CHARACTERISTICS OF GERMINATION AND BIOMASS PRODUCTION OF *Ocimum basilicum* L. CULTURED *IN VITRO*

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Abstract: The aim of this study was to investigate impact of nutrient medium on sweet basil (*Ocimum basilicum L.*) *in vitro* germination, early growth and biomass acumulation. Two different nutrient media (MS and B5 full and half of strait), most commonly used in tissue culture, demonstrated different impact on sweet basil. According to the results, B5 medium significantly outperformed all other media used in this study. We suggest that B5 medium are better for a variety of uses in sweet basil biotechnology, research and production system.

Key word: *Ocimum basilicum*, nutrien medium, germination, growth

Introduction

Ocimum basilicum L, commonly known as sweet basil, is annual herbaceous and aromatic plant worldwide cultivated commercially. Basil possesses potential medicinal, pharmaceutical, and food industrial interests. Biological properties of basil are well documented and most of these studies are regarding the content and composition of essential oils (Oxencham et al., 2005, Politeo et al., 2007; Hussain et al., 2008; Dambolena et al., 2010). The most of the commercial basils belong to the *Ocimum basilicum* species; however there is a significant amount of phytochemical diversity present among individual plants and plant population. Therefore, commercial products show high level of variability of various compounds. In addition, numerous chemotypes of these plant species are presented. Grayer et al., (1996) studied infraspecific taxonomy and essential oil chemotypes in *Ocimum basilicum* belonging to different varieties. These authors suggest that for comparative studies plant should be grown under as identical conditions as possible. In order to solve this kind of problems, tissue culture may serve for efficient and controllable production of active components. However, despite being one of the most exploited plants of the mint family, *Ocimum basilicum* has been less investigated *in vitro* in comparison to other Lamiaceae members, which make these species a potential leading plant for different studies (Srivastava *et al*., 2014).

The proper concentration of medium salt is one of the most important factors in controlling active component production (Yin et al., 2013). Although medium selection is mostly based on medium history or its prior use with many plant species and culture tissue systems, these selection may not give consistent result when evaluated with different plant species, different plant tissue or new applications (Greenway et al., 2012). In attempt to improve productivity together with better understanding of sweet

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basil physiology, we investigated impact of medium salt type and strength on *O. basilicum* seedlings cultured *in vitro*. Data obtained in this study may provide a significant contribution to the optimization of basil tissue culture together with diverse *in vitro* biotechnological applications.

Material and methods

The seeds of *Ocimum basilicum* were surface sterilized and inoculated in glass tubes containing Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) or Gamborg (B5) medium (Gamborg et al., 1968) full and half of strength (0.5 MS; 1 MS; 0.5 B5; 1 B5). All media were supplemented with 30 g l^{-1} sucrose, 0.1 g l^{-1} myo-inositol, 2 g l^{-1} casein hydrolyzate and solidified with 0.7 g l^{-1} agar. The pH of all media was adjusted to 5.8 before autoclaving at 121 °C for 15 min. After seedling inoculation, the cultures were incubated in a plant growth chamber at 25 ± 2 °C at 60 % relative humidity and 16/8h photoperiod.

To study characteristics of germination in different media, three characteristics of germination were determined according to Fernandez et al (2015): *Final Germination Percentage* (FGP), *Rate of Germination* (RG) and *Mean Time to Germination* (MTG). *Mean Time to Germination* (MTG) was calculated by equation:

$$\text{MTG} = \sum n_i x\, t_i \Big/ \sum n_i$$

where n_i is the number of newly germinated seeds in the time i and t_i is time from the start of experiment to the observation (in days) (Ranal et al, 2006).

Rate of Germination (RG) was estimated by using a modified Timson's index of germination velocity according to Fernandez et al (2015) with some modifications:

$$\text{Germination velocity} = \sum G / t$$

where G is the percentage of seed germination at one day intervals and t is the total germination period. The germination characteristics were determined by counting the number of germinated seeds along 7 days period at 24 h intervals. The emergence of the radicle (at least 2 mm) was considered as evidence of germination.

After 28 days in culture, in order to investigate medium effect on sweet basil growth and productivity the following parameters were measured: length of hypocotyl, length of root, fresh and dry weight (biomass accumulation) and seedling vigor index. The dry biomass was determined after drying the tissue at 105 °C for 24h. Seedling vigor index was calculated according to Kharb et al., (1994):

Seedling Length Vigor Index (SLVI) = mean shoot length + mean root length x FGP

Seedling Weight Vigor Index (SWVI) = mean seedling weight x FGP.

Results and discussion

Germination is one of the most critical processes in life cycle of the plants. In the field, this process often takes place in environmentally stressful conditions. Varied

responces regarding *Ocimum basilicum* seed germination percentage and characteristics of germination was observed in different nutrient medium used (Table 1). According to Fernandes et al., (2015) the lower MTG demonstrated the faster the germination. Also, the higher the RG represent the more rapid germination.

Table 1. Germination responces of Ocimum basilicum to different nutrient medium

	MTG	RG	FGP (%)
1MS	3,71 ± 0,48	44,44 ± 1,25	77,78
0.5 MS	4,44 ± 0,43	44,19 ± 0,85	77,46
1 B5	2,44 ± 0,23	62,71 ± 0,93	86,67
0.5 B5	3,41 ± 0,15	56,95 ± 0,98	81,48

In our work, the lowest value for MTG was obtained using B5 medium for *Ocimum basilicum* seed germination. Same is in the case of RG as well as FGP, the highest value was obtained also in the B5 medium. Faster and more rapid germination of seed in Gamborg B5 regarding MS medium may be due to differences mainly in salt components and proportions of some of the major jones (Djilianov et al., 2010).

The obtained results for *Ocimum basilicum* growth parameters measured after 28 days in *in vitro* culture are presented in Table 2. According to the results, B5 medium significantly outperformed all other media used in this study.

Table 2. Effect of nutrient medium on Ocimum basilicum growth parameters

	Shoot length (mm)	Root length (mm)	Fresh weight (mg)	Dry weight (mg)	Seedling Length Vigor Index (SLVI)	Seedling Weight Vigor Index (SLVI)
1MS	32,63 ± 1,54	56,13 ± 2,17	104,3 ± 1,72	12,15 ± 0,01	6903,75	8112,45
0.5 MS	28,80 ± 1,47	53,67 ± 2,56	87,12 ± 1,40	10,01 ± 0,02	6338,13	6748,32
1 B5	34,67 ± 1,53	58,07 ± 1,91	141,25 ± 1,68	17,85± 0,32	8037,77	12242,14
0.5 B5	25,07 ± 1,17	44,23 ± 1,69	71,3 ± 0,69	6,54 ± 0,07	5646,56	5795,67

MS and B5 are two most commonly used nutrient media. B5 medium contain a higher level of potassium nitrate than MS, which may be benefitial for sweet basil growth and development. On the other hand, MS contain ammonium nitrate in much higher concentration than in B5, which may not be optimal in the case of sweet basil.

Conclusion

The Gamborg B5 medium coul be used to improve the *Ocimum basilicum* tissue culture system for diverse applications. We suggest that B5 medium are better for a variety of uses in sweet basil biotechnology, research and production system.

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