IN VITRO PROPAGATION FROM SEEDS OF NIGELLA SATIVA L. AND RUBIA TINCTORUM L.

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Abstract: The article presents the results *in vitro* propagation of two types of the medicinal plants, such as Black seeds belonging to the genus *Nigella sativa L.*, family *Ranunculaceae* and Dyer's madder belonging to the genus *Rubia tinctorum L.*, family *Rubiaceae* in laboratory conditions. These cultures are used to grow from seeds in a nutrient medium. A nutrient medium with the following composition was prepared for the in vitro cultivation of *Nigella sativa L.* The composition of the nutrient medium was as follows: KNO_3-950 mg/l; NH_4NO_3-412 mg/l; $MgSO_4 \cdot 7H_2O$-185 mg/l; $CaCl_2 \cdot 2H2O$-440 mg/l; KH_2PO_4-68 mg/l; Fe-chelate, trace elements, mesoinositol - $C_6H_{12}O_6$ - 50 mg/l; thiamine - $C_{12}H_{17}N_4OS \cdot HCl$ - 0.2 mg/l; nicotinic acid - $C_6H_5NO_2$ - 0.2 mg/l; pyridoxine - $C_8H_{11}NO_3 \cdot HCl$ - 0.2 mg/l; indolyl acetic acid - $C_{10}H_9NO_2$ - 0.5 mg/g; ferulic acid - 1 mg/l; It consists of sucrose - $C_{12}H_{22}O_{11}$ - 10 g/l, agar-agar - $(C_{12}H_{18}O_9)n$ - 7.5 g/l, and the nutrient medium pH is 5,8.

Key words: Black cumin, *Nigella sativa L.*, Dyer's madder, *Rubia tinctorum L. in vitro*, explants, growth, development.

1. Introduction

The plant world of Uzbekistan is distinguished by its richness in a variety of medicinal trees, shrubs and herbs. Human life is inextricably linked with the plant world, as they are of incomparable importance in human life as a source of healing, construction and technical raw materials. Medicinal plants have been known since ancient times, and people have widely used plants not only as food, but also as a source of biologically active substances. Currently, according to the International Food and Agriculture Organization (FAO) in Uzbekistan, more than 50,000 medicinal plants are used for medicinal purposes in the world. Over the past fifteen years, the global biodiversity crisis has been intensifying in the world. This requires targeted, rapid and decisive measures aimed at the sustainable management and rational use of biological resources, the preservation of individual species and ecosystems. This requires further strengthening the capacity to study and systematically monitor biodiversity at the national and international levels, and improving the functional functioning of natural ecosystems [8].

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. So, many medicinal plants grow in Uzbekistan. For examples of these include *Nigella sativa L.* and *Rubia tinctorum L.* The similarity between these two plants is that they are inhibition and dissolution of phosphate kidney stones [2,3].

Nigella sativa L. is a very important minor seed spice crop. It is an annual and seasonal cross pollinated crop belonging to the family *Ranunculaceae,* commonly known as black cumin in Uzbekistan [3]. *Nigella sativa L.* is a hermaphrodite, erect, annual herb, with a more or less branched stem, pinnately dissected leaves; plant height 35-45 cm with determinate flowering patterns. The flower terminates the main shoot and ends with the flowers on the lowermost branches. The leaves are 2.5-5.0 cm in length, linear to lanceolate in shape; flowers are delicate, usually pale blue and white in colour, 2.0-2.5 cm across, solitary and peduncular. The seed bearing capsule is 1-1.5 cm long. The perianth is differentiated into an outer whorl of five, 15–20

mm long, whitish, petaloid sepals and an inner whorl of eight, 7–8 mm long, nectariferous petals. The flowers are protandrous with 5 to 10 petals and characterized by the presence of nectaries. Seeds are flat, oblong, angular, funnel shaped, size 0.20-0.25 cm long and 0.1-0.15 cm wide, dark black in colour, generally small (1- 5 mg) dark grey or black. The fruit is large and its inflated capsule contains numerous seeds [2,3,6,9]. It is cultivated as a winter crop with flowering and fruiting from January to April (Fig. 1).

A B C D

Figure 1. Morphology of *Nigella sativa L.*: A-plants; B-flowers; C-fruits; D-seeds.

Rubia tinctorum L. can grow up to 30-150 cm in height. The stem quadrangular, angles scabrid, other parts glabrous. The evergreen leaves are approximately 5-10 cm long and 2-3 cm broad, produced in whorls of 4-7 starlike around the central stem. It climbs with tiny hooks at the leaves and stems. The flowers are small, greenish-yellow, clustered in a semi-umbrella that grows from the leaf axils, forming a rosette-like inflorescence. Inflorescence indistinct, corolla 5, fused, funnel-shaped, paternal 5, maternal node 2-lobed, located below. Fruit 1-2 seeds, globose, first red, then black, juicy wet fruit 3.5-4 x 4-5 mm. It blooms in June-August, the fruits ripen in August-September [9]. The roots can be over a metre long, up to 12 mm thick and are the source of red dye (Fig. 2).

A B C D

Figure 2. Morphology of *Rubia tinctorum L.*: A-plants; B-flowers; C-fruits; D-seeds.

In this work was undertaken to develop the complete *in vitro* propagation protocol of *Nigella sativa L.* and *Rubia tinctorum L.*, as they a potential candidate for further pharmacological investigations.

2. Materials and Methods

2.1. Materials

The mature seeds of *Nigella sativa L.* and *Rubia tinctorum L.* which were used as explant source of this study are angular and globose in shape and dark black in color. So, seeds of

Nigella sativa L. and *Rubia tinctorum L.* were collected from the Tashkent Botanical Garden named after Academician F.N. Rusanov Tashkent, Uzbekistan.

2.2. Methods

Seeds were pre-treated with hot water 45 °C, sandpaper, GA3-1.0 mg/L, 10% v/v sulphuric acid - H_2SO_4, and 10% v/v hydrochloric acid - HCl, washed with sterilized water followed by surface sterilization of 3 min with mercuric chloride.After than, in order to grow and propagate seeds of *Nigella sativa L.* and *Rubia tinctorum L. in vitro*, they seeds were first chemically treated and sterilized. For this, the seeds of seeds of *Nigella sativa L.* and *Rubia tinctorum L.* were sterilized with a 75% aqueous solution of ethyl alcohol for 30 seconds, then left for 10 minutes in a 3% solution of sodium chlorate - $NaCl_3O$ and a 0.1% solution of polyoxyethylene sorbent Tween 20. After the treatment process was completed, the prepared seeds were sown in the appropriate nutrient medium. These cultures are used to grow from seeds in a nutrient medium. A nutrient medium with the following composition was prepared for the in vitro cultivation of *Nigella sativa L.* and *Rubia tinctorum L.* The composition of the nutrient medium was as follows: KNO_3-950 mg/l; NH_4NO_3-412 mg/l; $MgSO_4 \cdot 7H_2O$-185 mg/l; $CaCl_2 \cdot 2H2O$-440 mg/l; KH_2PO_4-68 mg/l; Fe-chelate, trace elements, mesoinositol - $C_6H_{12}O_6$ - 50 mg/l; thiamine - $C_{12}H_{17}N_4OS \cdot HCl$ - 0.2 mg/l; nicotinic acid - $C_6H_5NO_2$ - 0.2 mg/l; pyridoxine - $C_8H_{11}NO_3 \cdot HCl$ - 0.2 mg/l; indolyl acetic acid - $C_{10}H_9NO_2$ - 0.5 mg/g; ferulic acid - 1 mg/l; It consists of sucrose - $C_{12}H_{22}O_{11}$ - 10 g/l, agar-agar - $(C_{12}H_{18}O_9)n$ - 7.5 g/l, and the nutrient medium pH is 5,8.

3. Results and Discussions

The advantage of in vitro plant propagation is that genetically identical plants are planted and propagated, and it is possible to multiply a unique plant by several thousand. The transition of a plant from a juvenile - a seed-grown grass or a vegetative bud - to a reproductive stage is accelerated. In addition to accelerating the selection process, work is carried out on updating plant varieties and on large-scale propagation. Since this method is carried out in laboratory conditions, it allows plants to be propagated throughout the year, regardless of the season. The choice of nutrient medium is important when propagating plants in vitro.

Phytohormones were used to grow of *Nigella sativa L.* and *Rubia tinctorum L.* seeds (explants) *in vitro*, for which sterilized seeds were placed in MS (Murashige-Skoog, 1962) nutrient medium. Combinations of this medium containing 1-5 mg/l BAP+0.4 mg/l NAA and MS+2,4-D (1-5 mg/l) were tested. In the experiments, plant explants were placed in glass jars (275 ml) filled with 25 ml of MS nutrient medium, and the cultures were incubated at +20°C, +25°C for 4 weeks under 16 hours of light and 8 hours of darkness. It was found that the highest level of germination was achieved in samples of MS nutrient medium containing 0.5-2 mg/l BAP+0.25-1.5 mg/l NAA. A higher intensity of tumor formation was noted in the combination of MS nutrient medium BAP (0.5-2 mg/l) + NAA (0.1-1 mg/l) + kinetin (0.5-1 mg/l), and GA3 (0.5 mg/l) + adenine sulfate (40 mg/l) (Table 1).

Table 1.

Composition of the nutrient medium for *in vitro* cultivation of *Nigella sativa L.* and *Rubia tinctorum L.* seeds

№	Inorganic compounds	Amount of nutrient medium, mg/l	Organic compounds	Amount of nutrient medium, mg/l
1	KNO_3	950	Mesoinositol - $C_6H_{12}O_6$	50
2	NH_4NO_3	412	Thiamine - $C_{12}H_{17}N_4OS \cdot HCl$	0,2

3	$MgSO_4 \cdot 7H_2O$	185	Nicotinic acid - $C_6H_5NO_2$	0,2
4	$CaCl_2 \cdot 2H_2O$	440	Pyridoxine - $C_8H_{11}NO_3 \cdot HCl$	0,2
5	KH_2PO_4	68	Indole-3-acetic acid - $C_{10}H_9NO_2$	0,5
6	$MnSO_4 \cdot H_2O$	16,7	Ferulic acid	1
7	$ZnSO_4 \cdot 7H_2O$	8,7	Sucrose - $C_{12}H_{22}O_{11}$	10
8	H_3BO_3	6,2	Agar-agar - $(C_{12}H_{18}O_9)n$	7,5
9	$Na_2MoO_4.H_2O$	0,24	IBA	0,5-2
10	$CuSO_4 \cdot 5H_2O$	0,025	NAA	0,1-1
11	$CoCl_2.6H_2O$	0,025	Kinetin	0,5-1
12	KI	0,81	Adenine sulfate	40
	pH		5,8	

In plants, Mesoinositol has roles in the growth and development of plants, including cell wall biosynthesis, stress response, phosphate storage, Osmo tolerance, cell to cell communication, and storage and transport of plant growth regulators (PGRs) or plant hormones [7].

In plants, Thiamine is known to have its role as a cofactor for important metabolic activities. Thiamine is known to be an essential regulator that plays an important role in plant's primary regulatory system. Living organisms require the active form of thiamine which is known as thiamine pyrophosphate (TPP) in order to play the role as an important cofactor. TPP is a crucial component required in many metabolic activities such as Acetyl-CoA biosynthesis, amino acid biosynthesis, Krebs cycle and Calvin cycle [10].

Nicotinic acid represents an important connecting link between primary and secondary metabolism, because it is a basic element of the coenzymes NAD and NADP and on the other hand it leads into alkaloid metabolism. The ability of nicotinic acid to form conjugates is known for many plant species. In case of heterotrophic cell suspension cultures the rapid alternative conversion of exogenously applied nicotinic acid to either the N-methyl (trigonelline) or the N-glucosyl conjugate has been observed [4].

Vitamin B6 is a collective term for a group of six interconvertible compounds: pyridoxine, pyridoxal, pyridoxamine and their phosphorylated derivatives. Vitamin B6 plays essential roles as a cofactor in a range of biochemical reactions. In addition, vitamin B6 is able to quench reactive oxygen species in vitro, and exogenously applied vitamin B6 protects plant cells against cell death induced by singlet oxygen (1O2). These results raise the important question as to whether plants employ vitamin B6 as an antioxidant to protect themselves against reactive oxygen species [5].

Indole-3-Acetic Acid (IAA) is a natural auxin, a plant growth regulator or plant hormone that is required for the proper growth and development of plants. IAA promotes cell enlargement and division, tissue differentiation, and response to light and gravity. Stimulate root initiation and development. Helps establish apical dominance, plays a role in the differentiation of vascular tissues: xylem and phloem [7].

Agar to provide a solid medium for the growth and regeneration of plant cells, tissues, and organs. It is frequently employed for techniques like micro propagation and somatic embryogenesis [2,3].

Studies have shown that the intensity of root formation in ½ MS medium is relatively high in the combination of NAA (0.5-3 mg/l) + IBA (0.5-3 mg/l), as well as NAA (0.5-3 mg/l) + IBA

(0.5-3 mg/l) + activated carbon (3 g/l). *In vitro* propagation of *Nigella sativa L.* shown in Figure 3.

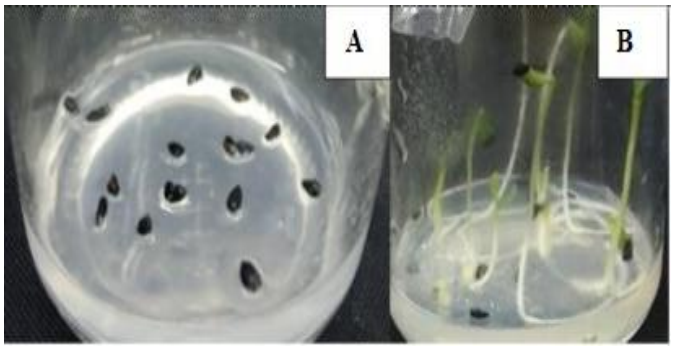

Figure 3. *In vitro* propagation of *Nigella sativa L.*:
A - root formation,
B - stem formation.

In vitro propagation of *Rubia tinctorum L.* shown in Figure 4.

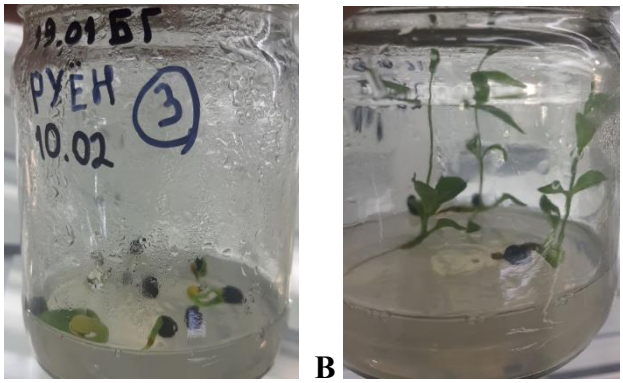

Figure 4. *In vitro* propagation of *Rubia tinctorum L.*:
A - root formation,
B - stem formation.

4. Conclusion

The current investigation has resulted in development of an efficient single-step protocol for *in vitro* propagation of *Nigella sativa L.* and *Rubia tinctorum L.* from seeds. Methods for in vitro propagation of *Nigella sativa L.* and *Rubia tinctorum L.* were developed. It was found that the optimum level of rooting of plant explants was achieved in the combination of 1 mg/l BAP + 1 mg/l NAA in the nutrient medium MS. The combination of BAP (2 mg/l) + NAA (0.1 mg/l) + GA3 (0.5 mg/l) showed a high intensity of root formation.

The quantification of secondary products is considered urgent to elucidate the exact amount of these metabolites in *in vitro* cultures compared with the intact plant, in order to produce them on a large scale, industrially. Therefore, optimizing the culture conditions via biotechnological techniques is needed in the future to support the industrial production of the most valuable secondary products of *Nigella sativa L.* and *Rubia tinctorum L.* through cultures.

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