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Optimization of Regeneration Conditions and *In Vitro* Propagation of *Sideritis Stricta Boiss & Heldr*

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ABSTRACT

In this study the micropropagation of endemic species *Sideritis stricta* was investigated. Leaf segments and shoot explants (hypocotyl, single node and shoot tips) taken from *in vitro* growing plantlets and cultured on MS and B5 media containing different growth regulators combinations BAP (0.0, 1.0, 2.0 and 3.0 mg/l) and NAA (0.0, 0.1 and 0.5 mg/l). MS and B5 media supplemented with BAP (1.0, 2.0 and 3.0 mg/l) and NAA (0.1 mg/l) combinations or only BAP and kinetin (2.0 and 3.0 mg/l) were used at the subculture experiments of shoots and MS and B5 media supplemented with different concentrations of IBA (0.0, 1.5, 3.0, 4.5 and 10.mg/l) were used at the rooting experiments. *S. stricta* seeds germinated at the rate of 100% when the seed coat was removed and endoperm with embryo part cultured on B5 medium. The single node explants taken from *in vitro* germinated and grown 30–40 days plantlets on B5 medium have been determined as the most successful explant at all used hormone combinations. B5 medium supplemented with 1.0 mg/l BAP + 0.1 mg/l NAA and 2.0 mg/l BAP + 0.5 mg/l NAA was determined as the most effective medium on shoot formation. At the first and second subculture, the highest shoot formation was maintained on medium supplemented with 1.0 mg/l BAP + 0.1 mg/l NAA and the number of shoots per explant were 4 and 2.11, respectively. The highest multiplication rate has been determined as 33.76 at the end of second subculture. The best rooting was achieved on B5 medium supplemented with 4.5 mg/l IBA. The rooted shoots were successfully acclimatized to outdoor conditions and survival rate was determined as 90%.

1. Introduction

Plants belonging to genus *Sideritis* L. (Lamiaceae) are represented by 54 in Turkey and 40 of them are endemic [1]. *Sideritis stricta* is an endemic medicinal herb of Turkey, endangered species (LR (cd) (Lower Risk, Conservation Dependent)) which grows in a limited area of the Mediterranean coasts and is exploited commercially. Endemic plants greatly contribute to the richness and diversity of the flora of Turkey [1,2]. Bajaj et al. [3] have been pointed out the growing interest worldwide in medicinal and aromatic plants. The satisfaction of the necessities can be achieved purely from wild resources therefore what is needed is the mapping out of an appropriate policy that will be based on the proper management of our natural resources [4]. Conservation of endemic, endangered [2], medicinal and aromatic plants is beyond regional scope and becomes of global significance. They should be protected by different methods including *in vitro* culture. *In vitro* propagation is a suitable method for plant regeneration, micropropagation and long-term storage of plant material. *In vitro* culture of these plants will prevent the destruction of the flora. There is a few information about *in vitro* culture of *Sideritis* species in the current literature [5–11]. Some species of these plants are well known in folk medicine and widely used as spices and herbal tea [12,13]. They are of great economic importance which is not only related to their use as a spice. In fact, as recent studies have pointed out, *Sideritis* is used traditionally in many other ways as their essential oils have antimicrobial, antifungicidal, cytotoxic, antiviral, nematicidal and antioxidant properties [14–16]. The chemical components found in *Sideritis* genus include terpenes, flavonoids, essential oil, iridoids, coumarins, lignanes and sterols, among others. Diterpenes, flavonoids and essential oil occur in almost every species and are the main compounds responsible for the observed *in vivo* and *in vitro* pharmacological activities. *In vitro* culture of the species as an approach for conservation of the natural habitats and provision of herb with high and permanent quality has also been important in order to increase the accumulation of biologically active compounds and to obtain herb with permanent and good quality [14,15]. This work attempts to develop optimal

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Fig. 1. *S. stricta* from a natural habitat.

protocol for *in vitro* propagation; *in vitro* clonal multiplication, rooting and acclimatization of this endemic species. *In vitro* propagation can ensure the availability of plant material throughout the year.

2. Materials and methods

Seeds of *S. stricta* were collected from a natural habitat in, C3 Antalya Saklıkent (Fig. 1). Seeds were sterilized by immersion for 25 min in 10% sodium hypochlorite (NaOCl), then rinsed three times with sterile water. MS [17] and B5 [18] hormone-free media were used for seed germination *in vitro*. Seeds were germinated in a growth chamber at $25 \pm 2\,^{\circ}C$, continuously dark conditions, during 21 days. At the end of this period seedlings were incubated for 30–40 days under a 16-h light/ 8-h dark photoperiod (40 $\mu mol/m^2/s/n$ light intensity) in the same media and conditions. Hypocotyl, shoot tips, leaf segments and single node explants (Fig. 2) excised from these seedlings were cultured on shoot formation-multiplication media; MS and B5 media supplemented with 6-benzyl amino purine (BAP) (0.0, 1.0, 2.0 or 3.0 mg/l) and naphthalene acetic acid (NAA) (0.0, 0.1 or 0.5 mg/l) combinations, 3% sucrose and 0.6% agar. Incubation conditions were the same as indicated for seedling development.

The multiplication rate, that is, the multiplication of average number of shoots after 4 weeks in culture, was determined for 40 explants per medium through 2 subcultures (Table 1). Elongated shoots excised from *S. stricta* multiple shoots cultures were rooted on B5 medium supplemented with auxin; IBA (0.0, 1.5, 3.0 and 4.5 mg/l). Results were recorded for 100 explants per medium after 4 weeks in culture. Plants were acclimatized in plastic containers filled with sterile soil + perlite (3:2). Relatively high numbers of explants were (40 per shoot multiplication medium and 100 per rooting medium) were statistically analyzed. The experiments were set up in a completely randomized design. Data were analyzed by analysis of variance (ANOVA) to detect significant differences between means. Means differing significantly were compared using Duncan's multiple range test (DMRT) at the 1% probability level. Mean values, standard deviations and Duncan's test were used for analysis and interpretation of the data. Shoot multiplication of *S. stricta* on B5 media containing different concentrations of macroelements and plant growth regulators was statistically analyzed globally by merging the results from all two subcultures, and also separately by comparing the results from the second subculture (Table 2).

3. Results

3.1. Initial culture and multiplication rate

Seeds cultured on B5 hormone-free medium were 100% germinated. The influence of different BAP and NAA combinations of B5 medium and various explant types on shoot differentiation of *S. stricta* are given in Fig. 3a–c. Media containing B5 macro and microelements were used for all stages of *S. stricta* culture. Single node explants are the best source for the highest shoot induction

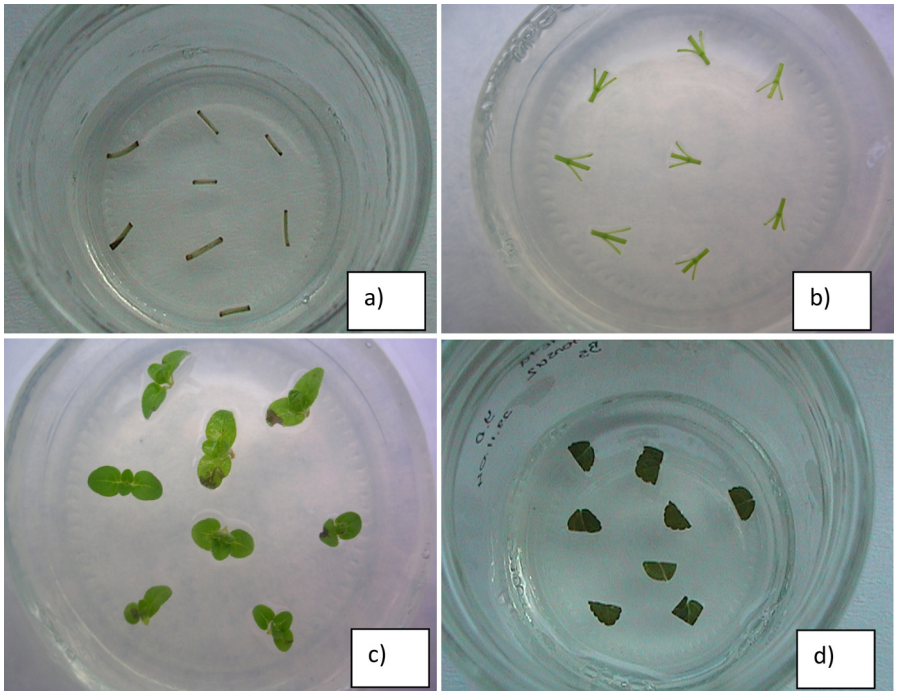

Fig. 2. Explants excised from seedlings of *S. stricta*: hypocotyl (a), single node (b) shoot tips (c) and leaf segments (d).