



## Enhancement Rutin Production from *Capparis spinosa* Plant by UV-C or Gamma Irradiation using In vitro Culture



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**I**N THE FRAMEWORK of climate changing adaptation and mitigation efforts, biotechnology can positively contribute to lowering the likelihood of environmental systems being affected by climate change's consequences. Caper bush is notoriously difficult to grow through traditional techniques, although despite the substantial social-economic and medical value of this species, its micropropagation has received very little study thus far. Therefore, in our study the internode segments were collected from the Southern of Sinai, and disinfected with various treatments of disinfectant agents and exposure times. Then the successive clean shootlets formed in the sterilization stage were separated and cultured on MS medium at various strengths of salts (Full,  $\frac{3}{4}$ ,  $\frac{1}{2}$  and  $\frac{1}{4}$  strength). The established micronodes were cut into small pieces, and re-cultured on MS medium supplemented with BAP or Kin each at 0.0, 1.0, 2.0 and 3.0 mg/l. The growing shootlets were removed individually and cultured on rooting MS medium supplemented with 0.0, 1.0, 2.0, 3.0 and 4.0 mg/l IBA or IAA for each, and finally the rooted explants were acclimatization in green house. UV-C rays exposure times (2, 4 and 6 hrs) or gamma radiation wave doses (0.5, 1.0 and 1.5 Gy) were applied. The successive rooted explants cultured on MS media containing IAA 4.0 mg/l (scored 41.66 % roots) were transferred on peat moss: vermiculite 2:1 which scored 41.66 % acclimatized plantlets. Also, gamma radiation at 1.0 Gy increased flavonoid and rutin content to 12.34 and 3.25 mg/g DW compared to 7.21 and 0.06 mg/g DW for control plants, respectively.

**Keywords:** *Capparis spinosa* L, In vitro, Micropropagation, UV-C, Gamma radiation, Flavonoids, Rutin.

### Introduction

Food security and health problems are often the most important dangers to human life, especially with an expected increase in population to over ten billion by 2050 (Gubser et al., 2021). In addition to the negative consequences of climate change, extra pressures have caused a 70% reduction in agricultural productivity overall (Sesana et al., 2021), which call for an increase in crop yield for each unit area (Raven and Wagner, 2021).

Agricultural crop growth and yields are hampered by the variability of climatic factors that generate problems with the environment such as flooding, severe drought, alkalinity, salinity, and, variations of light intensity, chilling, heavy metals toxic effects, and, low oxygen levels, as well as high air wind velocity (Malhi et al., 2021). Changes in the global climate system have also increased the length and frequency of many environmental stresses, which include a shortage of water and high light levels, which have caused considerable yield losses in a range of plants (Verma et al., 2020).

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In order to solve some of these problems, the agricultural biotechnology (plant cell culture) offers workable options for an environmentally eco-friendly supply of phyto-ingredients with reduced energy, carbon dioxide, and water impacts (Verma *et al.*, 2022). Additionally, by safe guarding uncommon, unique, rare or endangered species of plants (Krasteva *et al.*, 2021). In the past decades, there has been a significant increase in the growth of *in vitro* propagation techniques for plant species with potential for use in agriculture, pharmaceuticals, and, forestry (Gusain *et al.*, 2021). This is especially clear in perennial plants, because conventional methods of multiplication are not very effective for them (Moraes *et al.*, 2021).

The caper (*Capparis spinosa* L.) is an illustration of this, a shrubby plant, endemic to the Mediterranean and adjacent areas (Chedraoui *et al.*, 2017). Within Egypt, it is a very unusual plant that grows wild in North Sinai (Musallam *et al.*, 2011). It is a member of the Capparidaceae family and genus *Capparis*, which has over 250 species that are typically utilized for ornamental and decorative industry, culinary, cosmetic, pharmacological, and therapeutic reasons. Caper is distinguished by a great deal of intraspecific variation and taxonomy due to its strong morphological and ecological variety (Foschi *et al.*, 2022 a and b). Furthermore, it is suited to hot temperatures, bright sunlight, and changing climates, and it plays a significant role in socioeconomic activities in many countries' dry regions (Kereša *et al.*, 2019).

One of the biggest obstacles to the commercial spread of capers is the fact that the plants are often propagated by seed and stem cutting, both of which offer severe issues (Koufan *et al.*, 2022). Due to seed dormancy, caper seeds have a low ability to germinate, and their lignified and herbaceous cuttings have a major rooting problem (Sottile *et al.*, 2021). Thus, the development of an effective tissue culture propagation technique is necessary today for capers (Gianguzzi *et al.*, 2020). *In vitro* propagation has a great promise for the quick generation of caper plants (Gianguzzi *et al.*, 2019).

The caper plant's young fruits and unopened blossoms are grown for use in a variety of traditional dishes (Zarei *et al.*, 2021). Caper fruit could be produced by both wild and domesticated plants (Shahrajabian *et al.*, 2020b), from Turkey, Spain, and Morocco (Samari *et al.*, 2019).

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The caper bush has a high therapeutic value (Nowruzian and Aalami, 2022). It is abundant in bioactive substances that can be employed for therapeutic, culinary, and ornamental purposes, including flavonoids, glucosinolates, phenolic acids, and alkaloids (Shahrajabian *et al.*, 2021). Along these lines, numerous health-promoting qualities of caper extracts, including antioxidant and anti-cancer activities, were scientifically proven (Wang *et al.*, 2023). The ecological benefits of caper include sustaining biodiversity, reducing soil erosion, and maintaining soil water (Sadeghi and Taban 2021).

One of the major families of chemically active compounds found in caper is the flavonoid family, which makes it a great medicinal plant (Christodoulou *et al.*, 2022). Additionally, these flavonoids exhibit an amazing involvement in numerous pharmacological processes, such as anti-allergic, anti-inflammatory, and antioxidant actions (Kdimy *et al.*, 2022), as well as, may have an impact on how well the immune system functions (Valijanovich, 2021). Rutin made up the majority (average wet weight 0.23% for raw and 0.13% for pickled) of the flavonoids in capers (Saleem *et al.*, 2021; Roy *et al.*, 2022). Caper is an advantageous medicinal herb because of their flavonoid rutin content, which enhances capillary function and serves as a general antioxidant (Isagaliev *et al.*, 2022).

Radiation is a procedure where the extra energy of an unstable nucleus can be released to attain stability in the form of particles or waves (Dowlath *et al.*, 2021). It can be categorized as both ionizing radiation (IR), and, non-IR (Hosoda *et al.*, 2021). IR is high frequency, and, tremendous energy with possible negative consequences, including X-rays, gamma rays, and cosmic rays (Ofiaz *et al.*, 2022). Non-IR, which includes infrared, radio waves, ultraviolet waves (UV), microwaves, etc., is distinguished by low frequency and extremely low energy and is not directly damaging (Zhong *et al.*, 2021). Among other ways, the usage of UV-C light, and gamma radiation as a physical elicitor, has gained more attention because of their significant effects on phytochemicals in many varieties of medicinal herbs (Rai and Agrawal 2020). Mechanically, the strain that UV-C and gamma radiation cause increases plant defenses while also creating phytoalexin (Jaiswal *et al.*, 2022).

The identification of UV-C and gamma rays on cell receptors, along with their signaling cascades,

has also shed insight on the mechanisms that can boost the generation of secondary metabolites (Jaiswal et al., 2023). It has been shown that plant of which grow in harsh environments are better suited to manufacture these chemicals (Kataria et al., 2021). This happens as a result of secondary metabolic processes, which are more effective upon the occurrence of any biotic or abiotic stress, being a part of the common defense system (Pandey et al., 2021). As a result of creating these substances, plant factories produce more protective responses, such as antioxidant activity enzymes, secondary metabolism products, and alterations to cell walls, which help plants deal with the oxidative damage brought on by UV-C and gamma radiation by scavenging deadly reactive oxygen species (ROS) (Apoorva et al., 2021). Other advantages include stopping the deterioration of chlorophyll, eradicating infections, and improving nutritional characteristics (Abbasi et al., 2021).

Our objective was to investigate a practical procedure for caper plant *in vitro* propagation, as well as, study the impact of UV-C and gamma radiation as biotechnology instruments on the growth, and concentration of certain classes of antioxidant chemicals (flavonoid and rutin).

## **Materials and Methods**

### *Experiment, and plant materials*

This study took place in Tissue Culture & Germplasm Conservation Laboratory - Horticulture Research - Institute Agricultural Research Centre - Giza-Egypt. The stem cuttings of caper plant (*C. spinosa* L.) were collected, during June and July 2022, from different areas in the South-western part of Sinai, from young, healthy and disease-free shrubs, as explants for *in vitro* our study.

### *Micropropagation*

#### *Disinfecting stage*

Stem cuttings (explants) were washed under running tap water for 1 h, and then soaked in water containing hygienic soapy (Septol) for 30 min with continuous agitation. Under sterile conditions, the cuttings were transferred to 70 % alcohol for 1 min, and then transferred to various solutions of sodium hypochlorite 5.25 % (commercial bleaching compound, Clorox) at 10 and 20 %, and HgCl<sub>2</sub> 0.1 and 0.2 %, for 5 and 10 min for each solution with continuous agitation. A few drops of tween-20 were added to each used disinfection solution as a wetting agent (emulsifier). Finally, the cuttings were washed with sterilized distilled water three times for 5 min each.

### *Establishment stage*

Sterilized stem cuttings were cut into small explants containing one bud under sterile conditions and cultured in jars on MS (Murashige and Skoog 1962) basal medium with 3% sucrose, 0.8 % agar, and pH adjusted to 5.7±1, at four strengths (Full, ¾, ½ and ¼ strength). Five replicates with 10 explants per replicate were used for each strength. The jars were placed in growth room under 24±°C and 16 h light with a light intensity of 3000 lux growing conditions. Four weeks later the growing shootlets were removed from the jars, under sterile conditions. Four different types of culture medium composition (MS media in four strengths) are employed in this stage, along with four subcultures of explants on *in vitro*. Small explants with one bud were transferred into each jar in each subculture.

### *Shoot proliferation*

Nodal cuttings containing one bud obtained from *in vitro* culture plantlets were used as explants, and cultured on fresh MS medium (full strength) containing different concentrations of BAP and Kin at (1.0, 2.0, and 3.0 mg/l for each) to induce adventitious shoot formation. Measurements were taken at this stage 4 weeks after cultivation.

### *Root initiation and formation*

Another set of media MS (full strength) containing activated charcoal 2.0 g/l with different concentrations of IAA and IBA (0.0, 1.0, 2.0, 3.0, and 4.0 mg/l for each) were used to induce, and formation roots (using interior explants).

### *Acclimatization*

After root formation (8 weeks) the plantlets were washed with fungicide 0.1 % (Topsin) for 10 min and rewashing with water twice to remove fungicide residue, and planted in polypropylene bag containing mixtures of (Peat moss, sand, Vermiculite, Peat moss + sand 1:1, Peat moss + sand 2:1, Peat moss + vermiculite 1:1, and Peat moss + Vermiculite 2:1), and let them to grow for 4 weeks under greenhouse condition, therefore, the plants let to grow under normal light and environmental conditions.

### *Irradiation (UV-C & gamma) treatments*

To study the effects of UV-C, and gamma irradiation on the growth of caper shoots, single-node cuttings were placed in small jars containing basal MS medium (without growth regulators), and then subjected to irradiation treatments.

- a. The jars were exposed to UV-type-C rays for three times (2, 4, and 6 h). Employing model G15T8 ultraviolet light UV-C lamp: Philips -TuV- 15W- 54 V- 0.34 A, and long at 45 cm, diameter at 2.8 cm, containing 2.0 mg of mercury (Hg), and disinfects water air. UV-C light is a short-wavelength linear tube (254 nm). The distance of exposure was 10 cm from the lamps (Sztatelman *et al.*, 2016; Dwivedi *et al.*, 2021). The irradiation treatments were done at the Horticulture Research Institute, ARC, Giza, Egypt.
- b. Exposure to gamma rays with three concentrations (0.5, 1, 1.5 Gray), was performed at the National Centre for Research and Technology in Nasr City, Cairo, Egypt, using  $^{60}\text{Co}$ - $\gamma$  of India gamma cell at dose rate (0.782 Gy/h) (Bahmani *et al.*, 2016; Babina *et al.*, 2020).

#### *Morphological characteristics*

Four weeks after each treatment, various measurements were obtained, except for the attributes of the roots after eight weeks of culture, including the following:

- Establishment stage: decontamination %, survival %, bud sprouting %, number of shootlets / shoot, and the lengths were taken using a sterile thread and ruler.
- Shoot parameters: number of shoots, shoot length (cm), number of leaves per each shootlet, shoots fresh and dry weights.
- Root assessments: rooting %, root no/ shootlets, root length (cm).
- After irradiation: survival %, number of shoots, shootlets length (cm), number of leaves per each shootlets, shoots fresh and dry weights.

#### *Secondary metabolites analysis*

The secondary metabolites in leaves were extracted in accordance with Hakiman and Maziah (2009).

#### *Total flavonoids content*

The total flavonoids content was determined using the technique described by Marinova *et al.* (2005), and were calculated as mg/g DW.

#### *Rutin determination by HPLC*

Using HPLC Agilent Technologies 1260 Infinity (USA and Canada) had solvent delivery system quaternary pumps (61311B), including a diode array detector (DAD 61315D) with 10-

mm flow cell, the separation was achieved by a reversed-phase Zorbax SB-C18 column (3.5  $\mu\text{m}$  particle size, i.d. 4.6 mm  $\times$  250 mm). Injected volume was maintained at 5  $\mu\text{l}$ . A photo diode array UV detector run wavelength at 210 nm according to absorption maxima of analysed compounds was set to detect HPLC chromatogram with analysis total time per sample 25 minutes, performed method according to Mutuli *et al.* (2022).

#### *Statistical analysis*

Completely randomized block design (CRBD) had been utilised for all assessment tests. Five replicates with 10 explants per replicate were used for all experiments, except acclimatization one plantlets per pots were used and three replicates in the treatment. Analysis of variance, and LSD ( $P \leq 0.05$ ) (Steel and Tori 1980) have been conducted using CO- Stat version 5.1 statistical software (Co-Stat 1999).

## **Results**

### *Micropropagation*

#### *Disinfecting process*

The response of internodesegments of *Capparis spinosa* to different concentrations of detergents and exposure time was observed in Table 1. The highest antimicrobial effect of caper was  $\text{HgCl}_2$  at 0.2 for 10 min which scored 100 % of decontamination percentage but survival percentage of this treatment was 71.66 % and 65 % bud sprouting. Moreover, the highest survival 100 % and bud sprouting 85 % was observed for clorox 20 % for 5 min. These data explain the toxic effect of  $\text{HgCl}_2$  on bacteria and explants, but clorox was weak toxic effect of microbes but not toxic on explants.

#### *Media strength*

The different MS salts concentrations had significant influence on survival %, shoot number, and shoot length (Table 2). According to the results shown in Table 2 the highest survival percentage (93.33 %) was obtained for explants cultured on MS medium full strength, whereas using MS-strengths of  $\frac{3}{4}$ ,  $\frac{1}{2}$  and  $\frac{1}{4}$  decreased the survival percentage to 46.66, 33.33 and 13.33 %, respectively. Moreover, the results indicated that the nodal explants were more capable of producing the highest number of shoots (2.5 shootlets/ explant) in MS medium full strength than other strengths. Similarly, the length of formed shootlets have a significant effect for all treatments, but the longest explants formed was observed for the explants cultured on MS media

**TABLE 1.** Effect of various disinfectant agent concentrations and exposure time on decontamination, survival and bud sprouting percentages of *Capparis spinosa*.

Treatment	Decontamination %	Survival %	Bud sprouting %
Clorox 10 for 5 min	26.66	11.11	11.11
Clorox 10 for 10 min	40.00	61.11	33.33
Clorox 20 for 5 min	73.33	100.0	85.00
Clorox 20 for 10 min	80.00	50.00	16.66
HgCl <sub>2</sub> 0.1 for 5 min	26.66	52.77	66.66
HgCl <sub>2</sub> 0.1 for 10 min	66.66	70.00	11.11
HgCl <sub>2</sub> 0.2 for 5 min	86.66	33.33	11.11
HgCl <sub>2</sub> 0.2 for 10 min	100.0	71.66	65.00
<b>LSD 5%</b>	<b>36.03</b>	<b>32.11</b>	<b>16.32</b>

Treatments averages are compared to the LSD test (5 %).

**TABLE 2.** Effect of MS salts concentrations on survival %, shoots number and shoots length of *Capparis spinosa*

Treatment	Survival %	Shootlets No. / shoot	Shoot length (cm)
MS full strength	93.33	2.50	1.20
MS ¾ strength	46.66	1.72	0.50
MS ½ strength	33.33	0.66	0.50
MS ¼ strength	13.33	1.00	0.43
<b>LSD 5%</b>	<b>14.38</b>	<b>1.03</b>	<b>0.58</b>

Treatments averages are compared to the LSD test (5 %).

containing full strength (1.20 cm).

#### Shootlets proliferation

Data of Table 3 and Fig. 1-b display the influence of BAP and Kin concentrations on shoot no, shootlets length, leaves no/shootlet, shoots fresh and dry weights of caper plant. Most suitable shootlets proliferation on MS medium at various concentrations of BAP and kin explants of *Capparis spinosa* was achieved in MS medium supplemented with 3.0 mg/l BAP which scored the highest number of shootlets 5.66 per shoot. On the other hand, the tallest shootlet plant (3.20 cm) was obtained for those treated with kin 3.0 mg/l following by (3.03 cm) for explant cultured on the medium containing 3.0 mg/l BAP. Similarly, the average of leaves number per shootlet, shoots fresh, and dry weights gave 6.66 leaves/shoot, 2.12 (g), and 0.44 (g), as well as 6.33 leaves/shoot, 2.03 (g), and 0.33 (g) for the previous treatments, respectively.

#### Rooting induction

*In vitro* rooting from shoot is an essential step in micropropagation. Different concentrations of IAA (1.0, 2.0, 3.0 & 4.0 mg/l<sup>-1</sup>), and IBA (1.0, 2.0, 3.0 & 4.0 mg/l<sup>-1</sup>) were used for root formation. Table 4 and Fig. 1-c shows the significant response of rooting induction (rooting %, roots no/shootlets, and roots length) to administration of various IBA levels and various IAA concentrations. Considering IAA at 4.0 mg/l<sup>-1</sup> the greatest rooting percentage was discovered to be 41.66 %, as shown in Fig. 1-d following by IBA at 4.0 mg/l (37.03 %). The number and length of roots were the highest (4.26 and 2.96 cm, respectively) after 30 days of culture in MS medium supplemented with IBA 3.0 mg/l.

#### Acclimatization

For the establishment of plants, regenerated healthy rooted plantlets were placed at room temperature (25± 1 °C) for one week. Then the plantlets were removed from the culture bottle

and carefully cleaned to remove adhering agar, and they were planted on sterilized soil to observe the accomplishment of the plant in earthen pots. According to the data in Table 5, the plantlets were survived well in the following substrates: peat moss (37.03 %); peat moss with vermiculite at 2:1 (41.66 %) and peat moss with vermiculite

at 1:1 (29.62 %), as compared with vermiculite and sand alone. Similarly, the increasing of the shootlet length were scored high rate for the explants cultured in peat moss alone (5.43 cm) following by peat: vermiculite 1:1 (4.56 cm); then peat: sand. 2:1 (4.40 cm).

**TABLE 3. Effect of BAP and Kin concentrations on shoot number, shoot length, leaves number shoots fresh and dry weights of *Capparis spinosa***

Treatment PGRs	Shoot No	Shootlets length (cm)	Leaves no/shootlet	Shoots FW (g)	Shoots DW (g)
MS free (control)	2.50	1.20	2.66	0.79	0.03
BAP 1.0 mg/l	2.66	1.16	4.00	1.09	0.21
BAP 2.0 mg/l	3.33	1.46	5.00	1.21	0.22
BAP 3.0 mg/l	5.66	3.03	6.33	2.03	0.33
Kin 1.0 mg/l	3.00	2.06	4.33	1.11	0.04
Kin 2.0 mg/l	3.33	2.50	5.33	1.45	0.32
Kin 3.0 mg/l	3.33	3.20	6.66	2.12	0.44
LSD 5%	2.35	1.06	1.76	0.21	0.03

Treatments averages are compared to the LSD test (5 %), BAP: benzyl-6-aminopurine; Kin: kinetin; PGRs: Plant Growth Regulators.

**TABLE 4. Effect of IBA and IAA concentrations on rooting percentage, root length, and root number of *Capparis spinosa*.**

Treatment PGRs	Rooting %	Roots no/shootlets	Roots length (cm)
MS free (control)	0.00	0.00	0.00
IBA 1.0 mg/l	5.93	1.43	0.96
IBA 2.0 mg/l	22.22	3.46	2.46
IBA 3.0 mg/l	30.55	4.26	2.96
IBA 4.0 mg/l	37.03	3.76	2.93
IAA 1.0 mg/l	0.00	0.00	0.0
IAA 2.0 mg/l	17.03	1.76	1.23
IAA 3.0 mg/l	26.85	3.03	1.80
IAA 4.0 mg/l	41.66	3.73	1.83
LSD 5%	6.21	1.49	0.73

Treatments averages are compared to the LSD test (5 %), IBA: indole -3-buteric acid; IAA: indole -3- acetic acid



Fig. 1. (a): micronodes established on MS medium full strength; (b): shootlet proliferation on MS medium supplemented with BAP and Kinetin; (C): Rooting induction for the explants transferred on MS media supplemented with IBA and IAA (d): root formation from the explants cultured on media containing IAA 4.0 mg/l; (e): The explants after transferred on sterilization peat: sand at 1:1.

**TABLE 5.** Effect of various acclimatization media on survival %, and shoot length of *Capparis spinosa*.

Acclimatization media	Survival %	Shoot length cm
Peat moss	37.03	5.43
Vermiculite	00.00	0.00
Sand	00.00	0.00
Peat moss + vermiculite 1:1	29.62	4.56
Peat moss + vermiculite 2:1	41.66	4.16
Peat moss + sand 1:1	5.92	3.00
Peat moss + sand 2:1	15.74	4.40
LSD 5%	7.49	1.34

Treatments averages are compared to the LSD test (5 %).

#### *Irradiation effect*

##### *Growth*

Statistics in Table 6 illustrate the effect of UV-C and gamma irradiation on growth of *C. spinosa* plants (survival %, shoot length, shoot number, leaves number, shoots fresh, and dry weights) after exposed to various times, and doses. Regarding UV-C radiation exposure, it was found that there was not a significant difference in survival percentage (100 %) comparing the various times, and the control (non-radiation-treated). On the other hand, the effect of gamma radiation on survival %, showed another trend, as all gamma doses decreased it compared to control and UV-C treatments at different times. Whilst, raising the level of gamma rays from 0.5 to 1.0 Gy did not increase survival % significantly (the two doses gave 60% survival). Moreover, the lowest significant value, (survival 33.33 %) was detected for the exposure gamma rays at 1.5 Gy. On the other hand, shootlets number was the highest with UV-C treatment after exposure for 4 hours (4.33 shootlets/ explant). The highest elongation rate of shootlets, heaviest shoots fresh, and dry weights were observed in the explants exposed to UV-C at 6 hours, while the leaves formation was the highest (5.66 leaves/ shootlet) for control followed by 5.44 and 5.33 leaves/ shootlet for both UV-C exposure time at 2 and 4 hours, respectively. However, no marked effect was noticed with the exposure to gamma rays at different levels, in contrast to control, and UV-C treatments (exposure time at 2 and 4 hours).

##### *Total flavonoids and rutin content*

The overall effects of the two investigated radiation (UV-C and gamma) on the contents of flavonoid and rutin of caper plant (as mg/g DW) are presented in Fig. 3, 4, and 5. Based on the HPLC analysis, the retention time of rutin standard in acetonitrile eluent was  $\pm 2.08$  minutes. When the HPLC results of sample extracts were compared with the standard solution of rutin, it was concluded that all of the cultures treated with 0.5, 1.0 and 1.5 Gy of gamma radiation contained this compound with different concentrations. Progressive increase in flavonoid and rutin content (12.34 and 3.52 mg/g DW), respectively was performed due to increasing in gamma rays levels up to 1.0 Gy. Likewise, raising gamma rays levels to 1.5 Gy was ineffective in this situation, as compared to 1.0 Gy. As for the effect of UV-C radiation various times, obtained results showed that UV-C at 6 hrs produced the highest value of flavonoid and rutin contents (9.51 and 1.00 mg/g DW) followed by UV-C at 4 hrs (8.67 and 0.63 mg/g DW) then by UV-C at 2 hrs (7.42 and 0.35 mg/g DW), respectively. Nevertheless, the lowest value of flavonoid and rutin content (7.21 and 0.06 mg/g DW) was showed for the control plants (non-radiation-treated).

#### **Discussion**

Considering the significant trends in climate change that are currently evident, the likelihood of additional changes being generated, as well as an increasing number of potential climatic

**TABLE 6.** Effect of various doses and time of gamma and UV-C radiations on survival percentage, and shootlet growth of *Capparis spinosa*

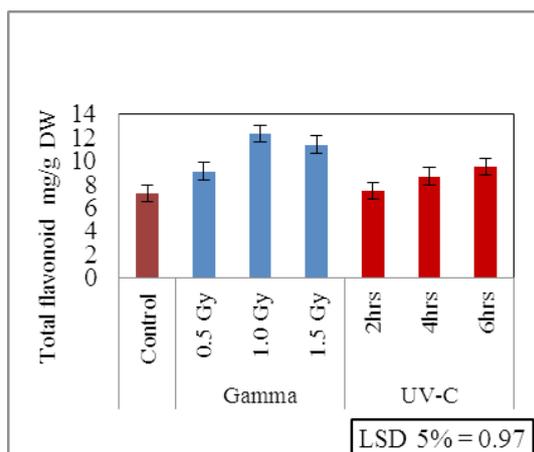
Irradiation treatment	Survival %	Shoots No.	Shootlets length (cm)	Leaves	Shoots	Shoots
				no/shootlet	FW (g)	DW(g)
Control	100.0	2.50	1.20	5.66	0.76	0.03
UV-B 2 hrs.	100.0	2.66	1.09	5.44	2.02	0.24
UV-B 4 hrs.	100.0	4.33	1.75	5.33	3.02	0.30
UV-B 6 hrs.	100.0	2.66	2.06	4.03	3.32	0.32
Gamma 0.5 Gy	60.0	0.60	0.67	3.22	1.02	0.27
Gamma 1.0 Gy	60.0	0.66	0.77	4.44	0.91	0.16
Gamma 1.5 Gy	33.30	0.20	0.54	1.21	0.82	0.18
LSD 5%	7.24	0.47	0.29	0.78	1.02	0.04

Treatments averages are compared to the LSD test (5 %).

**Fig. 2.** Growth of the explants cultured on MS medium after exposure to UV-C at different times and gamma radiation at various levels.

impacts, it is necessary to address adapting agricultural systems more forcefully (Habibur-Rahman et al., 2022). Furthermore, the fact that agriculture, in all of its forms and areas, continues to be highly vulnerable to climate change. It is contribute significantly to annual variations, and the continuous disturbance of environmental conditions, which negatively affects crop yield and productivity (Nguyen and Scrimgeour 2022). The primary objectives of agricultural biotechnology (tissue culture)

used are to enhance production, as well as maximizing the productive capacity of capers, and as a way to overcome climate changes (Arora et al., 2022). The natural environment presents numerous challenges for the seed, and stem cuttings -based growth of capers (Osman and Awal 2023). As was already indicated, *in vitro* propagation of seeds could be significantly helpful in overcoming these limitations (Kdimy et al., 2022).

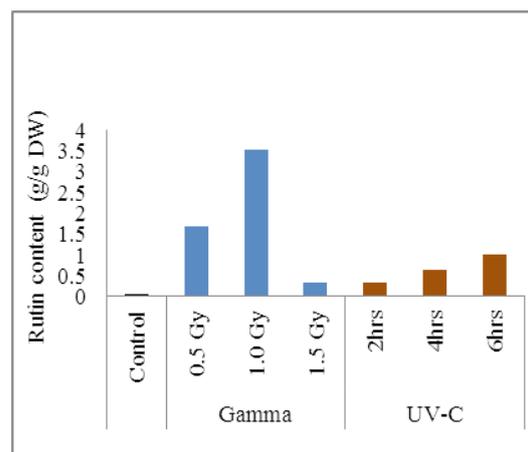


**Fig. 3.** Effect of gamma and UV-C radiation on total flavonoids and rutin content of the leaves in *Capparis spinosa*. Total flavonoids averages are compared to the LSD test (5 %)

In the present investigation, *Capers spinosa* plants were successfully established *in vitro*. The procedures applied for sterilization methods of nodal explants have been found to be effective, with a success rate of 80-100 % sterile explants.

HgCl<sub>2</sub> was applied at a concentration of 0.2 for 10 min, which resulted in 100 % disinfection, however the survival %, and bud sprouting only reached 71.66 % and 65.0 %, respectively. Contrarily, Clorox has a negative impact on microorganisms, while being positive for plant survival, and bud sprouting. The effect of HgCl<sub>2</sub> was caused by the Hg element being harmful to plant cells, which inhibits differentiation and division of cells (Sahu *et al.*, 2022). This action may also be related to chloride atoms and ions that can firmly adhere to proteins and exterminate organisms (Ahmed *et al.*, 2022). Hypochlorite is a well-known highly effective bactericide; even micromolar levels are adequate to significantly reduce the amount of bacteria present (Ahmadpoor *et al.*, 2022). Perhaps this effect is due to the fact that when the hypochlorite salts (NaOCl and Ca (OCl)<sub>2</sub>) are diluted in water, hydrogen chloride (HCl) is produced, and the concentration of HCl is related to bactericidal action, as well as possibly as a result of fatal DNA damage (Handayani *et al.*, 2022). These findings were in line with those published by Ghareeb and Taha (2018) who discovered that using Clorox 10 % for 3 minutes and mercuric chloride 0.1 % for 2 minutes led to the most significant percentages of disinfection for cultures, and explant survival (100 %) of *Antigonon leptopus* explants.

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**Fig. 4.** Effect of gamma and UV-C radiation on the rutin content of the leaves in *Capparis spinosa*

Use of an appropriate nutritive medium is crucial for the success of explants. Among the four tested mediums, MS full strength was determined to be the optimum basal medium for establishing shoot induction, and proliferation from nodal explants of caper plants. Furthermore, the survival %, shoot number, and length were dramatically decreased due to lower MS salt strength concentrations. Unsurprisingly, one of the most often used for cultivating plant tissue basal media is MS salt strength (Hajare *et al.*, 2021). It is a superior media for regenerating plants maybe as a result of the high nitrogen concentrations throughout both nitrate and ammonium compounds, as well as a comparatively high proportion of ammonium to nitrate (Phillips and Garda 2019). Similar outcomes were also noted with medicinal herbs including *Paederia foetida*, *Phyllanthus niruri* and *Codonopsis ...etc.*

As shown in Table 3, the patterns of behaviour of both *in vitro* multiplication shoot induction, and proliferation from nodal explants of *Capparis spinosa* were mirrored. The MS medium full strength supplementary with two cytokinins (BAP or Kin at 3.0 mg/l) during explant cultivation produced the longest shootlets, the greatest number of shoots, and the highest number of leaves per shootlet, as compared to control (non-cytokinin MS medium). These findings corroborated those proposed by Grzegorzczuk-Karolak *et al.* (2021) on *Salvia bulleyana* and Tikendra *et al.* (2022) on *Dendrocalamus latiflorus* they noted that cytokinin is one of the key hormones essential for development and growth of plants that is

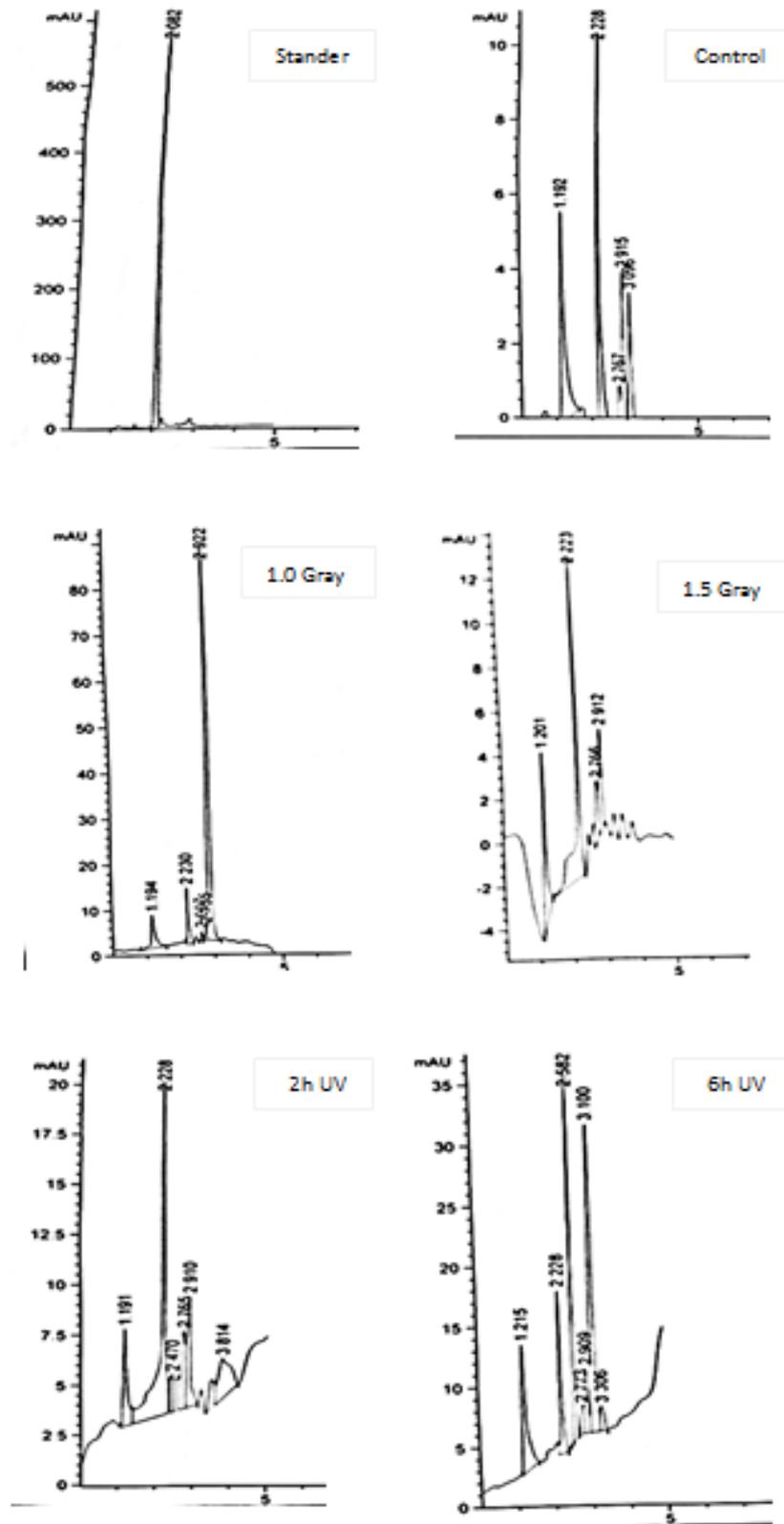


Fig. 5. Chromatogram of rutin determination by HPLC in radiation treatments (Gamma and UV-C)

considered to encourage the division of cells and differentiation. As discussed by Okello *et al.* (2021) cytokinins frequently assist in the multiplication and lengthening of shoots on *Aspilia africana*. These results may also be due to the main functions of cytokinins in forcing axillary buds to sprout, removing apical dominance (Mohamad *et al.*, 2022), and subsequently increasing the number of shoots /explant and leaves /shootlets.

Based on the findings of the root induction, IBA at 3.0 mg/l was considered to be the most effective auxin type for number of roots per shootlets, and root length, meanwhile, IAA at 4.0 mg/l produced the greatest rooting %, as compared to the control (non-auxin MS medium). These results are consistent with those of Sreekissoon *et al.* (2021) who found that IBA was the most suitable auxin for root formation from nodal explants of *Scelletium tortuosum*. Auxin acts by attaching to a receptor protein and initiating a signaling cascade that most likely includes the proteolysis of transcription regulators (AUX/IAA) through the ubiquitin-proteasome system, which un-represses genes (Nazir *et al.*, 2022). Furthermore, given that auxin has been demonstrated to play a key role in rooting apical organizing, it is conceivable to interpret the auxin oscillations from induction throughout formation as potentially connected to apical organizational processes (Nadal *et al.*, 2022).

Explant proliferation in a number of plant varieties is influenced by the quantities of exogenous and endogenous auxin, and cytokinin. A high auxin-to-cytokinin or cytokinin-to-auxin ratio, whichever is higher, encourages root, and shoot regeneration (Asghar *et al.*, 2023).

The effectiveness of the entire micropropagation technique is determined by the efficiency of the plantlet acclimatisation phase. When regenerants adapt to new growth circumstances, they experience biotic and abiotic challenges like water loss, tissue dryness, and a decrease in the synthesis process. Numerous publications, including Jagiełło-Kubiec *et al.* (2021) and Trasar-Cepeda *et al.* (2022), claim that the substrate which was used during acclimatisation had a substantial impact on the long-term survival and development of the plantlets that were regenerated via *in vitro* culturing. The results of this study demonstrated that caper plantlets cultivated in a peat moss + vermiculite combination or in just peat moss exhibited impressive *ex vitro* adaptation, as well as healthy growth, and development. The

physicochemical characteristics of the substrate can be used to explain its major effects on plant survival, and shoot length (Bertsouklis and Panagaki 2022). The substrate actually has to be permeable, and well-drained. Additionally, it ought to make it easier to obtain water and nutrients, and for gas exchange (Basiri *et al.*, 2022). The aforementioned will encourage plant survival, growth, and the establishment of a strong root and shoot-organisation.

Radiation has considerable effects on the growth and formation of plant-based products. One of these is UV-C rays, which regulates an expression of genes related to second-generation metabolism, and cell formation (Phanomchai *et al.*, 2021). Gamma rays are the second kind, and they hasten the growth process by rupturing the central lamella in the cell wall. They also affect how the plastid develops and functions, for example, by converting starch to sugar (Ulukapi 2021). The findings showed that cultures exposed to UV-C rays at 6 hrs had the best plant survival, and somewhat less shoot formation, nonetheless gamma radiation had no discernible effects on the aforementioned features. Previous studies have demonstrated that UV-C and gamma radiation have an impact on a variety of medicinal plants cultivated *in vitro*. The decrease in shoot formation at increased UV-C and gamma radiations may be due to oxidative stress induction and irrevocable toxic effects on cells that can promote the breakdown of cells in plant culture (Costa-Pérez *et al.*, 2023). Moreover, prolonged exposure to intense light stress causes plants' photosynthetic mechanisms to become photo-inhibited (Pujari *et al.*, 2021). The UV-C rays produced the best results when compared to the outcomes of exposing plants to gamma radiation *in vitro* because they can affect the synthesis of plant hormones, which in turn can indirectly influence how plants regulate their growth (Darras *et al.*, 2022).

The metabolism and physiological mechanisms of a plant have been known to change quickly and reliably when exposed to UV-C or gamma radiation (Rafi *et al.*, 2021). Thus, in recent years, UV-C and gamma irradiation has been more well-liked as a novel elicitation strategy for boosting the synthesis of secondary metabolism in plant tissues and cell cultures (Kyeré *et al.*, 2021). When UV-C or gamma rays are supplied, they interact with free radicals in cell membranes to form signal molecules that activate the defence mechanisms, and secondary metabolism

(Hashim et al., 2021). In this investigation, UV-C or gamma radiation exposure raised the yield of total flavonoids and rutin compared to the control (non- radiation-treated).

Likewise, on the basis of the data presented in Fig. 3 and 4, it was shown that total flavonoid, and rutin content in caper leaves declined as gamma radiation level increased. This might be the case because of the high amounts of ROS production, cellular membrane rupture, photo-inhibition, metabolism impairments, damages to DNA, and the second photosystem degradation (Mohamed et al., 2021). This is in line with Khalifa et al. (2022) who concluded that gamma radiation at 50 Gy accelerated the growth of *Silybum marianum* callus cultures, whereas the greater levels considerably inhibited the formation of secondary metabolites.

Under UV-C or gamma radiation, there was an increase in flavonoid and rutin contents, this may be as a result of alterations in the flavonoid, and rutin biosynthesis pathway's enzyme activities (Aly et al., 2022). Irradiation results in the radiolysis of water and contributes to the production of free radicals like as hydroxyl (OH•), and hydrogen peroxide radical (HOO•), as well as hydrated electrons. These radicals can breakdown the pro-cyanidin trimer, tetramer, and hexamer glycosidic links in plants, increasing the amount of phenolic and flavonoid compounds overall in radiation-exposed plants (Pelcaru et al., 2021), and similar results also those described by Abbasi et al. (2021).

According to our findings, exposure to gamma rays was more effective than exposure to UV-C radiation because gamma rays can pass through cells, whereas UV-C radiation cannot (Volkova et al., 2022).

### **Conclusion**

Caper plants typically are considered to be difficult to cultivate using traditional techniques. Based on the results presented here, it's clearly evident that caper micropropagation was successful technique to solve this issue, and also, the effectiveness of acclimatization in green house under Egyptian conditions. In actuality, the advancement of effective tissue culture propagation techniques has a direct impact on the growth of the many industrial uses of capers. So, appropriate growth regulators were found that can encourage microneodes to produce shoots and roots. The outcomes of this investigation, also demonstrated that radiation (UV-C or Gamma) might be utilised as a stimulant in low concentrations to enhance

the production of secondary metabolism compounds from caper plants, which might then be employed commercially to produce these essential substances for medicine.

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### *Conflict of interest*

There are no conflicts between the authors.

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## تحسين إنتاج الروتين من نبات *Capparis spinosa* بواسطة الأشعة فوق البنفسجية أو أشعة جاما باستخدام زراعة الأنسجة

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في إطار جهود التكيف مع تغير المناخ والتخفيف من آثاره، يمكن للتكنولوجيا الحيوية أن تساهم بشكل إيجابي في تقليل احتمالية تعرض النظم البيئية لعواقب تغير المناخ. من المعروف أن شجيرة الكبار يصعب نموها من خلال الطرق التقليدية، على الرغم من القيمة الاجتماعية والاقتصادية والطبية الكبيرة لهذا النوع، إلا أن تكاثرها الدقيق لم يتلق سوى القليل من الدراسة حتى الآن. لذلك في هذه الدراسة، تم تجميع البراعم من سيقان غضة من نباتات في شمال سيناء، وتعقيمها بمعالجات مختلفة النوع والتركيز ووقت التعقيم. تم زراعة النموات علي بيئه موراشيچ واسكوج بتركيز أملاح مختلفة (تركيز كامل، ثلاث اربع تركيز، نصف تركيز، ربع تركيز). تم تجزيته النموات الناتجة الي اجزاء اصغر ونقلها على بيئه موراشيچ واسكوج محتويه على هرمون بنزيل امينو ببورين، كينينين بتركيزات ١,٠، ٢,٠، ٣,٠، ٤,٠ مجم/لتر لكل منهما على حدا. تم نقل الفريعات الناتجة على بيئه موراشيچ واسكوج محتويه على هرمون اندول اسيتك اسيد واندول بيوتريك اسيد بتركيزات ١,٠، ٢,٠، ٣,٠، ٤,٠ ملجم/ لتر لكل منهما، وبعد ذلك تمت عملية الاقلمة. تم تعريض النباتات الناتجة من الاكثار للتشيع بالجاما بجرعات ١,٥، ١,٠، ٠,٥، ٠,٥، ١,٥ جرائ والاشعه فوق بنفسجيه لمده ٢، ٤، ٦ ساعات. اعطت المعامله بهرمون اندول اسيتك اسيد ٤ مجم/لتر اعلى نسبه تجذير ٤١,٦٦ ٪. كما اعطت الاقلمه على بيت موس والفرمكوليت بنسبه ١:٢ اعلى نسبه حيويه فى الصوب ٤١,٦٦ ٪. اعطى التشيع بالجاما بجرعه ١,٠ جرائ اعلى نسبه من الفلافونويد الكلية والروتين (١٢,٣٤ , ٣,٢٥ مليجرام/جرام وزن جاف) بالمقارنة بنباتات الكنترول (٧,٢١ , ٠,٠٦ مليجرام/ جرام وزن جاف، بالترتيب) .