that the studies of tissue on this plant very few *In vitro* for study this plant physiologically and biologically as medical plants and decoration plants. (Quazi *et al.,* 2011). The availability of tissue culture on the level of tissue, cell or part of the plant in unlimited area under controlled conditions regardless of the agricultural season, which leads to the possibility of development and the development of plants as decorative plants or extraction of pharmaceutical materials like Bufadienolides which known as chemical compound structuring same steroid in many form as bufadienolide glycosides, *Bryophyllum* has a special medical value to contain byrophilin A and byrophilin C The first has an effective anti - tumor, and the second has the properties of insecticide It also contains many other compounds that have many beneficial medicinal properties (Supratman *et al.,* 2001; Pattewar, 2012; Kumlesh *et al.,* 2016). For all these aims and there is no study on the effect of salt stress on the callus that affects the increase of secondary metabolites under stress and to develop the best conditions for the production of callus and regeneration, to a plantlet and find a lethal dose of salinity affect the *Bryophyllum* callus and its effect on the wet, dry weight of callus and its contains of bryrophilin A and byrophilin C under salt stress.

Material and Methods

Explants sterilization:

After the selection of the explants (leaves) in healthy condition and free from mechanical damage and to the running to water for half an hour and then treated with alcohol 95% for five seconds and then washed with distilled water and then transferred to the inside the laminar air flow and sterilized with mercuric chloride ($HgCl_2$) of 0.01% solution for 5 minutes, and then rinsed with distilled water for (4-5) times.

Media Preparation

Weight 4.6 grams of powder MS media (Plantigen HIMEDIA TS1068) was used for induction of callus and regeneration of plants, this media prepared as supplemented with proline,800 mgl^{-1} Casein hydrolysate, 500 mgl^{-1} vitamins and 500 mg Glutamine and source of carbohydrates sucrose 30 gl^{-1}. and modified with hormone 2,4-D (1,2,3) mgl^{-1} for induction of callus, for plant regeneration media modified with growth regulators Kn and IAA (0.5, 1.0) mg/l and (0.5, 0.1) mgl^{-1} respectively. The pH of the media was adjusted at 5.5 -5.8 by using pH meter. The media was

solidify with 8 gl^{-1} of agar after cooked until boiling, the media poured in to the culture tubes, bottles and autoclaved at 121 ^{0}C, 15Lbs for 15 minutes.

After 21 days of inoculation ex-plant on the medium with different concentrations of 2,4-D collected wet weight of the callus and then the callus produced was transferred to different concentrations of Kn and IAA to regeneration of callus to plants were transferred callus to different concentrations of salts to find out the effect of salinity callus of *Bryophyllum* after 28 days of inoculation, was the wet and dry weight as an indicator of the effect of salinity, Chemicals were evaluated via using Spectroscope.

Results

The 2,4-D most powerful Auxin in induction of callus is added to the appropriate concentration to be consistent with internal growth regulators, the table (1) indicated that best concentration to induction of callus 2 mgl^{-1}, which differed significantly from the concentrations (1, 3) mgl^{-1} which didn't differ significantly from each other in terms of weight, despite the difference in form and strength of callus (Hsieh *et al.,* 2013; Quazi *et al.,* 2011).

Table 1: effect of different concentrations of 2,4-D mgl^{-1} on the wet weight of callus (g) of Bryophyllum after 21 days of inoculation on MS media.

2,4-D concentration mgl^{-1}	wet weight Callus g
1	1.4
2	2.26
3	1.2

The process of plant tissue culture is incomplete if it does not end with the formation of whole plants, the use of a combination of growth regulators of cytokines and auxins (Kn, IAA) with suitable concentrations leads to a better regeneration of the plants and (Kn, IAA) (1.0, 0.1) mgl^{-1} respectively.

Table 2 : Effect of different concentrations of (Kn, IAA) mgl^{-1} regeneration callus to plantlets after 28 days of inoculation on MS media.

Kn	IAA	Number of plantlets
0.5	0.1	22
	0.5	17
1.0	0.1	47
	0.5	32

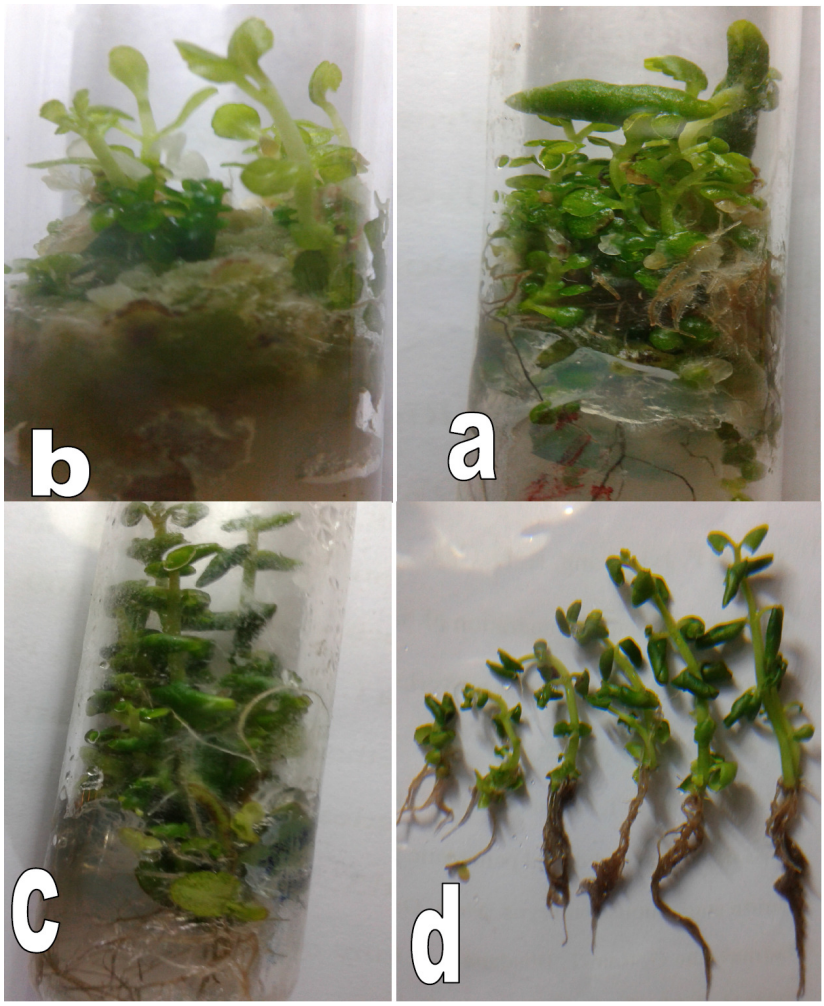

Fig 1 : a-Regeneration of shoot from callus. b- Rooting stage. c-increasing shoot and root group ready to acclimatization. d- *Bryophyllum* plantlets.

From table (3): the wet weight of callus was affected by the saline concentrations from the first concentration and then the effect increase with increased by the salinity concentration. However, the maximum tolerance of the saline concentration was 30%, as for the last concentration, it was unable to survive until the end of the incubation period.

The pathway results of the dry weight were not different with wet weight from the first concentration and continued to deteriorate until the last concentration. The lowest dry weight at 40% was 0.035g. *Bryophyllum* callus tolerant this high level of salinity due to genetic causes and may be due to its ability to withstand drought.

Table 3 : Effect of different salt concentrations on wet and dry weight (g) of callus after 28 days of inoculation.

NaCl concentration	Wet weight	Dry weight
0.0	2.213	0.147
10%	1.372	0.085
20%	0.872	0.063
30%	0.634	0.041
40%	0.414	0.035
L.S.D (0.05)	0.112	0.013

The results showed in table (4) effected of salt clear on bryophilin A from first concentration 10% it was differed significantly from control, although it was less amount among all treatments best result at 30% it was 4.18 which differed significantly from all treatments, best amount of bryophilin C was 2.12 at 20% concentration which differed significantly from all treatments lowest contain at 40% concentration was 1.68 but it was differed significantly from control.

Table 4 : Effect of different salt concentrations on callus contain of (Bryophilin A and Bryophilin C) µg/g after 28 days of inoculation.

NaCl concentration	Bryophilin A µg/g	Bryophilin C µg/g
0.0	2.22	0.58
10%	3.12	1.80
20%	3.48	2.41
30%	4.18	1.72
40%	3.40	1.68
L.S.D(0.05)	0.78	0.51

Conclusion

In this present investigation *Bryophyllum* developed callus induction, plant regeneration and screening of callus for salt tolerance, the salt tolerance callus for the useful for analysis of increasing concentration of medicinally important secondary metabolites.

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