In Vitro Root Induction and Culture of the Medicinal Plant *Capparis spinosa* L.

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Abstract

***Capparis spinosa* L. "Caper" is one of the known medicinal plants in Palestine. In Arabic traditional medicine every part of Caper has medicinal uses, and roots particularly posses anti-inflammatory activity and commonly used for the treatment of rheumatism and back and joints pain. For better utilization of *C. spinosa* and to protect it from genetic erosion, a tissue culture protocol was optimized for the *in vitro* production of roots. MS media supplemented with IAA, IBA and NAA at different concentrations (0.0, 0.1, 0.5, and 1.0 mg/L) were evaluated for the production of adventitious roots under *in vitro* conditions. Results revealed that the highest rooting percentage (62.5%) was observed on when adding 1.0 mg/L NAA to the media. At 1.0 mg/L NAA, leaf discs from *in vitro* growing plants resulted in 68.8% rooting while leaves from *ex vitro* plants gave less percentage (43.8%). The effect of carbon type (sucrose, sorbitol and fructose) and concentration (1.5 or 3.0% w/v) on the *in vitro* adventitious root induction was tested. Sorbitol resulted in no rooting at both concentrations. Sucrose at 3.0% gave higher (41.6%) root induction compared to fructose (25.0%) at the same concentration. After six weeks from the induction, well established roots were transferred to 1X MS liquid medium containing 1.0 mg/L NAA and under continuous agitation at 80 rpm. The maximum root growth was obtained after six weeks of culture and later at the 6th week the growth was slowly declined.**

Key words: *Capparis spinosa* L., anti-inflammatory activity, root culture.

I. INTRODUCTION

Capparis spinosa L. is one of the known medicinal plants in Palestine which has high pharmaceutical and ecological values [1]. Caper belongs to the genus *Capparis* of the Capparidaceae family. The genus *Capparis* has about 350 species including shrubs, trees and woody climbers [2]. *Capparis spinosa* was given a new synonym *Capparis hierosolymitana* Danin. or The Jerusalem Caper [3]. *C. spinosa* has a wide distribution in the Old World from South Europe, North and East Africa, Madagascar, Southwest and Central Asia to Australia and Oceania [4]. Caper is a dicotyledonous, spiny medium size perennial bush [4]. It may reach about one meter height [5, 6, and 7]. It grows spontaneously in cracks and crevices of rocks and stone walls. Caper adapts to poor soils where water and nutrients are major limiting factor [1, 6, and 8]. Caper has many pharmaceutical uses since it contains a wide range of phytochemicals like alkaloids and flavinoids. Although all parts of caper are pharmaceutically useful, roots are the principle material used in the traditional medicine in Palestine. Roots are mainly used to treat joints inflammations, and also to treat hypertension, arteriosclerosis, gout, rheumatism, and anemia [8, 9, and 10]. For better utilization of *C. spinosa* and to protect it from future overexploitation and genetic erosion, a tissue culture protocol was optimized for the *in vitro* production of roots.

II. MATERIALS AND METHODS

The experimental work in this study was conducted in the Plant Tissue Culture Laboratory, Biotechnology Research Center at the Palestine Polytechnic University in Hebron, Palestine.

A. Source of Plant Material

Seeds of *C. spinosa* were collected from wild plants growing in Hebron city in September 2008. Part of the seeds were germinated in pots and kept in the growth room for further growth. Explants like leaves were taken from the potted plants and used for further experiments. The remaining of the seeds were germinated on plated filled with MS media supplemented with 1.0 mg/L GA_3.

B. Media Preparation and Sterilization

The growth medium was prepared by dissolving the basal salt mixture and vitamins of Murashige and Skoog (1962) with 3.0% sucrose and pH was adjusted to 5.8 with NaOH and HCl. Plant growth regulators (PGR) were added to the media and finally brought to the final volume. Media was solidified with 6.0 g/L agar. All components were heated with continuous stirring until complete dissolution. Finally, medium was dispensed in autoclavable bottles for use in sterile polystyrene Petri dishes and in 50.0 ml Erlenmeyer flasks (8.0 ml). Media autoclaved at 121°C and 15 of Psi pressure for 20 min.

C. Growth Conditions

Cultures were incubated in the growth room at 24±1ºC with 16:8 light:dark photoperiod under cool-white fluorescent illumination of 40-45 µmol/m^2/sec photosynthetic photon flux density (PPFD).

D. *In Vitro* Root Culture

1. Adventitious Root Induction from Leaf Discs:

Roots were induced from sterilized leaf segments (0.8 cm × 0.6 cm) on 5.0 cm Petri-dishes filled with 8.0 ml of MS media according to the following treatments:

a. Treatment with NAA, IBA or IAA at the concentrations of 0.0, 0.1, 0.5, and 1.0 mg/L each.
b. Different carbon sources; sucrose, fructose and sorbitol at different concentrations (either 1.5 or 3.0% w/v) in combination with 1.5 mg/L NAA and 7.0 g/L agar.
c. NAA at 1.0 mg/L cultured by leaf explants obtained from two different sources; potted (*ex vitro* growing) and *in vitro* growing plants.

Experiments were carried out by placing the abaxial (lower) surface of the explants facing the medium. For each different treatment, four explants were cultured in the dark in the growth room. In treatment (a), four Petri dishes were kept in dark and the other four incubated under light. In treatment (b), three Petri dishes were used. Data were reported after six weeks of culture according to rooting percentage, root number and length.

2. *In Vitro* Root Growth in Liquid Media

The root growth was estimated by culturing leaf segments with adventitious roots in 8.0 ml of 1X liquid MS media supplemented with NAA at 1.0 or 2.0 mg/L in 50 ml Erlenmeyer flasks. The cultures were kept under continuous agitation at 80 rpm on a rotary shaker and covered with aluminum foil to ensure darkness. Six replicates for each treatment were evaluated. Regular measurement of root growth in different treatments was assessed in terms of root growth area at two weeks interval. The root growth area was determined up to a period of eight weeks.

E. Experimental Design and Statistical Analysis

All experiments were set at the Completely Randomized Design (CRD) and significance in data means was tested by the analysis of variance ANOVA with StatPlus 2007 Professional software. In the case of Significant ANOVA at p=0.05, means were separated by Fisher LSD test. Alternatively, when comparing between two means, statistical T-test was conducted at p=0.05. Data with unequal variance or not normally distributed, the non-parametric test of Kruskal-Wallis ANOVA was carried out.

III. RESULT AND DISCUSSION

In Vitro Root Culture

1. Adventitious Root Induction from Leaf Discs

The effect of auxin type on root induction from *C. spinosa* leaf discs was evaluated after six weeks from culture. Under light condition, no root induction was observed in all treatments. Under dark, adventitious roots were induced in different media types at different rates. The root induction response, roots number and length are presented in Table I. No root induction was observed in auxins deprived medium. The vital role of auxins on rooting has also been previously reported by [11] and [12]. MS medium supplemented with 1.0 mg/L NAA gave the highest root induction response (62.5%), followed by 0.1 mg/L NAA and 0.5 mg/L NAA (56.25% and 25%) respectively. On the other hand, IBA at different concentrations gave lower root induction response with less than 19%.

TABLE I: Rooting percentage, average number of roots, and root length of adventitious *in vitro* root induction from leaves of *C. spinosa* in dark condition in response to different treatments of auxins after six weeks of culture. Sample size (n) = 16.

Type and Concentration of Auxins (mg/L)	Percentage of Response (%)	Mean of Root Number / Culture (Mean ± SE)	Mean of Root Length / Culture (cm) (Mean ± SE)
NAA (0.1)	56.25	1.19 ± 0.42	0.56 ± 0.18
NAA (0.5)	25.00	0.94 ± 0.49	0.33 ± 0.16
NAA (1.0)	62.50	1.69 ± 0.50	0.57 ± 0.16
IBA (0.1)	18.75	0.25 ± 0.14	0.14 ± 0.09
IBA (0.5)	0	0	0
IBA (1.0)	12.50	0.19 ± 0.14	0.12 ± 0.08
IAA (0.1)	0	0	0
IAA (0.5)	0	0	0
IAA (1.0)	0	0	0`
Control	0	0	0

No effect of IAA at the concentrations used on root induction was observed. According to the number of roots and root length, the statistical significance between treatments was analyzed by Kruskal-Wallis ANOVA, the results have shown a statistically significant differences (p < 0.05) between the 10 tested media. The MS medium supplemented with 1.0 mg/L NAA gave the highest number of roots per culture and the longest root per culture compared with the other auxins at different concentrations. This result supports a similar approach report by [13] when showed an evidence of root induction on MS medium containing 4.5 mg/L NAA in *Rauwolfia serpentina*. The reasons of lower rooting efficiency from IBA and no rooting response in IAA treatments in caper plant could be attributed to factors like genetic variation between species in which different species responded to same type of plant growth regulator differently and some plants species were reported to response better in IBA or IAA treatments [11, 12, 13, and 14]. Also, [15] mentioned that IAA is better used to promote rooting of herbaceous species, while trees and woody plants respond better with IBA and NAA.

In vitro adventitious root culture has also been reported in many studies on other plant species. Leaf segments were the explants that gave highest root induction. In [13] study, root growth from the leaf segment of *Rauwolfia serpentine* on MS medium containing 4.5 mg/L NAA was obtained. Moreover, Reference [11] was able to derive roots from the leaves of *Vernonia amygdalina*. Direct root growth from the leaf and hypocotyls explants of *Cichorium*

intybus L. had also been reported by [14]. Furthermore, root induction had been obtained from the leaf of *Orthosiphon stamineus* cultured on MS medium supplemented with 3.0 mg/L of IAA in [12] study. Reference [12] justified that leaf segments are good starting tissue for the adventitious root induction. This could be due to the presence of cells associated with the leaf veins (vascular
tissue) which can be readily stimulated by exogenous auxin. Light played an important role in i*n vitro* adventitious root induction. The present result was agreed to [14] where root culture was produced more and faster adventitious roots under dark than light. Reference [15] also confirmed that light commonly has a negative effect on root formation. Moreover, reference [16] reported that root growth can be improved in dark induction.

Carbon source and concentration has a significant effect on root induction from leaves of *C. spinosa* (Table II). In this study, no root formation was observed in sugar deprived media and this demonstrates the necessity of the carbon source in adventitious roots induction. MS medium supplemented with 1.5 mg/L NAA and 3.0% w/v sucrose gave 41.6% rooting percentage, while media contained 3.0 or 1.5% w/v fructose produced 25% and 8.3% rooting percentage respectively. According to the number of roots and roots length, the significance between treatments was analyzed by Kruskal-Wallis. The results showed statistically significant differences ($p < 0.05$) between the tested media. MS medium containing 1.5 mg/L NAA supplemented with 3.0% w/v sucrose gave the highest number of roots and root length per culture compared with other carbon sources and concentrations. This result is agreed with [17] who showed that a medium containing 0.2 mg/L NAA and sucrose (3.0% w/v) produced 70% root induction and highest root length of *Centella asiatica* L. rather than other carbon sources like glucose and maltose. It is reported that carbon source (sucrose, fructose, glucose and maltose) may have limited influence on root induction in *Phoenix dactylifera* L. [18] where they influenced root length.

TABLE II: Percentage of root induction, average number of roots, and root length of *in vitro* root induction from of *C. spinosa* in dark condition in dark condition in response to different sugar types and levels used in MS media supplemented with 1.5 mg/L NAA after six weeks of culture. Sample size (n)= 12.

Carbon Source Types and Concentrations	Percentage of Root Induction (%)	Mean of Root Number / Culture (Mean ± SE)	Mean Root Length / Culture (cm) (Mean ± SE)
Sugar Free	0	0	0
Sucrose (3.0% w/v)	41.6	0.67 ± 0.26	0.13 ± 0.05
Sucrose (1.5% w/v)	0	0	0
Fructose (3.0% w/v)	25	0.42 ± 0.23	0.09 ± 0.05
Fructose (1.5% w/v)	8.3	0.17 ± 0.17	0.03 ± 0.03
Sorbitol (3.0% w/v)	0	0	0
Sorbitol (1.5% w/v)	0	0	0

According to the effect of explant source on root induction, leaf discs that were taken from the *in vitro* growing plants gave higher root induction percentage (68.8%) than *ex vitro* growing plants (43.8%). Also, the roots that were induced from *in vitro* leaves were longer and appeared as a fibrous root mass "fig. 1". This result can be explained by the fact that the root formation is more easily induced in juvenile than old plant parts [15]. Additionally, the surface sterilization of *ex vitro* leaves is negatively affects leaf structure and consequently it altered the root induction.

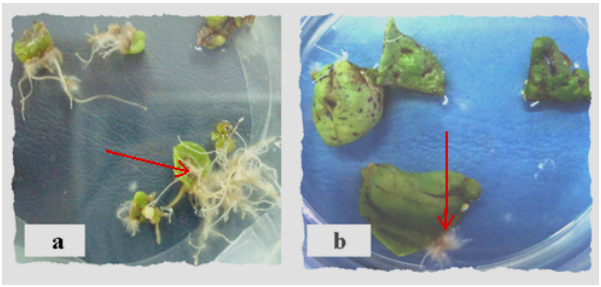

Figure 1: Adventitious roots (arrows) induced on MS medium supplemented with 1.0 mg/L NAA after six weeks of culture from, (a) *in vitro* leaves, (b) *ex vitro* leaves.

2. *In Vitro* Root Growth in Liquid Media

The effect of liquid media with NAA at 1.0 or 2.0 mg/L on root growth was tested by measuring the growth area of roots every two weeks of culture. The mean growth areas in cm^2 in two weeks interval during a period of eight weeks are presented in Table III.

TABLE III: the effect of NAA concentration on root growth from caper leaves in liquid media during eight weeks of culture. The difference column represented the root growth area increased from the time of culture starting to the six weeks of culture. Sample size= 6.

MEDIUM TYPE	MEAN ROOT GROWTH AREA (CM^2) ± SE					
	0 week	**2 weeks**	**4 weeks**	**6 weeks**	**8 weeks**	**Net Area Growth (0-6 weeks)**
1.0 mg/L NAA	4.67 ± 1.43	6.50 ± 1.95	7.96 ± 2.07	8.75 ± 2.12	7.96 ± 2.07	4.08 ± 1.13
2.0 mg/L NAA	1.71 ± 0.32	3.75 ± 0.51	4.04 ± 0.48	4.71 ± 0.52	4.54 ± 0.72	3.00 ± 0.43

Result did not show significant differences between the two tested media pertaining to their effect on root growth ($p > 0.05$). The MS liquid medium supplemented with 1.0 mg/L NAA gave higher growth than MS liquid medium containing 2.0 mg/L NAA where the mean root growth area was 4.08 ± 1.13 cm^2 when using 1.0 mg/L NAA compared to 3.00 ± 0.43 cm^2 when the 2.0 mg/L NAA was added to the medium. A root tissue (4.67 cm^2 ± 1.43) cultured in 8.0 ml full-strength MS liquid medium supplemented with 1.0 mg/L NAA and under continuous agitation (80 rpm) showed most significant root growth increase during the first four weeks of culture. The maximum growth area of roots (8.75cm^2 ± 2.12) was

obtained at the 6th week of culture and later at the 6th week the growth was slowly declined (7.96 $cm^2 \pm 2.07$) and roots began to appear brown in color "Fig. 2".

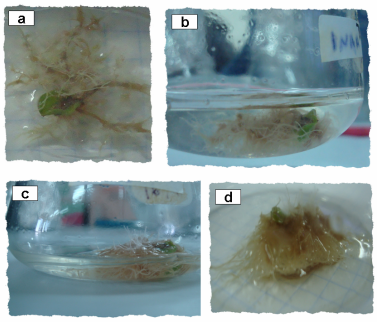

Figure 2: Root growth in MS liquid media supplemented with 1.0 mg/L NAA. (a) at starting day of culture, (b) after two weeks of culture, (c) after six weeks of culture, (d) after eight weeks of culture.

In full-strength MS liquid medium supplemented with 2.0 mg/L NAA, the most significant root growth expansion was during the first two weeks of culture. The maximum growth area of roots (4.71 $cm^2 \pm 0.52$) was obtained at the 6th week of culture and later at the 6th week the growth was gradually decreased (4.54 $cm^2 \pm 0.72$) and roots began to appear brown in color.

In summary, root growth in liquid media is better than in solid media. Reference [15] reported that the formation of adventitious roots especially in woody plants is generally poor on solid media and liquid medium is sometimes preferred in woody plants. In the present study, the root growth was measured by calculating the root growth area during growing period and the growth area was taken every two weeks until the eighth week of culture. This method was used instead of taking root fresh weight to avoid possible contamination during weighing and losing cultures. In the two media used, the most significant in increase root growth was observed during the first two weeks of culture. The maximum growth area of roots was measured at the 6th week of culture, and later on at the growth was slowly declined and roots began to browning. The decline of root growth might be due to the accumulated inhibitors and nutrients decline. Accordingly, it is necessary to change the growth media and do subculture every six weeks of root culture. Similarly, in [11] study, the *Vernonia amygdalina* root biomass was increased after five weeks of culture and the root culture was maintained up to six weeks. Also, Reference [14] reported that the biomass of root culture of *Cichorium intybus* was increased after six weeks of culture and the root culture was maintained up to the eight weeks.

To the best of my knowledge, no previous studies were conducted to put a protocol for the *in vitro* root induction and culture of *C. spinosa*. The present study is carried out based on the fact that although all parts of caper are pharmaceutically useful, roots are the principle material used in our traditional medicine to treat many diseases like rheumatism and back and joint pains [10]. In the present study, a reproducible method for high frequency root induction and growth from leaf segments without threatening the wild life and natural resources was established.

IV. CONCLUSION

In this study, an alternative propagation protocol for caper propagation via plant tissue culture was investigated. Furthermore, plant tissues that have medicinal activities were produced in a large scale. In the present study, The leaf discs from *in vitro* growing plants gave better adventitious roots compared to leaves from *ex vitro* growing plants. Moreover, the types and concentrations of carbon source have a significant effect on root induction from leaves of *C. spinosa*; MS medium containing 1.5 mg/L NAA and supplemented with 3.0% (w/v) sucrose gave the highest number of roots and root length per culture compared to other types and concentrations of carbon sources like fructose and sorbitol. In this study, MS liquid medium supplemented with 1.0 mg/L NAA was the optimum medium for root growth. The maximum growth area of roots was obtained at the 6th week of culture and later at the 6th week the growth was slowly declined.

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