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Effects of different nitrogen fertilizer sources on the yield, nitrate content and other physiological parameters in garden beans

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Abstract

Garden bean plants (*Phaseolus vulgaris* (L.) Savi.) cv. Xera were grown under glasshouse conditions at optimal fertilizer rates using mineral, organic, and foliar fertilizers previously established in a model pot experiment. It was shown the favorable effect of the foliar feeding on the biomass accumulation in the different vegetative organs and formation of fresh products with low levels of nitrates and nitrites. In the leaves of the plants with foliar feeding higher levels of nitrate reductase enzyme activity are observed which contributes to more intensive nitrogen uptake. No differences are established among the variants with different fertilizer sources regarding plastid pigment content.

Keywords: Garden beans; *Phaseolus vulgaris* (L.) Savi.; Fertilizer sources; Foliar feeding

1. Introduction

Forms and amounts of applied fertilizers influence to a great extent on the yield and quality of vegetable plants (Wery et al., 1988; Gunes et al., 1995; Sidiras et al., 1999). Vegetable plants response positively to organic and mineral fertilization. According to some reports (Rankov et al., 1993; Kovacheva et al., 1999; Panayotov, 2001) these cultures response positively also to foliar feeding. At times foliar fertilizers are vital supplements to standard plant nutrition, especially under unfavorable soil–climatic conditions and other stress circumstances.

The aim of our study is to establish the advantages of different fertilizer sources for the optimal yield formation with high quality of garden beans plants.

2. Material and methods

Garden bean plants (*Phaseolus vulgaris* (L.) Savi.) ssp. Nanus cv. Xera were grown under glasshouse conditions at optimal water regime without deficiency on glasshouse soil substrate with pH 6.3. A randomized block design with four replicates was used at plant density of 10 plants per 1 m^2. Each experimental plot was 6.72 m^2 in area and consisted of four rows. Plants

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черноземно-смолница надминава 6 милиона при целинните участъци. В обработваемите почви и особено при използване на азотни торове този вид микроорганизми се стимулира, при което количеството им многократно нараства.

Просмукващата се през почвения слой подхранваща инфилтрационна вода внася в подземните води продуктите от жизнената дейност на амонифициращите бактерии.

Нитратните йони са един от най-консервативните йони в състава на подземните води. Те не се разлагат, не участват в йонообменни реакции и при условията на забавения водообмен в плиоценските водоносни хоризонти имат свойството да се натрупват. В резултат на това концентрациите им достигат драстични стойности, многократно надвишаващи ПДК за питейните води - в някои от изследваните водоизточници съдържанието им надминава 1000 мг/л / при норма 50 мг/л /

Общата минерализация на водите от този тип най-често варира от 3 до 10 г/л, а в състава им се откриват и повишени магнезиеви концентрации /от порядъка на 100 - 170 мг/л /

Изучаването на състава на подземните води подпомага правилната им експлоатация и дава възможност за своевременното вземане на мерки срещу негативните въздействия на човешката дейност върху тях

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ВЛИЯНИЕ НА ИЗТОЧНИЦИТЕ И НОРМИТЕ НА АЗОТНОТО ТОРЕНЕ ВЪРХУ ДОБИВА И КАЧЕСТВОТО НА МАРУЛЯ

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Марулята е от листните зеленчуци, които натрупват най-големи количества нитрати. Някои автори (Geyer,1978)дори я поставят на първо място сред зеленчуците по акумулиране на нитрати. Martinetti (1996), Kalembosa and Peska (1996) , Gonnella et al (2000) са установили, че натрупването на нитрати зависи главно от азотното торене. Други автори (Kondela and Petrikova (1999), Slipka et al. (2000), посочват, че метеорологичните условия, периода на отглеждане, а също така и сорта оказват влияние върху съдържанието на нитрати в салата и маруля. Установена е зависимост между съдържанието на нитрати в растителната продукция и механичния и химичния състав на почвата (Geyer,1978). Запасеността на почвата с леснодостъпни азотни съединения оказва съществено влияние върху натрупването на нитрати в зеленчуковите растения. При спанака и марулята слабо акумулиране е отчетено при запас на азот в почвата до 3 meq/L (Райкова и Ранков, 1984). Основна цел на проведеното изследване, е да се установи концентрацията и източника на азот, оказващ най-благоприятно въздействие както върху добива, така и върху редица параметри на качеството на маруля.

Материал и методи

Опитът с маруля (сорт Paris Island cos.) е засят на 07.03.2003, разсадът е засаден на 03.04. 2003 и растенията са прибрани на 09.05.2003. Растенията са отгледани по едно в еднокилограмови съдове на алувиално ливадна почва –pH (H2O) = 7.2 в четирикратна повторност при следните варианти:

1. контрола /неторено/
2. 100 mg минерален N/kg почва
3. 300 mg минерален N/kg почва
4. 500 mg минерален N/kg почва
5. 4.2 g /kg почва оборски тор
6. 12.6 g /kg почва оборски тор
7. 21.0 g /kg почва оборски тор
8. листен тор Агролиф (Agroleaf)

Минералният азот при вариантите 2, 3 и 4 е внесен като амониев нитрат (NH_4NO_3) при фоново съзържание на фосфор, калий и магнезий както следва 300 mg/kg почва P_2O_5 373 mg/kg почва K_2O и 40 mg/kg почва Mg. Съставът на внесения полуразложен оборски тор е общ N –0.64%, общо съдържание на P_2O_5 –1.84% и общо съдържание на K_2O 0.51%. Листният тор Агролиф е произведен от американската компания Скотс. Той е високо пречистен с много добра разтворимост и съотношение на основните макроелементи N:P:K=20:20:20. Хелатираните микроелементи са представени в уникална М77 формула : 0.1% Fe, 0.06% Mn, 0.06% Cu, 0.06% Zn и 0.02% B. Растенията са третирани с 0.3% разтвор на Агролиф, три пъти през вегетацията през 10 дни започвайки от 3-та след разсаждането. Влажността на почвата е поддържана на 70% ППВ по тегловния метод с ежедневни поливки.

При прибирането на растенията са определени следните показатели:

1. Добив /свежа биомаса/, g/растение
2. Абсолютно сухо вещество -%
3. Витамин С – mg/100g свежо тегло по метода на Мури
4. Разтворими захари - mg/100g сухо тегло по метода на

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Biochemical Characteristics Used as Quality Parameters in White Head Cabbage at Different Nitrogen Rates, Sources and Ways of Application

Abstract

The study was performed to establish the effect of different nitrogen rates, sources and ways of nitrogen fertilizer application as an after - effect on the biochemical characteristics - considered as quality parameters of head cabbage. White head cabbage (*Brassica oleracea* var. capitata, cv. Kyosse -17) was grown as a second crop after potatoes without additional fertilization - after effect. Before the first crop sowing the following nitrogen rates had applied: N_0, N_{90}, N_{180} and N_{270} kg/ha at two nitrogen sources: ammonium nitrate and urea. Two ways of nitrogen fertilizer application were tested: single before the sowing and split application. The results from the study show determinative influence of fertilizer rates on the quality parameters, while the effect of nitrogen source and way of application were less effective. The content of nitrates in cabbage leaves was much lower beneath acceptable limit concentration. Similar values of quality parameters under application of urea and ammonium nitrate did not give a reason to determinate the advantage of the used nitrogen sources. Two appropriate ways to obtain good quality parameters for head cabbage - grown as a second crop were suggested: single application of urea and split application of ammonium nitrate.

Keywords: white head cabbage; quality parameters; after-effect, fertilizer application

Introduction

The head cabbage with its chemical composition, taste and nutritional value appeared to be among the most valuable vegetables and it is a good, dietary food (Alipieva, 1986). Cabbage is a good source for carbohydrates (mainly sugars), vitamins, minerals, amino acids and other biologically active substances. Vitamin C content is very high in cabbage leaves and especially varieties appropriate for storage are rich in vitamin C (Volodina, 1987). The head cabbage is a crop with high nutrient requirements to the nitrogen as well as potassium and phosphorus because it accumulates large vegetative biomass for a relatively short period. The right proportion between the nitrogen and other nutrients leads to the ballanced mineral nutrition. Unbalanced nitrogen application leads to the quality deterioration – crude protein increase, reduction of carbohydrates and accumulation of nitrates were observed, cabbage turned sleazy and disposed to bursting Bomme et al., 1987; Ezepchuk, 1990). Different opinions exist regarding sources and ways of nitrogen application. Some authors suggested split application of nitrogen fertililizer – before the sowing and dressing Volodina (1987), Amirov et al. (1990), Everaats (2000), while according to the others the nitrogen fertilizer split application did not increased the yields compared with the one time spread application before the sowing Barthacur et al. (1990).

The aim of the present study is to establish the effect of different nitrogen rates, sources and ways of nitrogen fertilizer application as an after - effect on the biochemical changes indicators for quality of white head cabbage grown as a second crop after potatoes.

Material and Methods

The field experiment was conducted on an alluvial-meadow soil (Fluvisol) in South Bulgaria with white head cabbage (*Brassica oleracea* var. capitata, cv. Kyosse –17) as a second crop after potatoes without additional fertilization – after effect. The experiment is a part of complex trials carried out by the scientific team from the N. Poushkarov Institute of Soil Science - Sofia with potatoes, cabbage and lettuce grown in compact vegetable rotation. The arable horizon has the following agrochemical characteristics: pH (H_2O) - 6.20, humus - 0.70%, N total - 0.052%, N mineral - 10.7 mg/kg and low cation exchange capacity -

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съответен израз от формула /3/ след деление на диференциала на времето dt.

Още от времето на Максуел е известна следната обща формула за интензитета на изпарение:

/4/ $$\frac{dW}{dt} = -\frac{k}{4}\frac{\delta}{\sin\theta}\frac{c}{r},$$

където с k е означен коефициент на дифузия на водните пари във въздуха.

По-долу ще разгледаме въпроса за време на изпарение при мод с постоянен контактен ъгъл.

В случай че при изпарение се мени само δ, във формулата /2/ делена на dt, за $\frac{dW}{dt}$ след анулиране на $\frac{d\theta}{dt}$ получаваме:

/5/ $$\frac{dW}{dt} = \frac{\rho\pi\delta^2}{16}(tg^3\frac{\theta}{2} + 3tg\frac{\theta}{2})\frac{d\delta}{dt}.$$

Вместо лявата страна на /5/ поставяме неговото равно според израза на Максуел във вида /4/:

/6/ $$-\frac{k}{4}\frac{\delta}{\sin\theta}\frac{c}{r} = \frac{\rho\pi\delta^2}{16}(tg^3\frac{\theta}{2} + 3tg\frac{\theta}{2})\frac{d\delta}{dt}$$

Можем да разглеждаме горния израз като диференциално уравнение с разделящи се променливи. Като прехвърлим неизвестната величина $\delta(t)$ и нейния диференциал $d\delta(t)$ от едната страна на равенството, а диференциала от другата страна и интегрираме получаваме:

$$\int_{\delta_0}^{\delta_1} \delta d\delta = -\frac{4k}{\pi\rho\delta}\frac{(c/r)}{(tg^3\frac{\theta}{2} + 3tg\frac{\theta}{2})\sin\theta}\int_{t_0}^{t_1} dt.$$

Тук δ_0, δ_1 са съответно диаметрите на контактната област на капката съответно в началния t_0 и крайния t_1 момент на изпарение.

След като интегрираме и заместим границите на интегриране получаваме за времето на изпарение $t_n = t_1 - t_0$ на тази мода:

/7/ $$t_n = \frac{\pi}{8}\rho\frac{(\delta_0^2 - \delta_1^2)}{kc/r}(tg^3\frac{\theta}{2} + 3tg\frac{\theta}{2})\sin\theta.$$

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ТОРЕНЕТО КАТО ФАКТОР ЗА ФОРМИРАНЕ НА ДОБИВА И КАЧЕСТВОТО ПРИ ГЛАВЕСТОТО ЗЕЛЕ

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Увод

Резултатите от продължителни научни изследвания показват, че за повечето зеленчукови култури, в зависимост от почвено- климатичните условия, торенето, респективно азотното торене се явява важен фактор за повишаването на добива (Георгиева и др.,1978; Журбицкий,1963).Без използването на азотни торове добивите в зеленчукопроизводството като цяло са по- ниски с 20-25% (Борисов и др., 1987).

Това има особено важно значение за главестото зеле, което е между най-взискателните към хранителния режим зеленчукови видове, и е на едно от първите места по изнесени от почвата хранителни елементи с добива (Петкова, 1984; Стойков и др.,2004; Atanasova et al., 2004).

Установено е, че за отглеждането на зелето между всички хранителни елементи азотът е от първи минимум (Борисов и др.,1987).

Прекомерното завишаване на азотните норми обаче, влияе отрицателно върху качеството на получената продукция като води до натрупване на нитрати и нитрити, влошава съхраняемостта и способността за консервиране и др.(Антонова и др.,1995; Петкова, 1984).

Съчетаването на двата критерия за добив и качество изисква продължителни изследвания във вегетационни и полски условия за оптимално съчетание на факторите определящи формирането на добива и допустимите стойности на показателите за качество.

Цел на иследването е да се установят източника и концентрацията на азот оказващи най-благоприятно въздействие както върху добива, така и върху някои характерни показатели за качеството при главесто зеле.

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Response of Inoculated Pea Plants (*Pisum sativum L.*) to Foliar Fertilizer Application with Elevated Concentrations

Abstract

A comparative effect of combined root and foliar applied mineral elements with increased foliar fertilizer concentrations (0.1-0.7%) on the plant growth and root nodulation at early steps of nodule formation was studied. Plants grown at combined root and foliar nutrition at foliar fertilizer concentration 0.3% showed highest values of the parameters related to biological nitrogen fixation: nodule number, nodule fresh weight, plant dry weight, shoot content of soluble sugars and total nitrogen.

Keywords: *Pisum sativum* L., foliar application, nodule formation, shoot to root dry weight ratio, total nitrogen

Introduction

The formation and functioning of nitrogen fixing nodules of legume roots is known to be very successful under nitrogen-limiting conditions. Many authors (Kijne et al., 1988; Smit et al., 1986; Smit et al., 1992) using *Rhizobium leguminosarum bv. vicae* have shown, that the conditions under which *Rhizobia* are grown are often of prime importance to their ability to attach to root hairs. The early steps of nodule formation are especially sensitive to the changes of mineral nutrition both under conditions of nutrient starvation (Thorne and Williams, 1997) and nutrient excess (Streeter 1988; Marschner 1995).

The negative effect of higher than 2 mM of soil NO_3^- - N on nodule nitrogenase activity and synthesis of transport products is also described (Streeter, 1988). A perspective way to reduce the inhibitory effect of soil supplied inorganic nitrogen on the nodulation and symbiotic nitrogen fixation is to change the direct place of plant N uptake and assimilation. In agriculture, a common method of fertilization is the application of nutrients by spraying a solution onto the leaves (foliar nutrition).

One of the established benefits of foliar fertilization is the increased uptake of nutrients from the soil because of the released more sugars and other exudates from the roots into the rhizosphere (Kuepper, 2003). Beneficial microbial population in the root zone is stimulated by the increased availability of these exudates. Although high rates of foliar applied N would cause serious leaf damage, the small rates could stimulate growth without inhibiting nodulation.

In these reason the aim of our study is to establish the most appropriate foliar fertilizer concentration that ensure normal plant growth and nodule development at early steps of nodule formation of the inoculated with a *Rh. leguminosarum bv. Vicae* pea plant seedlings.

Material and Methods

Seeds of garden pea *Pisum sativum* L. var. *Avola* were surface sterilized with 4% NaOCl and germinated in the Petri dishes at 25 °C. Three days old seedlings were inoculated with the bacterial suspension of *Rhizobium leguminosarum bv. Vicae* strain D 293 at approximately 10^9 cells per cm^3. On the 5th day the seedlings were transferred to 1.2 dm^3 pots (2 plants per pot) containing liquid nutrient solutions of Helriegel and were grown in the greenhouse until the 15th day. The solution was aerated continuously and replaced twice weekly. Nutrient solution of Hellriegel supplied to plants was with half strength of nutrients with the exception of the control variant, which contained full strength solution. The NO_3^- concentration was 0.5 mM in all-experimental variants. The complementation of nutrients with micronutrients was done according to Hoagland

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EFFECT OF DIFFERENT FOLIAR FERTILIZER CONCENTRATIONS ON PEA PLANTS NODULATION AT REDUCED MO SUPPLY

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ABSTRACT. Garden pea *Pisum sativum* L. var. *Avola* inoculated with bacterial suspension of *Rhizobium leguminosarum bv. Vicae* strain D 293 were grown in the greenhouse until the 15th day on liquid half strength nutrient solutions of Hellriegel, contained 0,5 mM NO_3^- in the all experimental variants. The complementation of nutrients with micronutrients was done according to Hoagland and Arnon excluding Mo from the nutrient medium. The following variants were tested: (1) control plants with root nutrition only and presense of Mo; (2) control plants with root nutrition only without Mo (3) plants with combined root and foliar nutrition (0,1%Agroleaf); (4) plants with combined root and foliar nutrition (0,3%Agroleaf); (5) plants with combined root and foliar nutrition (0,5%Agroleaf). Application of foliar fertilizer in elevated concentrations had a positive effect on the Mo deficient pea plants. An increase of nodule number, plant dry weight, total shoot nitrogen and leaf soluble sugars have been found. The highest nodule number and leaf soluble sugar content was observed at spraying with 0.3% concentration of Agroleaf.

KEY WORDS: *Pisum sativum* L, *Rhizobium leguminosarum bv. Vicae*, foliar fertilization, plants with reduced Mo supply.

INTRODUCTION

The most importance of molybdenum (Mo) for plants, animals and microorganisms has been known for a long time (Coughlan, 1980). Molybdenum is a

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Quality and Yield of Lettuce in Dependence of Different Fertilizer Sources

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Abstract

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The influence of different fertilizer sources on lettuce yield and quality was studied. The field experiment was carried out on alluvial meadow soil in Tsalapitsa experimental station. The experiment included three fertilizer sources: mineral fertilizer, farmyard manure and foliar fertilization. The highest yield values from lettuce were obtained in the variants with mineral fertilization. A beneficial effect of the lettuce quality was observed: high values of soluble sugars and vitamin C content, low level of cellulose and reduced nitrate content.
Key words: lettuce, fertilizer, nitrate content, cellulose, soluble sugars

Introduction

Application of different fertilizer rates and sources makes an impact in a different way on the yield and quality of leafy vegetables fresh production in dependence on soil-climatic conditions of growth. The nitrate content in lettuce leaves is mainly influenced by the nitrogen fertilization (Martinetti, 1996; Pavlovic, 1997;Gonnella et al. 2000). High rate of nitrate uptake and slow nitrate reduction in plants is due to the relatively short vegetative period of lettuce (Reinken, 1992). It was established in our previous studies carried out both under conditions of phytocamber (Stancheva and Mitova, 2002) and glasshouse (Stancheva et al., 2004) that foliar fertilization with Agroleaf resulted in high values of lettuce fresh and dry biomass with low nitrate content.

The aim of our study is to establish the advantages of different fertilizer sources (mineral, organic and foliar fertilization) on the quality and yield of lettuce under specific soil-climatic conditions.

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Response of Pea Plants (*Pisum sativum* L.) to Reduced Supply with Molybdenum and Copper

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ABSTRACT

The role of molybdenum (Mo) and copper (Cu) in regulation the activities of the enzymes involved in primary nitrogen assimilation in particular the nitrate reductase (EC 1.6.6.1) and glutamine synthetase (EC 6.3.1.2) was examined. Pea plants were grown in a phytotron chamber at 12 h photoperiod, day /night temperature 25/18 °C and photon flux density of 95 μmol m^{-1} s^{-1} until 21^{-st} day. Plants were grown at full strength Helriegel nutrient solution competed with micronutrients as in Hoagland and Arnon and reduced Mo and Cu concentrations. Although only extremely small amounts of Mo and Cu are required for normal plant growth, reduced supply with Mo and Cu to the growth medium decreased activities of the enzymes (nitrate reductase and glutamine synthetase) involved at initial steps of nitrate assimilation, fresh weight, and plastid pigment content (total chlorophyll and carotenoids). Accumulation of nitrates in plant tissues enhanced, especially in the variants with restrictive Cu concentration.

Key Words: Pea (*Pisum sativum* L.); Molybdenum; Copper, Nitrate reductase, Glutamine synthetase
Abbreviations: NR - nitrate reductase, GS- glutamine synthetase, FW-fresh weight

INTRODUCTION

Plants in balanced proportion require all the essential nutrients. Continuous shortage of a nutrient or nutrients might cause nutritional disorders and even plant death. Plant species differ regarding its Mo and Cu requirements (Gupta & Lipsett, 1981). According to Gupta (1997) pea requirement for Mo is medium, while for Cu is low (Ayala *et al.,* 1992). Molybdenum and copper are among the most recently recognized nutrient elements essential for the plant growth and development (Bottrill *et al.,* 1970; Brown & Clark, 1977). The role of Mo as a plant nutrient is related to its function as a metal component of some enzymes that catalyze nitrogen fixation, nitrate assimilation and reduction. (Kisker *et al.,* 1997; Campbell, 2001). Mo itself seems to be catalytically inactive in biological systems until is bound in complex to a unique pterin compound named Mo cofactor – MoCo. The presences of MoCo have been reported in seeds of pea (Svetsov *et al.,* 1992).

In spate of the known action of Cu on photosynthetic membranes (Quartacci *et al.,* 2001), on plasma membrane level and on its lipid composition, there is a lack of information regarding Cu effects at the function of copper in nitrogen uptake and assimilation in legumes. There are some reports about suppressed nodulation and nitrogen fixation when a copper is insufficient in the growth medium (Cartwright & Hallsworth, 1970). The study is aimed at establishing the physiological response of pea plants under conditions of reduced supply with Mo and Cu to the nutrient solution.

MATERIAL AND METHODS

Pisum sativum L. cv. Avola seeds were surface sterilized with 4% sodium hypochlorite (NaOCl) and germinated in thermostat at 25°C. Five-day old seedlings were transferred to a hydroponics system - 0.8 L pots with 5 plants per pot (4 replicates per variant). Plants were grown in a phytotron chamber at 12 h photoperiod, day/night temperature 25/18°C and photon flux density of 95 μmol m^{-1} s^{-1} until 21th day. Nutrient solution was prepared after Helriegel and completed with micronutrients as in Hoagland and Arnon (1950). The following variants were tested: (1) control plants – full strength nutrient solution; (2) full strength solution with twice reduced Mo concentration - 0.085 μM Mo; (3) full strength solution without Mo, (4) full strength solution with twice reduced Cu concentration – 0.15 μM Cu; (5) full strength solution without Cu.

Enzymes were extracted at 0-4°C from 1.0g leaves using mortars and pestles and 5 cm^3 of optimized extraction medium 50 mM Tris-HCl (pH 8.0), 1 μM Na_2MoO_4, 10 mM $MgSO_4.7H_2O$, 1 mM EDTA, 10 mM L-cysteine, 1% PVP-40, 1g Dowex (Frechilla *et al.*, 2000). The extracts were filtered through one layer of Miracloth, centrifuged at 20 000g for 20 min, and the supernatant used for the following respective assays. Nitrate reductase (NR: NADH, EC 1.6.6.2) activity was measured according to Hageman and Reed (1980). Glutamine synthetase (GS: EC 6.3.1.2) activity was determined by a biosynthetic assay based on γ-glutamil hydroxamate synthesis (O' Neal & Joy, 1973). Protein content was determined as in Bradford (1976) with BSA as a standard. The content of nitrates was determined by Nitrachek from Hawk Creek Laboratory Inc. USA and leaf tissue nitrate quick test was made.

Total chlorophyll and carotenoids in the pea leaves were exhaustively extracted in 80% acetone. The pigments

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Comparative Study of Some Quality Parameters of Lettuce in Dependence on Way of Cultivation

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Abstract

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The influence of different way of cultivation on lettuce yield and quality was studied. Two field experiments were carried out on alluvial meadow soil in Tsalapitsa experimental station. The experiments were performed with lettuce, grown as a spring crop and winter crop with application of three fertilizer sources: mineral fertilizer, farmyard manure and foliar fertilization.

Significantly higher yield values from lettuce were obtained when lettuce was grown both at spring and winter lettuce crop in the variants with mineral fertilization. The best quality parameters: high values of soluble sugars and vitamin C content, low level of cellulose and reduced nitrate content were observed in the variants with foliar fertilization.
Key words: lettuce, mineral fertilization, organic fertilization, foliar fertilization, quality parameters

Introduction

The optimum fertilizer rates application in vegetable growing are the main factors for receiving profitable production with low nitrates content without creating conditions for pollution of different components of the ecosystem. When the lettuce was grown as a third crop in the rotation has lead to maximum inclusion of nitrogen in the nutrients cycle was observed resulted in the restoration of nitrogen content in the soil near its background values (Stoicheva, et al., 2002).

The quality of vegetables is in close dependence on the way of cultivation. Application of different fertilizer sources influenced in a different way lettuce yield and quality which strongly depended on soil climatic conditions (Martinetti, 1996; Stancheva et al., 1997). The most favorable effect of foliar fertilization on the quality of lettuce grown during the spring was shown in our earlier work (Dimitrov et al., 2005).

In our previous studies with leafy vegetables the optimal fertilizer rates of mineral organic and foliar fertilizers that de-

The effect of inoculation of pea plants with mycorrhizal fungi and Rhizobium on nitrogen and phosphorus assimilation

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ABSTRACT

The study evaluated the response of pea (*Pisum sativum* cv. Avola) to arbuscular mycorrhizal fungi (AM) species *Glomus mosseae* and *Glomus intraradices* and *Rhizobium leguminosarum* bv. *viceae*, strain D 293, regarding the growth, photosynthesis, nodulation and nitrogen fixation activity. Pea plants were grown in a glasshouse until the flowering stage (35 days), in 4 kg plastic pots using leached cinnamonic forest soil (Chromic Luvisols – FAO) at P levels 13.2 (P1) and 39.8 (P2) mg P/kg soil. The obtained results demonstrated that the dual inoculation of pea plants significantly increased the plant biomass, photosynthetic rate, nodulation, and nitrogen fixation activity in comparison with single inoculation with *Rhizobium leguminosarum* bv. *viceae* strain D 293. On the other hand, coinoculation significantly increased the total phosphorus content in plant tissue, acid phosphatase activity and percentage of root colonization. The effectiveness of coinoculation with *Rhizobium leguminosarum* and *Glomus mosseae* was higher at the low phosphorus level while the coinoculation with *Glomus intraradices* appeared to be the most effective at higher phosphorus level.

Keywords: *Pisum sativum*; *Glomus mosseae*; *Glomus intraradices*; *Rhizobium leguminosarum*

The endomycorrhizal fungi produce a highly branched hyphal structure within the plant cell. This infection creates an absorptive structure with a very high surface area of transfer for nutrients between the plant and the fungus. Mycorrhizal fungi hyphae secrete acid and alkaline phosphatases (APA and ALP) into the rhizosphere. It was established that APA activity increases in roots growing under P stress (Woolhouse 1975). Therefore, the regulation of these enzymes is critical to a plant's survival in soils with limited P resources (Duff et al. 1991). There is extensive evidence for a decrease in the number of arbuscules under high external P (Bethlenfalvay et al. 1990, Smith and Smith 1996).

Phosphorus has a key role in the energy metabolism of all plant cells, and particularly in nitrogen fixation (Dilworth 1974). It was established that nodulating legumes require more P than legumes growing on mineral nitrogen (Al-Niemi et al. 1997). The AM fungi associated with legumes are an essential link for effective phosphorus nutrition, leading to enhanced nitrogen fixation that in turn promotes root and mycorrhizal growth. Synergistic effect of dual colonization of roots with AM fungi and *Rhizobium* on growth, nutrient uptake and nitrogen fixation in soybean (Bethlenfalvay et al. 1990) and pea (Xavier and Germida 2003) were reported. The effectiveness of the tripartite symbiosis – AM fungi, *Rhizobium* and plant, depends on the competition of the three symbionts for carbon (Jakobsen and Rosendahl 1990). Roots with AM fungi receive about 4–20% more photosynthates than comparable non-mycorrhizal roots (Smith and Reed 1997). Jakobsen and Rosendahl (1990) estimated that AM fungi could use up

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Методи за определяне на генотипове соя, толерантни към воден стрес

Methods for Determination of Tolerant to Water and Temperature Stress Genotypes of Soybean

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Abstract

Two types of pot experiments were carried out: vegetation glass house experiments, supported with two soil moisture regimes: 50% and 80% FC and in climatic chamber under 50% FC soil moisture and air humidity < 40% and air temperature 27-35 °C during the period "flowering- pod formation". The experiments were conducted with 10 genotypes: 5 resistant to water stress (dry resistant - DR) and 5 responsive to irrigation (RI). Prolin and electrolyte content in the plant leaves were determined to evaluate plant water stress.

The experimental results for DR- and RI- genotypes showed that the standard pot experiments in glass house under soil water deficit and those in climatic chamber under soil and air water deficit with parallel determination of prolin and electrolyte content in the plant leaves were one good system for screening of dry tolerant genotypes.

Keywords: water stress, genotypes of soybea, pot experiment

Сушата е един от най-важните фактори, лимитиращи производството на зърно от селскостопанските култури не само у нас, но и в световен мащаб. Усилията на голям брой учени понастоящем са насочени както към изучаване на физиологичната адаптация на растенията към воден стрес, така също и към създаване на ефективни методи за оценка на толерантността им в селекционните програми. Редица автори в чужбина и у нас съобщават за толерантни на суша образци соя [2, 7, 9, 14, 15]. Като сухоустойчиви се определят сортовете Evans u Hodgson от САЩ, украинските - Херсонская 2, Северная 5 [4], а през последните години и българските сортове Павликени 121 и Даниела 97 [7]. Направеният скрининг на 300 генотипове соя от Carter et al. [11] е единствен по рода си, въз основа на който се казва, че 416937(PI) е уникален по неговата възможност да задържа вода по време на засушаване във фаза наливане на зърното.

Bates et al. [10], Hanson et al. [13] определят степента на сухоустойчивост при растенията въз основа на концентрацията на натрупания пролин. По количествените изменения на съдържанието му в листата на растенията, страдащи от воден стрес, е разработен метод за определяне на сухоустойчивостта – "пролинов

EFFECTS OF COMBINED INOCULATION OF PEA PLANTS WITH ARBUSCULAR MYCORRHIZAL FUNGI AND *RHIZOBIUM* ON NODULE FORMATION AND NITROGEN FIXING ACTIVITY

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Summary. The response of pea (*Pisum sativum* cv. Avola) to arbuscular micorrizal fungi (AM) species *Glomus mosseae* and *Glomus intraradices* and *Rhizobium leguminosarum* bv. *Viciae*, strain D293 regarding growth, nodulation and nitrogen fixing activity was studied. Pea plants (*Pisum sativum* cv. Avola) were grown in a glasshouse until flowering stage (35 days) in 4 kg plastic pots using leached cinnamonic forest soil at low phosphorus level (60mg P_2O_5 kg $soil^{-1}$). The obtained results demonstrated that dual inoculation of pea plants increased plant biomass, nodulation parameters, N_2 fixation activity at varying levels compared to plants submitted to single inoculation with *Rhizobium leguminosarum*, strain D293, and depended on AM fungi species. Coinoculation increased significantly total P content in plant tissues and percentage of root colonization. Coinoculation efficiency of *Rhizobium* bacteria and *Glomus mosseae* was higher compared with *Glomus intraradices* regarding biological N_2 fixation and AM colonization at the tested P concentration.

Keywords: *Glomus intraradices, Glomus mosseae, Pisum sativum, Rhizobium leguminusarum*

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RESPONSE OF INOCULATED PEA PLANTS (*PISUM SATIVUM L.*) TO ROOT AND FOLIAR FERTILIZER APPLICATION WITH REDUCED MOLYBDENUM CONCENTRATION

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Summary. The effects of foliar absorbed nutrients on root processes related to assimilation of nitrogen under presence or absence of molybdenum (Mo) were studied. Pea plants (*Pisum sativum* L.), var. Avola were grown until the 15th day in a glasshouse on liquid nutrient solution both without and with Mo added to the media. Plants were inoculated with bacterial suspension of *Rhizobuium leguminosarum* bv. *Viciae* strain D 293 at approximately 10^8 cells per cm^3. Application of a liquid foliar fertilizer at a concentration of 0.3% was performed twice a week by spraying under high pressure. The effects of foliar supplied nutrients in addition to root nutrition on dry biomass accumulation, protein content, root nodulation and activities of the enzymes of primary nitrogen assimilation (nitrate reductase (NR – NADH, EC 1.6.6.1), and glutamine synthetase (GS: EC 6.3.1.2) during the early stage of nodulation were studied. Foliar application of nutrients had a positive effect on the activities of NR and GS enzymes in shoots of Mo-supplied plants. It was found that foliar application of nutrients reduced the inhibitory effect of Mo shortage on root nodulation, plant dry biomass and protein content. The negative influence of Mo exclusion from the nutrient media on biomass accumulation and nodule formation was diminished through the foliar absorbed nutrients.

Keywords: pea *(Pisum sativum L),* foliar fertilizer, nitrate reductase, glutamine synthetase, molybdenum.

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RESPONSE OF INOCULATED FOLIAR FED PEA PLANTS (*PISUM SATIVUM* L.) TO REDUCED MO SUPPLY

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The application of nutrients to the roots and leaves of inoculated pea plants grown under conditions of reduced Mo supply was studied. Pea plants *(Pisum sativum L.)* were grown on liquid nutrient solution excluding Mo from the media until the 35th day under glasshouse conditions. Plants were inoculated with the bacterial suspension of *Rhizobium leguminosarum Bv. Vicae,* strain D293 at approximately 10^8 cells per cm^3. The foliar fertilizer Agroleaf® was applied at 0.3% concentration. Changes in the root nodulation and the activities of the enzymes connected with nitrogen assimilation pathway (nitrate reductase – NR-NADH: EC 1.6.6.1; glutamine synthetase – GS: EC 6.3.1.2; glutamate synthase – NADH-GOGAT: EC 1.4.1.14 and nitrogenase – NG: EC 1.7.99.2) were observed. It was established that the foliar application of nutrients reduced the inhibitory effect on the root nodulation and nitrogen assimilatory enzyme activities due to the Mo shortage.

Keywords: pea – foliar fertilizer – nitrogen assimilation – molybdenum

INTRODUCTION

The most important highly regulated enzymes of N metabolism in higher plants are nitrate reductase (NR), glutamine synthetase (GS) and glutamate synthase (GOGAT) [18]. In legumes ammonia can be formed by the direct fixation of atmospheric dinitrogen atoms within root nodules [12]. With the addition of nitrates to the plant tissues ammonia is generated by the concerted reactions of nitrate reductase and nitrite reductase. It is established that the major pathway for ammonia incorporation into non-toxic organic compounds occurs through GS-GOGAT cycle [9, 16].

Changing the site of primary N assimilation can affect the activities of some key enzymes of nitrogen metabolism in the roots and shoots. Foliar application of nutri-

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Nitrogen Assimilatory Enzymes and Amino Acid Content in Inoculated Foliar Fertilized Pea Plants Grown at Reduced Molybdenum Concentration

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ABSTRACT

A possibility to improve nitrogen assimilation in nitrogen fixing Molybdenum (Mo) deficient pea plants was shown. The influence of foliar supplied nutrients in addition to root nutrition resulted in reducing the unfavorable effects of inorganic nitrogen on nodule function and Mo deficiency on the nitrogen assimilatory enzymes. Inoculated pea plants were grown on liquid nutrient solution both with and without Mo. The following variants were tested: Mo supplied plants with root nutrition (F1 + Mo); Mo supplied plants with root and foliar nutrition (F2 + Mo); Mo deficient plants with root nutrition (F1 − Mo); and Mo deficient plants with root and foliar nutrition (F2 − Mo). Foliar application of nutrients had a positive effect on the glutamine synthetase and glutamate synthase enzyme activities in the roots and nodules of Mo deficient plants. It was found that the foliar fertilization reduced the inhibitory effect of Mo shortage on the aspartate/asparagine content in the pea shoots.

Keywords: amino acids, foliar fertilizer, glutamate synthase, glutamine synthetase, molybdenum, pea *(Pisum sativum L)*

INTRODUCTION

Amino acids in plant tissues are determined by complex interplay of factors, which may be influenced by nutrition, developmental stage and by

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ОЦЕНКА НА ЗЕМИТЕ И УПРАВЛЕНИЕ НА ПОЧВЕНОТО ПЛОДОРОДИЕ

CONTENT OF NITROGENOUS FORMS AND AMINO ACIDS IN HEAD CABBAGE – CHANGES AS A RESULT OF NITROGEN FERTILIZATION

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Introduction

Nitrogen is very important element for plant growth and development and plays a key role in the synthesis of amino acids, proteins, pigments, hormones and DNA. Plants received nitrogen from the soil rather as NO_3-N than as NH_4^+-N. Forms and amounts of applied fertilizers influence to a great extent on the yield and quality of vegetable plants. Vegetable plants response positively to organic and mineral fertilization. The head cabbage with its chemical composition, taste and nutritional value appeared to be among the most valuable vegetables and it is a good, dietary food (Alipieva, 1986). Cabbage is a good source for carbohydrates (mainly sugars), vitamins, minerals, amino acids and other biologically active substances (Huxsoll et al., 1989). The head cabbage is a crop with high nutrient requirements to the nitrogen as well as potassium and phosphorus because it accumulates large vegetative biomass for a relatively short period. The right proportion between the nitrogen and other nutrients leads to the ballanced mineral nutrition. Unbalanced nitrogen application resulted in quality deterioration. The content of amino acids is a good indicator for the quality of vegetables. In most non-leguminous crop plants, the dominant organ in amino acid synthesis and distribution is the leaf (Noctor and al., 2002). Leaf amino acid content increase with enhanced supply of nitrogen during growth. In plants, fluctuations in the amino acid proportion might reflect changes in the source of nitrogen for growth (Peoples et al., 1987). The composition of amino acids is genetically determined, but the content varied in dependence on several factors as fertilization and different soil – climatic conditions (Shmatko et al., 1986). Some authors established significant changes in amino acid composition as a result of sources and rates of nitrogen fertilization (Atanasova and Bubarova, 1990, Alehina, 1992).

The aim of our study is to establish relationship between different nitrogenous forms (protein and non-protein N) and the content of amino acids synthesized in head cabbage leaves after treatment with different forms and amounts of nitrogen fertilizers.

Material and methods

Head cabbage plants, hybrid Orion F_1 are grown in 3 kg plastic pots under glasshouse conditions on an alluvial meadow soil from Chepinci – Sofia region until head formation stage. All treatments were carried out in four replications. Nitrogen was applied as ammonium nitrate and calcium nitrate in the following increased rates: 250, 500, 750 and 1000 $mg.kg^{-1}$ soil on the background of the control without fertilization. Potassium and phosphorus were applied at the rate $400mg.kg^{-1}$soil. Initial accessible NPK reserves are as follow: nitrogen (NH_4^+-N - 8.9 $mg.1000g^{-1}$ soil, NO_3- N – 26.5 $mg.1000g^{-1}$ soil), P_2O_5 - 16.6, K_2O – $25.5mg.100g^{-1}$ soil. Exchangeable Al is zero, i.e. no exchangeable acidity in this soil type was found. At cabbage harvest the following parameters were measured: total nitrogen – spectrofotometrically after Kjeldal digestion, protein nitrogen – after Bernstain, crude protein = protein nitrogen x 6.25. Total amino acid content (free and binded into proteins) was determined after preliminary acid hydrolysis with 6n HCl by the aminoanalytic chromatograph "Hromaspect" according to the method of absorption gromatography.

Data are expressed as means ± standard error where n=4. Comparison of means were performed by the Fisher LSD test ($P \leq 0.05$) after performing multifactor ANOVA analysis.

Results and Discussion

Total nitrogen content varied between 1.17% in the control variants and 3.22% in the variants with the highest rate of fertilization with NH_4NO_3 (table 1). The total N content increased with the increase of

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ФИЗИОЛОГИЧЕСКАЯ РОЛЬ НЕКОТОРЫХ МИНЕРАЛЬНЫХ ЭЛЕМЕНТОВ В ОБРАЗОВАНИИ КЛУБЕНЬКОВ И ФИКСАЦИИ АТМОСФЕРНОГО АЗОТА У БОБОВЫХ РАСТЕНИЙ

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Исследовано влияние дефицита и повышенного содержания фосфора (P), бора (B) и молибдена (Mo) на образование клубеньков и активность фиксации азота у гороха и сои. Установлена непосредственная связь между содержанием фосфора и бора в питательной среде и количеством специфических флавоноидов в корневых эксудатах, играющих роль индукторов для образования клубеньков у бобовых. Отрицательное влияние отсутствия молибдена в питательной среде на образование клубеньков, активность азотфиксации и содержание аминокислот в растительных тканях можно избежать при введении питательных элементов через листья.

Симбиотическая азотфиксация включает комплекс взаимодействий бобовых растений с почвенными бактериями 5 различных родов: *Azorhizobium, Bradyrhizobium, Mesorhizibium, Sinorhizobium и Rhizobium*. В результате симбиотических взаимодействий образуется новый орган — клубеньки. Этапы образования клубеньков очень чувствительны к уровню минеральных элементов [1] как в условиях недостатка [17], так и их избытка [13].

После азота фосфор является одним из основных биогенных элементов, регулирующих рост и развитие растений. Фосфор оказывает влияние на симбиотическую фиксацию азота, так как стимулирует рост растения-хозяина, а также образование нормально функционирующих клубеньков [8]. Фосфорный дефицит оказывает влияние на процессы инфицирования, поскольку задерживает выделение специфических флавоноидов в корневых эксудатах, играющих роль индукторов при образовании клубеньков, а также прикрепление бактерий к корневым волоскам [12]. Бор в структуре клеточной стенки [7], фенолпропаноидном пути [15] и фитогормональном балансе [10] играет существенную роль в симбиотических отношениях между азотфиксирующими бактериями и растением-хозяином. Молибден является важным компонентом кофакторов ферментов, катализирующих восстановление или фиксацию азота [14]. При внесении восстановленного или органического азота потребность в молибдене уменьшается или исчезает вовсе. Одним из способов, позволяющим избегнуть подавляющий эффект неорганического азота на образование клубеньков [3], является подача минеральных элементов через листья. Листовая подкормка стимулирует выделение сахаров в корневых эксудатах, что, в свою очередь, положительно влияет на развитие клубеньков [9]. В настоящее время проведено недостаточно исследований влияния P, B и Mo на образование клубеньков и фиксацию атмосферного азота у бобовых.

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Effect of different fertilizer sources on the qulaity of head cabbage

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Abstract

The influence of different fertilizer sources on head cabbage (*Brassica oleracea* var. *capitata*, cv. 'Pructor') yield and quality was studied. The field experiment was carried out on alluvial - meadow soil (*fluvisol –FAO*) pH 6.5. The trial included mineral fertilizer, farmyard manure and foliar fertilizer. The highest yield values were obtained with mineral fertilization. The best quality parameters in the cabbage leaves – dry weight, total soluble sugars, cellulose, vitamin C and nitrates content were obtained in the treatments with foliar fertilization followed by the treatments with organic fertilization. The observed decrease of N and K residuals after the harvest of head cabbage crop in comparison with the initial soil reserves indicated complete absorption of fertilizers supplied and this is a very important result from ecological point of view.

Key words: Head cabbage, quality parameters, foliar fertilization, fertilizer application

Introduction

Forms and amounts of applied fertilizers influence the yield and quality of vegetable plants to a great extent (Sidiras *et al.*, 1999). Vegetable plants response positively to organic and mineral fertilization. According to some reports (Kovacheva *et al.*, 1999; Panayotov, 2001) these crops also respond positively to foliar feeding. The head cabbage is considered among the most valuable vegetables becuase of its chemical composition, taste and nutritional value (Alipieva, 1986). Cabbage is a good source for carbohydrates (mainly sugars), vitamins, minerals, amino acids and other biologically active substances (Huxsoll *et al.*, 1989). Vitamin C content is very high in cabbage leaves and especially varieties appropriate for storage are rich in vitamin C (Volodina, 1987). The head cabbage is a crop with high nutrient requirements to the nitrogen as well as potassium and phosphorus because it accumulates large vegetative biomass in a relatively short period. The right proportion between the nitrogen and other nutrients leads to the ballanced mineral nutrition. Unbalanced nitrogen application resulted in quality deterioration - reduction of carbohydrates and accumulation of nitrates were observed, cabbage turned sleazy and disposed to bursting (Soyergin *et al.*,1999; Rozek *et al.*, 2001).

The aim of the present study was to investigate the effect of different fertilizer sources (mineral, organic and foliar fertilizers) on the yield and quality parameters of head cabbage.

Material and methods

White head cabbage (*Brassica oleracea* var. *capitata* cv. 'Pructor') plants were grown in a field experiment on alluvial – meadow soil (*fluvisol – FAO*) with pH 6.5 and low content of total nitrogen and humus – 0.052 and 0.70%, respectively. The alluvial – meadow soil is distinguished with relative density 2.53- 2.71 and volume density within the range 1.54-1.66g cm^{-3}.

Soil samples were collected both before the planting and after the harvest of the cabbage. The arable horizon (0-30 cm) had the following agrochemical characteristics: NH_4^+ - N = 12.35 mg kg^{-1} soil, N-NO_3^- =18.53 mg kg^{-1} soil. The content of movable P and K forms were P_2O_5 = 63 mg kg^{-1} and K_2O = 265 mg kg^{-1} soil. Soil samples have been collected for determination of soil mineral nitrogen (spectrophotometrically after Kjeldal digestion), phosphorus and potassium after acetate - lactate method (Ivanov, 1984).

A randomized block design with 4 replicates was used at plant density 33 x 10^3 plants per hectare. Each experimental plot was 40 m^2 in area and consisted of 12 rows (11 plants in row). Plants were grown at optimal fertilizer rates previously determined in model pot experiments (Mitova *et al.*, 2005). The following treatments were tested:

B_1- Control, without fertilization.

B_2- Mineral nitrogen, applied as NH_4NO_3, 150 kg ha^{-1} active substance, P as one time application as a triple super phosphate and K applied as a K_2SO_4 (100 kg ha^{-1} P_2O_5 and 100kg ha^{-1} K_2O, respectively). For the nitrogen and potassium fertilizers split application was performed: for NH_4NO_3 2/3 as a base fertilization +1/3 as a dressing and for K_2SO_4 1/2+1/2, , respectively.

B_3- Farmyard manure –24t ha^{-1}. (The composition of the farmyard manure was: total N – 0.64%, total P_2O_5 – 1.84% and K_2O - 0.51%.). The farmyard manure fertilized rate was calculated to be relevant to the NPK content in the applied mineral fertilizers.

B_4- Foliar fertilizer Agroleaf (Agroleaf total) from Scotts company, Ohio, USA and distributed by VLADI Company in Bulgaria. Agroleaf distinguishes with high purity, N:P:K –20:20:20 + all important microelements. Agroleaf chemical characteristics have been described in details in our previous study (Stancheva *et al.*, 2004).

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Effect of Soil Fertilizer, Foliar Fertilizer, and Growth Regulator Application on Milk Thistle Development, Seed Yield, and Silymarin Content

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Abstract: An important consideration for milk thistle (*Silybum marianum L.*) cultivation is regulating development to lengthen the reproductive stage and increase seed yield with high silymarin content. The treatment of milk thistle with foliar fertilizers and growth regulators—thidiazuron (Dropp®), 2,3,5-triiodobenzoic acid (Tiba®), mepiquat chloride (Pix®), and prohexadione-Ca (Regalis®)—resulted in an increase in the proportion of mature flower heads. Highest seed yield was observed in plants treated with Pix® and mineral soil fertilization, whereas in plants treated with foliar fertilizers, highest yields were observed with Pix® and Regalis®. The highest content of silymarin was found in plants treated with Dropp® and foliar fertilizer. Generally, treatment of milk thistle with plant-growth regulators in combination with soil or foliar mineral fertilizers increased the total amount of silymarin by increasing seed yield per hectare.

Keywords: Dropp®, Pix®, Regalis®, *Silybum marianum L.* silymarin, Tiba®

INTRODUCTION

Milk thistle plants produce seeds that are an important source of silymarin, which is used in modern pharmaceutical industries. Silymarin is a group of

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PROCEEDINGS

Influence of foliar fertilization and growth effector 5-tert-butyl-*N*-m-tolylpyrazine-2-carboxamide on the milk thistle (*Silybum marianum* L.) seed yield and quality

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ABSTRACT

The aim of this study was to evaluate the effects of foliar fertilization and a growth effector 5-tert-butyl-*N*-m-tolylpyrazine-2-carboxamide (MD148/II) on the growth, seed yield and silymarin content of field grown milk thistle (*Silybum marianum* L. Gaertn.) plants. Foliar fertilizer Agroleaf^{R} was applied at different plant developmental stages, with different proportions of N, P, K. Combined application of foliar fertilizer with MD148/II in concentration 1.10^{-3} M affected most positively growth, number of plant lateral shoots and flower heads per plant. Treatments with foliar fertilizer and MD148/II resulted in improvement of whole plant biomass, and seed yield and quality. The increase of seed yield per unit area was a result of the flowering rate improvement and flower head setting per plant. Dry seeds contained more total flavonoids and silymarin compounds. Analysis of the fatty acid composition of milk thistle seed oil showed some increase of the fraction of saturated over the polyunsaturated fatty acids and the content of high molecular fatty acids declined.

Keywords: milk thistle (*Silybum marianum* L. Gaertn.), 5-tert-butyl-*N*-m-tolylpyrazine-2-carboxamide, foliar fertilization, seed yield, silymarin, fatty acids

INTRODUCTION

Silymarin, derived from the seeds of milk thistle plant (*Silybum marianum* L. Gaertn.) has been used widely for the treatment of toxic liver damage (Dewick 1998). The dried seeds contain 1- 4% of silymarin flavonoids. Silymarin primarily consists of an isomeric mixture of six phenolic compounds: silydianin, silychristin, diastereoisomers of silybin (silybin A and B), and diastereoisomers of isosilybin (isosilybin A and B) (Lee et al., 2007). In our previous field experiments (Geneva et al. 2008) it was found that the treatment of milk thistle plants with thidiazuron and foliar fertilizer resulted in higher silymarin content in the seeds. Although the effects of growth regulators on the growth and development of plants are well stated, their applicability as plant secondary metabolite effectors is still unknown. Recently Dolezal et al., (2002, 2007), synthesized and tested biological activity of amides derived from substituted pyrazinecarboxylic acid. Additionaly, Tumova et al, (2005) found that the newly synthesized compound 5-tert-butyl-*N*-m-tolylpyrazine-2-carboxamide (MD148/II, Figure 1) increased flavonolignan production milk thistle suspension culture.

On the basis of the results of the previous studies regarding biological activity of the substituted pyrazine -2- carboxamides in a milk thistle callus culture we carried out experiments aiming to evaluate the combined effects of abiotic elicitor MD 148/II (3-methylphenylamide 5-tert-butylpyrazine-2-carboxylic acid) and foliar fertilization on seed yield, flowering rate, silymarin content and composition of fat in the seeds of milk thistle grown in field experiments.

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EFFECTS OF FORE-CROP FERTILIZATION ON THE YIELD AND QUALITY OF KIDNEY BEANS UNDER VEGETABLE CROP ROTATION CONDITIONS

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Kidney beans (*Phaseolus vulgaris* (L.) Savi.) ssp Nanus cv. Lodi were grown as a mid-early second crop after lettuce on an alluvial meadow soil with $pH_{(H2O)}$ = 6.4 and a plant density of 22 plants per m^2. The bean crop was grown without fertilization, but the lettuce crop was treated with mineral, organic or foliar fertilization. The application of different fertilizer sources to the fore-crop resulted in high yields of the subsequent bean crop, with good quality parameters and without polluting the arable soil layer with fertilizer residues. Foliar fertilization applied to the fore-crop resulted in a lower accumulation of nitrates in fresh beans in comparison with the other fertilizer sources. Organic fertilization supplied during the cultivation of the previous crop had a stronger effect than mineral fertilization on certain quality parameters of the kidney bean plants, such as dry matter, vitamin C content, and the accumulation of nitrates.

Key words: kidney beans, lettuce, fertilizer sources, crop rotation, quality parameters

Introduction

Several factors are responsible for the quality of fresh vegetable produce. Among these factors, fertilizer forms and rates play an important role in ensuring an optimal nutritional regime for successful plant development and in influencing the yield and quality of vegetables (Sidiras et al., 1999). Horticulture may result in the accumulation of nitrogen fertilizer residues in the root zone after the crop harvest. Eddy (2000) and Stancheva et al. (2004) reported on the effects of foliar fertilization on the duration of the plant growth period and on improvements in fruit quantity and quality. Reducing the supply of fertilizers and crop cultivation after a fore-crop is also a promising way to improve the quality of vegetables. When lettuce was grown as a third crop in the rotation, the maximum inclusion of nitrogen in the nutrient cycle was observed and the nitrogen content in the soil was restored to close to its background values (Stoicheva et al., 2002).

RESPONSE OF ALFALFA (*MEDICAGO SATIVA* L) GROWTH AT LOW ACCESSIBLE PHOSPHORUS SOURCE TO THE DUAL INOCULATION WITH MYCORRHIZAL FUNGI AND NITROGEN FIXING BACTERIA

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Summary. The study evaluated the response of alfalfa (*Medicago sativa* L) to arbuscular mycorrhizal fungi (AM) species *Glomus intraradices* and *Sinorhizobium meliloti*, strain 1021 regarding the dry biomass accumulation, mycorrhizal fungi colonization, nodulation and nitrogen fixation activity. Alfalfa plants were grown in a glasshouse until the flowering stage (58 days), in 4 kg plastic pots using leached cinnamonic forest soil (Chromic luvisols – FAO) at P levels 42 mg P_2O_5 kg^{-1} soil (applied as 133 mg kg $soil^{-1}$ tunisian phosphorite). The obtained results demonstrated that the dual inoculation of alfalfa plants significantly increased the percent of root colonization and acid phosphatase activities in the root tissue and in the soil in comparison with a single inoculation with *Glomus intraradices*. Coinoculation also significantly increased the total phosphorus and nitrogen content in plant tissues. Under conditions of dual inoculation high nitrogenase activity was established, especially in the floral budding stage as compared to the single inoculation

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EFFECTS OF *SINORHIZOBIUM MELILOTI* STRAINS (1021 AND NitR) ON NITROGEN ASSIMILATION OF ALFALFA PLANTS UNDER CONDITIONS OF MINERAL ELEMENTS SHORTAGE

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Summary. Two *Sinorhizobium meliloti* strains (1021 and NitR) were used for inoculation of alfalfa plants to study nitrogen assimilation under nutrient deficiency conditions in hydroponics experiments. The wild type *Sinorhizobium meliloti* 1021 was compared with a mutant strain – *S. meliloti NitR*. NitR protein was found to be a regulator of *S. meliloti hmgA* expression under nitrogen deprivation conditions, suggesting the presence of a *ntr*-independent nitrogen deprivation regulatory system. *nitR* insertion mutations were shown not to affect bacterial growth, nodulation of *Medicago sativa* plants, or symbiotic nitrogen fixation under the physiological conditions examined. The relationship between free living and symbiotic bacterial forms was revealed indirectly by the changes of nitrogen fixation and assimilation under conditions of nutrient deficiencies. Before seeds inoculation, bacteria were cultivated in Vincent minimal media with limited nitrogen source. The alfalfa plants were grown on a nutrient solution in the presence or absence of molybdenum. The differences between the two symbiotic systems were established by the variations of nodule formation and enzyme activities participated in nitrogen fixation and

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assimilation (nitrogenase – NG: EC 1.7.99.2; glutamine synthetase – GS: EC 6.3.1.2; glutamate synthase – NADH-GOGAT: EC 1.4.1.14 and nitrate reductase – NR-NADH: EC 1.6.6.1). Negative effects of Mo shortage on the rate of nitrogen fixation and nitrate reduction in both symbiotic systems were found. When plants were inoculated with strain *S. meliloti NitR* and grown under nitrogen limiting conditions, the highest stability of nitrogen fixation and nitrogen reduction was determined in conformity with characterization of this mutant strain.

Key words: alfalfa; strain effect; nitrogen assimilation, mineral elements shortage. VA multifactor analysis.

INTRODUCTION

The role of biological nitrogen fixation is well-known for a long time as a nonpolluting and cost-effective way to improve soil fertility. Rhizobia, such as *S. meliloti*, must be able to persist and compete for scarce nutrients in the bulk soil, compete for colonization of the rhizosphere and plant infection, and adapt their metabolism to the nutritionally more favorable, distinct conditions within the plant cells in the nodule. These three different modes of existence exemplify the need for a high degree of physiological adaptability, specific genetic mechanisms to sense changes in environmental conditions, and the ability to respond rapidly. These characteristics led to a search for *S. meliloti* genes specifically expressed under nutrient limitation conditions (Milcamps et al., 2001). Recently, a gene, named *nitR,* involved in direct or indirect regulation of *hmgA* gene expression in response to various stresses, including starvation, have been identified in *S. meliloti* (Davey and de Bruijn, 2000; Milcamps *et al.*, 2001). *hmgA* gene expression, is involved in the degradation of tyrosine as an alternative nitrogen source (Milcamps, A., and F. de Bruijn. 1999). The mutant strain referred to as NitR is with reduced expression of *hmgA* under nitrogen deprivation conditions.

It is well known that legumes possess a systemic regulatory control able to detect the presence of combined nitrogen in the rhizosphere and

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EFFECT OF FOLIAR FEEDING ON NITROGEN ASSIMILATION IN ALFALFA PLANTS AT INSUFFICIENT MOLYBDENUM SUPPLY

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The influence of foliar feeding on the nitrogen assimilation in alfalfa plants under conditions of Mo shortage was studied. It was established that foliar fertilization with 0.3% solution of Agroleaf® resulted in increase of nitrogen fixation and nitrogen assimilation in the absence of Mo. Insufficient molybdenum supply leads to significant reduction of plant Mo content and nitrogen-fixing activity, while stress induced amino acids as alanine, GABA, threonine, proline and serine increased repeatedly. The negative effect of Mo deficiency on the enzyme activities related to the primary nitrogen assimilation (NR, GS, GOGAT) and plant growth diminished due to the foliar absorbed nutrients.

Keywords: Alfalfa – Mo deficiency – foliar feeding – nitrogen assimilation – nitrogen fixation

INTRODUCTION

Molybdenum (Mo) is among the micronutrients that are very essential for the plant growth and is required in small amounts. The symptoms associated with Mo deficiency are closely related to nitrogen metabolism [6] and nitrogen assimilatory processes in plants tissues strongly depend on the plant Mo levels [13]. Mo is an important constituent of several enzymes catalyzing different chains of nitrogen metabolism: nitrate reductase (EC 1.6.6.1), nitrogenase (EC 1.18.6.1), xanthine dehydrogenase (EC 1.1.1.204) [17]. Loss of Mo-dependent activity (directly or indirectly through low internal Mo levels) impacts nitrogen metabolism and eventually plant development. Otherwise, it might be expected that when plants are supplied

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GROWTH AND NITROGEN FIXATION OF DIFFERENT *MEDICAGO SATIVA-SINORHIZOBIUM MELILOTI* ASSOCIATIONS UNDER CONDITIONS OF MINERAL ELEMENTS SHORTAGE

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ABSTRACT

Sinorhizobium meliloti is an α-proteobacteria of the family Rhizobiaceae that alternates between a free-living phase in soil and a symbiotic phase within the host plant cells, where the bacteria ultimately differentiate into nitrogen-fixing organelle-like cells, called bacteroids. The present study was designed to compare the difference in responses to nitrogen and carbon limitation in free living Sinorhizobium meliloti strains and their ability to form symbiotic association with alfalfa (Medicago sativa) plants. The effectiveness of observed symbiotic associations was evaluated by number of formed nodules, nitrogen fixing activity, and plant biomass production in control environmental conditions of a growth chamber. A wild type strain Sinorhizobium meliloti 1021 and two mutants - Sinorhizobium meliloti NitR and Sinorhizobium meliloti TspO were used in this research. Both mutant strains were previously created to study the general stress response in Sinorhizobium meliloti and its regulation mechanisms. The products of the genes named tspO and nitR, act either as direct or indirect regulators of gene expression in response to various stresses, including starvation. The up- and down regulated genes under conditions of nitrogen and carbon limitation were identified in free living forms of the three strains. Comparison of the genes differentially expressed in the wild type strain to those found in the tspO and nitR mutants showed no significant difference. In both starvation conditions the most effective symbiotic system was established between alfalfa and Sinorhizobium meliloti TspO, concerning nitrogen fixing capacity and plant biomass production.

Keywords: nitrogen fixation, starvation, strain effect

Introduction

The soil bacterium *Sinorhizobium meliloti* exist either as a free-living form or in symbiosis within nodules of alfalfa *(Medicago sativa*) plants, where nitrogen is fixed by the bacteria and released to the plant in exchange for photosynthates. Both in the soil and *in planta*, rhizobia are subject to stress and they developed specific genetic mechanisms to sense and respond to various stresses, such as nitrogen and carbon starvation, oxygen limitation etc (29). Some *Sinorhizobium* strains are more tolerant towards environmental stresses, whereas others are unable to develop an effective symbiosis under these conditions. Above-mentioned facts led to a search for *S. meliloti* genes specifically expressed under nutrient limitation conditions and creating of new strains overcoming the negative influence of the environment factors with successful nitrogen-fixation induction in symbiotic conditions. Both mutant strains used in this work were previously created to study the general stress response in *Sinorhizobium meliloti* and its regulation mechanisms. A gene, named *nitR* (member of the *arsA* family of regulatory genes), involved in direct or indirect regulation of *hmgA* gene expression in response to various stresses, including starvation, have been identified in *S. meliloti* (16, 17). The expression of *hmgA* is involved in the degradation of tyrosine as an alternative nitrogen source (18). The mutant strain referred to as NitR is with reduced expression of *hmgA* under nitrogen deprivation conditions. Identification of nutrient deprivation-induced locus in *Sinorhizobium meliloti* strain 1021 (*ndiAB*) by use of a *Tn5-luxAB* reporter gene transposon was reported by Milcamps et al (16) and Davey and de Bruijn (5). The expression of the *ndi* locus was found to be induced by carbon and nitrogen deprivation, osmotic stress, and oxygen limitation and during entry into stationary phase. A double-mutant strain was

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RESEARCH PAPERS

Antioxidant Capacity of Sage Grown on Heavy Metal-Polluted Soil[1]

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Abstract—Oxidative stress response and essential oil composition of sage (*Salvia officinalis* L.), grown on industrially polluted soil were studied. Sage plants were grown on the soil polluted with Cd, Cu, Pb, Zn, and non-polluted control soil. One-year-old sage possessed a high potential for heavy metal accumulation mainly in the roots. Heavy metal pollution resulted in root and shoot dry biomass inhibition. The increased levels of hydrogen peroxide and MDA showed that the heavy metal uptake caused oxidative stress. The increase towards the control was observed in the levels of glutathione, ascorbate, dehydroascorbate, catalase, dehydroascorbate reductase, and glutathione peroxidase. Weak activities of the most enzymes of the ascorbate–glutathione cycle allowed to suppose that H_2O_2 neutralization is rather non-enzymatic than enzymatic process. Observed decline in α- and β-thujones and elevated camphor content in the sage leaves did not indicate a deterioration of the essential oil quality. Sage grown on heavy metal-polluted soil successfully accumulated cadmium, lead, and zinc, which is resulted in plant biomass inhibition, but essential oil yield and quality was not declined.

Keywords: Salvia officinalis, heavy metals, antioxidants metabolites, antioxidant enzymes, essential oils.

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INTRODUCTION

Medicinal properties of the Lamiacea plants have been used since antique times for their healing properties. Garden sage (*Salvia officinalis* L.) essential oil is applied in the treatment of a large range of diseases, such as those of the nervous system, heart, blood circulation, and respiratory [1].

Some essential aromatic and medicinal plants are capable to accumulate heavy metals from contaminated soils [2]. All heavy metals, both essential (Cu, Zn) and non-essential (Cd, Pb) can cause toxic effects to plants and humans if found in high concentrations. In the field experiments conducted in the vicinities of the Non-Ferrous Metals Combine near Plovdiv. Zheljazkov et al. [2] reported about the reduction of essential oil yields of several medicinal plants and distribution of heavy metal accumulation in the plant organs. This study demonstrated that high concentrations of heavy metals in soil did not result in metal transfer into essential oil.

To combat the metal toxicity of several free radicals, there is a mobilization of the antioxidant reserves in the plant, which react both enzymatically and non-enzymatically with these toxic molecular species, making them less harmful. Non-enzymatic antioxidants include ascorbate (ASC) and glutathione (GSH), and enzymatic antioxidants are superoxide dismutase (SOD), various specific peroxidases, catalase (CAT) and enzymes of the ascorbate–glutathione cycle, as reviewed by Foyer [3]. It is well known that the ascorbate–glutathione cycle plays a vital role in ROS detoxification.

Several reports have indicated the development of oxidative stress under Cd toxicity [4, 5], Cu and Zn toxicity [6], and Pb toxicity [7]. The development of oxidative stress symptoms was judged by the accumulation of H_2O_2 and the oxidative damage to lipids.

In order to improve knowledge regarding the response generated by heavy metal producing oxidative stress, we investigated specific antioxidant protection and essential oil composition of one-year-old

[1] This text was submitted by the authors in English.

Abbreviations: APX—ascorbate peroxidase; ASC—ascorbate; CAT—catalase; DHAR—dehydroascorbate reductase; DHASC—dehydroascorbate; GPO–guaiacol peroxidase; GPX—glutathione peroxidase; GR—glutathione reductase; GSH—reduced gluthatione; GSSG—oxidized gluthatione; GST—glutathione S-transferase; MDHAR—monodehydroascorbate reductase.

Research Article

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Effects of foliar fertilization and arbuscular mycorrhizal colonization on *Salvia officinalis* L. growth, antioxidant capacity, and essential oil composition

Maria P Geneva,* Ira V Stancheva, Madlen M Boychinova, Nadezhda H Mincheva and Petranka A Yonova

Abstract

BACKGROUND: The effect of foliar fertilization and *Glomus intraradices* inoculation on the growth, qualitative and quantitative pattern of essential oil in *Salvia officinalis* was determined. Sage plants were grown in a glass house on a soil/sand mixture (w/w = 3 : 1). Agroleaf® total, N : P : K = 20 : 20 : 20 + microelements, was used at the whole vegetative growth stage as a 0.3% solution. Inoculation with *Glomus intraradices* was done at the sowing stage.

RESULTS: Application of foliar fertilization and/or mycorrhizal colonization improved dry biomass accumulation and increased the content of antioxidant metabolites (ascorbate and reduced glutathione). Applied treatments lowered the activities of the antioxidants enzymes catalase, ascorbate peroxidase and superoxide dismutase, while guaiacol peroxidase increased. The relative quantity of essential oil pattern was also altered as a result of the applied treatments. Combined application (FF+Gi) significantly promoted 1,8-cineole and α-thujone, mycorrhizal colonization enhanced bornyl acetate, 1,8-cineole, α- and β-thujones, while foliar fertilization increased bornyl acetate and camphor. The favorable effect of root colonization by *Glomus intraradices* was determined both on quantitative and qualitative pattern of sage essential oil.

CONCLUSION: We conclude that inoculation with *Glomus intraradices* resulted in improved essential oil yield and quality, while combined application of foliar fertilizer and mycorrhizal fungi predominantly enhanced shoot biomass accumulation.

Keywords: sage (*Salvia officinalis* L.); foliar fertilization; *Glomus intraradices*; antioxidant capacity; essential oil

INTRODUCTION

Essential oils of the plants belonging to the Lamiaceae family possess high biological activities and show antioxidant, antibacterial, antimicrobial and anti-inflammatory activities.[1,2] It is generally accepted that the medicinal properties of this family are due to the secondary metabolites as total phenols (including flavonoids and phenylpropanoids) as well as anthocyanins. There is increasing interest in the use of antioxidants in the food, pharmaceutical, and cosmetic industries. Usage of natural antioxidants as food preservatives is a very appropriate policy for many countries. Among these natural antioxidants, ascorbate, glutathione and vitamin E (tocopherols and tocotrienols) are of significant importance and have been extracted from a number of medicinal plant species.[3] Antioxidants and antioxidant enzymes function to interrupt the cascades of uncontrolled oxidation.[4]

Many species belonging to the Lamiaceae form arbuscular mycorrhizal (AM) associations. In addition to increasing uptake of poorly accessible nutrients[5] or conferring protection against pathogens,[6] AM fungi can also induce changes in the accumulation of secondary metabolites in host plant roots.[7] Relatively little is known about the effects of AM colonization on the accumulation of active compounds in shoots of medicinal plants, which are often the harvest products. However, it was recently reported that *Glomus mosseae* directly increases the essential oil content in shoots of different species of Lamiaceae medicinal plants.[8,9] Endomycorrhizal colonization of *Salvia officinalis* roots can provide an efficient and natural way of improving the growth of these medicinal herbs and increase essential oil yield. To the best of our knowledge there are no reports on variations of the antioxidant capacity and essential oil composition as a result of the foliar feeding with fertilizers containing balanced nutrients.

The aim of the present study was to establish the effects of foliar fertilization and AM fungus (*Glomus intraradices*) inoculation applied separately and in combination on *Salvia officinalis* growth, essential oil composition, level of secondary metabolites and antioxidant capacity.

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INFLUENCE OF FOLIAR FERTILIZATION AND GROWTH REGULATOR ON MILK THISTLE SEED YIELD AND QUALITY

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□ *The effects of foliar fertilization and a growth regulator 5-tert-butyl-*N*-m-tolylpyrazine-2-carboxamide (MD148/II) on the growth, seed yield, and silymarin content of milk thistle (*Silybum marianum *Gaertn.) plants were evaluated. The study was conducted over two years at an experimental field on a slightly acid-leached cinnamonic meadow soil. The MD148/II was applied in the beginning of milk thistle flowering stage. Foliar fertilizer was applied at different plant developmental stages with different proportions of nitrogen (N), phosphorus (P), and potassium (K). Treatments with foliar fertilizer and MD148/II resulted in improvement of plant biomass, number of plant lateral shoots, flowering rate, and seed yield and the content of some active substances in milk thistle seeds. A reduction of high molecular fatty acids was observed. The increase of seed yield was a result of the flower head setting enhancement. Therefore the combined treatment of foliar fertilizer and MD148/II was efficient in elicitation milk thistle production under field conditions.*

Keywords: 5-tert-butyl-*N*-m-tolylpyrazine-2-carboxamide, silymarin, fatty acids

INTRODUCTION

Silymarin, derived from the seeds of milk thistle plant (*Silybum marianum* Gaertn.) has been used widely for the treatment of toxic liver damage (Dewick, 1997). The dried seeds contain 1% to 4% of silymarin flavonoids. Silymarin consist primarily of an isomeric mixture of six phenolic compounds: silydianin, silychristin, diastereoisomers of silybin (silybin A and B), and diastereoisomers of isosilybin (isosilybin A and B) (Guz and Stermitz, 2000; Lee et al., 2007). In field experiments Geneva at al. (2008) reported

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SHORT COMMUNICATION

Growth-Regulating Activity of Three 4-Hydroxycoumarin Derivatives on Inoculated Soybean Plants

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Abstract Three 4-hydroxycoumarin derivatives (ethyl 2-[(4-hydroxy-2-oxo-2*H*-chromen-3-yl)(4-hydroxyphenyl)methyl]-3-oxobutanoate (SS-14), ethyl 2-[(4-hydroxy-2-oxo-2*H*-chromen-3-yl)(3-nitrophenyl)methyl]-3-oxobutanoate (SS-21), and 2-[(3,4,5-trimethoxyphenyl)(4-hydroxy-2-oxo-2*H*-chromen-3-yl)methyl]-3-oxobutanoate (T-2)] were tested for growth-regulating activity on nitrogen-fixing soybean plants in different concentrations: 10^{-5}, 10^{-4}, and 10^{-3} M. They revealed growth-regulating activity in a concentration-dependent manner. The most powerful suppression effect of T-2 on shoot and root fresh and dry biomass accumulation, length of roots, and height of plants was found. Shoot fresh biomass was suppressed in an equal extent at 10^{-3} M of the three compounds but the order of inhibition regarding the three applied concentrations was T2 > SS-14 ≈ SS-21. The compound SS-14 inhibited nodule number and nodule biomass mainly at the highest applied concentration, 10^{-3} M. The highest inhibition of nitrogenase activity was established at the three applied concentrations of the compound SS-14.

Keywords Plant growth inhibition · 4-Hydroxycoumarins · Nitrogenase · Soybean

Introduction

The coumarins are a widely spread group of natural compounds. They have anticoagulant, antibacterial, antiviral (anti-HIV), spasmolytic, and cytotoxic properties (Hayward 1984; Cespedes and others 2006). The influence of different coumarin derivatives (coumarin, 4-hydroxycoumarin, 7-hydroxycoumarin, psoralen, and xanthotoxin) was explored on root tips in onion plants (*Alium cepa*). Segregation of areas of the cytoplasm by sheets of endoplasmic reticulum and invaginations of plasmalemma in the meristematic cells (Podvielkowska and others 1996) was noted. Cytostatic activity of coumarins in plant cells in vitro (Gawron and Glowniak 1987) was discovered. Decreased growth of cells may be caused by lack of energy, and coumarins were found to inhibit and uncouple oxidative phosphorylation (Knypl 1969). Aleksieva and others (1995) showed the inhibiting activity of some synthetic phosphorus-containing coumarin derivatives on the growth of the shoots of pea, wheat, and cucumbers. Most known coumarins possess potent biological properties and strongly influence biosynthetic pathways (Seigler 1997). Coumarins were the most effective against Gram-negative bacteria (Cespedes and others 2006). The role of some synthetic dicoumarols as growth inhibitors on *Mimosa pigra* Linn. was shown by Chavasiri and others (2001). Some of these dicoumarols that contain hydroxy or methoxy groups as substituents on the benzene ring display high herbicidal activity.

The aim of the present study was to evaluate the effects of three 4-hydroxycoumarin derivatives on the plant growth and nodule parameters and nitrogen-fixing activity in soybean plants inoculated with *Bradyrhizobium japonicum*.

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Materials and Methods

Chemicals

The three tested 4-hydroxycoumarin derivatives have been synthesized by Stanchev and others (2008a, b). They are ethyl

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Essential Oil Variation of *Salvia officinalis* Leaves during Vegetation after Treatment with Foliar Fertilizer and Thidiazuron

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The essential oil yield and chemical composition of Salvia officinalis *L. (*Dalmatica *origin) have been analyzed. Leaf samples for essential oil analysis were harvested at different developmental stages after treatment with foliar fertilizer Agroleaf® and foliar fertilizer + thidiazuron. In total, 10 constituents were identified and quantified. The main compounds in the essential oil that increased during the vegetative to the fruiting-set stage are α-thujon and camphor, whereas borneol, viridoflorol, and manool decreased. The effect of thidiazuron applied together with foliar fertilizer was established mainly at the flowering stage, increased essential oil yield by 16% over the control, and positively affected the percentage of β-caryiophylene, α-humulene, viridoflorol, and manool. Application of foliar fertilizer resulted in a greater increase of essential oil yield at the flowering stage in the combined foliar and thidiazuron application over the control. Both treatments decreased camphor at flowering and fruiting stages.*

Keywords Chemical composition, essential oil, *Salvia officinalis* L., thidiazuron

Introduction

Garden sage (*Salvia officinalis* L.) of the family *Lamiaceae* is well known as a common medicinal and aromatic plant and is widely used in food and herbal products (Hohmann et al. 2003). *Salvia officinalis* essential oil is applied in the treatment of a large range of diseases, such as those of the nervous system, heart and blood circulation, and respiratory system (Duke 2001). Good-quality sage oil contains a high percentage (>50%) of the α- and β-thujones and low proportion (<20%) of camphor (Putievsky, Ravid, and Sanderovich 1992). It seemed likely that different developmental stages of *S. officinalis* would have different oil compositions because Mirjalili et al. (2006) reported the essential oil variation of sage aerial parts during its phenological

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Regulation of Nitrogen Assimilation in Foliar Fed Legume Plants at Insufficient Molybdenum Supply

Marieta Hristozkova, Maria Geneva, and Ira Stancheva

Contents

Abstract Formation and function of N_2-fixing systems between bacteria from *Rhizobiaceae* family and legume plants from *Fabaceae* family are especially sensitive to molybdenum (Mo) deficiency. The hypothesis of the present work was that nitrogen fixation and assimilation in Mo deficient pea and alfalfa plants are enhanced when the nutrients were supplied through the foliage. It was established that foliar fertilization resulted in the increase of nitrogen fixation and biomass accumulation in the absence of Mo. The positive effect of foliar fertilization at insufficient Mo supply on the nitrogen uptake is better expressed in garden pea than in alfalfa. Otherwise, alfalfa was more sensitive to Mo starvation than the pea plants. Insufficient Mo supply leads to significant reduction in plant Mo content and nitrogen fixing activity, while stress induced free amino acids increased repeatedly. The negative effect of Mo exclusion from the nutrient media on nitrogen assimilation and biomass accumulation diminished through the foliar absorbed nutrients.

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ORIGINAL ARTICLE

Accumulation of Cd, Pb and Zn in *Tribulus terrestris* L. Grown on Industrially Polluted Soil and Plant Antioxidant Response

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I. Stancheva, M.Geneva, P.Yonova and Yu. Markovska: Accumulation of Cd, Pb and Zn in *Tribulus terrestris* L. Grown on Industrially Polluted Soil and Plant Antioxidant Response

ABSTRACT

Possibilities for phytoaccumulation of heavy metals and antioxidant capacity of puncture vine, grown on industrially polluted with Cd, Pb and Zn soil were studied. Content of Cd, Pb and Zn in the polluted soil exceeded permissible concentrations 3, 4 and 2 times respectively. Puncture vine plants (*Tribulus terrestris* L.), were grown under glasshouse conditions on polluted and non-polluted control soil. Plants grown on heavy metal polluted soil accumulated in the aboveground parts 3.3 times more Cd, 4.3 times more Pb and 2.3 times more Zn, in comparison with the control plants. Heavy metals concentration in plant and soil samples were determined on the inductively – coupled Plasma Mass Spectrometer. Spectrophotometric quantification of ascorbate, reduced glutathione and vitamin E was performed through the formation of phosphomolybdenum complex. Total antioxidant capacity (free radicals scavenging activity) was measured from the bleaching of the purple-colored methanol solution of free stable radical (diphenylpycril-hydrazyl, DPPH$^{\bullet}$) inhibition. All antioxidant enzymes (ascorbate peroxidase, catalase, dehydroascorbate reductase, guaiacol peroxidase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase and monodehydroascorbate reductase) were assayed spectrophotometrically. Puncture vine plants possess good ability to accumulate heavy metals. Plants grown on heavy metal polluted soil accumulated heavy metals in both the shoots and roots. Cd and Pb accumulated more in the roots than in the shoots of plants both from the non - polluted and polluted soil. The observed levels of main contaminates in aboveground parts were 3.3 times more Cd, 4.3 times more Pb and 2.3 times more Zn, in comparison with the control plants. Heavy metals content in the roots of treated plants was 2.5, 2.8 and 1.4 times more than in the controls for Cd, Pb and Zn respectively. The levels of heavy metals accumulation in aboveground parts allowed supposing that *Tribulus terrestris* is a plant that could be used for phytoremediation, more over that higher Cd and Zn levels were found in the plants than in the soil. Absence of biomass reduction indicated that puncture vine plants tolerate the existing concentration level of Cd, Pb and Zn. The antioxidant potential of the puncture vine plants is defined by the content of antioxidant metabolites vitamin E, ascorbate, glutathione and total phenols and antioxidant enzyme activities of glutathione peroxidase, glutathione reductase and dehydroascorbate reductase. From the results we can conclude that soil Cd, Pb and Zn in concentrations far exceeded permissible limit concentrations influenced only a part of antioxidant capacity of *Tribulus terrestris* plants.

Key words: *Tribulus terrestris* L. - heavy metals - antioxidants metabolites – antioxidant enzymes

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(54) МЕТОД ЗА РЕГУЛИРАНЕ ДОБИВА И КАЧЕСТВОТО НА СЕМЕНА ОТНОСНО СЪДЪРЖАНИЕТО НА СИЛИМАРИН И НЕНАСИТЕНИ МАСТНИ КИСЕЛИНИ ПРИ КУЛТИВИРАНО ОТГЛЕЖДАНЕ НА МЕДИЦИНСКОТО РАСТЕНИЕ БЯЛ ТРЪН SILYBUM MARIANUM L.

(57) Изобретението се отнася до метод за регулиране на растежа, развитието и добива от семена и тяхното качество при отглеждане в полски условия на медицинското растение бял трън Silybum marianum L. Като лекарствено растение белият трън се използва заради семената си, в които се съдържат известни количества от биологично активни съединения от групата на флаванолигнаните, известни с общото название силимарин. Силимаринът е група от съединения с близък химичен строеж - силибин, изосилибин, силидианин и силикристин и техни изомери. Извлечен от семената, силимаринът се използва широко във фармацевтичната промишленост за получаване на лекарства със силни антиоксидантни свойства, използвани за лечение на увреждания на черния дроб. Предлага се метод за регулиране на добива от семена и тяхното качество при растението бял трън с помощта на оптимизирано листно минерално хранене и третиране с растежен регулатор. Комбинираното третиране на растенията с цитокининовия растежен регулатор тидиазурон през фаза розетка и листно подхранване с течен тор през вегетацията при различно съотношение на минералните елементи в него води до подобряване цъфтежната динамика, залагане на повече цветоносни стебла с по-големи цветни кошнички, които съдържат по-едри семена. Семената съдържат повече силимарин и ненаситени мастни киселини в липидната фракция след узряване.

2 претенции, 3 фигури

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Antioxidant activity of in vitro propagated *Stevia rebaudiana* Bertoni plants of different origins

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Abstract: An efficient in vitro protocol for propagation of *Stevia rebaudiana* Bertoni is described. Multiple shoots were induced in vitro from shoot tip and nodal segments on Murashige and Skoog medium containing 6-benzylaminopurine, zeatin, or thidiazuron alone and in combination with naphthalene acetic acid or indole-3-acetic acid. A high frequency of shoot induction as well as maximum number of shoots per shoot tip explant was observed on Murashige and Skoog medium supplemented with 6-benzylaminopurine (1.0 mg L^{-1}) alone and combined with indole-3-acetic acid (0.1 mg L^{-1}). For root induction, in vitro shoots were transferred to rooting media containing naphthalene acetic acid, indole-3-acetic acid, or indole-3-butyric acid. The highest rooting frequency and the highest number of roots was observed in half-strength Murashige and Skoog medium supplemented with 0.1 mg L^{-1} indole-3-butyric acid. The rooted in vitro plants were successfully acclimatized in a growth chamber and transferred to the field. Leaf extracts of plants propagated in vitro and adapted to field conditions are characterized by high levels of water-soluble antioxidant capacity (expressed as equivalents of ascorbic acid), phenols, and flavonoids, and therefore by high total antioxidant potential, expressed as DPPH radical scavenging activity.

Key words: Acclimatization, micropropagation, nodal segments, shoot tips

Introduction

Stevia rebaudiana Bertoni, belonging to the family *Asteraceae*, is a perennial sweet herb. It is a native medicinal plant of Paraguay and is a new alternative source of calorie-free sweetener having no carbohydrates. The leaves of this plant produce diterpene glycosides (stevioside and rebaudiosides). Pure stevioside is 30 times sweeter than sugar (1–4). Recently, food-derived antioxidants, such as vitamins and phenolic phytochemicals, have received growing attention because they are known to function as chemopreventive agents against oxidative damage (5). The dry extract from the leaves also contains flavonoids, alkaloids, water-soluble chlorophylls and xanthophylls, hydroxycinnamic acids (caffeic, chlorogenic, etc.), neutral water-soluble oligosaccharides, free sugars, amino acids, lipids, essential oils, and trace elements (6). Plants constitute an important source of active natural products, which differ widely in terms of structure, biological properties, and ways of propagation. Therefore, it is of great interest to evaluate the nonenzymatic antioxidants and the water-soluble and lipid-soluble antioxidant capacities (expressed as equivalents of ascorbate and α-tocopherol), total phenolic compounds, flavonoids, and free radical scavenging activity of *Stevia rebaudiana* Bertoni propagated in different ways. Although phenolic compounds do not have any nutritional function, they may be important to human health because of their antioxidant potential (7). Therefore, the study of the importance and role of nonnutrient

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Morphological evaluation and antioxidant activity of *in vitro*- and *in vivo*-derived *Echinacea purpurea* plants

Research Article

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Abstract: An effective *in vitro* protocol for rapid clonal propagation of *Echinacea purpurea* (L.) Moench through tissue culture was described. The *in vitro* propagation procedure consisted of four stages: 1) an initial stage - obtaining seedlings on Murashige and Skoog (MS) basal medium with 0.1 mg L^{-1} 6-benzylaminopurine, 0.1 mg L^{-1} α-naphthalene acetic acid and 0.2 mg L^{-1} gibberellic acid; 2) a propagation stage - shoot formation on MS medium supplemented with 1 mg L-1 6-benzylaminopurine alone resulted in 9.8 shoots per explant and in combination with 0.1 mg L^{-1} α-naphthalene acetic acid resulted in 16.2 shoots per explant; 3) rooting stage - shoot rooting on half strength MS medium with 0.1 mg L^{-1} indole-3-butyric acid resulted in 90% rooted microplants; 4) *ex vitro* acclimatization of plants. The mix of peat and perlite was the most suitable planting substrate for hardening and ensured high survival frequency of propagated plants. Significant higher levels were observed regarding water-soluble and lipid-soluble antioxidant capacities (expressed as equivalents of ascorbate and α-tocopherol) and total pnenols content in extracts of *Echinaceae* flowers derived from *in vitro* propagated plants and adapted to field conditions in comparison with traditionally cultivated plants.

Keywords: *Purple coneflower • In vitro shoots • Seedlings • Morphological traits • Antioxidant activity*

Abbreviations

MS - Murashige and Skoog medium;
PGR - plant growth regulator;
BAP - 6-benzylaminopurine;
GA_3 - gibberellic acid;
AA - ascorbic acid;
IBA - indole-3-butyric acid;
NAA - α-naphthalene acetic acid.

1. Introduction

Echinacea purpurea L. Moench (Asteraceae) or purple coneflower is a widespread medicinal plant used in diverse range of herbal products. It was proved that Echinacea is one of the most promising immune strengtheners and modulators, with numerous scientific studies and rich clinical evidence in its favor [1-3]. The increasing demand in *E. purpurea* needed development of methods for rapid multiplication of plants and faster introduction of new cultivars with desired traits [4]. However, *E. purpurea* plants produced highly heterozygous progeny in the field [5]. In this regard, *in vitro* tissue cultures are proved to be valuable technique to produce genetically homogeneous plant material. There were identified 58 unique germplasm lines based on screening for antioxidant activity and concentrations of caftaric acid, chlorogenic acid, cichoric acid, cynarin, and echinacoside from clonal propagated seedling-derived plants [6].

Several *in vitro* techniques were developed in *E. purpurea* [3,4,7-9] as some of the genotypes showed high coefficient of *in vitro* propagation [8,10,11]. The application of biotechnological techniques might offer the possibility of producing large amount of uniform high-quality plants in a short period of time and limited space for obtaining a biomass as a source of biological active compounds [3]. Nevertheless, many questions about its *in vivo* and *in vitro* culture remain still unsolved. In Bulgaria, *E. purpurea* is grown on limited area and the information

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AGRONOMIC CHARACTERISTIC AND ANTIOXIDANT ACTIVITY OF AN INTERSPECIFIC HYBRID LINE BETWEEN *HELIANTHUS ANNUUS* AND *HELIANTHUS MOLLIS*

Roumiana Vassilevska-Ivanova, Boris Kraptchev, Ira Stancheva, Maria Geneva

(*Submitted by Academician A. Atanassov on March 23, 2012*)

Abstract

Most of the important agronomic characters of a sunflower cultivar, such as yield, oil content, oil quality, and response to abiotic stress, and also many of the pathogen-resistance traits would benefit from wild *Helianthus* species. The strategy using wide hybridization between cultivated sunflower *Helianthus annuus* and many wild annual and perennial species in the genus *Helianthus* produced introgressed plants with a wide range of variability. The advanced interspecific line *H. annuus* × *H. mollis* represents a successful wide cross between two morphologically dissimilar species. A detailed morphological characteristic and analysis of antioxidant activity was made to illustrate the interspecific origin of the plants. Results have revealed that introgression between cultivated sunflower and wild *H. mollis* has probably occurred. It is believed that the results and interpretations are confident as well as applicable for plant breeding.

Key words: sunflower, wild sunflowers, biochemical markers, morphology, hybridization

Introduction. The present work is a portion of a sunflower research programme whose objective is the production and evaluation of new interspecific hybrids for transferring desirable traits from wild relatives to cultivated sunflower lines, developing germplasm pools having wild *Helianthus* genes in domestic background, and evaluating sunflower evolution and interspecific relationships. In our previous studies, we reported the results from hybridization of cultivated sunflower *H. annuus* L. with silver-leaf sunflower *H. Argophyllus* [21], wild perennials

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RESEARCH PAPERS

EDTA Reduces Heavy Metal Impacts on *Tribulus terrestris* Photosynthesis and Antioxidants[1]

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Abstract—The effects of EDTA application to heavy metal-polluted soil on phytoextraction of heavy metals, leaf anatomy, gas exchange parameters, enzyme activities of C4 carbon cycle, antioxidant defense, and active compounds of *Tribulus terrestris* L. were evaluated. The addition of EDTA to the soil polluted with Cd and Pb markedly increased dry weight and Pb, Zn, and Cd contents in shoots. Plants responded to the action of EDTA by an increased stomatal conductance, photosynthetic and transpiration rates, water use efficiency, chlorophyll and carotenoid contents. The activities of C4 carbon cycle enzymes simultaneously increased, thus concentrating CO_2 for enhanced CO_2 assimilation and providing NADPH for the antioxidant system. Antioxidants, such as ascorbate, reduced glutathione, and flavonoids, increased more in the shoots of *T. terrestris* after the addition of EDTA. The activities of guaiacol peroxidase, catalase, and the enzymes of the ascorbate–glutathione cycle enhanced significantly in the presence of EDTA. Increased activities of antioxidant enzymes suggest that they have some additive functions in the mechanism of metal tolerance. EDTA application lowered the activity of phenylalanine ammonia-lyase and the content of total phenols, MDA, hydrogen peroxide, dehydroascorbate, and lipid-soluble antioxidant capacity expressed as α-tocopherol. Increased levels of total radical-scavenging activity are in correspondence with the activity of water-soluble antioxidant compounds in *T. terrestris* tissues. The content of furostanol saponins protodioscin, prototribestin, and rutin increased as a result of EDTA addition. The results obtained allowed us to assume that applied EDTA reduced a negative heavy metal impact on puncture vine photosynthesis and antioxidant potential.

Keywords: Tribulus terrestris, C4 carbon cycle enzymes, EDTA, heavy metals, leaf anatomy, antioxidant capacity, furostanol saponins

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INTRODUCTION

Tribulus terrestris L. is a flowering plant of the family Zygophyllaceae, native to warm temperate and tropical regions of the Old World in southern Europe, southern Asia, throughout Africa, and Australia. *T. terrestris* plant extracts contain saponins, flavonoids, amides, and alkaloids [1]. The biologically active furostanol saponin fraction of this plant finds application in the contemporary medicine as a component of drugs effective in treatment impotence and other sexual disorders [2].

T. terrestris grown on industrially polluted soil showed good ability for Cd, Pb, and Zn accumulation and could be used for phytoremediation of polluted soils [3]. The plant biomass production and the heavy metal (HM) concentration in the harvestable biomass are important factors for the practical efficiency of phytoextraction [4]. One strategy to achieve the higher HM removal efficiency is to enhance the concentrations of soluble HMs in the soil with the application of complexing agents [5]. EDTA is widely investigated due to its high complexing capability towards Pb, Cu, Cd, and Zn. That leads to an increase in the metal uptake by plants like *Brassica juncea, Helianthus annuus*, and *Zea mays* [6]. The medicinal plants could be grown as alternatives to edible crops in HM-polluted agricultural soils, because possess a significant

[1] This text was submitted by the authors in English.

Abbreviations: AGC—ascorbate–glutathione cycle; ASC—ascorbic acid; CAT—catalase; DHASC—dehydroascorbic acid; GPX—glutathione peroxidase; GR—glutathione reductase; GSH—reduced; GSSG—oxidized glutathione, GST—glutathione S-transferase; HM—heavy metal; NADP-ME—NADP-malic enzyme; PAL—phenylalanine ammonia-lyase; PEPC—phosphoenolpyruvate carboxylase; PPDK—pyruvate orthophosphate dikinase; SOD—superoxide dismutase.

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A compact sunflower line produced after cross *Helianthus annuus* x *Verbesina encelioides*

Research Article

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Abstract: Intergeneric cross was made between the cultivated sunflower inbred line HA89 and an accession of wild *Verbesina encelioides* tolerant to drought and high temperature. The line was a BC_2F_5 progeny. The most remarkable feature of the plants was their compact architecture due to short petiole length and also, rather specific bright-yellow inflorescences. Similar plant architecture did not exist in either the wild or the cultivated parent. For sunflower, it is considered as a favourable and potentially useful adaptive trait. The line was multi-branched of medium type branching and possessed good agronomic characteristics. The overall characteristics of HA-VERBENC line make it a useful plant material for research on wide hybridization.

Keywords: *Antioxidants • Crown beard • Cultivated sunflower • Intergeneric cross • Introgressive hybridization • Wide hybridization*

1. Introduction

Wide hybridization is frequently used as a tool to improve valuable agronomic traits of cultivated sunflower *Helianthus annuus* [1-5]. The range of wild *Helianthus* available to use is extensive while the genus comprises 51 species, 14 annual and 37 perennial, which are diploids, tetraploids and hexaploids [6]. The majority of wide hybridization studies have examined interspecific hybridization between closely related *Helianthus* species, while limited information is available on intergeneric hybridization and the barriers that prevent hybrid production [7-11].

The present work is a portion of a sunflower research program. Its objective is production and evaluation of new interspecific and intergeneric hybrids for transferring desirable traits from wild relatives to cultivated sunflower lines, developing germplasm pools having wild *Helianthus* genes in domestic background, and evaluating sunflower evolution and interspecific/intergeneric relationships. *Verbesina encelioides* [(Cav.) Bentham & Hooker fil. ex Gray] or golden crown beard was chosen due to its drought tolerance, early flowering and resistance to high temperature. It is an annual species, propagating by seeds, which are produced in abundance. Its seeds exhibit remarkable endurance to climatic extremes and survive under extremely high temperatures (38-46ºC) and soil drought during which they lie dormant in soil desiccated to below 5% moisture content [12]. *V. encelioides* is differentiated from the cultivated sunflower by the opposite leaves on the lower part of the plant, as well as smaller flower heads [13]. Besides its ornamental value, some studies pointed out the medicinal and economical importance of various fractions of *Verbesina encelioides*, which demonstrate considerable antibacterial, antifungal, antiviral, antitumor, hypoglycemic and anti-implantation activities [14]. Intensive pharmacognostic and pharmacological investigations are in a progress to ascertain therapeutic properties and medicinal use of *V. encelioides* roots for diabetes [15].

In the course of our study on wide hybridization *Helianthus* x *Verbesina*, we obtained a suite of diverse recombinants which reveal intermediate morphology and phenotype [9], or even novel features such as tubular ray flowers [16]. Indeed, intergeneric hybridization involving *Verbesina* ssp. is a genuine source of new sunflower hybrid

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A comparative study on plant morphology, gas exchange parameters, and antioxidant response of *Ocimum basilicum* L. and *Origanum vulgare* L. grown on industrially polluted soil

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Abstract: The effects of Cd, Pb, and Zn uptake on plant morphology, photosynthetic parameters, antioxidant potential, and essential oil yield and quality in *Ocimum basilicum* L. and *Origanum vulgare* L. plants were evaluated. The plants were grown as a pot experiment in soil heavily polluted with Cd and Pb and on unpolluted soil. Both plants accumulated Cd, mainly in the roots, while Pb occurred in the oregano shoots only. The leaf blade thickness of both plants increased when grown in polluted soil. Basil plants responded to the action of heavy metals with increases in gas exchange, stomatal conductivity, and transpiration, but water-use efficiency declined. Gas exchange and transpiration were reduced in treated oregano plants, but stomatal conductivity and water-use efficiency increased significantly. In basil, the increased levels of low molecular weight antioxidants such as phenols and flavonoids were observed, while in oregano, ascorbate, glutathione, and phenols were enhanced. Enzymatic antioxidant defense was observed in both plants when grown in contaminated soil, mainly with glutathione peroxidase, quaiacol peroxidase, glutathione S-transferase, and glutathione reductase. Accumulated levels of Cd, Pb, and Zn in plant organs resulted in a reduction in essential oil yield in basil only.

Key words: *Ocimum basilicum* L., *Origanum vulgare* L., heavy metals, gas exchange parameters antioxidants, essential oils

1. Introduction

Heavy metal contaminated soils due to industrial activities are a major environmental problem that can reduce both the productivity of plants and the safety of plant products. Phytoremediation is a promising method in which plants are used for removal of heavy metals from soil (Salt et al., 1995). The success of phytoremediation depends on plant growth rates and obtaining high metal concentrations in plant shoots. Some plant species accumulate large amounts of heavy metals in their shoots, and show great potential for cleaning metal contaminated soils (Baker and Brooks, 1989; Xiong, 1997). Some aromatic plants possess significant phytoextraction potential, and metal content in essential oils prepared from them is negligible. That is why they could be grown as alternatives to edible crops in heavy metal polluted agricultural soils (Zheljazkov et al., 2006).

Environmental stresses, including heavy metal pollution, increase reactive oxygen species (ROS) production and oxidative stress in plants (Mittler, 2002; Sharma et al., 2010). Plants develop various cellular enzymatic and nonenzymatic antioxidative mechanisms for detoxification of elevated ROS levels (Procházková and Wilhelmová, 2010). Some plants increase antioxidant activity through the generation of antioxidants. In these plants, the stimulated generation of antioxidants signals exposure to suboptimal conditions. Aromatic plants are especially rich in phenolic antioxidant compounds; their antioxidant activity is due to redox properties and chemical structure, which can play an important role in neutralizing ROS such as free radicals, singlet and triplet oxygen, and peroxides (Zheng and Wang, 2001). Because of the carcinogenic potential of synthetic antioxidants,

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Utilization of related wild species (*Echinacea purpurea*) for genetic enhancement of cultivated sunflower (*Helianthus annuus* L.)

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Abstract: *Helianthus annuus* is one of the most important oil species, with a comparatively narrow genetic base. The development of new sunflower cultivars is the focus of many research and breeding programs. Intergeneric hybridization between *H. annuus* and *Echinacea purpurea,* a valuable medicinal plant, has not yet been utilized in cultivar development. This paper describes 2 advanced hybrid lines produced from an intergeneric cross between the cultivated sunflower inbred line 6650 and an accession of *E. purpurea*. The hybrid plants have successfully been grown in the field. The hybrids, showing expression of traits from both parental species, were intermediate between both parents for plant height, leaf shape, leaf color, floral characteristics, tocopherol content, and fatty acid profile. It could be postulated that the hybridization *Helianthus* × *Echinacea* offers opportunities for combining desirable traits that increase the variability of cultivated sunflower.

Key words: Antioxidant content, cultivated sunflower, *Echinacea purpurea*, fatty acid, *Helianthus annuus*, wide hybridization

1. Introduction

Wide hybridization, including both interspecific and intergeneric hybridization, is one of the most important strategies for creating variations in plant species since it has the potential to combine useful traits, i.e. favorable morphology, disease resistance, and some environmental tolerances of both parents that could not be achieved by crossing within a single species. Sunflower (*Helianthus annuus* L.) cultivars lack acceptable levels of genetic variation since hybrid breeding utilizes a comparatively narrow genetic base. Wild *Helianthus* species are a potential source of genes for resistance or tolerance to insects, disease, and pests; for early maturation and resistance in unfavorable environmental factors (soil salinity, acidity, and drought); and as sources of cytoplasmic male sterility and fertility restoration (Faure et al. 2002; Seiler and Gulya 2004). Successful interspecific transfer of traits from wild species to cultivated sunflower was a reason for attempts of wider crosses, including those between members of related genera.

Advances in intergeneric crosses in tomato (Rick et al. 1986), species of wild grasses from the genera *Aegilops*, *Agropyron*, and *Secale* (Fedak 1984); wheat; rapeseed (*Brassica napus*); and tobacco (*Nicotiana tabacum*) have been well documented (Goodman et al. 1987). There is evidence that intentional crosses between species in different genera have the potential for crop improvement and to increase the genetic variability of crop species, including for *H. annuus* in particular. It is plausible that gene transfer between members of different genera may complement the more classical hybrids that provide gene flow across interspecific crosses. Additionally, they have proved to be very useful for elucidating the evolutionary relationships between *H. annuus* and related wild species of *Asteraceae.*

The present work is a part of a sunflower research program with the objective of producing and evaluating new interspecific or intergeneric hybrids that provide novel combination of traits useful for plant breeding. In particular, the program combines interest from both plant breeding and academic research in the use of wide hybridization for transferring desirable traits from wild relatives to cultivated sunflower, developing germplasm pools having wild *Helianthus* genes in a domestic background, and for characterizing phylogenetic affinity. Hybrids between cultivated *H. annuus* and species of different genera of the family *Asteraceae* such as *Tithonia rotundifolia* and *Verbesina encelioides*, identified as having intermediate characteristics, have been reported in our previous studies (Christov and Vassilevska-Ivanova 1999;

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