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Axillary shoot proliferation from aseptic seedlings of *Nepeta nuda* Subsp. *albiflora*

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Abstract

The objective of the present work was to develop an alternative propagation procedure with *in vitro* germination and axillary shoot propagation for *Nepeta nuda* subsp. *albiflora* (Boiss.) Gams (*Lamiaceae*) has potential importance as medicinal plant. The seeds were surface sterilized and transferred to different *in vitro* media. At the end of the experiments, maximum germination percentage was obtained 85% in distilled water medium solidified with agar. Germinated seeds were transferred onto Murashige and Skoog and White media for further seedling development. MS medium was found to be a superior medium than White medium as seedling growth medium. The obtained shoots were transferred onto MS media containing Benzyladenine, Kinetin and Thidiazuron (0.1, 0.5 and 1 mgL⁻¹) for axillary shoot propagation. MS medium without plant regulator was used as a control. The highest shoot number per explant and maximum shoot length were obtained on MS medium supplemented with 0.1 mgL⁻¹Kin.

Keywords: *Nepeta nuda* subsp. *albiflora*, *in vitro*, germination, axillary shoots

Introduction

Biotechnological methods are widely used to obtain the desired compounds from plants. Plant tissue culture techniques, one of the biotechnological methods has been developed and reported for medicinal plants [1]. This methods enable biologically active compounds to be produced on a large scale and permit genetic manipulation of the desired compounds [2]. In addition, these methods reduce the pressure on natural populations and problems such as limitations due to disease and/or seasonal variation [3].

Seeds are favored as starting material in *in vitro* plant tissue culture studies [4]. In this way *in vitro* germinated seeds allow the production of a great number of sterile plants. Obtained seedlings can be manipulated to increase secondary metabolites and the quality and quantity of medically important compounds can be changed by applying stress conditions. In addition, with axillary shoot propagation, a large number of plants can be obtained in a short time and the pressure on the natural populations can be reduced.

Nepeta L. (*Lamiaceae*) genus has a single or perennial herbaceous plant that has spread throughout Asia, Europe and North Africa. *Nepeta* species on Earth 250 [5], represented by strain 33 in Turkey [6]. Some of the medicinal properties of *Nepeta* species occurring in the World and Turkey are well known [7] and antispasmodic, expectorant, diuretic, antiseptic, and anti-asthmatics are widely used among people as antitussives [8, 9, 10].

The pharmacological properties and various biological activities of *Nepeta* species are attributed to nepetalactone compounds found in etheric oils [7]. Etheric oils and plant extracts of plant species have great potential in medical procedures, pharmaceutical applications, food and cosmetic industries.

N. nuda subsp. *albiflora* (Boiss.) Gams nepetalactones and other components were reported by Kökdil et al. [11]. In the other study, the essential oil of *Nepeta nuda* subsp. *albiflora* was prepared by hydrodistillation and analyzed by GC/MS. The important components were nerobiol, spathulenol and 1,8-cineole [12]. An other study, essential oil of *N nuda* subsp. *albiflora* showed antibacterial activity against *Klebsiella pneumoniae* and *Salmonella typhi* [13]. However, no studies have yet been undertaken to replicate *in vitro* conditions and promote the biologically active components.

In this study, it was aimed to germinate *in vitro* conditions and to establish an alternative propagation procedure with axillary shoot proliferation of *Nepeta nuda* subsp. *albiflora* (Boiss.) Gams plant. Thus, tissue culture conditions will have made the first step in promoting the secondary metabolites.

Material and Methods

In our experiments, *Nepeta nuda* subsp. *albiflora* seeds were used as starting material. Seed sterilization was carried out by the method recommended by Kurt and Erdağ [14]. The sterilized seeds were cultured in 190 cc glass jars containing 50 mL of nutrient medium. Three nutrient media were evaluated for seed germination: Distilled water (DW), Murashige and Skoog (MS) [15] and White (WH) [16] media. Radicle emergences was the main criteria to evaluate seeds as “germinated”. Results were evaluated after 6 weeks of incubation.

All the germinated seeds were transferred onto MS and WH media under aseptic conditions for further seedling development. Four-week-old seedlings developed on MS medium were separated from primary roots and transferred onto MS basal medium supplemented with cytokinins (0.1, 0.5 and 1 mgL⁻¹ BA, KIN and TDZ) for axillary shoot propagation. All the media were adjusted to pH 5.8 and solidified with agar. The cultures were incubated in a growth chamber at 24 ± 2° C, under 16/8 -h photoperiod. At the end

of the experiments the number of shoots per explant and shoot length were evaluated for each cytokinin type and concentration. The experiments were carried out least 2 times using a completely randomized design. The data on the axillary shoot propagation were subjected to ANOVA and means were compared using Duncan’s Multiple Range Test at the 5% level of probability.

Results and Discussion

Surface sterilized seeds of *N. nuda* subsp. *albiflora* incubated on different nutrient media showed a varied germination percentages (Figure 1). Seed germination percentage of 85% were obtained on the DW media (Figure 2) and 25% on the MS media. The lowest rate was in the WH media (5%). MS and WH media have high salt content compared with DW medium. Generally media high in salt content reduced germination [17]. Water is a basic requirement for germination process. Mineral requirement during the seed germination is species-specific [18]. *N. nuda* subsp. *albiflora* seeds do not need nutrient to germinate.

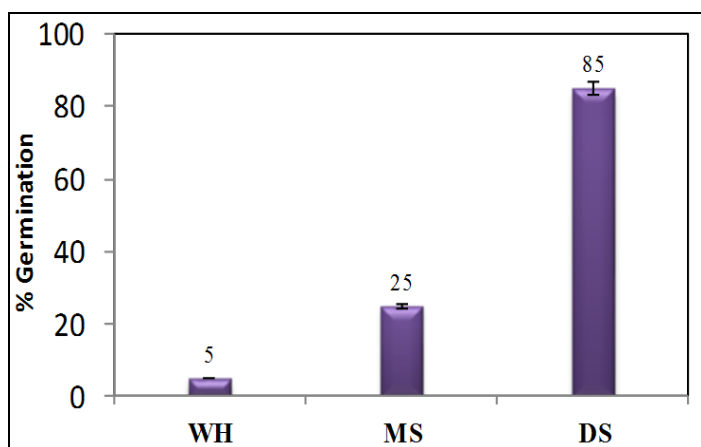


Fig 1: % germination in different nutrient media



Fig 2: Germinated seed on DW medium with solidified agar.

All the germinated seeds were transferred onto MS and WH media. MS medium revealed as the optimum medium for seedling development (considering the morphological criteria such as average number of leaves per seedling, average shoot length, average number of shoots per seedling, root length and

average number of roots per seedling; Figure 3 and 4). Media composition have a key role in *in vitro* plant morphogenesis. MS was the most commonly used basal medium for *in vitro* culture studies.

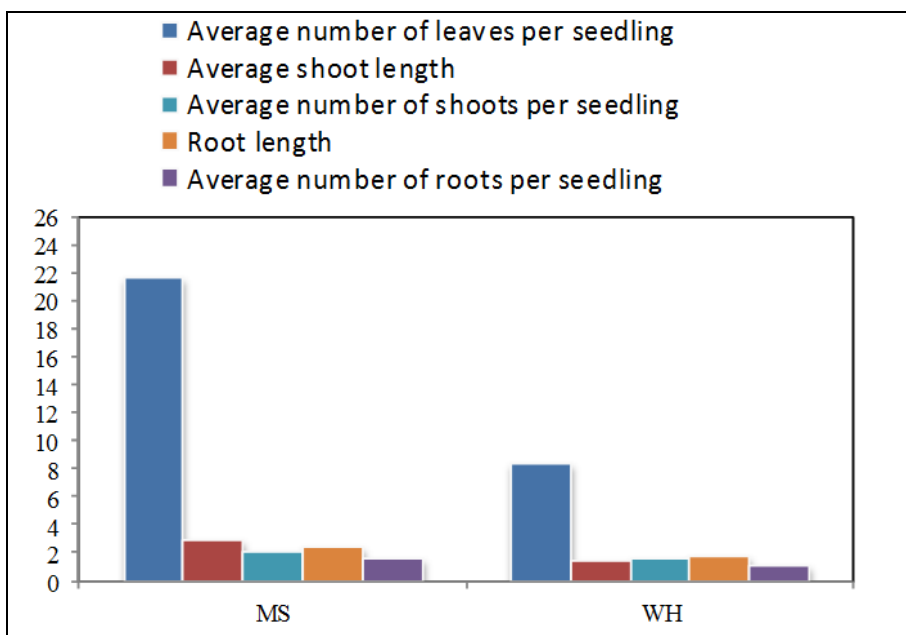


Fig 3: Seedling development on different media



Fig 4: Developed seedling on MS medium

In axillary shoot propagation experiments, the shoots obtained in the media supplemented with TDZ were hyperhydric, stunted and looked abnormal. For this reason, this cytokinin was not evaluated for axillary shoot experiment.

BA and KIN induced healthy shoots. In many studies were reported that amount of excess cytokinin concentration can induce the proliferation of shoots in *in vitro* cultured plants [19, 20, 21, 22]. In this study, a negative relationship was observed between the increasing concentration of the cytokinins (BAP or KIN). KIN was found to be a superior cytokinin than BA.

The maximum shoot number and highest shoot length per explant were recorded with MS media supplemented with KIN at optimal level of 0.1 mgL^{-1} (Figure 5, Table 1). This was followed by media containing 0.5 mgL^{-1} KIN. The highest number of shoots in BA-supplemented media was at a concentration of 0.1 mg L^{-1} .

In this paper, we reported on the germination and axillary shoot proliferation of *Nepeta nuda* subsp. *albiflora*. Thus, tissue culture conditions will have made the first step in promoting the secondary metabolites.

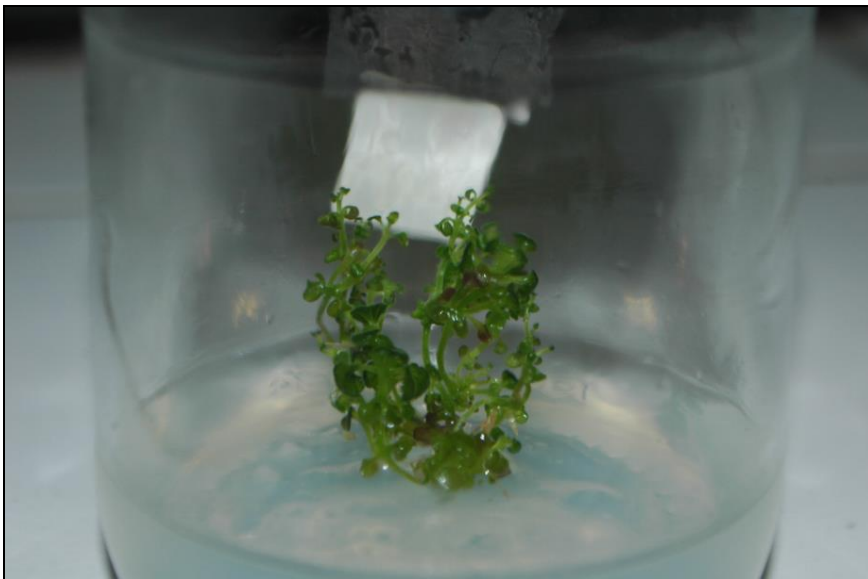


Fig 5: Axillary shoots on MS medium supplemented with 0.1 mgL^{-1} KIN

Table 1: Effect of cytokinins on axillary shoot number and shoot length of *N. nuda* subsp. *Albiflora*

BA (mgL^{-1})	KIN (mgL^{-1})	Number of shoots Mean \pm S.E	Shoot length (cm) Mean \pm S.E
-	-	2.00 ± 0.19 de	2.85 ± 0.19 ab
0.1	-	3.30 ± 0.38 bc	1.90 ± 0.15 c
0.5	-	2.20 ± 0.31 de	1.58 ± 0.14 cd
1	-	1.70 ± 0.22 e	0.80 ± 0.16 e
-	0.1	5.80 ± 0.42 a	3.20 ± 0.19 a
-	0.5	3.90 ± 0.24 b	2.60 ± 0.28 b
-	1	2.80 ± 0.19 cd	1.20 ± 0.21 de

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