

Root Colonization by Symbiotic Arbuscular Mycorrhizal Fungi Increases Sesquiterpenic Acid Concentrations in *Valeriana officinalis* L.

Authors

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Key words

- *Valeriana officinalis*
- Valerianaceae
- valerian
- arbuscular mycorrhiza
- sesquiterpenic acids
- *in vitro* propagation
- hydroxyvalerenic acid chemotype

Abstract

In some medicinal plants a specific plant-fungus association, known as arbuscular mycorrhizal (AM) symbiosis, increases the levels of secondary plant metabolites and/or plant growth. In this study, the effects of three different AM treatments on biomass and sesquiterpenic acid concentrations in two *in vitro* propagated genotypes of valerian (*Valeriana officinalis* L., Valerianaceae) were investigated. Valerenic, acetoxyvalerenic and hydroxyvalerenic acid levels were analyzed in the rhizome and in two root fractions. Two of the AM treatments significantly increased the levels of sesquiterpenic acids in the underground parts of valerian. These treatments, however, influenced the biomass of rhizomes and roots negatively. Therefore this observed increase was not accompanied by an increase in yield of sesquiterpenic acids per plant. Furthermore, one of the two genotypes had remarkably high hydroxyvalerenic acid contents and can be regarded as a hydroxyvalerenic acid chemotype.

Abbreviations

AM:	arbuscular mycorrhiza
AMF:	arbuscular mycorrhizal fungi
AVA:	acetoxyvalerenic acid
BAP:	6-benzylaminopurin
GABA _A receptor:	γ-aminobutyric acid activated type A receptor
HVA:	hydroxyvalerenic acid
IAA:	indole-3-acetic acid
MS:	Murashige & Skoog medium
SPMs:	secondary plant metabolites
SPSS:	Superior Performing Software System
VA:	valerenic acid

Supporting information available online at <http://www.thieme-connect.de/ejournals/toc/plantamedica>

received June 5, 2009
revised August 4, 2009
accepted Sept. 1, 2009

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DOI 10.1055/s-0029-1186180
Published online October 6, 2009
Planta Med 2010; 76: 393–398
© Georg Thieme Verlag KG
Stuttgart · New York ·
ISSN 0032-0943

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Introduction

Valeriana officinalis L. (Valerianaceae), valerian is a medicinal plant which is, due to its mild sedative and sleep-enhancing properties, used worldwide in traditional medicine [1]. For many years the biological activity in the underground parts of valerian has been attributed to valepotriates, essential oil compounds and sesquiterpenic acids [1–4]. However, recent findings attribute the main pharmacological effects to the sesquiterpenic acids such as valerenic acid (VA) and its derivatives, acetoxyvalerenic acid (AVA) and hydroxyvalerenic acid (HVA) [5,6]. Since they were thought to be species-specific VA, AVA and HVA were used for a long time as analytical markers for *V. officinalis* [7]; however, meanwhile, traces

of sesquiterpenic acids have also been found in *V. sitchensis* Bong [8]. For the commercialization of the valerian drug specific pharmacologically active compounds and certain quantities of them have to be achieved in the plant material. However, seasonal variations in the compound pattern and their quantities [1,9] and problems with heterogeneity between single plants [10] characterize and complicate valerian production. Thus, genetically homogeneous valerian plants, achieved either by rhizome cutting or *in vitro* propagation, are a precondition to test any treatment in order to alter the composition and amounts of the bioactive compounds. The arbuscular mycorrhizal (AM) symbiosis refers to an association between plants and soil-borne fungi, during active plant growth. The symbiosis

is characterized by the transfer of carbohydrates to the fungus and nutrients to the plant, mainly phosphorus (P). Furthermore, AM plants often show an increased growth and yield, and exhibit an improved fitness against biotic and abiotic stress [11].

Beside these effects AM root colonization can alter the biosynthesis of secondary plant metabolites (SPMs) resulting in altered levels of SPMs not only in the roots [12–14] but also in the aerial plant organs [15–18].

In the present study we produced as a first step by *in vitro* propagation a genetically homogeneous plant material of two valerian genotypes in order to test the effect of root colonization by AM fungi (AMF) on biomass parameters and on the levels of the bioactive sesquiterpenic acids in the rhizome, coarse and fine root fractions.

Materials and Methods

Biological material and *in vitro* propagation

Seeds of *Valeriana officinalis* L. (clone 1 purchased from Eifler company; clone 2 cultivated in the medicinal plant garden of the Department of Pharmacognosy, Vienna, Austria) were surface sterilized with 10% ethanol for 1 min, followed by a 10-min treatment with an aqueous sodium hypochlorite solution (1.6% active chlorine). After three rinses with sterile distilled water, seeds were transferred to test tubes (165 × 23 mm) containing 13 mL of modified MS [19] semisolid medium (1/2 MS) lacking ammonium nitrate and with the other macronutrients at half strength, and containing 10 g·L⁻¹ sucrose, 50 mg·L⁻¹ *myo*-inositol, 3 g·L⁻¹ Gelrite (Carl Roth GmbH) and 10.5 µg·L⁻¹ indole-3-acetic acid (IAA; Sigma-Aldrich). After germination, seedlings were transferred to glass jars (110 × 60 mm Ø) with 40 mL of modified MS medium containing 30 g·L⁻¹ sucrose, 100 mg·L⁻¹ *myo*-inositol and 3 g·L⁻¹ Gelrite, and supplemented with 1.1 mg·L⁻¹ 6-benzylaminopurin (BAP; Sigma-Aldrich) to adapt the seedlings.

Generally, the pH of the media was adjusted to 5.7 ± 0.1 with 1 M KOH or 1 M HCl before autoclaving for 20 min at 121 °C. All cultures were maintained at 25 ± 1 °C under a 16-h photoperiod (50 µmol·m⁻²·s⁻¹) provided by cool white fluorescent tubes (Sylvania GRO-LUX®). Subsequently, for rapid propagation of the valerian clones the BAP concentration was increased to 5 mg·L⁻¹ according to the protocol of [20]. Every 30 day the newly formed shoot-tuffs were divided and transferred to fresh medium. After 16 weeks of *in vitro* propagation shoots of two clones (clone 1, clone 2; each originating from one single seed) were transferred back to medium with lower BAP concentration (1.1 mg·L⁻¹) for shoot elongation and root formation.

After 4 weeks, rooted plantlets were transferred into pots containing an autoclaved mixture of peat and sand (2:1) and kept in a mist chamber at 80% initial relative humidity, which was gradually lowered to 50%. Two months later the plantlets were transferred to greenhouse conditions for the experimental setup.

Growth conditions and experimental setup

The plantlets were transferred into pots (2 L volume) containing an autoclaved substrate mixture of sand, soil and expanded clay (1:1:1; v/v/v). Plants were grown in a random design in the greenhouse with a day/night cycle of 16 h at 22 °C and 8 h at 19 °C (relative humidity: 50–70%). The experimental setup consisted of three different AM treatments (two pure inocula and one fungal mixture), a negative control and furthermore, of a positive control treatment with additional P to differentiate P-mediated

effects from effects of AMF. The pure inoculum of *Glomus mosseae* (BEG 12) and *Glomus intraradices* (BB-E) were purchased from BIORIZE/AGRAUXINE. The inoculum “Symbivit®” was purchased from the company “Symbio-m” and consisted of 6 different *Glomus* species (*G. mosseae*, *G. intraradices*, *G. cladoideum*, *G. microagregatum*, *G. caledonium* and *G. etunicatum*). The inocula consisted of lyophilized mycorrhizal roots containing sporocarps, spores and hyphae of the particular fungi, blended with silica sand.

The experimental treatments were:

- ▶ Control without AMF and without additional P,
- ▶ Symbivit without additional P,
- ▶ *Glomus mosseae* without additional P,
- ▶ *Glomus intraradices* without additional P,
- ▶ Phosphorus (136 mg KH₂PO₄ L⁻¹) without AMF.

The experiment was conducted with 5 replicates per treatment. When the valerian plantlets were transferred to the sterile substrate mixture, 5 g of the granular AMF inoculum was added into the plant hole of each plantlet in the mycorrhizal treatments. During the growth period of 6 months plants were watered with a nutrient solution [15] supplemented with (136 mg KH₂PO₄ L⁻¹) or without phosphorus depending on the treatment. The plants were harvested and the roots were gently cleaned from soil by washing. Roots and shoots were separated. Root segments were cut for the estimation of the root colonization. Remaining roots and underground parts were dried at 35 °C. Dried shoots were separated into stems and leaves, and dried underground parts into rhizomes, coarse and fine (diameter < 1 mm) roots. The three root fractions were taken for estimation of sesquiterpenic acids. Leaves and coarse roots were taken for the P estimation.

Estimation of the mycorrhization

The degree of mycorrhization was estimated on defined fresh root segments of 1 cm length, starting 10 cm downwards the shoot basis, after staining [21] according to the procedure of McGonigle et al. [22].

Determination of sesquiterpenic acids

The sesquiterpenic acid concentrations were separately determined for the rhizome, coarse and fine root fractions. One g of ground sample was extracted and analyzed according to the method of Bos et al. [3] with some minor modifications. HPLC conditions: RP Luna C₁₈ column (5 µm, 150 × 4.6 mm; Phenomenex) and Luna C₁₈ guard column (Phenomenex); Solvent system: A – 800 g water and 156.4 g acetonitrile, B – 200 g water and 625.6 g acetonitrile, solvent pH 2.7 (phosphoric acid); 46–100% B over 19 min, 100% B for 2 min; Flow rate: 2 mL·min⁻¹; Injection volume: 15 µL; DAD condition: 220 nm.

Chemicals

Valerenic acid (purity >98%), acetoxyvalerenic acid (purity >99%) and hydroxyvalerenic acid (purity >99%) were obtained from Apin Chemicals. Solvents for extract preparation were of analytical grade and solvents for HPLC analysis of HPLC grade.

Estimation of P concentration

Leaves and coarse roots were ground, oven-dried for 4 hours at 105 °C and solubilized with a triple acid mixture for the analysis of P with the ammonium-vanadate-molybdate method [23].

Table 1 Degree of mycorrhization, biomass parameters and phosphorus concentrations (mean \pm SEM).

Parameter	Clone 1					Clone 2				
	Control	Symbivit	<i>G. moss.</i>	<i>G. intra.</i>	Phosphorus	Control	Symbivit	<i>G. moss.</i>	<i>G. intra.</i>	Phosphorus
Degree of mycorrhization (%)	0 a	42.4 \pm 2.2 b	30.8 \pm 6.4 b	70.4 \pm 4.0 c	0 a	0 a	60.0 \pm 4.1 b	55.4 \pm 6.2 b	64.8 \pm 1.2 b	0 a
Total biomass (g DW)	33.48 \pm 1.24 b	27.30 \pm 1.98 ab	31.88 \pm 0.91 b	23.76 \pm 1.88 a	48.20 \pm 2.31 c	28.55 \pm 1.15 b	16.52 \pm 1.67 a	20.49 \pm 0.79 a	18.74 \pm 1.54 a	36.38 \pm 1.24 c
▶ shoots (g DW)	11.11 \pm 0.46 a	11.84 \pm 0.92 a	12.51 \pm 0.37 a	9.30 \pm 0.87 a	21.27 \pm 1.22 b	12.18 \pm 0.62 b	7.37 \pm 0.74 a	9.54 \pm 0.38 a	9.09 \pm 0.73 a	17.76 \pm 0.49 c
▶ leaves (g DW)	6.81 \pm 0.28 ab	7.72 \pm 0.60 ab	8.21 \pm 0.25 b	5.64 \pm 0.53 a	14.53 \pm 0.83 c	7.47 \pm 0.38 b	4.81 \pm 0.48 a	6.26 \pm 0.25 ab	5.52 \pm 0.44 a	12.13 \pm 0.34 c
▶ underground parts (g DW)	22.37 \pm 0.90 b	15.46 \pm 1.44 a	19.37 \pm 0.58 b	14.48 \pm 1.09 a	26.93 \pm 1.18 c	16.37 \pm 0.71 b	9.15 \pm 1.06 a	10.95 \pm 0.95 a	9.67 \pm 1.26 a	18.61 \pm 0.91 b
▶ rhizome (g DW)	3.36 \pm 0.10 bc	1.98 \pm 0.20 a	2.44 \pm 0.10 ab	2.21 \pm 0.47 ab	4.10 \pm 0.42 c	1.58 \pm 0.07 a	0.84 \pm 0.13 a	1.09 \pm 0.12 a	0.94 \pm 0.11 a	3.10 \pm 0.62 b
▶ coarse root fraction (g DW)	11.18 \pm 0.65 b	8.42 \pm 0.97 ab	9.12 \pm 0.41 ab	6.91 \pm 0.40 a	13.94 \pm 0.96 c	8.62 \pm 0.38 b	5.10 \pm 0.56 a	5.87 \pm 0.56 a	5.77 \pm 0.47 a	9.82 \pm 0.46 b
▶ fine root fraction (g DW)	7.83 \pm 0.58 b	5.06 \pm 0.30 a	7.80 \pm 0.42 b	5.35 \pm 0.70 a	8.90 \pm 0.46 b	6.17 \pm 0.35 b	3.21 \pm 0.37 a	3.99 \pm 0.30 ab	2.94 \pm 0.87 a	5.71 \pm 0.94 b
Leaf phosphorus concentration (mg/g DW)	1.29 \pm 0.09 a	1.72 \pm 0.06 b	1.40 \pm 0.03 a	1.66 \pm 0.07 b	2.74 \pm 0.04 c	1.04 \pm 0.03 a	1.70 \pm 0.08 bc	1.49 \pm 0.09 b	1.82 \pm 0.05 c	2.44 \pm 0.08 d
Root phosphorus concentration (mg/g DW)	0.65 \pm 0.02 a	1.36 \pm 0.09 c	0.91 \pm 0.05 b	1.34 \pm 0.11 c	2.75 \pm 0.06 d	0.76 \pm 0.01 a	1.51 \pm 0.08 c	1.12 \pm 0.08 b	1.70 \pm 0.08 c	2.23 \pm 0.05 d

Different letters within one line and genotype denote significant differences ($p < 0.05$)

Statistical analysis

Effects of genotypes and treatments were determined by two factorial analysis of variance. For treatment effects within one genotype one-way analysis of variance was used. Significant differences, expressed in different letters, were confirmed with multiple comparisons by Tukey's-b test for a significance level of 5%. All statistical analyses were performed with SPSS.

Supporting information

Results of the two factorial analysis of variance are available as Supporting Information.

Results

The *in vitro* propagation rates were satisfactory with a multiplication rate of 4.0 for clone 2, and 4.2 for clone 1, both within a 4-week culture period. However, the shoots were small and truncated. Upon subsequent transfer to MS medium with lowered BAP content ($1.1 \text{ mg} \cdot \text{L}^{-1}$), the shoots recovered quickly on the medium with lowered cytokinin content. All *in vitro* multiplied plantlets could be successfully adapted to *ex vitro* conditions, and further used in our study.

AMF successfully colonized the valerian roots in both genotypes. In clone 1 *G. intraradices* obtained a 1.7-fold higher colonization than Symbivit and a 2.3-fold higher colonization than *G. mosseae*-inoculated plants. All AM treatments of clone 2 reached approxi-

mately 60% degree of mycorrhization. No AMF colonization was observed in the control and P-fertilized plants (Table 1).

The lowest total biomass was observed for the AMF-treated compared to control plants in both genotypes (minus 19–35%), except for the *G. mosseae*-treated plants of clone 1. Highest shoot and leaf biomasses were found for P-treated plants in both genotypes (Table 1). Significant interactions between genotypes and treatments for the shoot and leaf biomasses were observed (Supporting Information, Table 1S). In both genotypes the biomasses for the underground parts decreased significantly by 0.6- to 0.7-fold for AM compared to control plants, except for the *G. mosseae*-treated plants in clone 1.

In all treatments the biomass of the rhizome fraction varied between 10 and 17%, the coarse root fractions between 47 and 62% and fine roots between 28 and 40% of the underground part (Table 1). In both genotypes generally the lowest rhizome, coarse and fine root biomass was found in the AM treatments (Table 1).

In leaves and roots the P concentrations were significantly increased in all treatments compared to control plants, except for the leaf P concentration of *G. mosseae*-treated plants in clone 1 (Table 1). Strong interactions between the two factors (genotype and treatment), with the main focus on the treatments, were observed for P concentrations in leaves and roots (Supporting Information, Table 1S).

In both valerian genotypes the total sesquiterpenic acid concentrations in the drug (mixture of rhizome, coarse and fine roots) were significantly increased for the Symbivit and *G. intraradices*-

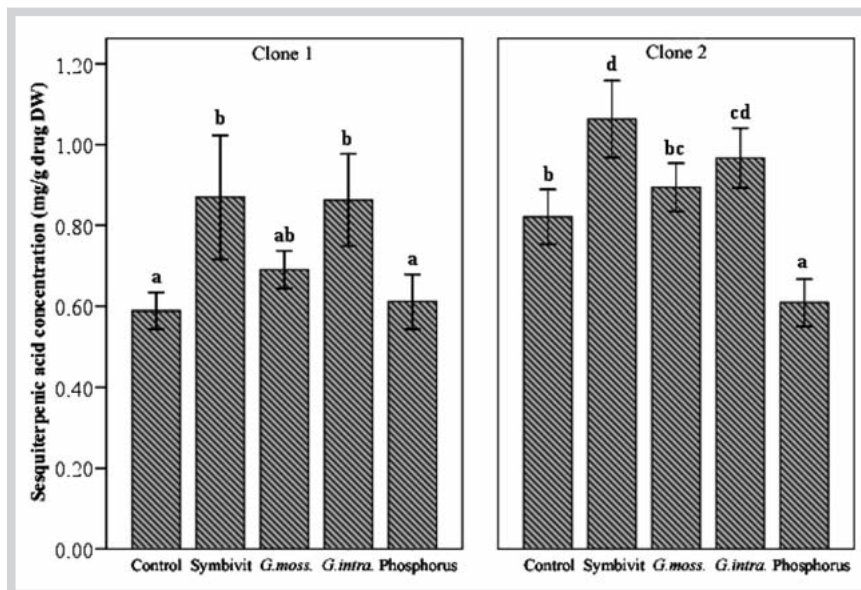


Fig. 1 Total sesquiterpenic acid concentrations in the valerian drug of the two genotypes (mean \pm SEM). Different letters within one genotype denote significant differences ($p < 0.05$).

treated plants compared to the controls. In clone 1 these increases were around 48%, in clone 2 the Symbivit treatment showed an increase of 29% and *G. intraradices* of 18%. The phosphorus treatment of clone 2 showed a decrease of 26% of the total sesquiterpenic acids per drug, while phosphorus did not reduce the total sesquiterpenic acids in clone 1 (● Fig. 1).

The chemical composition of sesquiterpenic acids (AVA:VA:HVA) in percentage resulted in some differences for both genotypes over all treatments. In clone 1 the rhizome composition (69:26:5; AVA:VA:HVA) was quite similar to the coarse and fine roots composition (70:27:3; AVA:VA:HVA). In clone 2, however, the composition of the rhizome (82:18:0; AVA:VA:HVA) varied strongly from the composition of the coarse and fine roots (72:27:1; AVA:VA:HVA).

In all AM treatments of clone 1 the AVA concentration was increased compared to the control; however, significant increases were only found in the coarse and fine root fractions (plus 45 and 66%, respectively). In clone 2 the AVA concentration of the rhizome significantly increased in the Symbivit (45%) and the *G. intraradices* treatments (51%). In the coarse and fine root fraction significant increases were found in the Symbivit treatment (20 and 26%, respectively). All AM treatments significantly increased the VA concentrations between 24 and 43% in the coarse roots of clone 1, but only Symbivit significantly increased the VA concentration by 23% in clone 2. In the rhizome of clone 2 significant increases in VA concentration were observed for the Symbivit and *G. intraradices* treatments (● Fig. 2). Slight but significant interactions between the genotype and treatments were observed for VA in the fine root fraction (Table 1S, Supporting Information). Neither significant differences nor interactions were observed for the HVA concentrations between the genotypes and treatments (● Table 2; Table 1S, Supporting Information). The sesquiterpenic acid yield per plant (sum of products of sesquiterpenic acid concentration multiplied by biomass for single fractions) did not show significant differences between the genotypes and treatments. However, the yield output was higher for clone 1 than for clone 2. In clone 1 the highest yield was observed for P-treated plants with 17 mg/plant, while the yield of AM and control plants varied between 13 and 14 mg/plant. Clone 2 showed the highest sesquiterpenic acid yield in the control treatment with 13 mg/plant, the AM treatments resulted in 9–10 mg/plant.

Discussion



In the present study the major effects of AMF colonization on plant growth and sesquiterpenic acid contents in two genotypes of valerian, a traditionally used and well-known medicinal root drug, were determined.

The main precondition for this study about AM symbiosis effects on valerian was the use of genetically homogeneous experimental plants. Since a pre-experiment (data not shown) resulted in a high genotypic variance in the chemical content and the composition of sesquiterpenic acids between single plants grown from commercially available seeds, a clonal propagation strategy using two genotypes (individual plants of different accessions) for the experiment was chosen to better observe effects of the plant-fungus interaction.

Interestingly the root morphology of valerian seems to be an important factor for the drug production. The underground part of valerian, apart from the rhizome, is dominated by coarse roots (averaged 55%) followed by fine roots (averaged 35%). Commercial drugs consist of a mixture of rhizomes and larger root parts, fine roots are mostly lost in the harvest and post-harvest procedures. Modifications of the harvest and post-harvest procedures reducing losses of fine roots could substantially increase drug quantity.

High P levels in the growth substrate are known to inhibit root colonization by AMF [24]. In our study the water-soluble P fraction of the substrate was high enough that control plants grew without any visible deficiency symptoms and low enough to reach high root colonization levels by AMF. Once colonized by AM fungi in general the P uptake of the mycorrhizal plants is drastically increased, thus resulting in an improved plant growth [24]. In our experiment the P concentrations in the root and shoot were clearly increased in all mycorrhizal treatments compared to the control treatment without AMF.

It is known from numerous reports that the AM association leads to an increased biomass production [24]. Several published data about medicinal and aromatic plants confirm an increase in P in the plant as well as an increase in biomass growth [15–18, 25]. In our study, despite the increased P concentration, a reduction in all biomass parameters of the AM-treated plants was observed, showing that under our experimental conditions the AM associ-

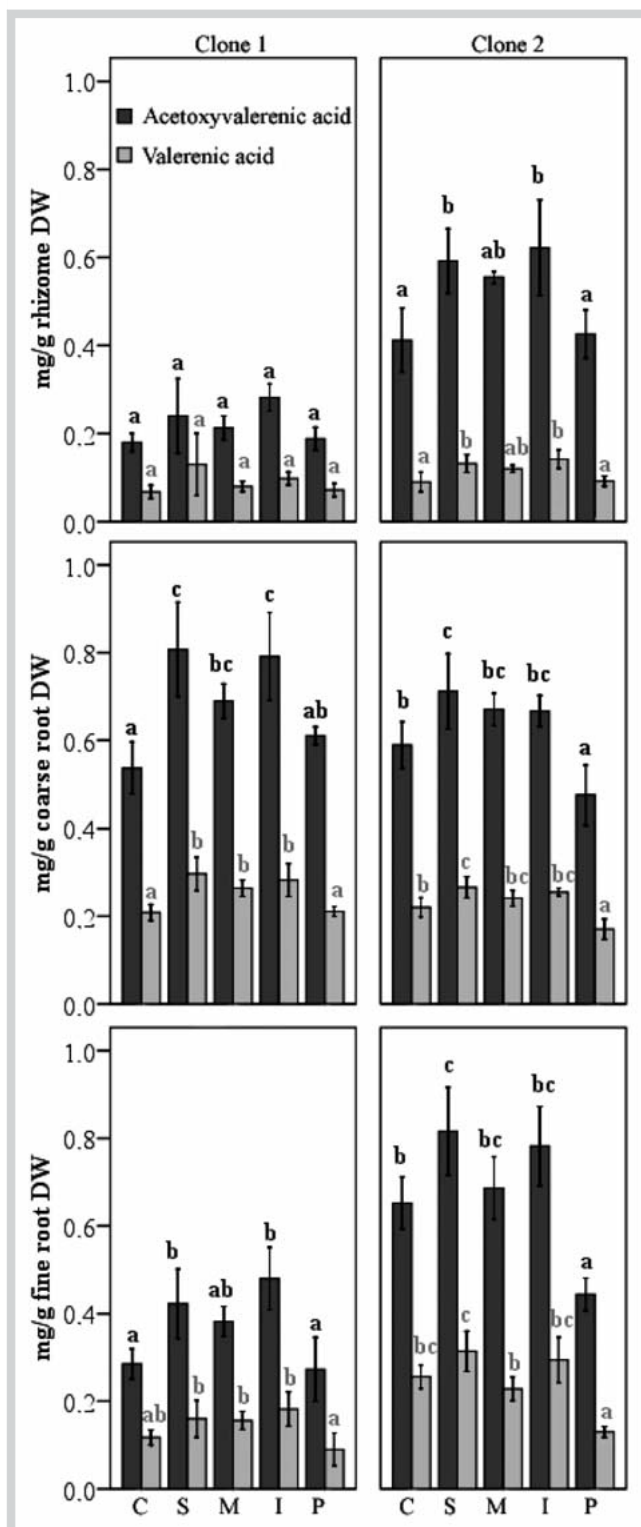


Fig. 2 Acetoxyvalerenic acid (dark-colored bars) and valerenic acid concentrations (light-colored bars) of rhizome, coarse and fine root fractions (mean \pm SEM) for control (C), Symbivit (S), *G. mosseae* (M), *G. intraradices* (I) and phosphorus (P) treatments. Different letters within one compound and genotype denote significant differences ($p < 0.05$).

ation was negatively influencing biomass production. The reason may be found in the loss of carbon to the fungi [26], relying obligatory on photosynthates from the host. Mycorrhization acts not only on growth but also on the secondary metabolism of plants, sometimes causing positive changes in the levels of compounds

of pharmaceutical interest [17,18,27]. In the present investigation we found that the valerian drug showed enhanced concentrations of sesquiterpenic acids, which were not P mediated but rather mediated by the AMF itself because P fertilization did not result in an enrichment of sesquiterpenic acids. Moreover, we found a significantly increased sesquiterpenic acid content in valerian in two out of three AM treatments. The increase of the sesquiterpenic acid content seems independent of whether plants are colonized by one or several AM fungi, as the sesquiterpenic acid concentration was increased to a similar level with a mixture of different AM fungi (the Symbivit treatment) and with the AM fungus *G. intraradices* alone. The proportion of single sesquiterpenic acids between the treatments was the same, but the content was increased. The European Pharmacopoeia regulates the total sesquiterpenic acid concentration in the drug. A minimum of 0.10% sesquiterpenic acids for cut drugs is requested [2]. Problems with variations in the content and composition of secondary metabolites in valerian have been reported [1,9]. In valerian production AMF treatment specifically with Symbivit or *G. intraradices* could be an environmental friendly alternative to reach the limits of the Pharmacopoeia.

In our study the predominant sesquiterpenic acid in *V. officinalis* was AVA which is in agreement with previous findings [7]. VA is present in about one-fourth of the sesquiterpenic acids with one exception for the rhizome of clone 2. Hänsel and Schulz reported VA contents between 18 and 44% of the total cyclopentan sesquiterpenes (including minor amounts of valeranal) in *V. officinalis* roots [7]. Existing literature often presents the total sesquiterpenic acid concentration and not single parts of AVA, VA and HVA, which would be more expedient since Trauner et al. [5] experienced that VA in combination with low AVA concentrations has a more pronounced effect than combined with high amounts of AVA [5]. Furthermore, the VA content in valerian extracts is related to the extent of the GABA_A receptor modulation [5]. The relation of AVA and VA was not changed by AMF, therefore no direct quality improvement due to AM can be deduced.

Quite big differences in the HVA concentration between the two investigated genotypes were observed. Clone 1 contained HVA in all fractions with most abundant amounts in the coarse root fraction. In the rhizome of clone 2, HVA was missing but in coarse and fine roots traces of HVA were found. In previous experiments only low HVA contents [8,9] or even a complete absence of HVA [7] was observed. Degradation processes of AVA during drying and/or storage are considered to be responsible for the formation of HVA [3,7,28,29]. The artefact formation agrees well with the absence of HVA in fresh samples but its presence in all stored valerian samples of Wills and Shoheta [30]. From our findings we assume that additionally to the post-harvest HVA formation this substance may already be present in higher amounts in the plant. Its presence is genetically determined since we only found HVA in one genotype and both genotypes were equally treated in cultivation, sampling and sample preparations. It remains to be elucidated whether the presence of HVA in the plant is an enzymatic reaction or not.

The present study shows that the AM symbiosis increases the sesquiterpenic acid content in two genotypes of *V. officinalis*, originating from two different provenances without affecting their composition. However, the sesquiterpenic acid yield per plant could not be enhanced due to a growth reduction of mycorrhizal plants. Possibly a regular biomass growth could be achieved through a slightly improved P fertilization thus allowing only a moderate AMF root colonization. In this way enhanced valerian drug biomass production could be combined with elevated

Table 2 Hydroxyvalerenic acid concentrations in rhizome, coarse and fine root fractions of the two genotypes (mean ± SEM).

Under-ground part	Hydroxy valerenic acid concentration (mg/g DW)									
	Clone 1					Clone 2				
	Control	Symbivit	G. moss.	G. intra.	Phosphorus	Control	Symbivit	G. moss.	G. intra.	Phosphorus
Rhizome fraction	0.020 ± 0.007 a	0.017 ± 0.003 a	0.010 ± 0.001 a	0.020 ± 0.004 a	0.013 ± 0.007 a	n. d.	n. d.	n. d.	n. d.	n. d.
Coarse root fraction	0.051 ± 0.004 a	0.064 ± 0.005 a	0.062 ± 0.008 a	0.065 ± 0.007 a	0.062 ± 0.005 a	0.006 ± 0.001 a	0.009 ± 0.001 a	0.010 ± 0.001 a	0.006 ± 0.001 a	0.007 ± 0.002 a
Fine root fraction	0.017 ± 0.006 a	0.015 ± 0.005 a	0.012 ± 0.006 a	0.018 ± 0.004 a	0.020 ± 0.006 a	0.008 ± 0.002 a	0.022 ± 0.005 a	0.012 ± 0.004 a	0.024 ± 0.012 a	0.024 ± 0.002 a

Different letters within one line and genotype denote significant differences ($p < 0.05$). n. d., not detected

levels of sesquiterpenic acids in the drug and AM symbiosis would be an effective, ecological approach for yield optimization of sesquiterpenic acids in valerian.

Acknowledgements

This study was funded by the Austrian Science Fund (FWF; project L194-B06). The authors thank Mrs. Brigitte Grauwald and Mrs. Hong Ling Liu for technical and practical assistance.

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