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# Ameliorative effects of hydrogel polymer on micropropagation performance of *Russelia equisetiformis* Schltdl. & Cham. under salinity stress

**Nora M. Youssef[1], Lobna S. Taha[1], Soha A. Murad[2], Hagar M. Abdel-Magied[1]**
[1]*Ornamental Plants and Woody Trees Department, Agriculture and Biological Research Institute, National Research Centre (NRC), Giza, Egypt*
[2]*Plant Biochemistry Department, Agriculture and Biological Research Institute, National Research Centre (NRC), Giza, Egypt*

Micropropagated *Russelia equisetiformis* Schltdl. & Cham. were examined using two different types of culture media (MS and WPM) at full strength to optimize the suitable proliferation medium. The optimized culture medium (MS medium + 0.5 $mgl^{-1}$ of BA) was selected for *in vitro* shooting and root formation. Based on the tested hydrogel polymer at five rates (0, 12.5, 25, 37.5, and 50% instead of agar percentage), the addition of 25% hydrogel polymer (2 $gl^{-1}$) caused an increase in all shooting characters. Using hydrogel at 25% in the culture medium under various salinity levels (NaCl at 0, 1000, 2000, 3000, and 4000 ppm) showed a positive response of all recorded characters (shooting and rooting) and in the estimated pigments content in shootlets grown on each salinity level. The secondary metabolites (total tannins, flavonoids, and phenols) as well as proline were highly influenced by saline conditions. Meanwhile, using 25% hydrogel polymer in the culture medium individually or in combination with various salinity levels decreased these compounds. The increased antioxidant activity was obtained at 4000 ppm NaCl and 0% hydrogel polymer, while unstressed shootlets on MS culture medium with added 25% hydrogel caused the lowest antioxidant activity using DPPH or phosphomolybdenum. Using hydrogel led to alleviating salinity stress on the micropropagated russelia plant, and this finding is relatively new.

**Keywords:** *Russelia equisetiformis*, micropropagation, salinity, hydrogel polymer, bioactive compounds, antioxidant activity

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**Hagar M. Abdel-Magied**,
Ornamental Plants and Woody Trees Department, Agriculture and Biological Research Institute, National Research Centre (NRC), Giza, Egypt
Email: hagar_nrc@yahoo.com
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## INTRODUCTION

*Russelia equisetiformis* Schltdl. & Cham. is a flowering and multi-branching shrub that reaches up to 1.5 m in height. It belongs to the Scrophulariaceae family, recently introduced into the new family *Plantaginaceae* (Ahmed et al., 2016). It is known as a fountain plant; its species name refers to this plant's resemblance to a horsetail. It is native to Mexico and Colombia and adapts to sunny and dry locations (Kreissig, 2019). *R. equisetiformis* is found in the Mediterranean region as an ornamental plant; it is a weeping shrub that has flowers with a tubular corolla that presents an attractive look all year round, thus encouraging gardeners to implant it as an ornamental plant (Riaz et al., 2012). Also, it looks decent for rock gardens commonly used to hide unattractive walls or fences due to its quickly growing evergreen and blooming bright-colored flowers. Additionally, *R. equisetiformis* has various medicinal uses, where the plant is used to treat pain, inflammation (Olorunju et al., 2012), cancer, and malaria, leukemia, and diabetes, and enhances healthy hair growth (Awe et al., 2010; Kolawole and Kolawole, 2010). *R. equisetiformis* extracts have antioxidant and antimicrobial properties with active phytochemicals such as alkaloids, saponins, flavonoids, tannins, terpenoids, and steroids (Riaz et al., 2012).

In most cases, the *in vitro* culture and reproduction of plants are performed in solid nutrient media. The high-cost agar substrate is commonly used in this process (Meshaal et al., 2018; Kaur and Mudgal, 2021). Due to the needs of the agro-market to proliferate plants commercially, the agar substrates are used in large quantities. So, alternative methods must be sought to avoid these high costs. There is a great need to replace agar with cheaper disposable materials, such as gum (Jain and Babbar, 2002), or develop an environmentally friendly solid substrate with ideal physicochemical and functional characteristics suitable for numerous utilizations (Kernosenko et al., 2023). Hydrogel polymers are characterized by high absorbency, strength, good salt resistance, nontoxicity, and biodegradability (Li et al., 2009). These polymers are vastly used in agriculture for their ability to hold higher soil water maintenance for longer periods and slower release of water and nutrients to plants, serving as buffers against abiotic stress (drought, salinity) to reduce the risk of plant damage during crop establishment, and they can alleviate soil salinity (Costa et al., 2021), reducing the rate of evapotranspiration, promoting growth and development of the plant, and obtaining higher productivity and quality of plants (Abedi-Koupai et al., 2008). These requirements may be met via hydrogels based on spatially cross-linked hydrophilic polymers like polyacrylamide (Kernosenko et al., 2023). It has been

used as a water-retaining material in arid and semiarid regions and salinity conditions that affect negatively the plant (Montesano et al., 2015; Abobatta, 2018).

Salt stress affects 20% of the world's arable lands and is constantly increasing due to human activities, in particular climate change and human activities (Arora, 2019). Moreover, it has been reported that 100 million hectares of soil (about 11% of the world's irrigated land) have become saline due to irrigation with water containing salts (Chartres and Noble, 2015). Currently, the increment of soil salinity has been a great problem in Egyptian soils. Most of the newly reclaimed lands located in the west and east of the Delta and west of the Nile Valley in Upper Egypt are suffering from salinity (Karajeh et al., 2011). About 30 to 40% of Nile Delta soils are classified as salt-affected soils (Hammam and Mohamed, 2020; Masarmi et al., 2023). Soil salinity affects plant growth through increasing osmotic stress, nutrient imbalance, ionic toxicity, or a combination of these factors affecting plant growth, physiological and biochemical metabolism, and reducing biological yield (Ashraf et al., 2023, Budran et al., 2023 Farghali, 2023; Taha et al., 2023).

The aim of this study was as follows: (1) to establish an efficient protocol for micropropagation of *Russelia equisetiformis* starting from the sterilization process until obtaining rooted plants, (2) to develop the agar substitute for enhancing *in vitro* plant growth with low-cost, and (3) to evaluate the efficiency of a hydrogel in mitigating the negative effect of salt stress on plant growth *in vitro*.

## MATERIALS AND METHODS

The present study was conducted at the Tissue Culture Technique Laboratory, Department of Ornamental Plants and Woody Trees, National Research Centre (NRC), Egypt, during the years 2022 and 2023.

### Procedure Layout

**Plant Material and Surface Sterilization:** *Russelia equisetiformis* fresh and recent shoots were taken from shrubs, maintained in the nursery of the Botanical Garden Research Department, Horticulture Research Institute, Agriculture Research Centre, Giza, Egypt. Nodal explants (5-8 cm length) were washed for one hour under tap water with adding a few drops of liquid soap. Explants have been rinsed with sterile demineralized water three times. All surfaces were sterilized under aseptic conditions in a laminar air flow hood. Initially, the nodal explants were disinfected for 30 sec by stirring in 70% (v/v) ethanol solution. After that, 15% (v/v) commercial sodium hypochlorite solution (NaOCl 5.25%) was shaken for 7 min followed by rinsing three times with autoclaved distilled water. Afterwards, they were immersed for 5 min in 0.1% (w/v) mercuric chloride ($HgCl_2$), and finally the nodal explants were rinsed three times with autoclaved distilled water.

**Culture Medium and Incubation Condition:** The sterilized nodal explants were cultured on MS (Murashige and Skoog, 1962) medium-free hormones for four weeks as an establishment stage. Two different types of culture media (MS and woody plant medium (WPM)) at full strengths were used for optimizing the suitable proliferation medium, supplemented with either benzyladenine (BA) or 6-(Y,Y-dimethylallylamino)purine (2iP) at two concentrations (0.5 or 1.0 $mgl^{-1}$). Survival rate of explants (%), formation of shootlets (number/explant), shootlets length (mm), and number of leaves/shootlet as well as rooting criteria (rooting percentage (%), number of roots/shootlet, and root length (mm)) were recorded after two months. All used media were supplemented with sucrose at 25 $gl^{-1}$ and solidified with agar at 8 $gl^{-1}$ which was adjusted to 5.7 ± 0.2 pH medium. All culture media were incubated in a growth chamber at 25 ±2 ˚C under a 16 h photoperiod of fluorescent light at 30 µmol $m^2sec^{-1}$. From the previously tested multiplication media, the optimized culture medium (MS medium + 0.5 $mgl^{-1}$ of BA + 25 $gl^{-1}$ sucrose) was selected for *in vitro* shooting and root formation. Five treatments of hydrogel polymer (commercial polymer (Barbary®)-acrylamide sodium acrylate, 40% hydrogel polymer, 6.5% N, 4.8% P, and 8.2% K) at 0, 12.5, 25, 37.5, and 50% instead of agar percentage were added to the optimized culture media, and after two months the performance of the shootlets (i.e., survival percentage (%), number of shootlets per explant, length of shootlets (mm), number of leaves per shootlet, rooting percentage (%), number of roots per shootlets, and length of root (mm)) were evaluated.

### Micropropagation Ability under Salinity Stress

MS medium supplemented with BA at 0.5 $mgl^{-1}$ +25 $gl^{-1}$ sucrose +25 % (2 $gl^{-1}$) hydrogel polymer +6 $gl^{-1}$ agar was used for testing the *in vitro* micropropagation ability under five levels of NaCl (0, 1000, 2000, 3000, and 4000 ppm), and data were recorded after two months.

**Biochemical Analysis**

**Photosynthetic Pigments:** Chlorophyll a, b and total carotenoids were determined according to Saric et al. (1967).

**Shootlet Extraction:** 5g of shootlets were soaked in 50 ml of ethanol 80 % and were shaken for 48 h at room temperature, and then the extracts were filtered and extracted twice.

**Phenolic Compounds:** The final extract was used for the assay of the phenolic compounds. Total phenols were assayed using Folin–Ciocalteu's reagent, according to Singleton and Rossi (1965). The tannins content was determined according to Tambe and Bhambar (2014). Total flavonoids were assayed by the method of Zhishen et al. (1999).

**Proline Content:** Proline content was measured in dried leaves using the method by Bates et al. (1973).

**Antioxidant Activity**

**Antioxidant Property by DPPH Radical Scavenging Activity:** Free radical scavenging of leaves extract of *R. equisetiformis* was tested using a 1, 1-diphenyl 2-picryl hydrazyl (DPPH) technique. The formula of Valko et al. (2007) was used to compute the percentage of antioxidants. Antioxidant activity (%) = [(Ac−As) ÷Ac] × 100, where Ac is control reaction absorbance and as is testing specimen absorbance.

**Total Antioxidant Activity using the Phosphomolybdenum Technique:** The approach was outlined by Prieto et al. (1999). The sample's absorbance at 695 nm was used to express the total antioxidant activity. Greater antioxidant activity was indicated by the higher absorption value (Prasad et al., 2009).

**Statistical Analysis**

MSTAT Computer Program (MSTAT Development Team, 1989) was used for comparing the treatments' means significance by Duncan's new multiple range test at a 5% level of probability. The proliferation stage and the evaluation of treatments of hydrogel polymer were designed as one factor in a completely randomized block design. For micropropagation ability and the biochemical changes under salinity stress, analysis of variance was estimated as factorials for two factors in a randomized complete block design. The data were statistically analyzed according to Steel and Torrie (1980).

## RESULTS

**Proliferation Optimized Condition**

The separated grown shootlets on MS culture medium free of hormones were used for a comparative study of *R. equisetiformis in vitro* culture responses to two different media types (MS and WPM) and cytokinins (BA and 2iP) which were used at three concentrations (0, 0.5, and 1.0 $mgl^{-1}$). The results in Table 1 and Figure 1a and 1b revealed that all shootlet explants could survive at 100% on the tested culture media containing the above-mentioned cytokinin treatments comparatively with those introduced in media free of hormones. The highest formation of shootlets and leaves was obtained significantly (7.67 and 51.67, respectively) from MS culture medium supplemented with 0.5 $mgl^{-1}$ of BA which also led to somewhat high rooting % and roots number (77.33% and 2.33, respectively), whereas the highest rooting percentage, roots number, and length were highly promoted on MS medium plus 0.5 $mgl^{-1}$ 2iP which gave 88.67%, 3.00, and 50.67 mm, respectively. Meanwhile, the most elongated shootlets (88.67 and 83.33 mm, respectively) were recorded on WPM supplemented with 0.5 or 1.0 $mgl^{-1}$ of 2iP. The *in vitro* surviving and rooted plantlets of *R. equisetiformis* were successfully acclimatized in a mixture of clay, peat moss, and sand (1: 1: 1 v/v/v) with a high survival percentage reaching 80%. From the present results, it could be proved that MS medium supplemented with a low concentration of BA (0.5 $mgl^{-1}$) was the suitable and optimized one of all other culture media that were examined for more proliferated and rooted shootlets.

**Effect of Five Different Levels of Hydrogel Polymer on Proliferation and Rooting of *Russelia equisetiformis***

Results in Table 2 and Figure 1c indicated the response of proliferated shoots to adding hydrogel polymer at various ratios (0, 12.5, 25, 37.5, and 50%) instead of agar percentages that were added to the optimized culture media. Addition of 25% hydrogel polymer (2 $gl^{-1}$) caused increase of all shooting characters (surviving explants, shoot number, and length as well as leaves number) to the highest values (100%, 8.00, and 80.00 mm, as well as 54, respectively). Also, this treatment led to the highest rooting % (93.33%) and roots number (4.33) as compared to control and other treatments.

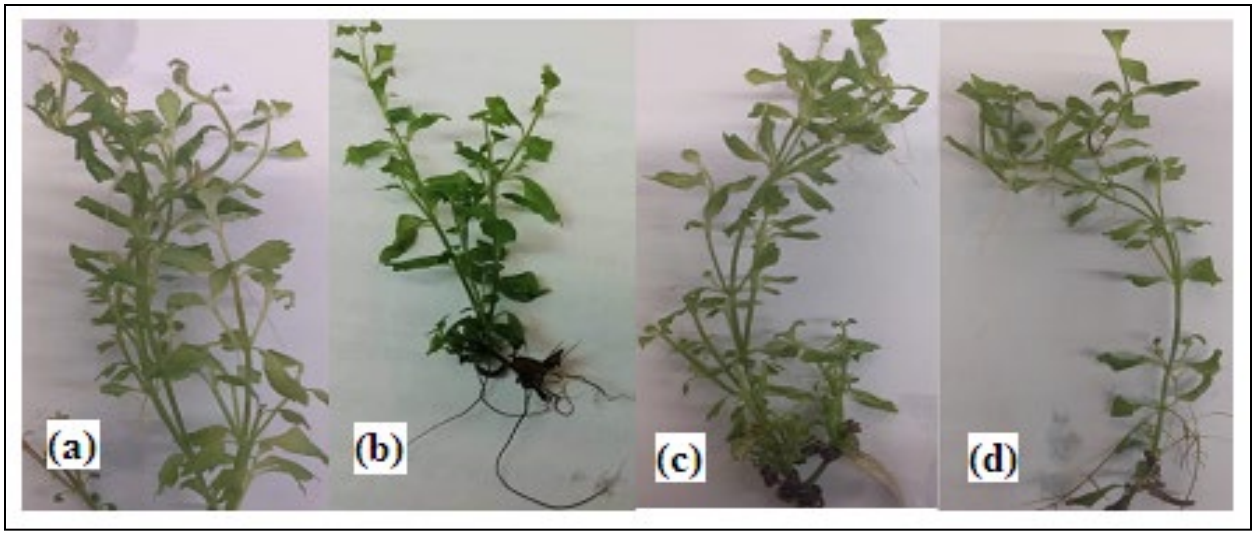


**Figure 1.** *In vitro* shooting and rooting ability of *R. equisetiformis*: (a) shootlet development that was cultured on MS supplemented with 0.5 $mgl^{-1}$ of BA (optimized culture medium), (b) rooting of shootlets cultured on the optimized culture medium, (c) shootlets cultured on MS + 0.5 $mgl^{-1}$ BA + 6 $gl^{-1}$ agar + 2 $gl^{-1}$ hydrogel polymer (25%), and (d) shootlets grown under salinity at 1000 ppm + 2 $gl^{-1}$ hydrogel polymer (25%).

**Table 1.** Effect of culture media (MS and WPM) and cytokinin (BA and 2iP) types on proliferation and rooting of *Russelia equisetiformis.*

| Parameter / Treatments | Survival % | Shootlet number/ explant | Shootlet length (mm) | Leaves number /shootlet | Rooting % | Roots number | Roots length (mm) |
|---|---|---|---|---|---|---|---|
| MS free | 80 C | 3.00 CD | 69.00 EF | 22.67 G | 66.67 C | 2.00 B | 30.00 CD |
| MS + 0.5 $mgl^{-1}$ BA | 100 A | 7.67 A | 74.00 CDE | 51.67 A | 77.33B | 2.33 AB | 30.00 CD |
| MS + 0.5 $mgl^{-1}$ 2iP | 100 A | 3.67 CD | 79.00 BC | 37.00 C | 88.67A | 3.00 A | 50.67 A |
| MS + 1 $mgl^{-1}$ BA | 100 A | 5.00 B | 60.00 G | 42.00 B | 0.00 G | 0.00 D | 0.00 F |
| MS + 1 $mgl^{-1}$ 2iP | 100 A | 3.33 CD | 68.00 F | 34.00 CD | 50.00 D | 1.00 C | 25.00 DE |
| WPM | 86.33 B | 2.33 D | 76.00 CD | 25.00 G | 66.67C | 2.00 B | 31.67 C |
| WPM + 0.5 $mgl^{-1}$ BA | 100 A | 3.00 CD | 72.00 DEF | 32.00 DE | 0.00 G | 0.00 D | 0.00 F |
| WPM + 0.5 $mgl^{-1}$ 2iP | 100 A | 2.67 CD | 88.67 A | 28.00 F | 49.33 D | 2.00 B | 40.00 B |
| WPM + 1 $mgl^{-1}$ BA | 100 A | 4.00 BC | 67.33 F | 35.00 CD | 16.67 F | 1.00 C | 20.33 E |
| WPM + 1 $mgl^{-1}$ 2iP | 100 A | 2.67 CD | 83.33 AB | 29.33 EF | 38.33 E | 1.00 C | 36.00 BC |

Different letters in columns show a significant difference between treatments based on Duncan's multiple range test.

**Table 2.** Effect of five different levels of hydrogel polymer on both proliferation and rooting parameters of *R. equisetiformis*.

| Parameters / Treatments | Survival % | Shootlet number/explant | Shootlet length (mm) | Leaves number/shootlet | Rooting % | Roots number | Roots length (mm) |
|---|---|---|---|---|---|---|---|
| A + 8 $gl^{-1}$ agar + 0 $gl^{-1}$ hydrogel (0%) | 100 A | 5.67 C | 70.00 C | 45 BC | 70.00 C | 2.00 B | 38.33 BC |
| A + 7 $gl^{-1}$ agar + 1 $gl^{-1}$ hydrogel (12.5%) | 100 A | 6.00 B | 73.33 B | 47 B | 80.00 B | 2.33 B | 50.00 A |
| A + 6 $gl^{-1}$ agar + 2 $gl^{-1}$ hydrogel (25%) | 100 A | 8.00 A | 80.00 A | 54 A | 93.33 A | 4.33 A | 42.33 B |
| A + 5 $gl^{-1}$ agar + 3 $gl^{-1}$ hydrogel (37.5%) | 100 A | 5.00 D | 60.00 D | 43 C | 85.00 B | 2.67 B | 40.00 BC |
| A + 4 $gl^{-1}$ agar + 4 $gl^{-1}$ hydrogel (50%) | 100 A | 3.00 E | 53.33 E | 30 D | 78.33 B | 2.00 B | 38.33 BC |

**A:** (MS+ 0.5 $mgl^{-1}$ BA). Different letters in columns show significant differences between treatments based on Duncan's multiple range test.

### Micropropagation Ability of *R. equisetiformis* under Salinity Conditions

The micropropagation ability was examined under five salinity levels by adding NaCl (0, 1000, 2000, 3000, and 4000 ppm) in optimization culture medium (MS + BA at 0.5 $mgl^{-1}$) enriched with zero or 25% hydrogel polymer (2 $gl^{-1}$) (Table 3 and Figure 1d). The explants could survive at 100% under various salinity levels. However, increasing this saline condition above 1000 ppm gradually decreased the observedshoot number, leaves number, and rooting percent. The stimulation effect of low salinity level (1000 ppm) was observed on elongated shoots and roots. The highest formation of roots (3.33 and 3.67) was also reported on saline conditions at 1000 and 2000 ppm with no significance between them and those grown under non-saline condition (control). The positive response of all recorded characters (shooting and rooting) was observed by using hydrogel at 25% in the culture medium which was also noticed when they interacted with various salinity levels.

**Table 3 .**Micropropagation ability of *R. equisetiformis* under various salinity levels

| Treatments | | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|
| NaCl ppm (A) | Hydrogel % (B) | Survival % | Shootlet number/ explant | Shootlet length (mm) | Leaves number/ shootlet | Rooting % | Roots number | Root length (mm) |
| Control | 0 | 100 A ±0 | 6.33 AB ±0.58 | 66.00 B ±1.00 | 41.33 B ±0.58 | 73.33 CDE ±3.06 | 2.00 DE ±1.00 | 28.00 DEF ±2.00 |
| | 25 | 100 A ±0 | 7.00 A ±1.00 | 70.00 B ±5.00 | 47.33 A ±2.52 | 93.33 A ±2.89 | 4.67 A ±0.58 | 56.67 A ±2.89 |
| 1000 | 0 | 100 A ±0 | 4.67 C ±0.58 | 58.33 CD ±2.89 | 35.00 C ±0.00 | 75.00 CD ±5.00 | 3.00 BCD ±0.00 | 50.00 B ±5.00 |
| | 25 | 100 A ±0 | 5.67 B ±0.58 | 81.67 A ±2.89 | 41.00 B ±1.73 | 83.33 B ±2.89 | 3.67 ABC ±0.58 | 52.33 AB ±2.52 |
| 2000 | 0 | 100 A ±0 | 3.00 EF ±0.00 | 47.67 EF ±4.04 | 20.33 E ±2.52 | 69.67 DEF ±4.73 | 3.33 BC ±0.58 | 43.33 C ±2.89 |
| | 25 | 100 A ±0 | 4.33 CD ±0.58 | 60.00 C ±0.00 | 30.67 D ±3.06 | 78.67 BC ±2.31 | 4.00 AB ±0.00 | 48.33 BC ±2.89 |
| 3000 | 0 | 100 A ±0 | 2.67 F ±0.58 | 42.33 F ±2.52 | 17.33 F ±1.15 | 66.00 EF ±1.73 | 2.00 DE ±0.00 | 27.67 EF ±2.52 |
| | 25 | 100 A ±0 | 4.00 CD ±0.00 | 53.33 DE ±2.89 | 23.00 E ±1.73 | 71.67 CDE ±2.89 | 3.00 BCD ±1.00 | 33.33 D ±2.89 |
| 4000 | 0 | 100 A ±0 | 2.67 F ±0.58 | 36.67 G ±2.89 | 15.00 F ±1.00 | 56.67 G ±5.77 | 1.67 E ±1.15 | 23.33 F ±2.89 |
| | 25 | 100 A ±0 | 3.67 DE ±0.58 | 50.00 E ±5.00 | 21.33 E ±1.15 | 62.67 FG ±3.06 | 2.67 CDE ±0.58 | 30.00 DE ±0.00 |
| Mean (A) | 0 | 100 A | 6.67 A | 68.00 A | 44.33 A | 83.33 A | 3.33 AB | 42.33 B |
| | 1000 | 100 A | 5.17 B | 70.00 A | 38.00 B | 79.17 B | 3.33 AB | 51.17 A |
| | 2000 | 100 A | 3.67 C | 53.83 B | 25.50 C | 74.17 C | 3.67 A | 45.83 B |
| | 3000 | 100 A | 3.33 C | 47.83 C | 20.17 D | 68.83 D | 2.50 BC | 30.50 C |
| | 4000 | 100 A | 3.17 C | 43.33 C | 18.17 D | 59.67 E | 2.17 C | 26.67 D |
| Mean (B) | 0 | 100 A | 3.87 B | 50.20 B | 25.80 B | 68.13 B | 2.40 B | 34.47 B |
| | 25 | 100 A | 4.93 A | 63.00 A | 32.67 A | 77.93 A | 3.60 A | 44.13 A |

Different letters in columns show significant differences between treatments based on Duncan's multiple range test.

**Table 4.** Photosynthetic pigments content (mg 100 $g^{-1}$ F.W.) in the proliferated shootless under the effect of culture media (MS and WPM), with different concentrations of cytokinin types (BA and 2iP).

| Treatments | Parameters | | |
|---|---|---|---|
| | Chlorophyll-a | Chlorophyll-b | Carotenoids |
| MS free | 30.91 EF | 12.47 E | 20.78 DE |
| MS + 0.5 $mgl^{-1}$ BA | 47.63 A | 17.26 A | 28.18 A |
| MS + 0.5 $mgl^{-1}$ 2iP | 39.17 BC | 14.62 B | 24.48 C |
| MS + 1 $mgl^{-1}$ BA | 37.96 C | 13.75 CD | 21.42 D |
| MS + 1 $mgl^{-1}$ 2iP | 40.07 B | 17.15 A | 24.32 C |
| WPM | 29.75 F | 13.48 CD | 20.82 DE |
| WPM + 0.5 $mgl^{-1}$ BA | 39.09 BC | 13.34 D | 26.02 B |
| WPM + 0.5 $mgl^{-1}$ 2iP | 31.09 E | 11.74 F | 21.32 D |
| WPM + 1 $mgl^{-1}$ BA | 33.11 D | 13.34 D | 24.85 C |
| WPM + 1 $mgl^{-1}$ 2iP | 24.64 G | 14.04 BC | 20.05 E |

Different letters in columns show significant differences between treatments based on Duncan's multiple range test.

**Photosynthetic Pigments Content in the Proliferated Shootlets of *R. equisetiformis***

As shown in Table 4, *R. equisetiformis* shootlets grown on MS culture medium supplemented with 0.5 $mgl^{-1}$ of BA contained the highest photosynthetic pigments content (chlorophyll-a, b and carotenoids) with values of 47.63, 17.26, and 28.18 mg/100 g F.W., respectively. Also, using MS supplemented with 1.0 $mgl^{-1}$ 2iP caused a significant effect of chlorophyll-b (17.15 mg 100 $g^{-1}$ F.W.).

**Photosynthetic Pigments Content in the Proliferated Shootlets under Salinity Condition**

The inhibition effect of increasing saline concentration above 1000 ppm (Table 5) gradually decreased the content of the pigments in russelia shootlets (Chl-a, b, and carotenoids) to attain the lowest values (22.95, 9.22, and 17.67 mg 100 $g^{-1}$ F.W., respectively) in shootlets grown on culture medium supplied with 4000 ppm of NaCl. The positive effect of 25% hydrogel polymer was recorded for these pigments which led to the highest values (31.96, 12.29, and 21.77 mg 100 $g^{-1}$ F.W., respectively) as compared to control. This stimulation effect of hydrogel polymer (25%) was also clearly observed in the estimated pigments content in shootlets grown on all applied salinity levels (1000, 2000, 3000, and 4000 ppm).

**Secondary Metabolites (Total Tannins, Flavonoids, and Phenols) and Proline Content**

The secondary metabolites (total tannins, flavonoids, and phenols) as well as proline showed a positive response to saline condition (Table 6), as the highest content of these compounds (10.53 mg/g, 79.77 mg QE/g, 40.83 mg GAE/g, and 44.00 µg/g, respectively) were found in shootlets grown under the highest salinity level (4000 ppm) while using 25% hydrogel in

the culture medium individually or in combination with various salinity levels (1000, 2000, 3000, and 4000 ppm) decreased the values of studied parameters.

**Antioxidant Activity**

**The Antioxidant Property by DPPH Radical Scavenging Activity:** Salt stress is one of the major environmental stresses that affect plant growth and development. It increases intracellular osmotic pressure and can cause accumulation of both sodium and reactive oxygen species (ROS) to toxic levels (Hasanuzzaman and Fujita, 2022). Plants overcome oxidative damage by activating antioxidants through enzymatic and non-enzymatic mechanisms (Shaheen et al., 2013). Table 7 illustrated that the highest antioxidant activity (86.58%) was noticed at 4000 ppm NaCl and 0% hydrogel while the lowest one (68.09%) was obtained with DPPH at 0 ppm NaCl and 25% hydrogel.

**Total Antioxidant Activity by using the Phosphomolybdenum Technique:** Table 7 showed different concentrations of antioxidant activity, and the maximum total antioxidant activity (62.95%) was attained at 4000 ppm NaCl and 0% hydrogel.

## DISCUSSION

Results in Table 1 proved that the MS medium supplemented with a low concentration of BA (0.5 $mgl^{-1}$) was the optimized one of all the other culture media that were examined, and this may be due to the fact that the composition of a culture medium is one of the most significant components of propagation, as it is essential for appropriate morphogenesis and propagation rate (Sudheer et al., 2022). Several media have been developed during the last several decades for various plant species.

Murashige and Skoog (1962) developed the most widely used medium which has a high ion concentration as compared to other media, particularly nitrogen, potassium, zinc, and chlorine (et al., 1995). Woody plant medium (WPM), which has low amounts of nitrogen, and potassium salts (Lloyd and McCown, 1980), explained the low response of *R. equisetiformis* explant to this medium. Aside from the mix of macro- and microelements in a culture medium, woody plants are frequently grown in more diluted media, such as the presence and concentration of growth regulators are essential factors utilized to control the in vitro grown plant. Similar results were noticed on the same plant (*R. equisetiformis*) which showed an inhibitory response with increasing BA concentration (Mahipal and Manokari, 2015). Also, Abdel-Magied et al. (2023) on micropropagated *Moringa oleifera* reported that using MS plus BA caused the maximum shoot multiplied per in vitro cultured shoot explant.

Cytokinins stimulate cell division, induce bud formation, and promote proliferation (Van Staden et al., 2008). Benzyladenine (BA) is a cytokinin that is often employed during the multiplication phase (Teixeira da Silva, 2012).

Concerning the effect of hydrogel polymer proliferation and rooting, there are valuable reviews on culture substrates for tissue culture. A previous study on *in vitro* rooting of pineapple showed that hydrogel polymer treatments at different concentrations used as a gelling agent referred to increments in all growth criteria. Agar contributes about 70% of the costs of constituent media in micropropagation, while hydrogel decreases this cost without negative effects on plant growth (Hassan et al., 2018). Adding hydrogel, instead of agar in *in vitro* culture media, enhanced *Cannabis sativa* growth and rooting, thereby obtaining higher proliferation in comparison with agar (Kernosenko et al., 2023). Hydrogel polymer can be utilized as a plant culture substrate, where it may help to overcome the drawbacks of conventional media, and present its functionality and potential, where it has solid-like properties, thermostability, and biodegradability, besides it can supply plants with water, air, and nutrients; hence it can be a plant growth culture substrate (Ma et al., 2023).

Regarding the micropropagation ability of *R. equisetiformis* under salinity conditions and using hydrogel polymer as alleviator to salinity, it seemed that the explants could survive under all tested salinity levels but increasing this level above 1000 ppm caused a decline of the shoot and leaves number and rooting%; however, using a hydrogel showed a positive response of shooting and rooting ability (Table 3). This adverse impact caused by the increase of salt concentration was confirmed by Ljubojevic et al. (2017), on three *Salvia* species, in which the plants treated with NaCl showed a negative response, while addition of hydrogel polymer resulted in clear positive impacts on the vegetative growth and the root system. The hydrogel alleviated the unfavorable influence of higher salt levels, by enhancing plant vitality, where the

**Table 5.** Photosynthetic pigments (Chl-a, b and carotenoids) content (mg 100 $g^{-1}$ F.W.), in the proliferated shootlets under effect of different salinity levels and hydrogel polymer.

| Treatments | | Parameters | | |
|---|---|---|---|---|
| NaCl ppm (A) | Hydrogel % (B) | Chlorophyll-a | Chlorophyll-b | Total carotenoids |
| Control | 0 | 38.80 B ±0.32 | 14.59 B ±0.41 | 24.15 B ±0.20 |
| | 25 | 40.92 A ±0.13 | 16.65 A ±0.36 | 26.28 A ±0.68 |
| 1000 | 0 | 30.20 E ±0.60 | 11.89 D ±0.23 | 20.82 D ±0.20 |
| | 25 | 35.78 C ±0.70 | 13.48 C ±0.50 | 22.75 C ±0.25 |
| 2000 | 0 | 27.00 G ±0.50 | 9.297 F ±0.14 | 18.33 F ±0.65 |
| | 25 | 30.58 D ±0.41 | 10.55 E ±0.39 | 21.00 D ±1.00 |
| 3000 | 0 | 25.32 H ±0.64 | 8.22 G ±0.70 | 19.33 E ±0.65 |
| | 25 | 29.06 F ±0.09 | 11.24 DE ±0.24 | 20.48 D ±0.45 |
| 4000 | 0 | 22.44 J±0.31 | 8.92 FG ±0.07 | 17.00 G ±1.00 |
| | 25 | 23.46 I ±0.42 | 9.51 F ±0.07 | 18.34 F ±0.64 |
| Mean (A) | 0 | 39.86 A | 15.62 A | 25.21 A |
| | 1000 | 32.99 B | 12.69 B | 21.78 B |
| | 2000 | 28.79 C | 9.92 C | 19.67 C |
| | 3000 | 27.19 D | 9.73 C | 19.91 C |
| | 4000 | 22.95 E | 9.22 D | 17.67 D |
| Mean (B) | 0 | 28.75 B | 10.58 B | 19.93 B |
| | 25 | 31.96 A | 12.29 A | 21.77 |

Different letters in columns show significant differences between treatments based on Duncan's multiple range test.

**Table 6.** Effects of salinity levels and hydrogel polymer on secondary metabolites and proline contents of the proliferated shootlets of *R. equisetiformis*.

| Treatments | | Parameters | | | |
|---|---|---|---|---|---|
| NaCl ppm (A) | Hydrogel % (B) | Total tannins (mg/g) | Total flavonoids (mg QE/g) | Total phenols (mg GAE/g) | Proline (µg/g) |
| Control | 0 | 8.91 BCD ± 0.09 | 41.00 H ±1.00 | 23.50 H ±0.50 | 23.26 G ±0.26 |
| | 25 | 8.35 CD ±0.32 | 38.05 I ±0.93 | 25.47 G ±0.47 | 21.32 H ±0.54 |
| 1000 | 0 | 9.26 BC ±0.26 | 57.42 F ±0.54 | 29.18 F ±0.18 | 30.79 E ±0.21 |
| | 25 | 8.29 D ±0.71 | 50.95 G ±0.07 | 25.04 G ±0.97 | 27.13 F ±0.31 |
| 2000 | 0 | 9.33 B ±0.32 | 70.65 D ±0.31 | 31.38 E ±0.65 | 36.51 D ±0.50 |
| | 25 | 8.79 BCD ± 0.86 | 61.30 E ±0.61 | 25.89 G ±0.11 | 31.51 E ±0.52 |
| 3000 | 0 | 9.59 B ±0.00 | 77.00 C ±0.00 | 34.33 C ±0.62 | 41.54 C ±0.52 |
| | 25 | 8.37 CD ±0.00 | 71.43 D ±0.16 | 33.06 D ±0.19 | 35.67 D ±0.33 |
| 4000 | 0 | 12.24 A ±0.00 | 81.15 A ±0.29 | 44.53 A ±0.41 | 44.82 A ±0.82 |
| | 25 | 8.82 BCD ±0.19 | 78.38 B ±0.49 | 37.13 B ±0.13 | 43.18 B ±0.27 |
| Mean (A) | 0 | 8.63 B | 39.52 E | 24.49 E | 22.29 E |
| | 1000 | 8.78 B | 54.19 D | 27.11 D | 28.96 D |
| | 2000 | 9.06 B | 65.98 C | 28.63 C | 34.01 C |
| | 3000 | 8.98 B | 74.21 B | 33.69 B | 38.60 B |
| | 4000 | 10.53 A | 79.77 A | 40.83 A | 44.00 A |
| Mean (B) | 0 | 9.87 A | 65.45 A | 32.58 A | 35.38 A |
| | 25 | 8.53 B | 60.02 B | 29.32 B | 31.76 B |

Different letters in columns show significant differences between treatments based on Duncan's multiple range test.

**Table 7.** Effect of salinity levels and hydrogel polymer on DPPH radical scavenging and total antioxidant activity in the proliferated shootlets extract of *R. equisetiformis*

| Treatments | | Parameters | | | |
|---|---|---|---|---|---|
| NaCl ppm (A) | Hydrogel% (B) | DPPH antioxidant (200 µg/ml) antioxidant % | Total antioxidant capacity antioxidant % | | |
| | | | 150 µg/ml | 200 µg/ml | 250 µg/ml |
| Control | 0 | 70.00 H ±2.00 | 19.33 H ±2.52 | 30.26 H ±0.65 | 27.67 H ±2.08 |
| | 25 | 68.09 I ±1.01 | 16.85 I ±0.15 | 26.09 J ±0.10 | 22.46 I ±0.76 |
| 1000 | 0 | 72.56 G ±0.44 | 22.48 G ±0.45 | 32.58 G ±0.42 | 33.71 F ±1.80 |
| | 25 | 69.83 H ±0.28 | 17.97 HI ±0.04 | 29.21 I ±0.21 | 31.46 G ±1.55 |
| 2000 | 0 | 84.00 C ±1.00 | 34.33 E ±0.32 | 43.82 E ±0.40 | 46.07 D ±1.91 |
| | 25 | 80.81 E ±0.81 | 25.55 F ±0.39 | 35.45 F ±0.51 | 39.55 E ±1.45 |
| 3000 | 0 | 85.23 B ±0.27 | 42.70 C ±0.22 | 55.05 B ±0.11 | 52.81 B ±2.06 |
| | 25 | 79.59 F ±0.42 | 37.47 D ±0.50 | 46.07 D ±0.93 | 48.33 C ±1.53 |
| 4000 | 0 | 86.58 A ±0.42 | 50.56 A ±0.44 | 58.42 A ±0.54 | 62.95 A ±1.08 |
| | 25 | 81.51 D ±0.49 | 48.32 B ±0.49 | 51.87 C ±1.10 | 53.15 B ±0.79 |
| Mean (A) | 0 | 69.04 D | 18.09 E | 28.17 E | 32.58 D |
| | 1000 | 71.19 C | 20.23 D | 30.89 D | 42.81 C |
| | 2000 | 82.40 B | 29.94 C | 39.63 C | 50.57 B |
| | 3000 | 82.41 B | 40.08 B | 50.56 B | 58.05 A |
| | 4000 | 84.05 A | 49.44 A | 55.15 A | 32.58 D |
| Mean (B) | 0 | 79.68 A | 33.88 A | 44.03 A | 44.64 A |
| | 25 | 75.96 B | 29.23 B | 37.74 B | 38.99 B |

Different letters in columns show significant differences between treatments based on Duncan's multiple range test.

hydrogel stimulated available ions at enough that is commonly restricted and in competition with excessive $Na^+$ ion amounts. Previous studies provided insights into the potential benefits of hydrogel application in improving plant performance and discussed the effects of hydrogel on various aspects of plant growth, physiological attributes, and tolerance to saline conditions (Nascimento et al., 2021; Sousa, et al., 2022; Kernosenko et al., 2023).

From Table 4, using cytokinins (BA and 2iP) obtained valuable photosynthetic pigments content (chlorophyll-a, b and carotenoids), and this simulative effect of cytokinins was due to enhancement of chloroplast biogenesis (Cackett et al., 2022), where cytokinins play a main role in the chlorophyll biosynthesis function and development by regulating more than 100 genes related to photosynthesis (Cortleven and Schmulling, 2015). The positive effects of cytokinins on photosynthetic pigments content might be attributed to their improving effects, particularly 2ip in shootlets tissues (Nowakowska et al., 2021; Youssef et al., 2021). Meanwhile, photosynthetic pigments content decreased by increasing the salt level (Yildirim et al., 2023), due to an increment in chlorophyllase and chlorophyll-degrading enzymes activity (Reddy and Vora, 1986), as well as the accumulation of ions in leaves which have a negative impact on photosynthetic pigments (Yeo and Flowers, 1983). Hydrogel polymer under abiotic stress could enhance chlorophyll biosynthesis and reduce the degradation of chlorophyll through providing the plant with water and nutrients (Tongo et al., 2014; Abd El-Aziz et al., 2022).

The positive responses of secondary metabolites (total tannins, flavonoids, and phenols) as well as proline to the high salinity level (4000 ppm) (Table 6) could suggest that the plant reduces salinity stress by balancing intracellular concentrations of phytochemicals such as tannins, phenols, and antioxidants, which conserve plant cells from oxidative damage (Gupta and Waoo, 2022). Plants modify metabolic pathways by changing gene expression levels which show tannins, flavonoids, phenols, and antioxidants accumulation (Kumar et al., 2017). There are increments in phytochemicals concentration that led to mitigating the unfavorable impacts of salinity stress (Hirayama and Shinozaki, 2010). Also, proline is an amino acid that accumulates during osmotic changes as a physiological response in plants exposed to abiotic stresses (Kaur and Asthir, 2015). Treatment with hydrogel polymer on plants grown under saline conditions significantly enhanced the parameters affected by high salinity and reduced phenolic compounds and proline (Canan et al., 2008; Sajjadi et al., 2021).

The free radical DPPH is a quick, easy, and affordable way to test a compound's antioxidant potential. It is frequently used to assess a compound's capacity to function as a hydrogen source and a free-radical scavenger. Numerous plant extractions have demonstrated the ability to counteract the DPPH radical scavenging activity *in vitro* (Aini et al., 2019; Kurniawan et al., 2021; Amrulloh et al., 2021).

The increased rate of antioxidant activity might play a role in self-defense against the effects of oxidative stress (Smirnoff, 1995). Tissues presenting great antioxidant activities could be additionally resistant to oxidative stress due to the ability of antioxidants to scavenge reactive oxygen species more than tissues with low antioxidant potential (Hasanuzzaman et al., 2020).

## CONCLUSION

*Russelia equisetiformis* plants could be grown under saline conditions, and the stimulated parameters presented in micropropagation, photosynthetic pigments, and bioactive compounds as total tannins, flavonoids, phenols, and proline were highly affected by using hydrogel polymer that could mitigate the negative effect of high salinity level.

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