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Improvement of caper (*Capparis spinosa* L.) propagation using *in vitro* culture and gamma irradiation

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ABSTRACT

Some of the factors influencing the propagation of caper (*Capparis spinosa* L.) plants *in vitro* and germination of the seed were studied. The number of adventitious shoots emerging from caper stems cultured *in vitro* increased from 2.2 shoots per explant when the growth medium contained 2 mg/L of gibberellic acid (GA3) to 5.5 when the growth medium contained 2 mg/L zeatin riboside (ZR) and 1 mg/L naphthalene acetic acid (NAA). The best medium for callus formation from leaf and stem parts contained the growth regulators 1 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L NAA and the best medium for plant regeneration contained 1 mg/L kinetin and 0.1 mg/L indole-3-acetic acid (IAA). The effect of gamma irradiation on the growth of caper shoots *in vitro* was also studied. A 10 Gy dose of gamma irradiation stimulated growth of shoots up to 200% and increased shoot rooting percentage from 75 to 100%.

Methods of scratching the seed coat with iron particles and treating the whole seeds with concentrated H_2SO_4, ultrasonic waves and gamma rays were employed for breaking the seed dormancy. Treating the seed with H_2SO_4 for 20 min along with scratching was very effective in stimulating germination (46% as compared with 0% for the control). Irradiating caper seeds with 100 Gray (Gy) dose of gamma rays led to 50% seed germination *in vitro* and 70% on peatmoss one month after culturing.

1. Introduction

Caper (*Capparis spinosa*) is a perennial spiny shrub that belongs to the genus *Capparis* and the *Capparaceae* family. It is believed that there are 350 species in the genus *Capparis* L. (Lawrence, 1951). Caper is a native Mediterranean plant but its distribution stretches from the Atlantic coasts of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, and eastward to the Caspian Sea and into Iran (Alkire, 2000).

The commercially valuable parts of caper are the immature flower buds, which are pickled in vinegar or preserved in granular salt. Semi-mature fruits (caper berries) and young shoots with small leaves may also be pickled for use as a condiment.

Capers contain substantial amounts of the antioxidant bioflavonoid rutin, which makes them favorable for use in the food industry. Capers are said to reduce flatulence and have antirheumatic effects (Demir et al., 2008). Capers are reported to be hepatic stimulants and protectors, and to improve liver function. Capers have also been reported to be useful in treatment of arteriosclerosis, as diuretics, kidney disinfectants, vermifuges and tonics. In addition to this, infusions and decoctions from caper root bark have been traditionally used for dropsy, anemia, arthritis and gout. Caper extracts and pulp have also been used in cosmetics (Bond, 1990).

Newly harvested seed germinate rather quickly but at a low rate due to the mucilage which develops when the seed is placed in contact with water and imposes an effective barrier against the diffusion of oxygen to the embryo (Olmez et al., 2004). However, when the seed are dry, they undergo dormancy and become very difficult to germinate and require special treatments to break dormancy (Bond, 1990; Olmez et al., 2004; Al-Oudat, 2008). Some of the treatments aim at softening the hard seed coat using acids (Olmez et al., 2004) or making scratches on the coat using a scalpel (Chalak et al., 2003).

Propagation of caper using stem cuttings is difficult, especially rooting, and not commercially feasible (TansÚ and Kocabaa, 1997; Alkire, 2000). Tissue culture techniques have been used in vegetatively propagated plants and for seed propagated plants that have germination problems (Al-Safadi, 2006). *In vitro* techniques have been used to produce disease-free plants by means of meristem cultures (Bertaccini et al., 1986; Conci and Nome, 1991; Zhen, 1991). Tissue cultures are also being used to facilitate selection of mutants. Many traits may be investigated *in vitro*, including tol-

Abbreviations: 2,4-D, 2,4-Dichlorophenoxyacetic acid; BAP, 6-Benzylaminopurine; GA3, Gibberellic acid; Gy, Gray (100 rad); IAA, Indole-3-acetic acid; KIN, Kinetin; NAA, Naphthalene acetic acid; ZR, Zeatin riboside; Z, Zeatin.

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Table 1
Plant growth regulators used in the growth media to induce the formation of adventitious shoots, callus formations or regeneration of plants from callus.

Medium	Purpose	Concentration of plant growth regulators (mg/L)							
		IAA	BA	GA3	NAA	2,4-D	KIN	ZR	Z
MS1	Adventitious shoots	0	0	0	0	0	0	2	0
MS2	Adventitious shoots	0	0	2	0	0	0	0	0
MS3	Adventitious shoots	0	0	0.1	1	0	0	2	0
MS4	adventitious shoots	0	0	2	1	0	0	0	0
Ms5	Regeneration	0	0	0	0.1	0	1	0	0
Ms6	Regeneration	0.1	0	0	0	0	1	0	0
Ms7	Regeneration	0	0	0	0.1	0	0	0	1
Ms8	Regeneration	0.1	0	0	0	0	0	0	1
Ms9	Callus formation	0	1	0	0.1	0	0	0	0
Ms10	Callus formation	0	0	0	0	2	0.5	0	0
Ms11	Callus formation	0	2	0.1	1	0	0	0	0
Ms12	Callus formation	0	0	0.1	1	2	0	0	0
Ms13	Callus formation	0	0	0	0.1	0	0	0	2
Ms14	Callus formation	0	0	1	0	0	0	0	2
Ms15	Callus formation	0	0	0	0	2	0	0	0
Ms16	Callus formation	0	2	0	0	0	0	0	0

erance to high and low temperature, water deficit, salinity and herbicides, and resistance to fungal diseases (Barlass, 1985; Ingram and MacDonald, 1985; Dami and Hughes, 1997; Al-Safadi and Arabi, 2003, 2007).

Low doses of ionizing radiation have been used to stimulate plant growth *in vitro* and *in vivo* and in many crops such as carrots, potato, beans and others (Maherchandani, 1975; Kuzin et al., 1986; Al-Safadi and Simon, 1990; Al-Safadi and Simon, 1995; Al-Safadi and Arabi, 2003).

Commercial cultivation of caper in Syria is still in the infancy with the possibility of future expansion owing to its economic importance which can contribute to livelihood of many small farmers due to its low cultivation requirements and its tolerance to adverse environmental conditions. Thus, the objectives of the current study were first to develop a protocol to obtain caper plantlets *in vitro* which can be acclimatized and planted in the field. The second objective was to study some of the factors that can help in enhancing seed germination.

2. Materials and methods

2.1. Plant material

Seeds, immature fruits, and stem cuttings of caper (*C. spinosa* L.) were collected from different areas in the south-western part of Syria. The collection areas included Aldimas, Erna, and Surghaya which are located between 35.52E and 36.06E and between 33.20N and 33.35N. The altitude of the collection areas ranges between 1100 and 1300 m above sea level and these areas receive an average amount of annual rain between 500 and 650 mm.

Stem cuttings and immature fruits were collected during June and July 2008 and the seeds were collected during August 2008 when the fruits were ripe.

2.2. Stem cuttings

2.2.1. Sterilization

Stem cuttings collected from the field were washed under running water for 1 h, and then soaked in water and soap for 30 min with continuous agitation. They were then soaked in water supplied with 0.5% systemic fungicide benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate, 50% active ingredients) for 15 min. Under sterile conditions, the cuttings were transferred to 70% alcohol for 1 min and then transferred to 1% sodium hypochlorite for 3 min with continuous agitation. Finally the cuttings were washed with sterile distilled water three times for 5 min each.

2.2.2. Tissue culture

Sterilized stem cuttings were divided into small explants containing one bud under sterile conditions and cultured in test tubes containing either Rugini (Rugini and Verma, 1983) or MS media (Murashige and Skoog, 1962). Four replicates with 15 explants per replicate were used for each medium. The tubes were placed in an incubator under 24 °C and 16 h light growing conditions. Four weeks later the growing shoots were removed from the tubes, under sterile conditions, and their lengths were taken using a sterile thread and ruler. The shoots were then divided into explants containing one bud and transferred to fresh MS medium (MS1, MS2, MS3, and MS4) containing different growth regulators (Table 1) to induce adventitious shoot formation. Another set of media (MS5, MS6, MS7, MS8, MS9, MS10, MS11, and MS12), also differing in type and concentration of growth regulators, were used to induce callus formation (using stem and leaf explants) and regeneration of plants from this callus (Table 1). Three replicates with 10 explants per replicate were used for last 2 experiments.

To study the effects of low doses of gamma irradiation on the growth of caper shoots, single-node cuttings were placed in test tubes containing MS medium (without growth regulators) and subjected to 10, 15, and 20 Gy of gamma radiation. The irradiated cuttings were immediately transferred to fresh medium, to avoid the effects of irradiation, and let to grow under the aforementioned conditions.

2.3. Fruits

The unripe fruits were sorted according to their length into 3 categories: less than 2 cm, 2–4 cm, and more than 4 cm. The fruits were cut into cross-sections 2 cm wide and placed on Petri dishes containing MS medium with different growth regulators (MS9, MS10, MS11, MS12, MS13, MS14, MS15, and MS16) as shown in Table 1 to test the ability to form callus on these pieces. Both peeled and non-peeled fruits were used in the experiment.

2.4. Seeds

Various treatments were tested to break dormancy of the seed and promote growth of the shoots. Four replicates with 10 seeds per replicate were used for each of the following treatments: