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MICROPROPAGATION OF *LEONURUS CARDIACA* – INFLUENCE OF AUXINS AND CYTOKININS

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Summary. The effect of purine cytokinin BA (N^6-benzyladenine) and auxin IBA (indole-3-butyric acid) on the development of *in vitro* cultured *Leonurus cardiaca* L. was studied. We have shown that when Murashige and Scoog culture medium was supplemented with either BA or IBA, the physiological features, such as the number of shoots and shoot fresh and dry weights were influenced. Low concentrations increased the shoot number per explants while higher concentrations stimulated callusogenesis. The pharmacological effects of *Leonurus cardiaca* L. have mainly been attributed to the phenolic substances content. The quantity of phenolic substances was determined and discussed regarding the break and growth of axillary buds.

Key words: secondary metabolites, *in vitro* propagation, *Leonurus cardiaca*, N^6-benzyladenine, indole-3-butyric acid.

INTRODUCTION

Plants have been used for medicinal application ever since man has begun caring for his body and health. For centuries, the world has depended on the valuable properties of plant as a source of healing. Based on the considerable knowledge about these plant species from the ancient books, the modern systems of medicine have enabled these plants to find a place in the commercial market. The majority of natural products used medicinally in plants are secondary metabolites such as terpenoids, phenols, steroids, cardenolides, quinine lignans, flavonoids or alkaloids. Medicinal plants have gained pharmaceutical importance due to the combination of secondary metabolites present in them. These metabolites are often differentially distributed among limited taxonomic

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groups within the plant kingdom and participate in interesting biological activities that can have high therapeutic value. One such medicinal plant species that has gained pharmaceutical importance is *Leonurus cardiaca* L., commonly known as motherwort. *Leonurus cardiaca* grows wild in Bulgaria. Flowers appear in leaf axils on the upper part of the plant and it blooms between June - August. It can be found along roadsides and in vacant fields and other waste areas. This plant was first described in medicinal literature in the 10th century as a remedy for healing nervous and functional cardiac disorders (K. Milkowska-Leyck et al., 2002). *L. cardiaca* is used for healing cardiac diseases in Germany, France, Russia, Hungary, Bulgaria and some other countries (S. Mills et al., 2000). The English name 'motherwort' is indicative of the other uses of *L. cardiaca*. Motherwort is predominantly a womb remedy. A combination of relaxant and uterotonic effects induced by alkaloids (stahydrine, etc.) gives motherwort a useful role in facilitating child-birth. *L. cardiaca* might be prescribed for palpitation, to stimulate heart function, especially in conditions when the heart is weak (S. Mills et al., 2000). *L. cardiaca* herbs synthesize flavonoids, alkaloids, iridoids, diterpenoids, cardenolids such as glycosides, tannins and other constituents in lower amounts (G. Papanov et al., 1998; G. Papanov, B. Rodriguez et al., 1998).

Motherwort has been used in the traditional medicine against nervous and functional cardiac disorders since the 15th century and now is described in pharmacopoeias (BHP, 1992) for producing sedative, hypotensive and cardiotonic pharmacological effects. Motherwort is a mild cardiac drug containing bufadienolide glycosides as active compounds as well a number of flavonoid and phenolic glycosides. Motherwort is historically revered as a calmative agent for the heart, especially palpitations (Milkowska-Leyck, 2002). It is also found in many menopausal formula and was typically combined with another components as a superior antispasmodic and nervine, however, contemporary research is lacking on efficacy and safety.

Micropropagation is a powerful tool for ex situ conservation programs of the rich flora, especially for the species with the reduced populations. It is also irreplaceable for low seed producing plants and for rapid multiplication of species producing important secondary metabolites or possessing other valuable traits. The type and concentration of plant growth regulators affect the capacity of *in vitro* propagation since they play a major role in cell division, differentiation and morphogenesis in plant tissue cultures. Axillary bud outgrowth, which is considered as a process of apical dominance release, can be enhanced in response to exogenous cytokinins (Philips, 1975; Bollmark, 1995). The successfull micropropagation mostly depends on the quality of the explants source and on the plant growth regulators used in the culture medium.

In the present study, we have tested the influence of different cytokinin and auxin concentrations on variety of features. BA and IBA were applied at different concentrations and their ability to affect shoot number, shoot growth, dry weight, the amount of phenolic compounds and callusogenesis was studied.

MATERIALS AND METHODS

Plant material from the Bulgarian population of *L. cardiaca* was collected near the village of Vrazhdebna, Sofia. The voucher N 105806 has been deposited in the Herbarium of the Department of Botany, Faculty of Biology, Sofia University. *L. cardiaca* was propagated *in vitro* on basal Murashige and Scoog (MS) culture medium. The explants isolated from these cultures - axillary buds from the 3rd and 4th position with a small part of shoots (single nodes) were grown on the same medium, supplemented with BA (N^6-benzyladenine) and IBA (indole-3-butyric acid), applied in the concentration range from 0.1 mg l^{-1} to 1 mg l^{-1}. Growth conditions were 25°C and 16 h of light (60 µmol m^{-2} s^{-1} photosynthetic photon flux density, Philips TLD-33). The results were obtained after five weeks of cultivation and are presented as a percent of control plants (numbers of callus, average number of shoots and length of shoots), grown on hormone-free MS.

The amounts of total phenols was determined according to the method of Swain and Hillis (1959).

For statistical significance the data were processed and assessed by LSD at a 5% level of probability and SEM, which was the standard error of M_n; $n \leq 10$.

RESULTS AND DISCUSSION

Plant growth regulators are known to control physiological and biochemical processes through regulating primary and secondary metabolism (Normanly et al., 1995; Mohr et al., 1995; Heldt et al., 1997). Auxins refer to an important group of phytohormones that has been implicated in most of the quantitative growth changes that occur during a plant's life cycle. The effects of auxin are many and diverse, and have been difficult to separate. They can, however, be divided into two broad categories: effects on cell expansion and effects on cell division. Evidence has been obtained also that auxin might have morphogenetic properties that are analogous to chemicals found in the animal kingdom, but the ability of auxin to change directly the developmental fate of cells has not yet been conclusively demonstrated. Compounds are generally considered auxins if they can be characterized by their ability to induce cell elongation in stems and otherwise resemble indole-3-acetic acid (the first auxin isolated) in physiological activity. Auxins usually affect other processes in addition to cell elongation of stem cells, but this characteristic is considered critical of all auxins and thus "helps" define the hormone (Arteca, 1996; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992). Cytokinins are considered as an important factor in controlling and breaking the dormancy and apical dominance (Cline, 1994; 1997). A number of physiological effects of natural and synthetic cytokinins are well documented, but the mechanism through which these plant growth regulators control the processes of growth and development remain still not quite clear. Auxins regulate not only vegetative growth, but also organ growth, whereas the cytokinins facilitate cell division and sprouting (Pan, 2001). Our results are shown as percent of the corresponding parameters observed in plants grown on a basal medium as control. In our experiments the application of higher concentrations of IBA increased the dry weight, which

means that biosynthesis of primary and secondary metabolites was enhanced and this is to be considered as a tendency (Fig. 1). Compared to control plants, dry weight was highest at a concentration of 0.4 mg l^{-1} IBA. At this concentration the habitus of plants was the best and we consider it as optimal for *in vitro* cultivation of

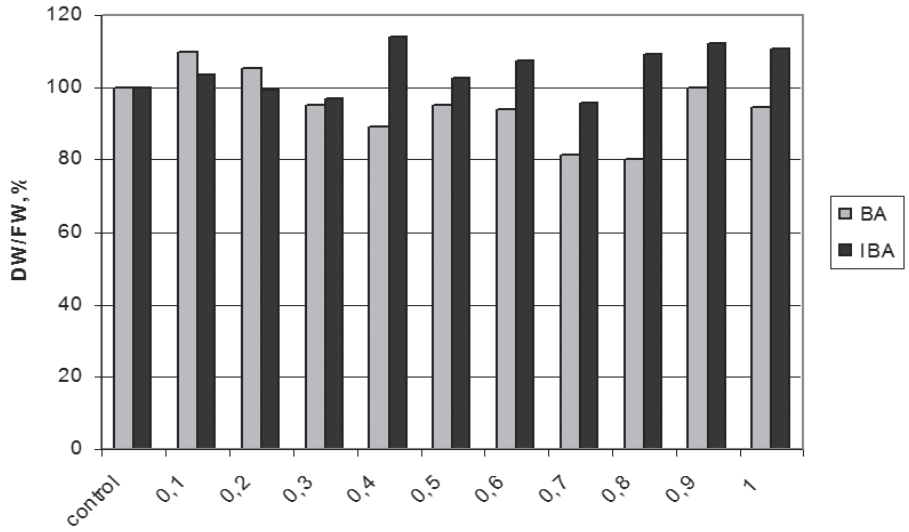

Fig. 1. Effects of different concentrations of BA and IBA (0.1-1.0 mg l^{-1}) on the dry weight of shoots of *in vitro* cultured *Leonurus cardiaca*. Data are expressed as percent of control.

L. cardiaca. Callusogenesis was lower at all concentrations tested as compared to the control plants and this is to be expected, considering that IBA promotes organ growth and possibly inhibits excessive cell division of undifferentiated cells (Fig. 2). Generally, the application of higher concentrations of BA decreased the dry weight and it can often lead to vitrification. Vitrification was sporadically observed, mostly at the highest concentrations of BA. On the other hand, callusogenesis was

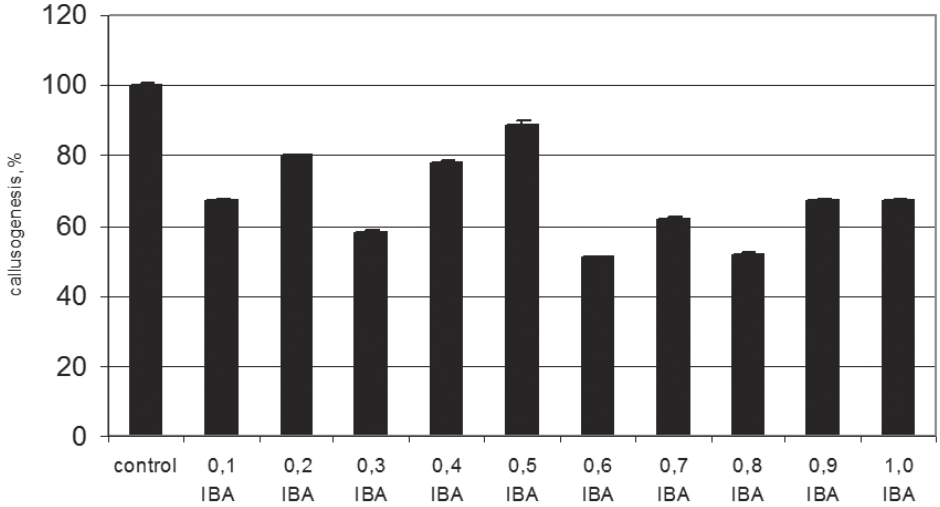

Fig. 2. Effects of different concentrations of IBA (0.1-1.0 mg l^{-1}) on callusogenesis of *in vitro* cultured *Leonurus cardiaca*. Data are expressed as percent of control.

stimulated at all concentrations of BA and it was remarkably high at 0.4 mg l^{-1} (Fig. 3). The number of shoots per explant was found to be dependent on both cultivar and BA concentrations. It was shown that higher concentrations of BA increased shoot number, but diminished the length of stems (Fig. 4). The concentrations of BA between 0.4 and 0.8 mg l^{-1} had the highest shoot inducing capacity. Further increment of concentrations, however, showed a negative impact on plant habit and vitality. The concentration of 0.8 mg l^{-1} caused the highest level of shoots per explant but plant habit was optimal at a concentration of 0.3 mg l^{-1} BA. These data corresponded to the

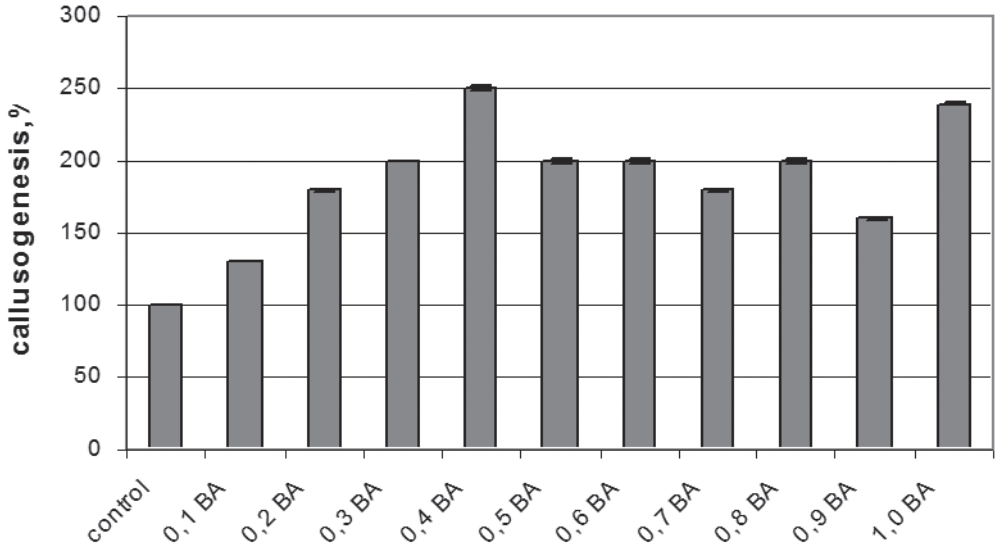

Fig. 3. Effects of different concentrations of BA (0.1-1.0 mg l^{-1}) on callusogenesis of *in vitro* cultured *Leonurus cardiaca*. Data are expressed as percent of control.

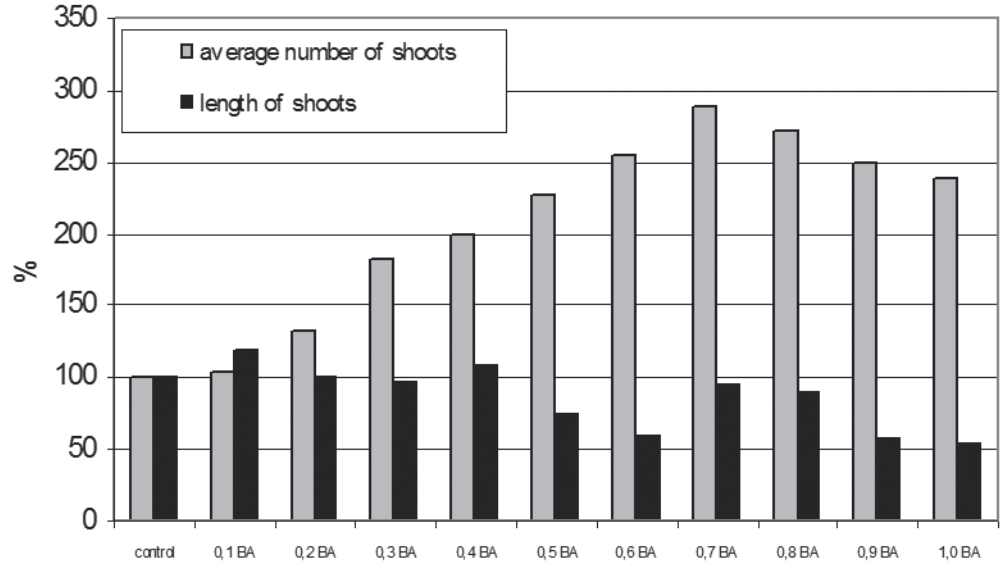

Fig. 4. Effects of different concentrations of BA (0.1-1.0 mg l^{-1}) on shoot number and length of shoots of *in vitro* cultured *Leonurus cardiaca*. Data are expressed as percent of control.

observation of Usman (2005) in explants of *C. halimii* where the highest number of shoots was observed at 1.0 mg l^{-1} BA. In the case with IBA and its impact on shoot number and stem length, tendencies were blurred and speculative (Fig.5). In general, the application of BA and IBA enhances the secondary metabolism, which leads to increased total phenols. When compared to BA, IBA appears to be a stronger activator of the secondary metabolism. Our results showed that the optimal concentrations leading to a higher total phenol amount and possibly higher amount of other secondary metabolites varied between 0.3 and 0.7 mg l^{-1} IBA. At these concentrations the overall amount of phenolic compounds appeared to be approximately 1.5-fold higher compared to the control plants (Fig.6).

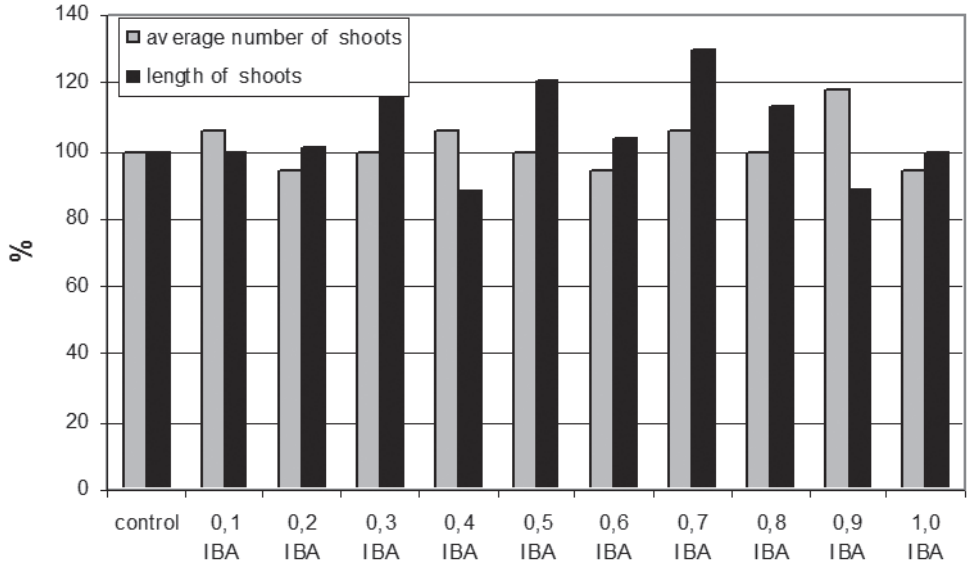

Fig. 5. Effects of different concentrations of IBA (0.1-1.0 mg l^{-1}) on shoot number and length of shoots of *in vitro* cultured *Leonurus cardiaca*. Data are expressed as percent of control.

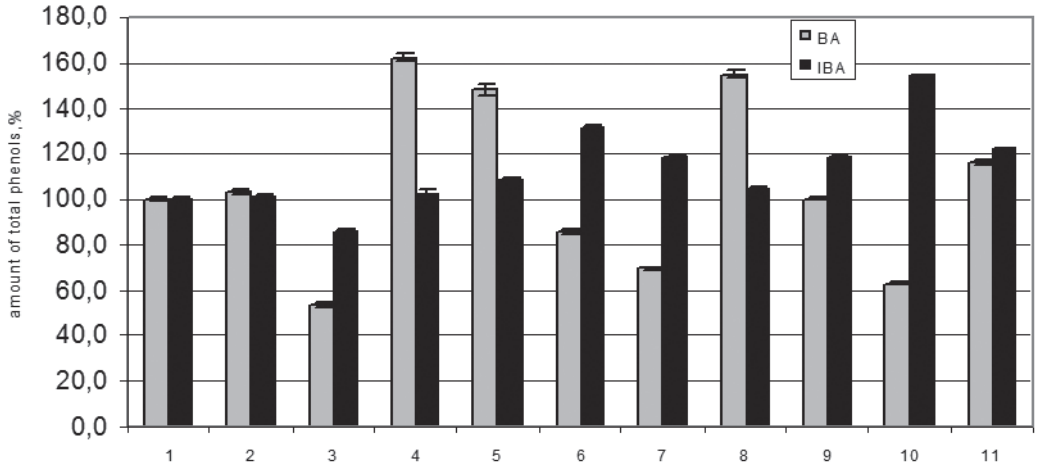

Fig. 6. Effects of different concentrations of BA and IBA (0.1-1.0 mg l^{-1}) on the amount of total phenols of *in vitro* cultured *Leonurus cardiaca*. Data are expressed as percent of control.

Conclusions

The growth regulator treatments resulted in a significant increase of shoot number and affected dry weight, callusogenesis and production of secondary metabolites. We established optimal concentrations of the growth regulators for *Leonurus* species. Further experiments are being conducted and other traits will be examined such as amount of pigments, total sugars and flavonoids.

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