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AN EFFICIENT MICROPROPAGATION OF *RESEDA LUTEA*: A RARE PLANT OF SAUDI ARABIA

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Abstract

The goal of this work was to look at the propagation of *Reseda lutea* L. by organogenesis in tissue culture. Explants from *In vitro* grown seedlings were taken from the axillary bud. After seven days of culture on MS medium supplemented with 1.0 mg/L BAP, the adventitious buds developed. After three weeks of culturing on MS medium supplemented with 1.5 mg/L BAP, the maximum multiplication of shoots (16.12 shoots/explant) was discovered, with an average (7.37 cm) shoots/explant. These shoots were sub-cultured on MS media with varying concentrations of NAA and IBA for root initiation. The MS medium combined with IBA produced the greatest percentage of root development (92%) and the greatest number of roots (7.37 roots/plant). In MS media supplemented with 0.5 NAA, the longest roots (3.08 cm) were found. After 17 days in a glasshouse, the plantlets were acclimatized in pots containing Peat moss and pearlite, 98 percent of the plantlets were acclimatized. To get a plant in a pot, the complete procedure took about 75 days. The technique proposed could aid in the preservation of the plant both *In vivo* and *In vitro*.

Key words: Micropropagation, *Reseda lutea* L.*, In vitro*, Organogenesis, Tissue culture, Acclimatization.

Introduction

Reseda lutea L., a member of the Resedaceae family, is a medicinal plant that is also used for natural dyes, cattle feed, and honey. In the world, there are 12 genera in the Resedaceae family (Christenhusz & Byng, 2016). Nearly 60 species of *Reseda* may be found all over the world (Davis, 1965; Davis *et al*., 1988; Ozhatay *et al*., 1994). *Reseda lutea* can be found in southern, central, and western Europe, including Norway, Finland, the United Kingdom, and Sweden; Anatolia and the Mediterranean Basin; Southwest Asia, the former Soviet Union, Chile, Afghanistan, Australia, the United States, New Zealand, and North and South Africa; and Southwest Asia (Dogan, 2001). A.G. Miller, *R. pentagyna* Abdallah & *R. lutea, R. arabica Boiss., R. alba, R. muricata, R. arabica Boiss., R. arabica Boiss., R. arabica Boiss., R. arabica Boiss., R. arabica Boiss., R. arabica Boiss., R. arabica Boiss., R. arabica Boiss*. In Saudi Arabia, *C. Presl, R. sphenocleoides Deflers*, and *R. aucheri* Boiss (Chaudhary, 1999) were discovered.

R. lutea is a short-lived, biennial, or perennial herbaceous plant that thrives in uncultivated fields, rocky slopes, and roadsides (Dogan *et al*., 2008). This plant grows in areas with sandy-clayey-loam and sandy-loam with a pH of medium alkaline to slightly alkaline (Dogan, 2001).

However, *Reseda lutea* has a high valued medicinal plant. It is also used in the carpet industry where it is used together with *R. luteola* as a source of a natural dye (Dogan, 2001; Anon., 1991). According to the Jablonski *et al*., (1992), *Reseda lutea* is used for the perfection of apiculture. In Iran and Australia, cattle breeders have been using *R. lutea* for grazing, as a dry or fresh food source (Moghaddam, 1977; Heap *et al*., 1995). This plant is used to prevent erosion of soil due to its roots that growing fast (Heap *et al*., 1995). Pagnotta *et al*., (2020) reported that the *R. lutea* attracts the honey bees and pollinators of the wild. Furthermore, *R. lutea* is one of the 10 important plants that feed *Apis mellifera* honey bee (Wood *et al*., 2015), as well as *R. lutea*, *was* known for its cure properties in pharmacotherapy from ancient times. Therefore, *R. lutea* has been recorded to the lists of interesting plant species for ethnopharmacological properties (Cakilcioglu *et al*., 2011). The roots of *R. lutea*, on the other hand, contain diuretic and diarrheal characteristics, according to McIntryre *et al*., (1988). It is also utilized as an antibacterial and anti-inflammatory agent (Bremner *et al*., 2009).

R. lutea is commonly used for tumor reduction, according to Radulovi *et al*., (2014).

The preservation of medicinal, imperiled, endangered, and indigenous plant species is critical to humanity's survival. The flora of Saudi Arabia is extremely diverse, with numerous rare and endangered plant species. Furthermore, these plants are under constant threat due to harsh environmental conditions and anthropogenic activity. As a result, the number of vulnerable plant species is growing every year (Khan *et al*., 2012).

Plant tissue culture and biotechnology, on the other hand, offer us plants that are free of contamination and explants that can be used for biotransformation, propagation, cryopreservation, and germplasm conservation (Al-Qurainy *et al*., 2018). The tissue culture technique has recently had a large impact on the agricultural industry, and the following are some of the characteristics that demonstrate its relevance (Jain, 2007; Zaid & Wet, 1999): Plants may be easily transported from the nursery to the field, and this technique can be utilized to improve tolerance to environmental variables and disease. Furthermore, the plants created using this process are disease-free. The propagation of unusual genotypes in the laboratory is possible (Ferry, 2011). The potential of meristematic tissue to generate new branches is exploited in direct organogenesis, and without a callus stage, these plants are assumed to be true-to-type (Kunert *et al*., 2003). Several research organizations have worked on organogenesis as a technique of propagation, although this approach is slow and produces few compared to

somatic embryogenesis. However, in terms of genetic variants, it is seen as less significant (Al-Khateeb, 2008; Bekheet *et al*., 2001).

There is relatively little information available regarding *Reseda lutea* propagation and multiplication in the field. Furthermore, there may be no information concerning this plant's propagation and regeneration *In vitro*. As a result, it's critical to look into alternate propagation strategies and the development of this strategy for propagating this crucial plant. To improve the number of *In vitro* ways that can be used to conserve, mass regenerates, and extend the value of this plant. The major goal of this study was to construct and develop an *In vitro* propagation strategy for *Reseda lutea* plants employing direct organogenesis that was highly repeatable.

Materials and Methods

The present study was performed at Laboratories of Tissue Culture and Molecular Biology, Botany and Microbiology Department, Science Faculty, King Saud University, Riyadh, Saudi Arabia.

Plant material: The shoots with flowers of the plant *Reseda lutea* were obtained from an overland population in Saudi Arabia's Tabuk region and authenticated by the Botany and Microbiology Department of King Saud University's Science Faculty. The seeds of *Reseda lutea* were removed from the fruits and dried for 96 hours in an open clean petri dish at room temperature, after which they were stored in a clean covered tube refrigerated at 4°C for four months.

Seeds germination: The seeds of *Reseda lutea* were taken out from the refrigerator and washed for 10 minutes in running tap water containing a few drops of tween 80. Furthermore, the seeds were sterilized for 10 minutes in the Laminar Air Cabinet under aseptic conditions using 15% sodium hypochlorite and moderate shaking at regular intervals. To remove the bleach, the seeds were rinsed three times with double distilled water for five minutes each time. Ten seeds of *R. lutea* were cultured on agar MS media and incubated in the culture room at 25 °C in the dark until seedlings with small four-leaved stages appeared (after one month, only three seeds were germinated). After that, we chose one plantlet as the explant source.

Media of culture: We employed the ready MS medium with vitamins (Sigma-Aldrich Chemicals Company) in this investigation, which comprises all nutrient elements (Murashige & Skoog, 1962). All vitamins (0.5 mg/L Nicotinic acid, 0.5 mg/L Pyridoxine HCI, Myo-inositol, 2 mg/L Glycine, 0.1 mg/L Thiamine HCL) are also included.

The sucrose was added at a rate of 20 g/l. Plant growth regulators were added as needed depending on the stage of propagation. The pH (pH meter 526 multical ® WTW) was adjusted to 5.6 using 0.1 N NaOH or 0.1N HCl, and 7 g/L Agar was added to solidify the MS media. The MS medium was autoclaved for 20 minutes at 121°C and 1x105 Pa to sterilize it (1.1 kg cm-2).

Shoot induction: Explants for shoot induction were the nodal segment of shoots (axillary bud) from *Reseda lutea* seedlings cultivated *In vitro*. The nodal segments were grown on MS agar media with varied PGR concentrations (0.0, 0.1, 0.5, and 1.0 mg/L BAP). For three weeks, cultures were incubated in the dark at 27 ± 2°C. The number of adventitious buds was calculated after three weeks of culture and the number of induced adventitious buds was measured by daily monitoring of the cultures.

Shoot multiplication: The MS agar media was supplemented with varying doses of 6-(γ,γ-Dimethylallylamino) Purine 2ip (0, 0.1, 0.5, 1.5, and 5 mg/L) and concentrations of benzyl amino purine (BAP) (0, 0.1, 0.5, 1.5, and 5 mg/L) were employed in this work. The adventitious buds were separated into tiny lumps, each comprising at least two buds, and grown in Magenta vessels on MS media. The cultures were incubated for two weeks at 27 ± 2°C. The number of multiplexed shoots was counted after two weeks of cultivation. In addition, the lengths of the shoots were calculated.

Rooting: Individually, multiplied and propagated shoots with length (2.0–3 cm) with at least 2–3 leaves were detached and cultivated on a fresh MS medium with varying concentrations of Indole-3-butyric acid (IBA) and α-Naphthaleneacetic acid (NAA) (0.0, 0.1, 0.5, 1.5, 3.0 mg/L). For four weeks, the cultures were incubated at 27 ± 2°C. The proportion of rooted plantlets, the number of roots per plantlet, and the length of roots were all measured after four weeks.

Acclimatization: Plantlets with good roots, a height range of (5-7) cm, and at least four leaves were chosen for acclimatization. To remove any trace of media adhering from the root system, these plantlets were rinsed with tap water. The plantlets were transplanted into containers containing a sterilized soil combination of peat moss and perlite (2:1). The plantlets were covered with a transparent plastic bag until the high humidity was maintained, then the cover was removed for half an hour every day for one week, then the length of cover removal was gradually increased until the cover was totally removed at the end of the second week. Watering was done every three days or as needed depending on the potting mix quality.

Culture condition: The cultures were incubated in a culture room set at 27 ± 2°C, under a photoperiod of 8 h dark/ 16 h light and light intensity 30–40 µmol m^{-2} s^{-1}, the relative humidity was maintained at 60%.

Design of the experimental and statistical analysis: The trials were designed and carried out as factorial experiments, which were entirely randomized. The tests were carried out in triplicate for each treatment. The statistical analysis was carried out with the use of the SPSS software program (version 11, SPSS Inc., Chicago, USA) and one-way analysis of variance (ANOVA) Duncan's test was used to compare mean averages of the main effects at the $p<0.05$ level (results were represented as means ± SE).

Results and Discussion

Shoot induction: After three weeks of growth on MS media, the effect of various concentrations of BAP (0.0, 0.1, 0.5, and 1.0 mg/L) on the induction of adventitious buds from *R. lutea* explants was studied. (Table 1 & Fig. 1A) indicate that there is a direct link between the number of buds and the concentration of BAP, with the number of buds increasing up to 1 mg/L BAP. Following that, the number of buds dropped as the BAP concentration grew, despite the fact that the PGR concentration increased by 1.5 mg/L. However, the optimal BAP concentration employed in this investigation was 1.0 mg/L, which produced the most buds (6.75 ± 0.35 buds/explant) with a significant difference compared to the other treatments. The findings of this investigation corroborate previous findings (Johnson *et al*., 2007) *Passiflora mollussima, Passiflora mollussima, Passiflora mollussi* (Raja & Arokiasamy, 2008) *Mentha viridis, Mentha viridis, Mentha viridis* (Swaroopa *et al*., 2011) (Kumar *et al*., 2016) *Trichosanthes dioica*, (Srikun, 2017) *Strobilanthes tonkinensis*, and *Reseda pentagyna* (Al-Qurainy *et al*., 2018).

The explants cultivated in MS media without PGRs, on the other hand, showed no reaction. The phytohormone BAP is known to stimulate adventitious buds. According to Jun-jie *et al*., (2017), different internal factors influence cell activities during adventitious buds regeneration, one of the cytokinins, which could explain changes in internal structure and chemical nature. However, the BAP's exact mechanism of action is unknown (Montalbán *et al*., 2013). Furthermore, Alansi *et al*., (2020) observed that adding cytokinins to the MS medium activates the adventitious buds by enhancing DNA replication, which leads to cell division. It is worth mentioning that during the stage of adventitious buds break, in the first week we saw little callus formed on the edge of the cut surface (Fig. 1A).

Table 1. Effect of different concentrations of BAP on the induction of adventitious buds in *R. lutea*.

Concentration of BAP	Number of adventitious buds Mean ± SE
0.0 mg/L	0.00 ± 0.00 a*
0.1 mg/L	2.75 ± 0.36 b
0.5 mg/L	4.62 ± 0.29 c
1 .0 mg/L	6.75 ± 0.35 d
1.5 mg/L	5.75 ± 0.31 e

*Data that hold the same alphabets are not significantly different, according to the test of Duncan at level (p<0.05)

Shoot multiplication: To optimize the concentration of BAP and 2iP for *R. lutea* shoot multiplications and plant growth, the adventitious buds were subcultured on MS media supplemented with two distinct phytohormones (BAP or 2iP) at varying concentrations (0.0, 0.1, 0.5, 1.5, 5 mg/L). The data in Table (2) and (Fig. 1) reveal that BAP, as well as 2ip, have a substantial effect.

The highest number of shoots (16.12 shoots/explant) is induced at 1.5 mg/L BAP, although the maximum number of shoots (13.87 shoots/explant) is generated at 5.0 mg/L BAP. However, up to 1.5 mg/L of BAP on the MS medium, the concentrations of BAP were directly connected to the number of shoots as well as the length of plantlets (Fig. 1F & H). The quantity and length of plantlets decreased as the concentration of BAP rose up to 5 mg/L (Table 2). As a result, at the same concentration of BAP (1.5 mg/L), which gave the largest number of multiplied shoots (16.12 ± 0.54) and gave the highest length of multiplied shoots (7.37 ± 0.16). In the case of 2ip, the maximum number of multiple shoots (13.87 ± 0.51) was obtained at the concentration of 5 mg/L 2ip (Fig. 1G), while the maximum length of shoots (6.02 ± 0.28) was obtained at the concentration of (1.5 mg/L) 2ip. It's worth noting that the plantlets' shoots grew quickly in all of the concentrations employed in this investigation, therefore the data were recorded after two weeks of culturing. In addition, there was a substantial difference between all of the treatments. These findings are consistent with those of our previous study on *Reseda pentagyna* (Al-Quriny *et al*., 2018), as well as Jose & Satheesh, (2004), who found that 5 mg/L of 2 iP offered the greatest outcomes in date palm in their study on *O. mungos* (Saheem *et al*., 2008). Our findings, on the other hand, contradicted those of Al-Taha *et al*., (2011), who discovered that a concentration of 4 mg/L of 2iP was optimal in the multiplication stage of date palm.

Rooting: Any *In vitro* plant regeneration protocol must include a roots system that is both efficient and effective (AL-Qurainy *et al*., 2015). As a result, the influence of two auxins at varying concentrations on root induction and growth was investigated. The rooting of *R. lutea* plantlets regenerated through organogenesis was investigated. In general, the findings in Table (3) reveal that the IBA and NAA have no significant effect on the quantity and length of roots.

On the other hand, there was a substantial difference in the number and length of roots between the phytohormone utilized in this study and MS media (control). The NAA, on the other hand, produced a higher mean number of roots (3.125 ± 0.410) and longer roots (2.378 ± 0.214 cm) than the IBA, which produced a mean number of roots (2.500 ± 0.212) and root length (2.140 ± 0.110 cm) with no significant effect. Our findings contradict those of Wang *et al*., (2020), who claimed that the IBA was the best for rooting in *Pseudostellaria heterophylla* shoots. In terms of the influence of different concentrations of IBA or NAA on the number and length of the roots, the data in (Table 3) reveals that varying concentrations of IBA or NAA have a substantial effect on the number and length of the roots (Fig. 2).

Furthermore, the optimum IBA concentration was 1.5 mg/L, which produced the most roots (7.37 ± 0.15/explant), the longest roots (2.92 ± 0.15 cm), and the highest percentage of rooting (92%) (Fig. 2D). The optimal NAA concentration was 0.5 mg/L, which produced the most roots (6.02 ± 0.26) and the longest roots (3.08 ± 0.14 cm), as well as the highest percentage of rooting (90%) (Fig. 2C). Our findings are consistent with those of Saheem *et al*., (2008), who found that 0.5 mg/L NAA was the optimum for roots in date palm shoots regenerated from adventitious buds. However, Al-

Qurainy *et al*., (2015) reported that when *Tamarix nilotica* shoots were cultured on MS medium with 100 mM IBA for 5, 10, and 15 days, they were rooted. 1.5 mM IBA was shown to be the best for rooting *R. pentagyna*, according to Al-Qurainy *et al*., (2018). Auxins (especially IBA) play a substantial role in root stimulation for *Turnera ulmifolia* and *R. pentagyna* shoots regenerated *In vitro*, according to Shekhawat *et al*., (2014) and Al-Qurainy *et al*., (2018). IBA was also utilized to root *Trichosanthes dioica* shoots *In vitro* (Kumar *et al*., 2016) and *Strobilanthes tonkinensis* shoots *In vitro* (Kumar *et al*., 2016) (Srikun, 2017). For acclimation, thirty rooted plantlets with five to seven complete leaves were chosen, then cleaned of traces of agar and put in pots containing peat-moss and perlite (2:1) for seventeen days. These plantlets were effectively acclimatized, with a survival rate of 98 percent (Fig. 2F, G, and H).

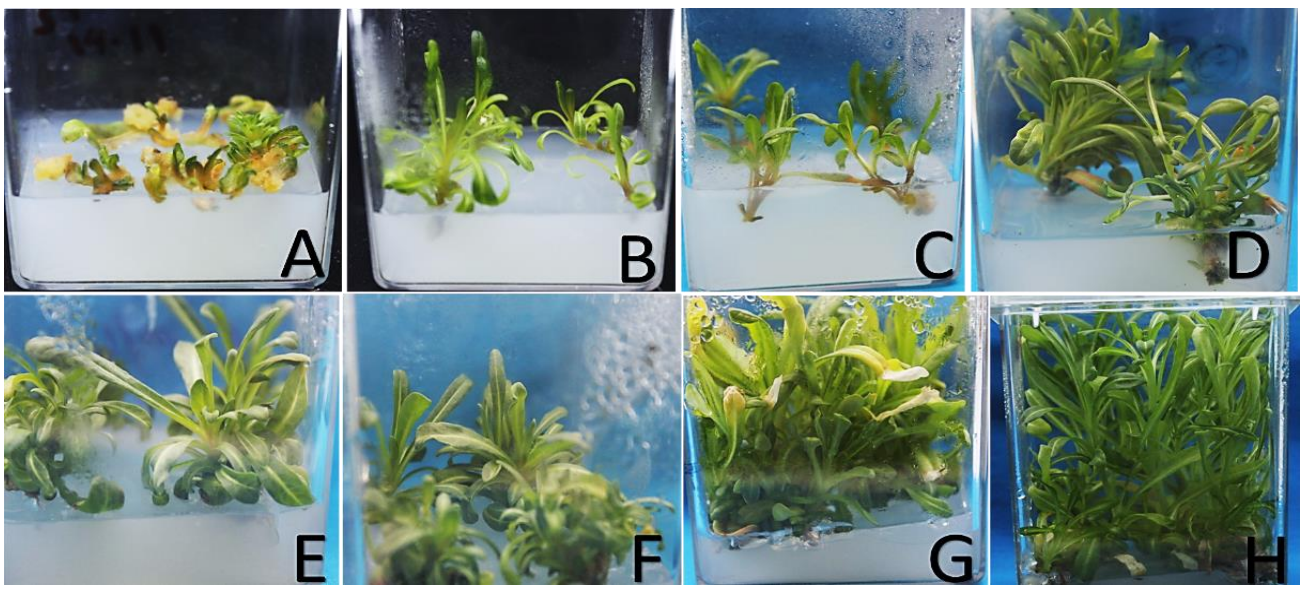

Fig. 1. *In vitro* multiplication of *Reseda lutea* A: Adventitious bud induction on MS medium supplemented with 1.0 mg/L of BA, B and C: Individual shoots cultured on MS medium supplemented with BA for multiplication, D: Shoots multiplication on MS medium containing 0.1 mg/L of BA, E: Shoots multiplication on MS medium containing 0.1 mg/L of 2iP, F: Shoots multiplication on MS medium containing 0.5 mg/L of BA, G: Shoots multiplication on MS medium containing 5 mg/L of 2iP, H: Shoots multiplication on MS medium containing 1.5 mg/L of BA.

Table 2. Effect of different concentrations of BAP and 2iP on the multiplication of indirect buds of *R. lutea*.

Con. of BAP and 2ip	Average no. of multiple buds ± SE	Average shoot length ± SE
0.0 BAP mg/L	3.75 ± 0.45 a*	3.06 ± 0.23 a
0.1 BAP mg/L	6.00 ± 0.42 b	5.25 ± 0.25 c
0.5 BAP mg/L	13.75 ± 0.77 f	6.62 ± 0.40 d
1.5 BAP mg/L	16.12 ± 0.54 j	7.37 ± 0.16 e
5 BAP mg/L	9.87 ± 0.47d	4.91 ± 0.22 bc
0.1 2ip mg/L	5.00 ± 0.46 ab	4.56 ± 0.22 bc
0.5 2ip mg/L	7.62 ± 0.41 c	5.11 ± 0.12 c
1.5 2ip mg/L	11.87 ± 0.51 e	6.02 ± 0.28 d
5 2ip mg/L	13.87 ± 0.51 d	4.18 ± 0.20 b

*Data that hold the same alphabets are not significantly different, according to the test of Duncan at level (p<0.05)

Table 3. Effect of different concentrations of IBA or NAA on the number and length of roots of *R. lutea* (The data are Mean ± SE).

Con. of (PGRs) mg/L	No. of Roots/ plantlet ± SE	Root length (cm) ± SE	Rooting %	Average no. of roots/plantlet ± SE	Average of root length (cm)(A)
(**MS**) Control	3.06 ± 0.27 a*	0.67 ± 0.25 a	10%	0.612 ± 0.175 a	0.675 ± 0198 a
0.1 IBA mg/L	5.25 ± 0.24 d	1.63 ± 0.07 ab	72%		
0.5 IBA mg/L	6.62 ± 0.41 e	2.33 ± 0.11 bc	87%	2.500 ± 0.212 b	2.140 ± 0.110 b
1.5 IBA mg/L	7.37 ± 0.15 f	2.92 ± 0.15 c	92%		
3 IBA mg/L	4.91 ± 0.19 bc	1.66 ± 0.11 ab	79%		
0.1 NAA mg/L	4.56 ± 0.17 bc	1.81 ± 0.97 b	35%		
0.5 NAA mg/L	6.02 ± 0.26 e	3.08 ± 0.14 c	90%	3.125 ± 0.410 b	2.378 ± 0.214 b
1.5 NAA mg/L	5.11 ± 0.13 d	2.23 ± 0.09 bc	80%		
3 NAA mg/L	4.18 ± 0.19 b	2.37 ± 0.91 bc	85%		

*Data that hold the same alphabets are not significantly different, according to the test of Duncan at level (p<0.05)

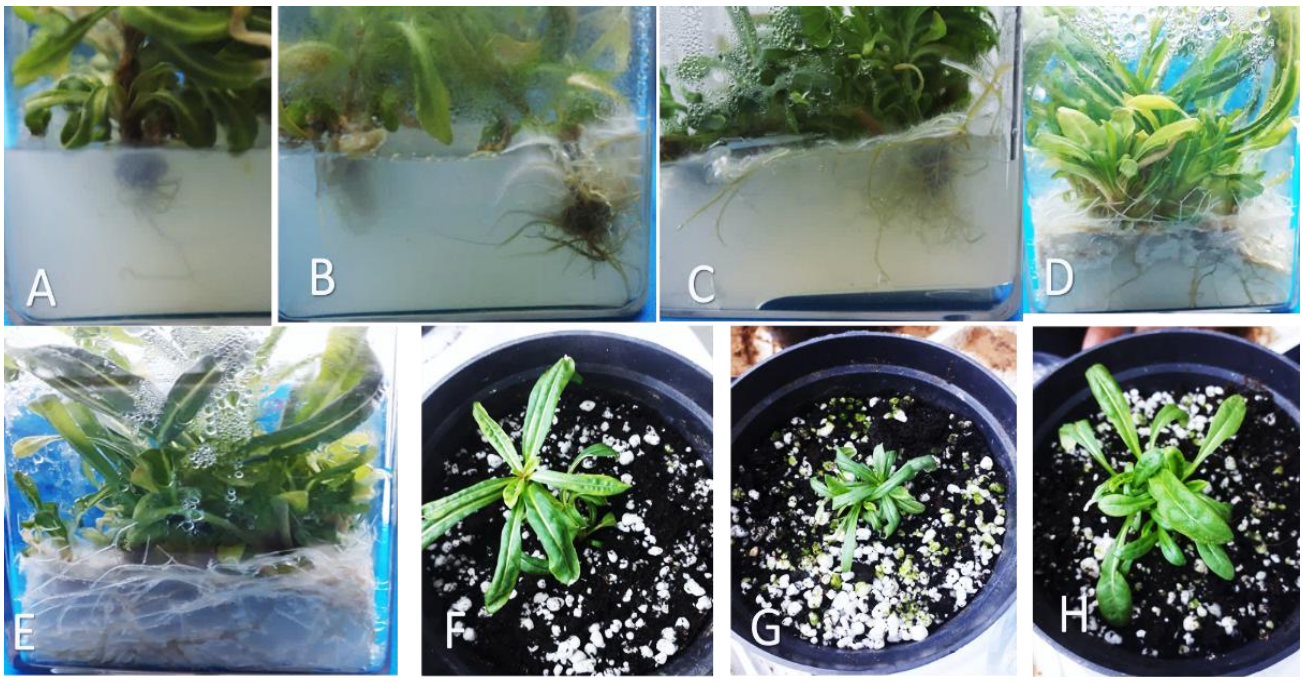

Fig. 2. Rooting and acclimatization of Reseda lutea A and B: Rooted plantlet cultured on MS medium containing 0.1 mg/L of NAA, C: Rooted plantlet cultured on MS medium containing 0.5 mg/L of NAA, D: Rooted plantlet cultured on MS medium containing 1.5 mg/L of IBA, E: Rooted plantlet cultured on MS medium containing 0.5 mg/L of NAA after four weeks, F, G and H: Acclimatized and hardened plant of *Reseda lutea*.

Conclusions

The current study described a successful technique for *R. lutea* shoot regeneration via direct organogenesis that is simple, quick, and adaptable. As a result, the methodology devised could aid in plant conservation and regeneration. It could also be employed in student practical studies, genetic investigations, and the extraction of active ingredients for medications from *In vitro* cultures.

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