P3: *Capparis spinosa* L., "Caper": *In Vitro* Propagation, Callus Culture and Secondary Metabolites Production and Bioassay

Ghada Abu Khalaf [1] and Rami Arafeh [1]

[1] Biotechnology Training and Research Unit, Palestine Polytechnic University. P.O. Box 198, Hebron, Palestine.

Introduction

There are more than 700 plant species in Palestine used for their medicinal properties [7]. Many of wild plants are becoming endangered due to rural and urban extension, uncontrolled deforestation and illegal collection, pollution, and low level of awareness in the Palestinian population. To counter overexploitation of natural resources and consequent threats to biodiversity an alternative biotechnological methods and sustainable practices must be implemented. The advancements in biotechnological methods of culturing plant tissues and cells provide new means for conserving and rapid propagation of valuable, rare and endangered plants.

Capparis spinosa L., "Caper", is one of the known medicinal plants in Palestine. It is a perennial subshrub about one meter height. It grows spontaneously in cracks and crevices of rooks and in stone walls. The plant has orbicular leaves and white fading pinkish purple flowers [8]. The medicinal properties of *C. spinosa* includes antifungal [4], anti-inflammatory [2, 5], anti-rheumatoid [1, 2, 3], antioxidant, diuretics [1, 2, 6, 8], and antidiabetic activity [2]. In traditional medicine, it is used to treat back pain, deafness and female infertility [7].

Capparis spinosa contains a wide range of phytochemical compounds that isolated from different plant parts like roots, leaves fruits, floral buds and stems.

In order to produce high number of *in vitro C. spinosa* plants, this project will focus on the following approaches:

- studying the chemical and physical factors affecting *in vitro* propagation of *C. spinosa*
- establishing callus tissue and cell culture of *C. spinosa*

After achieving the two above objectives, the biological activity of the material obtained will be examined.

Materials and Methods;

- Two sources of explant were used to establish *in vitro* mother stocks; seeds were collected from wild plants in Hebron area, and axillary buds from plants growing in pots in the growth room. Surface sterilization of the starting material was pursued by washing the explants for 15min. in 20% v/v commercial bleach followed by 3 times washes with sterile distilled water (SDW). Additional wash with 70% Ethanol for 30sec. was carried out followed by 2 times washing with SDW. Seeds and axillary buds were inoculated on the surface of different media supplemented with various concentrations and types of plant growth regulators.
- Callus induction and culture: callus was induced from leaf discs (5.0mm) on media supplemented with different levels of 2, 4-D. Callus was subcultured every 4 weeks on media with different PGR levels and callus growth curve was plotted.

The produced *in vitro* plants and callus will be the material for secondary metabolites extraction and bioassay.

Results

The overall seed germination percentage was 2.0%. No effect on seed germination was observed either by changing media type and constituents, or by placing seeds under dark or light conditions.

Auxiliary buds were best grown on 1/2 MS media with 15g/l sucrose, 1.0 mg/l GA_3, 0.5mg/l IAA, and 0.5 mg/l BA and resulted in maximum shoot proliferation.
Callus was successfully inducted on MS media + 0.5 mg/l 2,4- D, and subcultured on MS media with 1.0 mg/l 2-4D and 1.5mg/l BA.

References

Demir Y., Gungor A.A., Duran E. D. and Demir N. (2008). Cystenine Protease (Capparin) from Capsules of Caper (Capparis spinosa). Food Technol. Biotechnol, **46** (3):286-291.

Kusmenoglu S., Toker G., Can Baser K.H. and Koca U. (1997). Composition of the Fruit Oils of Capparis Species. Acta Pharmacutica Turcica XXXIX **(**2): 55-57.

Pu Fu,X , Wu T., Abdurahim M., Su Z., Hou X.L, Aisa H.A. and Wu H. (2008). New spermidine alkaloids from Capparis spinosa roots. Phytochemistry Letters, **1**: 59–62.

Ramezani Z., Aghel N. and Keyghobadi H. (2008). Rutin from Different Parts of Cappris spinosa Growing Wild in Khuzestan/Iran. Pakistan Journal of Biological Sciences, **11**(5):768-772.

Romeo V., Zino M., Giuffrida D., Condurso C.and Verzera A. (2007). Flavour profile of capers (Capparis spinosa L.) from the Eolian Archipelago by HS-SPME/GC-MS". Food Chemistry **101**:1272-1278.

Sakcali M.S, Bahadir H. and Ozturk M. (2008). Eco-Physiology of Capparis spinosa L.: A Plant Suitable for Combating Desertification. Pak. J. Bot. **40** (4):1481-1486.

Said O., Khalil K.,Fulder S. and Azaizeh H. (2002). Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and West Bank region. Journal of Ethnopharmacology**, 83:**251-265.

Satyanarayana T, Anjana A.M.and Vijetha P. (2008). PHCOG REV.: Plant Review Phytochemical and Pharmacological Review of Some Indian Capparis Species**.** Pharmacognosy Reviews [Phcog Rev.] -Supplement**, 2** (4): 36-45.