

**EXAMINATION OF DIFFERENT VITAMIN COMPLEXES  
IN IN VITRO  
MULTIPLICATION OF *RUBIA TINCTORUM***

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**ABSTRACT.** The influence of different vitamins in the nutrient media in multiplication of *Rubia tinctorum* was studied in the Biotechnology laboratory of IPGR-Sadovo. Included were vitamins from the following media *MS*, *White*, *Nitsch*, *Cheng* to a modifying media in individual variants. A nutrient media free of vitamins was used as a standard.

The results from the observations show that choosing the appropriate vitamin complex during the proliferation phase is very important not only for reaching a high multiplication coefficient but also for formation of buds with intensive growth and no physical deviations.

**KEY WORDS:** *Rubia tinctorum*, nutrient media, different vitamin compound, proliferation

**INTRODUCTION**

The species *Rubia tinctorum* is a medicinal plant that belongs to the family *Rubiaceae*. The most valuable parts are the roots used mainly in the phytotherapy. The species occurs rarely in our country in small populations and belongs to the endangered plant species (2). This calls for searching new opportunities for its multiplication and restoration in its natural habitats, which is one of the main tasks of the experimental work in the IPGR-Sadovo (1, 3, 4).

**MATERIAL AND METHODS**

The study was carried out in the Biotechnology laboratory of IPGR-Sadovo. The influence of different vitamins in the nutrient media in multiplication of *Rubia tinctorum* was investigated. Included were vitamins from the following media *MS*, *White*, *Nitsch*, *Cheng* to a modifying media in individual variants. A nutrient media free of vitamins was used as a standard (5). The composition of the examined nutrient medias differ in the number and the concentration of the individual compounds, included in the vitamin complexes. The nutrient media *Cheng* consists of only two

vitamins whereas the other medias differs in the number of each compound as well as the concentration.

With aim to assess the influence of the vitamins during the proliferation phase the following parameters were estimated: number formed buds, number buds developed in the sprouts, percent of the sprouts compared to the total number of buds. The nutrient media was autoclaved at 120°C and the plants were cultivated in growth cells at 26°C ( $\pm 1^\circ\text{C}$ ). The photoperiod lasted 16 hours per day and the illumination was 3000 lux. The trial was carried out in 3 replications with 30 micro plants in each replication.

## RESULTS AND DISCUSSIONS

The influence of the vitamins in the micro-multiplication of *Rubia tinctorum* is insufficiently studied. The researchers usually used different vitamin complexes or independently supplemented to the nutrient media for a normal plant growth.

The results obtained show that the multiplicative process proceeds normally with a great number of buds formed per explant on a nutrient media free of vitamins. Insignificant differences were established in comparison with the data received from the variants with the vitamin complexes and the standard (fig. 1). Number of buds was less on the nutrient media *White* compared with the standard one. Probably this is due to a certain extent to the ingredients of this nutrient media – there are a less number and concentration of compounds belonging to the vitamin's group. The observations made demonstrate that the complex of fixed vitamins have an effect on the plant growth and development. More intensive developing of the formed buds was observed under the influence of the vitamins in the nutrient media *MS*, which ensure average 38,81 % of the sprouts.

The results obtained during the budding show that the exclusion of the vitamins from the nutrient media has unessential effect on the bud proliferation but they are very important for the growth and the development of buds. A less number of formed sprouts were estimated on a nutrient media free of vitamins – average 10,25 % from the total number of buds per explant.

The independent influence of the most important compounds belonging to the vitamin's group was examined. It was established that the proliferation was comparatively good on a nutrient media with thiamine supplemented in concentration 0,5 mg/l. On average a total of 11,4 new buds were formed from per explant. The influence of glycine was similar but lower in comparison with the thiamine. The other examined compounds such as riboflavine, piridoxin, ascorbic acid that were supplemented independently to the nutrient media have no practical value for the proliferation of *Rubia tinctorum*.

Differences between the quality parameters of the sprouts in the examined variants with vitamins were observed. On the nutrient media consisting piridoxin and glycine (independently supplemented) the formed sprouts characterized with thin stems while mesoinosit stimulate bud's growth and the sprouts have strong stems and intensive green colour. Choosing the appropriate vitamin complex during the proliferation phase is very important not only for reaching a high multiplication

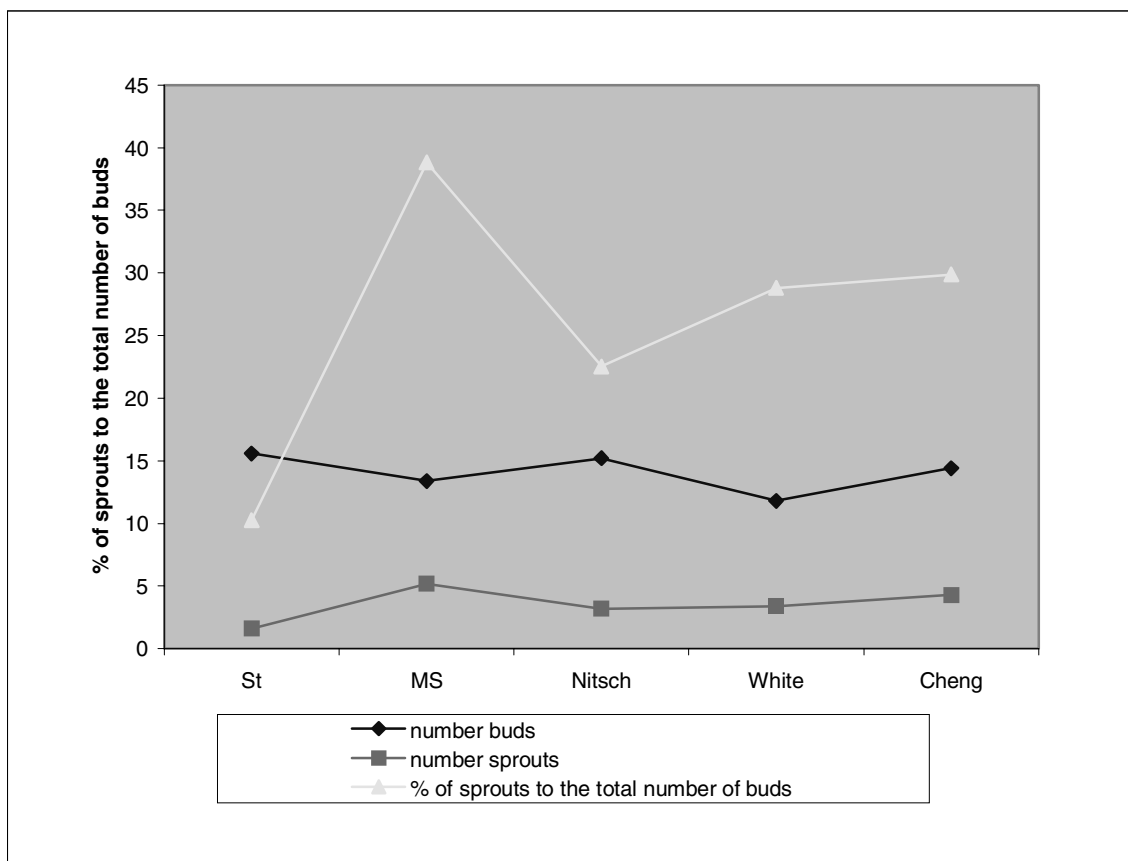
coefficient but also for formation of buds with intensive growth with no physical deviations.

As a result of the presented study the following conclusions can be made:

1. The exclusion of vitamins in the nutrient media has unessential effect on the bud formation process but they are essential for their growth in the sprout.
2. The appropriate vitamin compounds in the nutrient media are very important not only for reaching a high multiplication coefficient but also for formation of buds with intensive and normal growth.

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**Figure 1.** The influence of different vitamin complex during the proliferation phase of explants of *Rubia tinctorum*