International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2018; 6(6): 106-109
Received: 11-09-2018
Accepted: 15-10-2018

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In vıtro propagatıon of *Nepeta nuda* Subsp. *Lydıae* Ph Davıs

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Abstract
Due to increased interest in biologically active ingredients, the use of plant sources is increasing day by day. Unconscious harvesting of plants from their natural habitat increases the oppression on endemic plants and biotechnological methods are used to prevent them. The objective of the present work was to develop a simple micropropagation system for *Nepeta nuda* L. subsp. *lydiae* PH Davis an endemic plant, thus allowing its exploitation without threat to local biodiversity.
Seeds were surface-sterilized and incubated on different *in vitro* media for germination. Paper bridges and glass beads were used to provide physical support for the seeds. The cultures were maintained at 24 ± 2 º C temperature with a 16-h light period. The results of the experiments have shown that the maximum germination percentage was observed in distilled water (60%). In axillary shoot propagation experiments, the maximum shoot number per explant was obtained on MS medium supplemented with 0.1 mgL^{-1} Kinetin (5.30 shoot/explant). The highest shoot lenght was obtained on MS medium supplemented with 1 mgL^{-1} N^6-Benzyladenine (7.40). The maximum rooting percentage was obtained on MS medium supplemented with 1 mgL^{-1} IBA.

Keywords: *Nepeta nuda* subsp. *lydiae*, endemic, *in vitro*, germination, micropropagation

1. Introduction
Genus *Nepeta* L. *(Lamiaceae*) contains more than 250 species spread out in Europe, Asia and North Africa China, India, North and Central America [1, 2, 3]. There are 33 species (38 taxa) in the genus *Nepeta* were recorded in Flora of Turkey [4]. As a result of taxonomic studies made after the writing 7 th volume of Flora of Turkey, the number of species increased to 37 (44 taxa) [5, 6]. The endemic *Nepeta nuda* subsp. *lydiae* PH Davis is one of the four subspecies of *Nepeta nuda* L.
Nepeta species have expectorant, antispasmodic, diuretic, antiasthmatic, antiseptic, febrifuge, and antitussive activities are widely used in folk medicine [7, 8]. Phenolic compounds of *Nepeta* nuda subsp. *lydiae* leaves were identified by LC- MS/MS analysis [9]. Rosmarinic acid, chlorogenic acid and quinic acid were found in the methanol extract of leaves. In addition, lesser quantity of p-coumaric acid, kaempferol, apigenin, tr-caffeic acid, rhamnetin and luteolin were identified. Leaves of the species have great potential of phenolic ingredients that mainly based upon with biological activities were recorded.
Due to the mentioned properties the *Nepeta* species are collected uncontrollably from the nature. *In vitro* propagation techniques have been used as an alternative way to propagate of rare and endemic plant species. *In vitro* propagation studies of *Nepeta nuda* subsp. *nuda* L. [10, 11] and *N. nuda* subsp. *albiflora* (Boiss.) Gams [12] have also been reported. However, no *in vitro* tissue culture studies on *Nepeta nuda* subsp. *lydiae* were performed up to date.
The objective of the present work was to develop a simple micropropagation protocol for *Nepeta nuda* L. subsp. *lydiae* Davis an endemic plant, thus allowing its exploitation without threat to local biodiversity.

2. Materıal and Methods
In our experiments, *Nepeta nuda* subsp. *lydiae* seeds were used as starting material. Seed sterilization was carried out by the method recommended by Kurt and Erdağ [13]. Seeds sterilized were transferred to glass jars with 50 mL of culture media (Distilled water (DW), Murashige and Skoog [14] (MS) and ½ MS). All media were liquid. Because of using liquid medium, paper bridges and glass beads were used to provide physical support to the seeds.
The seeds were evaulated germinated when radicula emergenced. Germination were evaulated in percentage 6 weeks after culture initation. Plantlets obtained from germination of seeds were transferred onto solidified MS medium (with 0.8% agar-agar, Sigma) for seedling development.

After 4 week, seedlings were separated from primary roots and transferred onto MS medium without and with cytokinins. Effect of different concentrations (0.1, 0.5 and 1 mgL^{-1}) of N^6-Benzyladenine (BA) or Kinetin (KIN) were tested.

Axillary shoots were transferred onto MS medium without and with various concentrations (0.5, 1.0 and 2.0) of α-naphthalene acetic acid (NAA) and indole-33-butyric acid (IBA) for rooting.

All cultures were maintained at 24 ± 2 ºC and 16-h light period provided by cool white florescent lamps at 40 µE m^{-2} s^{-1}.,The experimental design were randomized with two replicates with twenty explant.

The axillary proliferation finding were statistically analysed by using statistical package SPSS version 16.0 in which data subjected to ANOVA. The means were compared using Duncan's Multiple Range Test ($p \leq 0.05$) and reported as ± Standart Error. The germination and rooting experiments were evaluated as percentage.

3. Result and Dıscussıon

Sterilisation procedure was convenient. 100% sterile cultures were obtained by the applied sterilization procedure. No contamination was encountered during experiments. The maximum germination percentage (60%) was observed on distilled water media (Figure 1).

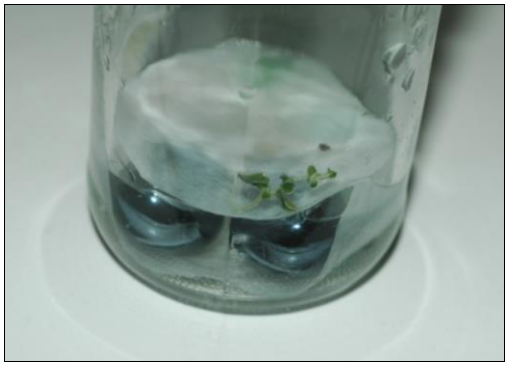

Fig 1: *In vitro* germinated seed on Distilled water media.

Second the best germination percentage (48%) was obtained on ½ MS media. MS media showed the lowest germination response (36%) (Figure 2). Every species has specific requirements for germination. MS medium is widely used in *in vitro* plant tissue culture studies. However, it is normal to inhibit germination due to high salt content. Therefore, germination in distilled water is widely used in endemic plant germination experiments [12, 15, 16].

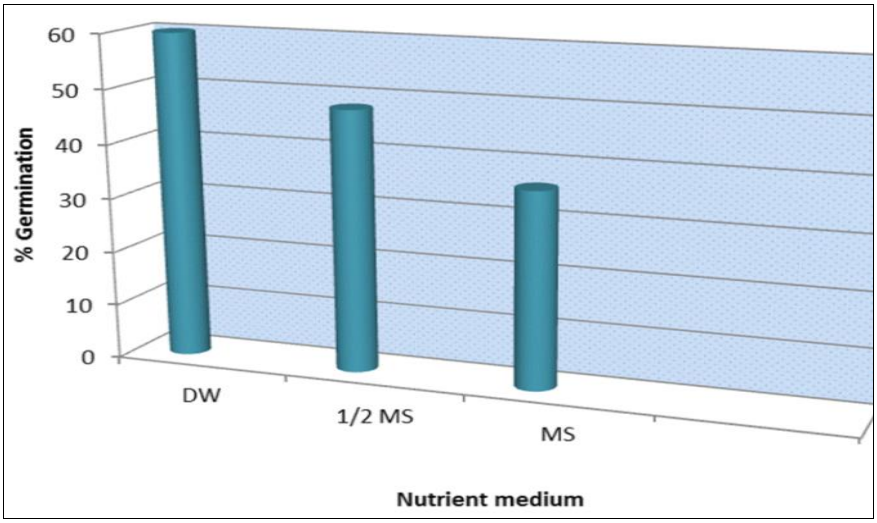

Fig 2: Germination percentages on different nutrient media

Seedlings which were obtained from germinated seeds were transferred onto MS medium for seedling development. After six weeks incubation, developed seedlings in this media were transferred onto MS media supplemented with different cytokinin type and concentration for axillary shoot propagation. Axillary shoot propagation was performed in all media including control medium without plant growth regulator. Number of axillary shoots per explants ranged 1.80 to 5.30. The best results in terms of the number of axillary shoots per explant was observed on MS medium supplemented with 0.1 mgL^{-1} KIN (Table 1, Figure 3).

Table 1: Effect of KIN and BA on axillary shoot proliferation and shoot lenght

BA (mgL^{-1})	KIN (mgL^{-1})	Number of shoots/Explant Mean ± S.E	Shoot length (cm) Mean ± S.E
-	-	2.50 ± 0.23 cd	2.60 ± 0.21 c
0.1	-	3.20 ± 0.35 bc	3.00 ± 0.33 c
0.5	-	2.75 ± 0.45 bcd	5.20 ± 0.61 b
1	-	1.80 ± 0.22 d	7.40 ± 0.48 a
-	0.1	5.30 ± 0.53 a	5.00 ± 0.63 b
-	0.5	3.60 ± 0.26 b	5.80 ± 0.38 b
-	1	2.40 ± 0.21 cd	6.20 ± 0.74 ab

The means with different letter(s) are significantly different at the 0.05 probability level using Duncan's multiple range test, ±Standart Error

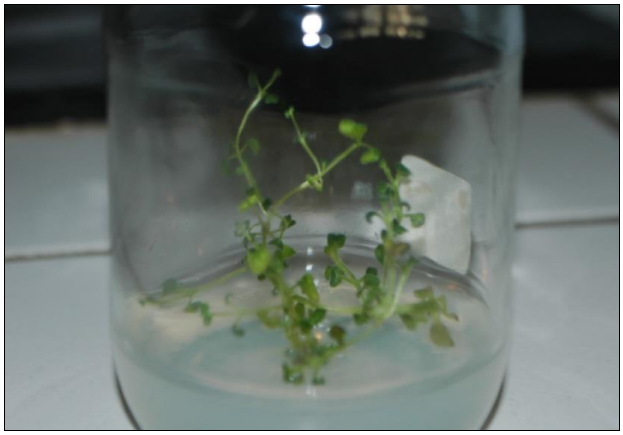

Fig 3: Axillary shoots proliferated onto MS medium supplemented with 0.1 mgL^{-1} KIN

With the increase of cytokinin concentrations, the number of axillary shoots decreased. The lowest number of axillary shoots was obtained MS medium supplemented with 1 mgL^{-1} BA. Nedelkova *et al*. were reported that 0.8 mgL^{-1} BA was the most effective plant growth regulator concentration on axillary shoot proliferation for *Nepeta nuda* subsp. *nuda* [10]. This situation was the exact opposite of our results. Our results show parallelism with results on axillary shoot number of *Nepeta nuda* subsp. *albiflora* [12]. In this study, researchers were reported that the maximum shoot number and shoot lenght obtained on the MS media supplemented with 0.1 mgL^{-1} KIN. In our study, a negative connection between the number of axillary shoots and the axillary shoot length was recorded. The highest shoot lenght was obtained on MS medium supplemented with 1 mgL^{-1} N^6-Benzyladenine (7.40). Increased cytokinine concentration was also increased in shoot length.

In rooting experiments IBA is more effective than NAA. The maximum rooting percentage (60 %) were obtained on MS medium with 1 mgL^{-1} IBA (Figure 4). There was no response on MS without auxin. IBA is an effective plant growth regulator of rooting of *Lamiaceae* members [16, 17, 18, 19].

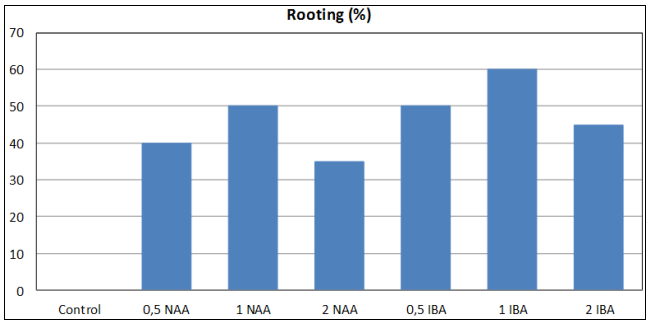

Fig 4: Effect of auxins on rooting of *Nepeta nuda subsp. lydiae*

4. Conclusion

In recent years, biotechnological methods have been widely used to obtain the desired compounds from plants. The *in vitro* micropropagation methods of a great number of plants showing important biological activity has been developed. Aseptic seeds are favourited as starting material for *in vitro* culture studies. *In vitro* germinated seeds allow the production of numerous steril plants to be inoculated in *in vitro* tissue culture experiments. However, in order to obtain seed-derived sterile seedlings, an efficient germination protocol needs to be determined. The sterile seedlings thus obtained can be manipulated to increase secondary metabolites and the quality and quantity of medically important compounds can be changed by applying stress conditions. 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.

5. Acknowledgement

We would like to give special thanks to Assoc. Prof. Dr. Tuncay Dirmenci for providing the seed material of this study. Abstract part of the study was presented as poster presentation at the 1st International Health Science and Life Congress, 2-5 May 2018, Burdur, Turkey.

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