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RESEARCH PAPER

Effect of 6-Benzylaminopurine on micropropagation of *Nuphar lutea* as an endangered species

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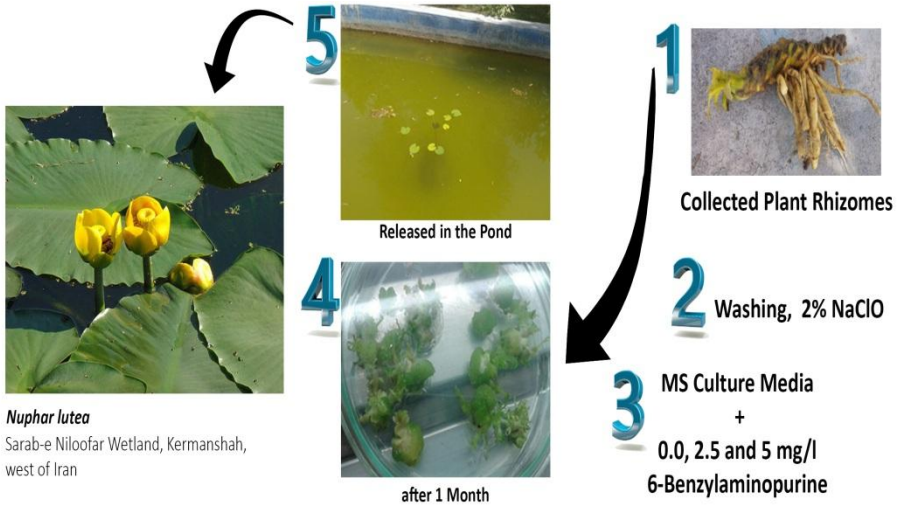
Highlights

- *Nuphar lutea* can be micro-propagated by rhizome.
- In *Nuphar lutea*, 2.5 mg/l BAP was known as the appropriate concentration for the micropropagation.
- It is better to use a plant growth regulator to micro-propagate the *Nuphar lutea* plant.
- *Nuphar lutea* Sarab-e Niloofar Wetland native plant is in danger of extinction and in addition to reproduction, it is necessary to take other necessary measures.

Graphical Abstract



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Abstract

Lotus is one of the most beautiful flowers that due to its sanctity, has a special place among other flowers. Nuphar lutea is one of the most beautiful lotus species that is found in Sarab-e Niloofar Wetland, Kermanshah, west of Iran. Due to the decrease in rainfall, droughts and uncontrolled water withdrawal from the lands around Sarab-e Niloofar Wetland, during the last decade, this wetland completely dried up and all its lotus also disappeared. Due to the above, which shows the very high value of this plant, special attention should be paid to its preservation and survival. After many studies, a plant of N. lutea was found and propagated by tissue culture method in the laboratory of Zagros Bioidea Company and now there are about 200 plants of this species. In this project, first, after finding an N. lutea plant, in the next stage, the collected plant rhizomes were isolated. Then, the rhizomes were washed and disinfected with 2% sodium hypochlorite. Then under the laminar hood was washed five times with sterile distilled water. Then it was cultured on MS culture media with different concentrations (0.0, 2.5 and 5 mg/l) 6-Benzylaminopurine (BAP) in three replications. The cultured rhizomes were then transferred to growth chamber conditions. After about a month, seedlings of cultured rhizomes appeared, which were transferred to pots and the pots were placed in water. After sufficient growth, they were transferred to ponds. Statistical analysis showed that 2.5 mg/l BAP had the greatest effect on the propagation of this plant so that 12 seedlings were produced from each piece of the cultured rhizome. Meanwhile, one seedling was produced in the control and 5 seedlings were produced in the amount of 5 mg/l of BAP. For this reason, 2.5 mg/l of BAP hormone was used to propagate the plant.

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1. Introduction

Among these floral symbols is a symbol that is constantly repeated throughout the art and mythology of the Orient. This flower is a lotus, oriental lily and has been used as a symbol in most Asian countries (Prigioniero et al., 2020). Since this mystery is rooted in religious beliefs and myths, over time, changing customs, beliefs and social conditions not only did not diminish its presence but also continued to appear more strongly and established its strength, firmness and stability (Ivanova et al., 2018). The emergence of the lotus from the primary waters, which is free from any pollution, is a sign of purity and potential force from which the sacred force of life, knowledge and knowledge emerges. The lotus is a symbol of peace and royal gifts to offer to be honored. The first tribes to use flowers as gifts were Iranians, and the lotus flower was used for this purpose. The lotus flower is carved in Persepolis and its reliefs. In the carvings of Kermanshah Taq-e Bostan, the lotus flower related to the Sassanid era can be seen (Roos, 1970; Amiri, 2019). To propagate the lotus, it is possible to plant the seeds, but because it takes four years to plant the lotus seeds until flowering, they are less likely to plant the seeds, unless they want to get a new variety. The simplest and easiest way to propagate this flower is to divide its underground stems in spring (Hongpakdee et al., 2018). But the tissue culture method is not only the fastest method of lotus propagation but also is not limited to the season and can be propagated in any season of the year (Dixit, 2020; Ghorbani et al., 2017; Pourjabar et al., 2019).

In this study, the tissue culture method was used for micropropagation of *N. lutea*. In 2009, El-On et al., studied the anti-leishmaniasis effects of lotus (El-On et al., 2009). In 1994, Lakshmanan investigated the reproduction of a species of lotus in vitro (Lakshmanan, 1994). In 2007, Chomchalow and Chansilpa investigated the possibility of hybridization between lotus species (Chomchalow and Chansilpa, 2007). In 2007, Udom and Tantiwiwat investigated the possibility of tissue culture and reproduction of some lotus species (Udom and Tantiwiwat, 2007). So far in Iran, no research has been reported on the tissue culture of any lotus and this research is the first report. Recent *N. lutea* related issues include genetic diversity, plant extracts, and reproduction (Cires et al., 2020; Lebedeva et al., 2020; Vyšniauskienė et al., 2020; Winer et al., 2020).

Tissue culture and cell culture are important pillars of biotechnology as one of the advanced sciences in the world (Akbari et al., 2019). Knowing that each of the undifferentiated plant cells can become a complete plant, a new window was opened for scientists and researchers in the biological sciences, including medicine, agriculture and pharmacy, that compared to traditional plant breeding methods, there was a considerable acceleration in the implementation of breeding programs and it also made it possible to have intercourse (Ghorbani et al., 2017; Motamedi et al., 2011). In addition to the maintenance of hereditary reserves, the production of virus-free plants and the production of haploid plants are other important applications of tissue culture and plant cells (Kahrizi et al., 2018; Vanaei et al., 2008). Conservation of plant species is very important in any country and the extinction of a species is irreparable damage (Awan et al., 2020; Molsaghi et al., 2014; Saffariha et al., 2021). Therefore, the main purpose of this study is to prevent the extinction *of N. lutea* via micropropagation. The detailed objectives of this study were to investigate the possibility of *N. lutea* in vitro propagation, to investigate the effect of BAP as a shoot induction hormone on *N. lutea* propagation and to study the possibility of adaptation of propagated plants in natural waters. So far, no research or report on the tissue culture of any lotus has been reported in Iran, and this research is the first report.

2. Materials and Methods

In this project, first, after finding an *N. lutea* plant from the Sarab-e Niloofar Wetland area of Kermanshah (34.4046° N, 46.8574° E), in the next stage, the collected plant rhizomes were separated. Then, the rhizomes were washed and disinfected with 2%sodium hypochlorite. Then under the laminar hood was washed five times with sterile distilled water. It was then cultured on MS media that solidified with 7 g/l agar with different amounts of BAP hormone. For this purpose, 0.0, 2.5, and 5.0 mg/l of BAP were used in three replications to determine the best concentration of hormone for the propagation of the *N. lutea* plant. The statistical population in this study includes three treatments with three replications. That is, a total of 9 experimental units were used in this study (Sarker et al., 2021; Sepahvand et al., 2021).

The cultured rhizomes were then transferred to growth chamber conditions. After about two months, seedlings of rhizomes appeared that were transferred to the pot and the pots were placed in water. After sufficient growth, they were transferred to ponds. For tissue culture, the method of Buddhist was used for rhizome cultivation; most of the upper part of the rhizome will be used (Grabovac et al., 2011). Because this area has more buds (Fig. 1).

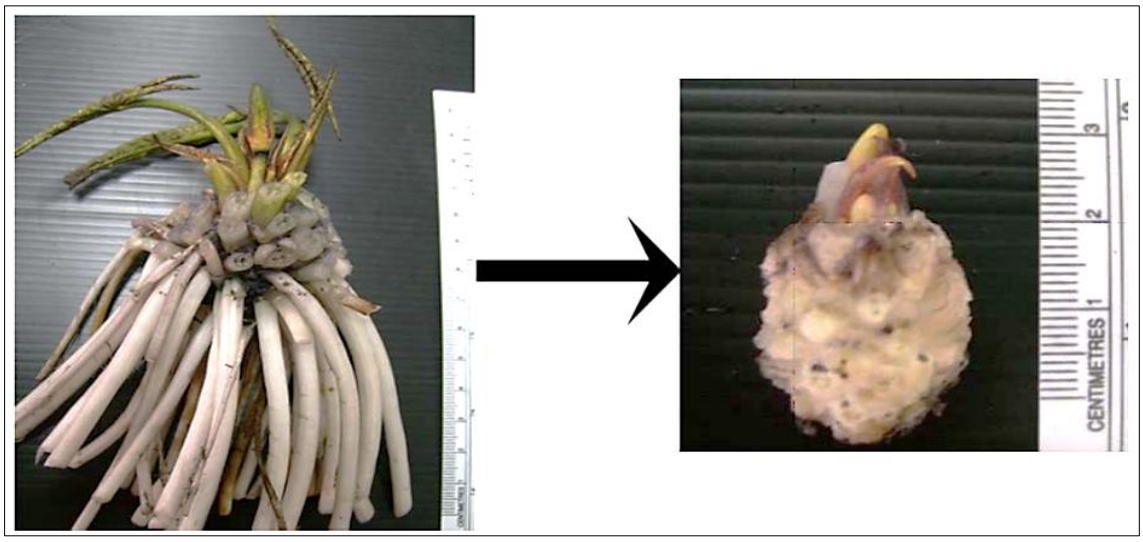

Figure 1. The appearance of a lotus rhizome after removing the leaves and roots attached to it.

The culture medium was prepared in the laboratory and the rhizomes were cultured under a laminar hood under completely sterile conditions. After planting the rhizomes on the culture medium, we kept them in a controlled growth room with 16 hours of light, 8 hours of darkness and a temperature of 25 °C. After growing the plants in the growing room, we transferred them to pots and pots in a bucket of water.

3. Results and Discussion

The results of statistical analysis showed that there was a significant difference between the three concentrations of BAP hormone ($P< 0.05$) (Table 1) and the comparison showed that 2.5 mg/L BAP hormone had the greatest effect on the micropropagation of this plant, so that showed that 12 seedlings were produced from each planted rhizome piece (Table 2). Meanwhile, one seedling was produced in the amount of 0.0 (control) and 5 seedlings in the amount of 5 mg/l BAP hormone. For this reason, 2.5 mg/l BAP hormone was used to propagate the plant.

Table 1. Effect of different concentrations of BAP hormone on *N. lutea* micropropagation.

Source of variation	Df	Mean of squares
BAP	2	90.94*
Error	6	20.00
CV (%)	12	

Where, Df (degree of freedom), CV (coefficient of variations), * (significant: $P< 0.05$).

Table 2. Mean comparison of the effect of different concentrations of BAP hormone on *N. lutea* micropropagation conditions by Duncan method at 5% probability level (with standard error).

BAP concentration	Mean of shoot production
0.0	1 ± 0
2.5	12 ± 3
5.0	5 ± 2

Considering that *N. lutea* species was only specific to Sarab-e Niloofar Wetland, Kermanshah, west of Iran and this wetland also suffered from severe drought for several years, the rapid propagation of this species from the remaining single plant is a very valuable work and maintained the survival of this species. You can see the different stages of micropropagation of this plant in Figs. 2 to 7.

Figure 2. The isolated rhizome from the plant for micropropagation.

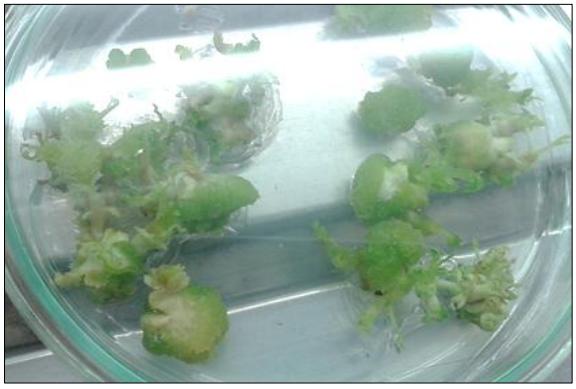

Figure 3. Rhizome fragments that reproduced seedlings on the culture.

Figure 4. A sample of a lotus micro-propagated and ready to be transferred to the water.

Figure 5. Transfer the lotus to a bucket of water.

Figure 6. Lotus released in the pond.

Figure 7. Flowering of *N. lutea*.

So far, no report has been made regarding tissue culture and micropropagation of *N. lutea* in Kermanshah, and this is the first report in the country.

4. Conclusion

Kermanshah *N. lutea* can be propagated under tissue culture conditions and the amount of 2.5 mg/l BAP hormone showed the greatest effect on the propagation of this plant.

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