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RESEARCH ARTICLE

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Anatomy, antiangiogenesis activity of wild, tissue culture and salt stress plants of *Brassica oleracea* L.

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ABSTRACT

The aim of this study was to optimize cultural conditions required for *in vitro* seed germination, micropropagation with normal growth regulator medium and salt stress medium. Successfully 96% germination of seed was achieved in MS basal medium, and 92% in MS medium with NaCl 20 mM concentration. The bud explant cultured in MS medium augmented with BAP (11.10 µm) + KIN (1.16 µm) and NAA (0.98 µm) induced 91% shoots whereas the MS medium fortified with BAP (11.10 µm) + KIN (1.16 µm) + NAA (0.98 µm) and NaCl (20mM) produced 96% shoots. In the same medium the root formation occurs simultaneously along with shoots during subculture. The anatomical features of normal cells and tissues intact with normal size and shape of the cells. The shoots developed through *in vitro* showed presence of tannins and cells are enlarged deviating from normal spherical shape to spindle shape. Also less amount of cytoplasm and large number of vacuoles compared with filed grown. Secondary growth is very slower on salt induced experimental group when comparing with experimental and normal control. Further, vascular tissues is not fully regenerate in the experimental salt induced growth probably due to salt stress. Natural, *in vitro* and salt stress induced plant extracts showed the higher angiogenic activity 75.0 ± 1.42, 85.7 ± 1.24 and 80 ± 1.62 at the concentration 1000 µg/ml respectively.

Keywords: Salt stress, Tissue culture, Anatomy, Angiogensis, *Broccoli, Brassica oleracea*

1. INTRODUCTION

The beginning of 21st century is marked by global scarcity of water resources, environmental pollution and increased salinization of soil and water. Increasing human population and reduction in land available for cultivation are two threats for agricultural sustainability [1]. Abiotic stresses such as drought, salinity and high and low temperatures limit crop productivity, and play a major role in determining the distribution of plant species across different type of environments [2]. Plant tissue - culture is an important aspect of plant biotechnology because genetic manipulation is now necessary to harness its potential to overcome crop yield losses due to biotic and abiotic stresses. Salinity is a serious problem that reduces growth and productivity of vegetable crops in many salt-affected areas. About 20% of cultivated lands and 33% of irrigated agricultural lands Worldwide are affected by high salinity [3]. One of the most effective ways to overcome salinity problems is the use of tolerant

species and varieties could produce from plant tissue culture [4]. Angiogenesis is the sprouting of new vessels from pre-existing ones and is a normal physiological process in embryogenesis and development. In contrast, pathological angiogenesis is observed in several diseases, including rheumatoid arthritis, atherosclerosis, diabetic retinopathy, psoriasis, and cancer [5].

Brassica oleracea L. var. *italica*, commonly known as broccoli belongs to the family Brassicaceae, which is nutritionally rich, high in vitamin A and C, soluble fiber and also contains the compound glucoraphanin, leading to an anticancer compound sulforaphane [6]. Broccoli as a source of bioactive compounds such as phenolics, flavonoids and glucosinolates, which possess antioxidant and anticancer effects [7,8,9]. Apart from other nutritional antioxidants, broccoli is also a significant source of calcium, folic acid, carotenoids, ascorbic acid and known to reduce risk of breast and prostate cancer [10,11]. Further, has antiseptic [12], antiulcer [13] and hypoglycemic activities [14]. The goal of this study is to standardize normal and salt stress medium for mass multiplication and to compare the anatomical and angiogenic activities of normal tissue cultured and salt stress plants.

2. MATERIALS AND METHODS

2.1 Plant Collection

The plants of Brassica oleracea L. Brassicaceae family were collected from Ooty, Tamilnadu, India.

2.1.1 Explants selection and mode of sterilization

The flower bud and seed explants were harvested from in vivo plants were treated with detergents (teepol, tween 20, domestos), fungicides (bavistin-methyl-3-benzimidizole carbomate solution), antibiotics (rifampicin, streptomycin) and surface sterilants namely 70% alcohol and mercuric chloride (HgCl2) with various concentrations at different time for sterilization. The surface sterilized explants were trimmed gently with the help of sterile surgical blade (Lisyter No: 10) and aseptically inoculated on MS medium with growth regulators.

2.1.2 Culture media employed and their composition

MS [15] basal medium in full strength (MS) was employed in the present study.

2.1.3 NaCl (Salt) medium

MS medium supplemented with various growth regulators like BAP and NAA along with NaCl (20 mM) were prepared for abiotic stress shoot and root induction.

2.1.4 Multiple shoot medium

MS medium supplemented with various growth regulators like BAP, NAA, KIN, GA3 and IAA along with at various concentrations were prepared for multiple shoot formation.

2.1.5 Culture conditions

All the cultures were maintained in the culture room at a temperature of 25±2°C and relative humidity of 65-70%. The cultures were kept under white light at intensity of 3000 Lux provided from white fluorescent lamps with 14/12 hours photoperiodic duration.

2.2 Preparation of Plant Extract

A 10 g powder of natural, in vitro normal and salt stress induced plants were extracted using 50 ml of ethanol for each sample by using the cold extract for 24 hrs. The extracts was filtered through Whatmann No.1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent and stored at 40 C used for further experiments.

2.3 Anatomical Studies

In order to identify and compare with natural and in vitro normal and salt stress induced plants stem anatomical studies were performed. In vitro plant material was sampled after 45 days of culture and natural plant from the field were carefully uprooted and gently cleaned with tap water. Both the samples were hand sectioned with a razor blade, and then stained with safranin and astra blue [16]. For starch grains iodine potassium iodine [17] stain was used and observed under a light microscope. Photographs were taken using an optical microscope (Olympus CH 30, Tokyo, Japan) equipped with a photographic camera (Canon Power Shot S50 AIAF, Tokyo Japan).

2.4 Chicken Egg Chorioallantoic Membrane (CAM) Assay

Antiangiogenic activity of crude extracts of Brassica oleracea natural and in vitro normal and salt stress induced plants were conducted on fertilized eggs by modified CAM assay method [18]. Fertile white Leghorn chicken eggs (Gallus domesticus) were obtained from a local hatchery with 3 days incubation. The eggs were incubated at 37ºC in humified incubator for 48 h, placed in horizontal position and rotated several times. The eggs were

returned to the incubator after the filter paper discs (100micrograms of extract) of ethanol extract is placed on blood vessels of embryo using sterile forceps. After 48 h of incubation on 8th day photographs of embryos were taken to obtain the image of CAM after treatment with various extracts. At least six eggs were used for each extract dose. The percentage inhibition was calculated.

RESULTS

3.1 *In Vitro* Micropropagation

3.1.1 Seed germination

Seeds were inoculated for germination in Water Agar Medium, MS basal (½ MS and Full MS), MS basal with growth regulators (BAP+NAA+KIN) and MS + growth regulators with NaCl (20 mM). The relative effectiveness of water agar medium, MS basal medium and MS basal medium with various concentration plant growth regulator and NaCl salt in the *in vitro* seed germination of *Brassica oleracea* is summarized in table 1 and Plate 1. Among the basal medium and basal medium with various growth regulators concentration used, the best *in vitro* seed germination percentage (96± 0.91) was observed in full strength MS basal medium, followed by MS medium and NaCl (20 mM) concentration with 92±1.28 percentage. MS medium with germination percentage 02±22.10 was observed in water agar medium. Increasing in BAP concentration from 2.22 to 8.88µm and also along with NaCl (20 mM) the seed germination percentage also increased from 41±2.05 to 85±1.23, 35±31.44 to 80±1.72 respectively.

3.1.2 Shoot formation

The morphogenetic response of bud explants to growth regulators (BAP, NAA, KIN, TDZ, GA_3 and IAA) are summarized in table 2. Best shoot sprouting frequency (91%) however occurred on MS+BAP (11.10 µm) + KIN (1.16 µm) and NAA (0.98 µm). followed by MS+BAP (8.88 µm) + KIN (1.16 µm) and NAA (0.98 µm) with 84% (Plate 2). Among the different combination of growth regulators the highest percentage of shoot sprouting frequency (91±0.09), highest number of shoot formation (12±0.35) highest number shoot formation during sub culture (26±0.38) in MS +BAP (11.10 µm) + NAA (0.98mm) and KIN (1.16 µm) concentration. The second best combination was BAP (8. 88 µm) + NAA (0.98µm) and KIN (1.16 µm) with 84 ± 0.19 shoot sprouting frequency, 9±0.37 shoots formation in initial stage and 16±0.24 shoots during subculture (Plate 2). Among the different combination of growth regulators and NaCl induced highest percentage of shoot sprouting frequency (96±0.19), highest number of shoot formation (10±0.19) highest number shoot formation during sub culture (18±0.29) was observed in MS +BAP (11.10 µm) + NAA (0.98mm) and KIN (1.16 µm) combination (Table 3). The second best combination was BAP (8. 88 µm) + NAA (0.98µm) and KIN (1.16 µm) with 83±0.36 shoot sprouting frequency, 08±0.18 shoots formation in initial stage and 10±0.19 shoots during subculture (Table 3 and Plate 2). During subculture root formation occur simultaneously along with shoot formation without using separate root induction medium. The rooting percentage 96±0.12, 93±0.14, mean number of roots 11±0.27, 8±0.21 and 4.3±0.22, 5.3±0.18 mean root length was observed in MS + growth regulators and MS + growth regulators with NaCl respectively.

3.2 Anatomy

The anatomical features of normal cells and tissues intact with normal size and shape of the cells constituting various tissues such as epidermis, cortex, vascular tissues and pith. Shoots developed through *in vitro* showed presence of tannins. Further, cells are enlarged deviating from normal spherical shape to spindle shape. Also less amount of cytoplasm and large number of vacuoles compared with normal control (Filed grown). Early onset of secondary growth in experimental control whereas secondary growth is very slower on salt induced experimental group when comparing with experimental and normal control. Further, vascular tissues (VB) is not fully regenerate in the experimental salt induced growth probably due to salt stress.

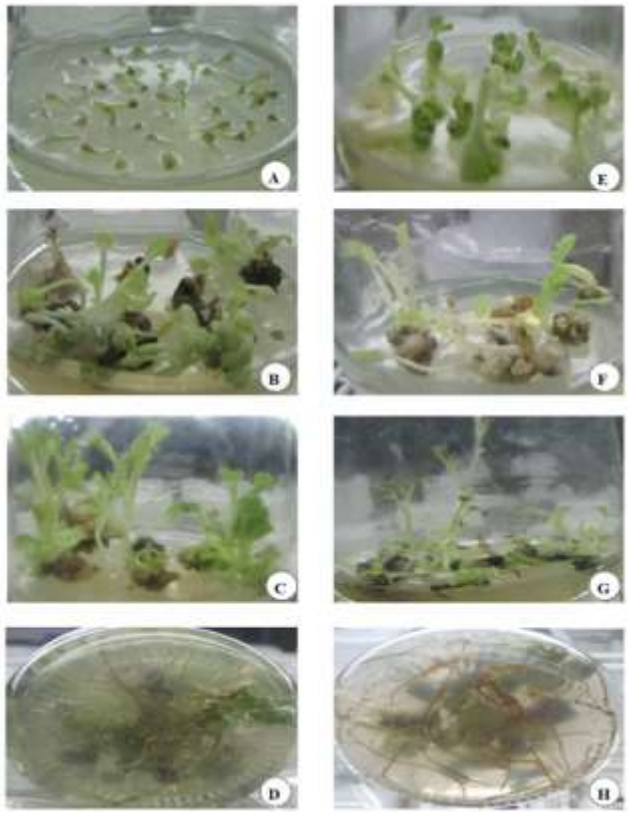
3.4 Antiangiogenesis activity

Antiangiogenic activity of various sample ethanol extracts such as natural, *in vitro* normal and salt induced plants were tested through *in vivo* CAM model. The 8^{th} day old embryo after treatment for number of blood vessels and their reduction was examined. The inhibition percentage is show in the table 4. All three samples showed the higher angiogenic activity 75±1.42, 85.7±1.24 and 80±1.62 at the concentration 1000 µg/mL respectively. The Plate 3 respects normal vascularation in the treated CAM which consists of primary secondary and tertiary micro vessels. The CAM treated samples displayed distorted vascularation as well as perturbation on existing vasculature Plate 3. The second best angiogenic activity was observed at 500 µg/mL as 70±0.11, 70±0.31 and 66.6±2.43% natural and *in vitro* normal and salt induced samples respectively against control (85.7±1.25) NaOH.

Table 1. Germination percentage of *Brassica oleracea* seeds under different media conditions

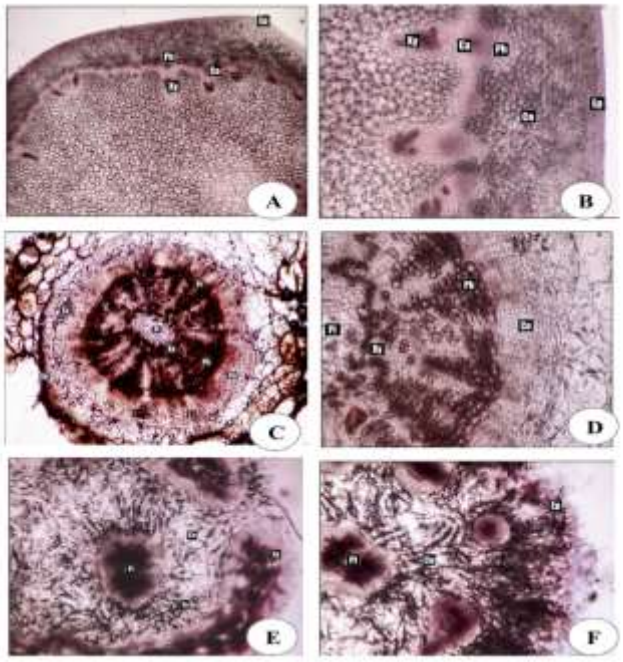
S. No	Treatments	Germination %	Treatments	Germination %
1	Water Agar Medium	02 ± 22.10	Water Agar Medium +20 mM NaCl	01 ± 2.11
2	½ MS	43 ± 13.41	½ MS + 20 mM NaCl	39 ± 3.41
3	Full MS	96 ± 0.91	Full MS +20 mM NaCl	92 ± 1.28
4	½ MS +BAP (2.22 µM) + KIN (1.16 µM) + TDZ (0.98 µM)	85 ± 1.23	½ MS +BAP (2.22 µM) + KIN (1.16 µM) + TDZ (0.98 µM) +20 mM NaCl	80 ± 1.72
5	½ MS +BAP (4.44 µM) + KIN (1.16 µM) + TDZ (0.98 µM)	76 ± 0.61	½ MS +BAP (4.44 µM) + KIN (1.16 µM) + TDZ (0.98 µM) + 20 mM NaCl	73 ± 2.09
6	½ MS BAP (6.66 µM) + KIN (1.16 µM) + TDZ (0.98 µM)	69 ± 1.39	½ MS BAP (6.66 µM) + KIN (1.16 µM) + TDZ (0.98 µM) + 20 mM NaCl	69 ± 0.19
7	½ MS BAP (8.88 µM) + KIN (1.16 µM) + TDZ (0.98 µM)	62 ± 0.46	½ MS BAP (8.88 µM) + KIN (1.16 µM) + TDZ (0.98 µM)+ 20 mM NaCl	62 ± 2.31
8	Full MS +BAP (2.22 µM) + KIN (1.16 µM) + TDZ (0.98 µM)	59 ± 0.48	Full MS +BAP (2.22 µM) + KIN (1.16 µM) + TDZ (0.98 µM) + 20 mM NaCl	57 ± 1.45
9	Full MS BAP +(4.44 µM) + KIN (1.16 µM) + TDZ (0.98 µM)	53 ± 1.10	Full MS BAP +(4.44 µM) + KIN (1.16 µM) + TDZ (0.98 µM)+ 20 mM NaCl	48 ± 0.44
10	Full MS BAP +(6.66 µM) + KIN (1.16 µM) + TDZ (0.98 µM)	46 ± 0.01	Full MS BAP +(6.66 µM) + KIN (1.16 µM) + TDZ (0.98 µM)+20 mM NaCl	41 ± 0.33
11	Full MS BAP +(8.88 µM) + KIN (1.16 µM) + TDZ (0.98 µM)	41 ± 2.05	Full MS BAP +(8.88 µM) + KIN (1.16 µM) + TDZ (0.98 µM) + 20 mM NaCl	35 ± 0.44

Plate 2. Shoot formation of *Brassica oleracea* from MS medium and MS + NaCl medium



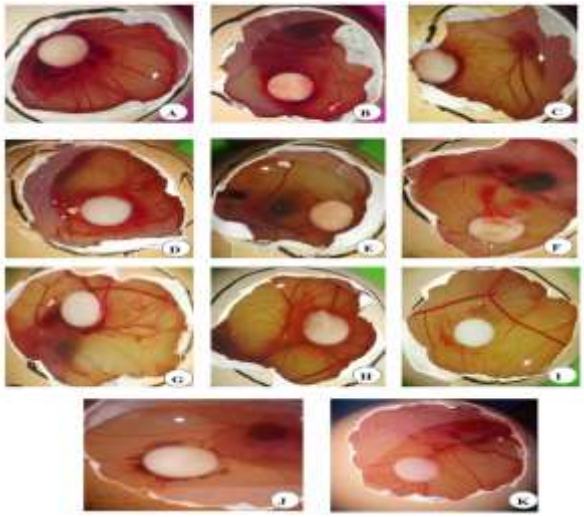
A, B, C & D - MS medium with growth regulators (BAP, KIN & NAA)
E, F, G & H - MS medium, growth regulators (BAP, KIN & NAA) with NaCl (20 mM).

Plate 3. Anatomical study of natural, *invitro* and salt stress plants of *Brassica olercea*



A&B – Wild plant, C&D – Tissue culture plant, E&F – Salt stress tissue culture plant

Plate 4. Antiangiogenic activity of natural, *in vitro* normal and salt stress plants ethanol extracts of *Brassica oleracea. L*



A, B & C - Natural plant; D, E & F – *In vitro* normal plant; G, H & I - *In vitro* plant with salt; J – Positive control (250 µg/ml NaOH) & K- Negative control (250 µg/ml DMSO).

Table 2. Effect of BAP, KIN, TDZ, GA_3 and IAA on initiation and multiple shoot induction from nodal explants of *Brassica oleracea* cultured on MS medium

S. No	BAP µM	KIN µM	NAA µM	TDZ µM	GA_3 µM	IAA µM	Shoot sprouting frequency %	Shoot No/explant	Shoot No/explants Subculture	Shoot length (cm)	Rooting %	Mean number of roots	Mean root length (cm)
1	2.22	-	-	-	-	-	24±0.12	4±0.22	9±1.23	1.7±0.28	24±0.21	3±0.23	1.1±0.14
2	4.44	-	-	-	-	-	31±0.18	4±0.18	10±0.42	1.4±0.19	29±0.19	4±0.38	1.3±0.24
3	6.66	-	-	-	-	-	36±0.16	5±0.43	10±0.35	1.9±0.16	32±0.16	4±0.15	1.5±0.32
4	8.88	-	-	-	-	-	48±0.27	6±0.58	12±0.20	2.1±0.05	46±0.23	5±0.32	2.3±0.15
5	11.10	-	-	-	-	-	51±0.36	6±1.88	14±0.39	2.7±0.38	57±0.21	6±0.36	2.5±0.61
1	2.22	1.16	0.98	-	-	-	57±0.10	5±0.92	10±0.42	2.6±0.27	64±0.16	5±0.21	2.3±0.37
2	4.44	1.16	0.98	-	-	-	59±0.61	7±0.17	11±0.37	2.9±0.45	78±0.35	7±0.38	2.2±0.06
3	6.66	1.16	0.98	-	-	-	70±0.42	8±0.26	13±0.28	3.4±0.58	82±0.27	7±0.19	2.7±0.17
4	8.88	1.16	0.98	-	-	-	84±0.19	9±0.37	16±0.24	4.5±0.72	93±0.28	9±0.54	3.6±0.09
5	**11.10**	**1.16**	**0.98**	**-**	**-**	**-**	**91±0.09**	**12±0.35**	**26±0.38**	**5.2±0.29**	**96±0.12**	**11±0.27**	**4.3±0.22**
1	2.22	-	-	1.13	-	-	42±0.54	5±0.61	11±0.35	1.9±0.30	58±0.31	5±0.28	2.4±0.18
2	4.44	-	-	1.13	-	-	49±0.37	6±0.20	12±0.38	2.4±0.47	63±0.41	6±0.11	2.7±0.45
3	6.66	-	-	1.13	-	-	54±0.39	6±0.62	13±0.28	3.2±0.26	69±0.29	5±0.15	3.5±0.62
4	8.88	-	-	1.13	-	-	65±0.18	7±0.16	15±0.45	3.7±0.32	75±0.09	7±0.30	3.2±0.36
5	11.10	-	-	1.13	-	-	77±0.05	8±0.18	17±0.38	4.6±0.18	86±0.57	8±0.26	3.8±0.63
1	2.22	-	-	-	0.25	0.25	43±0.17	4±0.36	10±0.37	2.7±0.49	47±0.61	4±0.73	2.1±0.21
2	4.44	-	-	-	0.25	0.25	48±0.29	5±0.28	11±0.29	3.6±0.51	53±0.28	5±0.20	2.4±0.43
3	6.66		-	-	0.25	0.25	57±0.39	6±0.54	13±0.24	3.8±0.27	58±0.32	5±0.09	2.7±0.26
4	8.88	-	-	-	0.25	0.25	63±0.46	7±0.28	15±0.09	4.1±0.20	69±0.17	6±0.38	3.5±0.25
5	11.10	-	-	-	0.25	0.25	68±0.29	7±0.73	17±0.05	4.3±0.17	72±0.33	7±0.28	3.6±0.17
Basal medium		-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Effect of BAP, KIN and TDZ on initiation and multiple shoot induction from nodal explants of *Brassica oleracea* cultured on MS + NaCl medium

S. No	BAP µM	KN µM	NAA µM	TDZ µM	NaCl (mM)	Shoot sprouting frequency %	Shoot No/explant	Shoot No/explants subculture	Shoot length cm	Rooting %	Mean number of roots	Mean root length (cm)
1	2.22	-	-	-	20	40±0.21	04±0.11	07±0.27	2.6±0.47	35±0.05	2±0.03	1.7±0.21
2	4.44	-	-	-	20	58±0.19	05±0.26	09±0.19	2.8±0.26	36±0.15	3±0.28	1.9±0.32
3	6.66	-	-	-	20	63±0.32	06±0.23	11±0.37	3.1±0.27	42±0.04	3±0.111	2.4±0.16
4	8.88	-	-	-	20	72±0.24	07±0.14	9±0.36	3.7±0.29	58±0.13	4±0.14	2.8±0.27
5	11.10	-	-	-	20	75±0.52	07±0.15	15±0.37	4.6±0.26	65±0.11	5±0.33	3.5±0.32
1	2.22	1.16	0.98	-	20	53±0.16	6±0.74	10±0.29	3.5±0.42	46±0.17	4±0.61	3.1±0.41
2	4.44	1.16	0.98	-	20	58±0.18	7±0.37	11±0.16	4.5±0.26	51±0.26	4±0.27	3.3±0.06
3	6.66	1.16	0.98	-	20	69±0.03	08±0.27	14±0.04	5.2±0.28	65±0.25	5±0.15	4.5±0.16
4	8.88	1.16	0.98	-	20	83±0.36	08±0.18	15±0.26	5.1±0.25	78±0.25	6±0.32	4.9±0.32
5	11.10	1.16	0.98	-	20	96±0.19	10±0.19	18±0.29	5.8±0.47	93±0.14	8±0.21	5.3±0.18
1	2.22	-	-	1.13	20	49±0.17	05±0.19	11±0.29	3.4±0.36	52±0.24	4±0.26	3.2±0.53
2	4.44	-	-	1.13	20	57±0.26	06±0.15	11±0.28	4.1±0.38	58±0.35	5±0.31	3.8±0.26
3	6.66	-	-	1.13	20	71±0.36	05±0.25	12±0.17	4.4±0.31	64±0.53	5±0.42	4.2±0.43
4	8.88	-	-	1.13	20	75±0.04	07±0.95	13±0.36	5.2±0.27	67±0.26	6±0.06	4.5±0.28
5	11.10	-	-	1.13	20	82±0.27	08±0.15	15±0.46	5.3±0.10	74±0.38	7±0.45	4.8±0.16
Basal medium		-	-	-	-	-	-	-	-	-	-	-

Table 4. Antiangiogenic activity of natural, *in vitro* normal and salt stress induced plant ethanol extracts of *B. oleracea*

Sample	Concentration (µg/mL)	No. of vessels in treated CAM	% of vessels Inhibition	% Inhibition (Mean ± SED)*
Natural Plant ethanol extract	1000	12±2.45	9±2.51	75±1.42
	500	10±2.02	7±2.34	70±0.11
	250	13±3.61	7±0.31	53.8±2.33
Normal *in vitro* plant ethanol extract	1000	7±2.12	6±2.66	85.7±1.24
	500	10±0.67	7±1.65	70±0.31
	250	7±2.14	4±3.22	57.1±2.43
Salt *in vitro* plant ethanol extract	1000	5±1.02	4±0.21	80±1.62
	500	3±0.31	2±3.22	66.6±2.43
	250	7±0.31	4±0.43	57.1±0.12
NaOH	250	7±1.11	6±2.17	85.7±1.25

Three eggs used for each samples. Mean ± SD was calculated for % inhibition of each sample.

3. DISCUSSION

A wide range of plants have now been successfully propagated by using plant tissue culture techniques. Most of the medicinal plants do not germinate in the soil. The mass propagation is possible through plant tissue culture. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species. Due to the presence of inhibition during seed germination, non-availability of the elite germination and poor seed germination due to short term viability. In some taxa *in vitro* regeneration was succeeded by using various explants [19, 20, 21, 22].

Seed germination patterns should vary depending on the environmental characters. Geophytes native to temperate zone often have dormancy, which require exposure to low temperature to break dormancy [23]. Seed germination under a range of environmental conditions is essential for plant species inhabiting wide altitudinal ranges and this combination of conditions is powerful for detecting selective effects on seed germination patterns. Increasing BAP concentration help for the enhancement of both germination rate and percentage but there was no significant difference among the various BAP concentrations attempted. Exogenous application of 1 mg/l and 2 mg/l BAP for 24 h increased the germination rate to a level comparable to that achievement with stratification in Petri dishes for 45 days. BAP has high activity in dormancy and germination control compared with GA3 in this case, but it is contrast with some plants breaking seed dormancy and stimulating germination by GA3. *In vitro* seed germination percentage was significantly progressed when MS media addition with NaCl (20 mM) on 30th day as maximum 92% of germination. The species of *C. mahanalei* from Western Ghats have reported only 4% of *in vitro* seed germination [24]. In all the treatments the germination rate was positively correlated with germination percentage. Therefore, rapid germination was associated with high germination percentage. It can be concluded that, full strength MS basal medium was suitable for seed germination and has a larger effect than the other treatments applied in this study.

The effect of BAP on shoot initiation and multiple shoot formation has been demonstrated in many cases using different varieties [25,26,27,28,29]. In the present study also BAP along with NAA and KIN produced maximum number of shoots. This results were in confirmation with the observation reported by [30] and [31] against hypocotyl, leaf and petiole morphogenic response of *B. oleracea* varieties respectively.

In the present study regeneration frequency decreased in MS with BAP+KIN+NAA along with NaCl when compared to without NaCl medium. In *B. oleracea* var. Botrytis also the increasing concentration of NaCl reduces the regeneration capacity [32]. This may be due to the excess soluble salts leads to osmotic stress [33]. High percentage of root regeneration was also observed on MS medium containing BAP+KIN+NAA. [34] used half strength MS medium containing 0.1mg/l NAA for regeneration of roots. [34] also used different concentration of NAA for rooting and found 88 % root regeneration on medium containing 0.3mg/l NAA. In the present study also presence of NAA in the shoot induction medium induces root formation simultaneously.

Transverse section of stem showed considerable anatomical alterations due to salt stress. In experimental control that is shoots develop through *in vitro* showed presence of tannins. Further, cells are enlarge deviating from normal spherical shape to spindle shape. Also less amount of cytoplasm and

large number of vacuoles compared with normal control (Filed grown). Early onset of secondary growth in experimental control whereas secondary growth is very slower on salt induced experimental group when comparing with experimental and normal control. Further, vascular tissues (VB) is not fully regenerate in the experimental salt induced growth probably due to salt stress. It is understand from the above observation that increase salt concentration reduced the growth of vascular tissues whereas in turn influence translocation of salt across the plant body. Also increase concentration of tannin observed may reduce cell physiology leading to reduction in the size of the tissues. The phyto hormones auxins, cytokines and gibberellins are major regulators of cell proliferation, cell specification and also cell elongation and differentiation [35]. It has been proposed that, changes in the cell wall structure and functions are the primary effects of salt stress leading to a cascade of secondary effects in growth and metabolism of plants. The difference in the cross section of normal, experimental control and experiment studied in this investigation clearly showed that this type of selection in cauliflower is very useful to generate abiotic stress resistant genotypes like in other *Brassica* species [36, 37]. Angiogenesis is essential in tumor growth and metastasis as the process provides necessary oxygen and nutrients for the growing tumor [38]. The present results showed that natural, *in vitro* normal and salt stress plant extracts changed the vascularization pattern; extract inhibited the new blood vessels formation in the treated CAMs as well as distortion of existing vasculature. Our result was confirmed by other earlier report on medicinal plants like *Ceropegia pusila* [39], *Baccharoides anthelmintica* [40], *Bouerhaavia diffusa* [41].

Conflicts of Interest

There are no conflicts of interest.

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