Int. J. Mendel, Vol. 35(1-2), 2018 & 36(1-2), 2019 125-127 ISSN0970-9649

In Vitro MORPHOGENETIC STUDIES FOR CONSERVATION OF LOTUS : EFFECT OF GROWTH HORMONES ON SEED GERMINATION

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Key words : Morphogenesis, tissue culture, conservation, Lotus, germination, hormones.

At the time of independence of India, there were 24,00,000 ponds or water bodies but the number was reduced to 4,70,000 functional ponds or water bodies in 2000-2001 (Chaturvedi, 2014, Bimal et al. 2014). The disappearance of 20,00,000 ponds or water bodies in just 53 years is an indicator of huge loss of aquatic biodiversity at an alarming rate cannot be redeemed. Keeping this in view and also continuing our efforts to develop technologies for in vitro conservation of aquatic plants in our laboratory, sacred LOTUS (Nelumbo nucifera Gaertn.) was selected which is an important system for establishing protocols for raising plants through tissue culture. The 1/4th MS medium supplemented with NAA (2mg/l) and Kinetin (2mg/l) was found to be the best germination promoting medium for Lotus in terms of shoot length as well as growth and differentiation in our preliminary screening for obtaining explants for tissue culture and micropropagation experiments

INTRODUCTION

Lotus is the sacred flower of India and is one of the most important aquatic plants playing a critical role in wetland, lake and pond ecoystems. The growth of lotus is also affected by water level and its fluctuations (Wang and Zhang, 2005). Recently, Lotus has been included in the list of endangered species in China (Dong and Zheng, 2005), New Jersey and Pennsylvania and threatened in Michigan and Delaware (Sayre, 2004).

It is a perennial water plant and is found throughout India, but is widely found in the Bandhavgarh National park and Eravikuluam National park in India. In a successfully pollinated flower the seeds are embedded in hard receptacle. The hard brown seed about 1 cm (0.4 inches) in diameter survives for long periods of time. In order to establish a reliable and reproducible Lotus plant regeneration system, it is necessary to work out the protocol for rapid germination of seeds. In the present paper effect of growth hormones on germination of Lotus seeds have been described.

MATERIALS AND METHODS :

The experimental plant *Nelumbo nucifera* (Lotus) belonging to family Nymphaeaceae is perennial aquatic plant grown for ornamental as well as great commercial and medicinal value. The plant normally grows upto a height of about 150 cm and a horizontal spread of up to 3 meters. The leaves may be as large as 60 cm and flower 20 cm in diameter. The plants were collected from Zoology Department pond and University residential pond from B.R.A. Bihar University campus, Muzaffarpur in the month of June-August. The seeds possess very hard seed coats so the seeds were properly surface sterilized and mechanically scarified (Fig.1). Two types of seeds were selected for experiment (a) mature seeds with black and hard shell, and (b) mature seeds with green seed coat i.e. fresh seeds.

The scarified seeds were cultured in double distilled sterile water as well as Murashige and Skoog's medium (1962) supplemented with plant growth hormones such as Napthalene acetic acid (NAA), 6-Benzyl aminopurine (BAP) and Kinetin (KN).The nutrient medium was regularly changed. The cultured seeds were transferred to culture room maintained at 25°C ± 2°C with a relative humidity of about 50-60% and under continuous fluorescent light (approximately 100 lux).

RESULTS AND DISCUSSION :

The scarified seeds cultured in sterile water showed first sign of growth as enlargement of scars on the seed surface exposing the folded embryo from 3rd day of culture. The emergent embryo showed further growth and attained a height of about 20 cm with open leaves in 7-8 days of culture. The roots also developed almost simultaneously (Figs. 2-5).

The scarified seeds cultured on MS (1/4th strength) medium supplemented with plant hormones NAA + BAP and NAA + KN showed similar initial morphogenetic responses in terms of swelling of seeds and exposure of embryo. However, in the presence of NAA (1mg/l) and KN (1mg/l) the rate of shoot elongation was rapid and about 8 cm long, curved shoots were observed in 3

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days of culture (Fig.6). A fully grown shining green shoot with coiled stem (Fig.7) and incurved leaves is formed in 4 days of culture. The rate of growth decreased in the third week of seed culture and later the shoot started turning light yellow and finally the shoot decayed. In the presence of ¼MS medium supplemented with NAA (2mg/l) and KN (2mg/l), the Lotus seeds showed strange morphogenetic responses. The scarified seeds swelled in 2 days of soaking in the nutrient medium and broke open on the third day to expose folded embryo. The growing shoot attained the length of 8 cm on 6th day and grew 2 cm more on 7th day of culture. The shoot attained the length of 25 cm in 10-11 days of culture. During shoot development various patterns of curvature, coiling as well as elongation in shoot was observed in the next 2-3 weeks of culture. Besides high degree of coiling, spiralling and curvatures other important observations were recorded during experiment such as swelling in stem, formation of white outgrowths on shoots in large numbers (Fig.8). These white crystalline globular structures were individual entities and were not produced as a continuous structure. Other important structures observed on shoots were tiny dark brown or black heads (Fig.9) which were easily detachable, such structures were not observed in the shoots developing from seeds soaked in water. The scarified seeds cultured on 1/4th MS medium supplemented with NAA (2mg/l) and BAP (5mg/l) showed swelling accompanied with expansion of sutures on seed surface and exposing folded embryo inside in 2-3 days of culture. The growing shoot attained a height of 8 cm in 3-4 days of culture. The growing stem and folded leaves were green, thick, stout and showed highly folded condition (Fig.10). The plants did not grow further and turned yellowish.

N. nucifera is usually propagated vegetatively through rhizome or tuber production but normally with low propagation growth rate. Lotus is also multiplied through seeds, however low seed setting under natural conditions is a major problem (Khatfan et al.,2014). The Lotus seeds are obtained with difficulty for experiments so the present paper reports a highly efficient method for in vitro germination of seeds to produce explants for micropropagation and in vitro conservation.

ACKNOWLEDGEMENTS

The authors thank the Head of the University Department of Botany, B.R.A. Bihar University for facilities. All authors have contributed equally in the paper.

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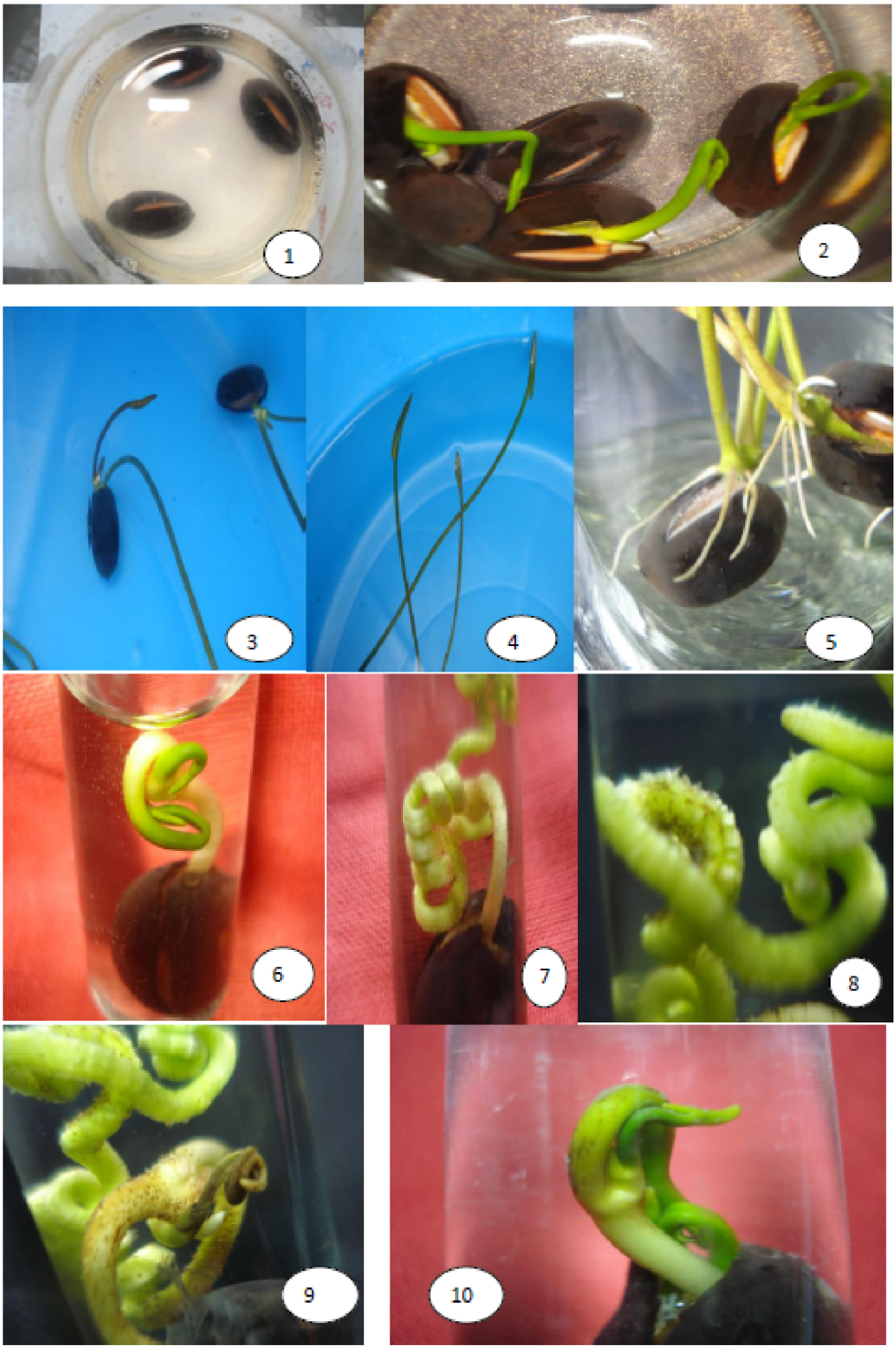

Fig.1 Scarified seeds showing scars made on the surface. Figs.2-5 Different stages of seed development under natural conditions in lab. Figs.6-7 Germination of Lotus seeds cultured on 1/4th strength MS medium supplemented with NAA+KN (1mg/l each). Figs. 8-9 Lotus seeds cultured on 1/4th strength MS medium supplemented with NAA+KN (2mg/l each) showing germination and stages of shoot morphogenesis. Fig. 10 Lotus seed cultured on 1/4th strength MS medium supplemented with NAA (2mg/l)+BAP(5mg/l) showing development of short, stout shoot.