

# 培养基组分对沙打旺 (*Astragalus adsurgens* Pall) 组培根增殖的影响及其培养滤液提取物的化感活性

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**摘要:**采用  $L_9(3^4)$  正交设计, 研究了  $B_5$  培养基营养成分对沙打旺组培根增殖的影响; 并采用玻璃皿滤纸培养法, 对其培养滤液提取物进行生物测定以验证沙打旺组培根的化感活性。结果显示: 培养基的所有营养成分中,  $Fe^{2+}$  对沙打旺组培根增殖的影响最大, 蔗糖、 $H_2PO_4^+$ 、 $Mg^{2+}$ 、 $Mn^{2+}$ 、 $Cu^{2+}$ 、 $Zn^{2+}$ 、 $BO_3^-$ 、 $Co^{2+}$ 、 $I^-$ 、 $C_8H_{12}ClNO_3$  +  $C_{12}H_{18}Cl_2N_4OS$  +  $C_6H_5O_2N$  +  $C_6H_{12}O_6$  的影响次之, 氮、 $Ca^{2+}$ 、 $MoO_4^{2-}$  和 NAA 的影响最小。根据不同养分条件下沙打旺组培根干重的极差分析, 筛选出适宜沙打旺组培根快速增殖的优化培养基。培养滤液提取物的生物测定结果表明沙打旺组培根培养过程中可能产生化感物质; 化感作用强度的差异预示营养胁迫可能影响其化感物质的产生。研究为沙打旺组培根再生与繁殖提供一定依据, 并揭示养分条件可能是该植物表达化感作用的影响因素。

**关键词:** 沙打旺; 组培根; 培养基组分; 培养滤液提取物; 化感作用

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## Effect of growth medium composition on the propagation of cultured milk vetch (*Astragalus adsurgens* Pall) roots and the allelopathic activity of extracts from the culture filtrate

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**Abstract:** The objective of this research was to determine effects of growth medium composition on the propagation of cultured milk vetch (*Astragalus adsurgens* Pall) roots. An orthogonal design was used to test multiple components in the

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growth medium. A second objective was to measure allelopathic activities of cultured milk vetch roots by conducting a bioassay using filtered extracts from the growth medium after culturing the roots. The results indicated that among all the nutritional components,  $\text{Fe}^{2+}$  had the greatest effect on the propagation of cultured milk vetch roots; the effects of sucrose,  $\text{H}_2\text{PO}_4^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{BO}_3^{3-}$ ,  $\text{Co}^{2+}$ ,  $\text{I}^-$  and  $\text{C}_8\text{H}_{12}\text{ClNO}_3 + \text{C}_{12}\text{H}_{18}\text{Cl}_2\text{N}_4\text{OS} + \text{C}_6\text{H}_5\text{O}_2\text{N} + \text{C}_6\text{H}_{12}\text{O}_6$  were intermediate; and the effects of nitrogen,  $\text{Ca}^{2+}$ ,  $\text{MoO}_4^{2-}$  and NAA were lowest. A recommendation was made regarding the optimum nutrient content of growth medium for the propagation of cultured milk vetch roots. A bioassay using filtered extracts from the growth medium indicated that milk vetch roots might have produced allelopathic chemicals during their culture. Differences in the degree of allelopathic effects suggest that nutrient stress may influence the production of allelopathic chemicals. This study provides a basis for the improvement of milk root regeneration and propagation and suggests that nutrition may influence the production of allelopathic chemicals by this plant.

**Key Words:** milk vetch; cultured root; medium composition; culture filtrate extracts; allelopathic effect

Milk vetch (*Astragalus adsurgens* Pall) is a perennial pasture plant that is native to China. Milk vetch grows well under a wide range of conditions. Its high protein content makes it a desirable forage plant for livestock. Consequently, milk vetch has been planted in dry areas of northern China for over 100 years.

In recent decades, milk vetch has been widely studied (eg. biological characteristics<sup>[1,2]</sup>, physiology and ecology<sup>[3-6]</sup>, pests and diseases<sup>[7-10]</sup>, heredity and breeding<sup>[11,12]</sup>, cell, tissue and organ culture<sup>[13-16]</sup>, planting techniques and utilization<sup>[17, 18]</sup>). These findings have promoted the use of milk vetch as a forage and green manure crop. Milk vetch has also been used for ecological restoration<sup>[19]</sup>.

One specific finding from the studies listed above was that the introduction of milk vetch resulted in a rapid shift from a Mongolian thyme (*Thymus mongolicus* Ronn) community to a Bunge needlegrass (*Stipa bungeana* Trin) community<sup>[19-21]</sup>. Banded sowing of milk vetch led to an increase in the yield of air-dry herbage, a decrease in poisonous plants, and an increase in the proportion of legumes<sup>[22]</sup>. Moreover, the establishment of milk vetch enhanced the degree of vegetative cover and altered the community composition of pastures on steep slope<sup>[22]</sup>. Guan *et al.*<sup>[23]</sup> observed that short-rooted cereal pastures grew well after milk vetch pastures declined, however, long-rooted legume forages grew poorly. Liu *et al.*<sup>[24]</sup> found that yield and biomass of millet [*Setaria italica* (L.) Beauv] was significantly less in fields infested with milk vetch compared to fields that had previously been planted to smooth brome grass (*Bromus inermis* Leyss). Du *et al.*<sup>[25]</sup> emphasized that there was a sharp decline in forage yield when milk vetch was planted for more than three consecutive years in agricultural areas with rainfall of 300 mm. The observations described above have traditionally been attributed to competition between species. However, recent studies suggest that allelopathy could be a contributing factor<sup>[26]</sup>.

Allelopathy is an interspecific relationship between plants and other organisms. It is a common phenomenon in nature and plays a significant role in the existence and multiplication of some plant species<sup>[27]</sup>. Allelopathy in plants is influenced by environmental factors<sup>[28]</sup>, especially soil microorganisms<sup>[29,30]</sup>. The presence and effect of microorganisms complicates allelopathic research. Sterile plant material eliminates environmental effects caused by microorganisms and therefore is considered ideal for the analysis of allelopathy<sup>[31,32]</sup>. Hence, we used sterile cultures of milk vetch roots in this study.

The climate is dry in the main milk vetch growing areas of northern China. The aerial parts of the plant are harvested two or three times per year. This suggested to us that the release of bioactive substances from milk vetch shoots might be limited. We hypothesized that the majority of bioactive chemicals were released from underground parts of milk vetch. This was another important reason why we used cultured roots but not other cultured tissues.

The objective of this experiment was twofold. First, we wanted to determine the influence of growth medium compositions on the propagation of cultured milk vetch roots. Second, we wanted to analyze the allelopathic effects of milk vetch roots. The latter objective was pursued by treating radish (*Raphanus sativus* L.), wheat (*Triticum aestivum* Linn) and milk vetch seeds with filtered extracts from the growth medium that had been used to culture milk vetch roots and then measuring the germination and seedling growth of the three species. This research should provide a basis for determining the allelopathic effect of milk vetch on other plant species.

## 1 Materials and methods

### 1.1 Establishment of sterile and stable milk vetch root cultures

Milk vetch seeds were surface sterilized in 72% (m/m) ethanol for 3 min and then washed three times with autoclaved distilled water. The seeds were then put into 1% NaClO for 5 min and washed three times with autoclaved distilled water. Surface sterilized seeds were cultured for 12 days in a 200 ml flask containing 50 mL of MS medium (pH 5.7) with agar. The flask was kept in an incubator at 25°C with  $(40 \pm 2) \mu\text{mol}/\text{m}^2 \cdot \text{s}$  of light.

Seedling roots were excised and put into a 200 mL flask containing 50 mL of B<sub>5</sub> medium (pH 5.7) with 2.0% sucrose and 2.148  $\mu\text{mol}/\text{L}$  of NAA. The excised roots were cultured in the dark on a rotary shaker ( $(70 \pm 1) \text{ r}/\text{min}$ ) at 25°C and sub-cultured at three week intervals. After six months of sub-culture, the propagated cultures of milk vetch root were stable.

### 1.2 Test to determine the effect of medium composition on the propagation of cultured milk vetch roots and the preparation of culture filtrate extracts

This experiment was designed to determine the effect of medium composition on the propagation of cultured milk vetch roots. The basic medium for the propagation of cultured milk vetch roots was B<sub>5</sub>. Adjustments in all compositions content of the medium were made according to the descriptions in Table 1. There were nine treatments arranged in an orthogonal design<sup>[33]</sup> (Table 2). Each media treatment contained the same components, but the levels of the components varied among treatments.

For each treatment, a 0.1 g (fresh weight) segment of sterile milk vetch root was transferred to a 100 ml Erlenmeyer flask containing 25 ml of improved B<sub>5</sub> medium (pH 5.7). After incubation in a rotary shaker ( $(70 \pm 1) \text{ r}/\text{min}$ ) at 25°C for 18 d, the cultured roots were collected, freeze-dried and weighed. Least significant differences (LSD) in the dry weight of cultured roots were determined using JMP 4.0 software. In addition, the dry weight of the cultured roots in each treatment was compared by range analysis to select the optimum improved medium for the propagation of cultured milk vetch roots.

Used medium from each treatment was vacuum filtered and then partitioned three times with ethyl acetate [culture filtrate: ethyl acetate = 1:1 (V/V)]. The ethyl acetate phase was collected from each kind of culture filtrate and then evaporated to dryness with a rotary evaporator. The dry substance remaining in the evaporation flask will be referred to as the culture filtrate extract throughout the rest of this paper.

### 1.3 Test to determine the effect of culture filtrate extract on seed germination and seedling growth of other plants

The purpose of this part of our experiment was to determine if milk vetch roots had an allelopathic effect on the germination and growth of radish (cv. Xinong-Qingfengdong), wheat (cv. Xiaoyan No. 22) and milk vetch (collected from Guyuan, Ningxia Hui Autonomous Region in October, 2005).

Solutions of the culture filtrate extracts (SCFE) were prepared by dissolving the culture filtrate extracts described in the previous section in a small amount of acetone. Distilled water was added to the samples to bring them up to the same volume as that of the culture filtrate before it was partitioned with ethyl acetate. There were nine SCFEs. A solution containing distilled water was also prepared as a control (CK). This made a total of ten SCFE

表 1 内蒙古锡林河流域 3 个草原类型 4 个退化梯度的 12 个样地的基本情况

Table 1 Geographical and basic information for the 12 sampling sites along the Xilin River Basin of Inner Mongolia

样地名 Sampling site	原生群落类型 Primary steppe type	土壤类型 Soil type	退化强度 Degeneration degree	地理坐标 Geographic coordinate	海拔 (m) Altitude	土地利用类型 Soil usage type	优势种 Dominant species	地上生物量 (g/m <sup>2</sup> ) Biomass productivity
羊草样地 <i>Leymus chinensis</i> plot	羊草典型草原 <i>L. chinensis</i> typical steppe	暗栗钙土 Dark chestnut soil	UD	N 43°33'02.4" E 116°40'30.0"	1277	围封 22a 样地 Enclosed grassland for 22 years	羊草 <i>Leymus chinensis</i> , 羽茅 <i>Achnatherum sibiricum</i> , 大针茅 <i>Stipa grandis</i>	179.16 ± 27.91
			LD	N 43°33'18.9" E 116°40'26.7"	1258	围封轮牧样地 Enclosed and seasonal grazed	大针茅 <i>S. grandis</i> , 糙隐子草 <i>Cleistogenes squarrosa</i> , 羽茅 <i>A. sibiricum</i>	195.48 ± 26.47
			MD	N 43°34'01.0" E 116°40'40.1"	1238	中度放牧 Medium-grazed	大针茅 <i>S. grandis</i> , 糙隐子草 <i>C. squarrosa</i> , 星毛委陵菜 <i>Potentilla acaulis</i>	106.80 ± 20.93
			HD	N 43°34'27.4" E 116°41'17.0"	1239	重度放牧 Heavily-grazed	星毛委陵菜 <i>P. acaulis</i> , 糙隐子草 <i>C. squarrosa</i> , 大针茅 <i>S. grandis</i>	55.39 ± 17.13
大针茅样地 <i>Stipa grandis</i> plot	大针茅典型草原 <i>S. grandis</i> typical steppe	典型栗钙土 Typical chestnut soil	UD	N 43°32'24.5" E 116°33'19.6"	1193	围封 22a 样地 Enclosed grassland for 22 years	大针茅 <i>S. grandis</i> , 羊草 <i>L. chinensis</i> , 黄囊苔草 <i>Carex korshinskyi</i>	137.52 ± 27.65
			LD	N 43°33'59.3" E 116°36'05.6"	1225	围封割草场 Enclosed and harvest grassland	大针茅 <i>S. grandis</i> , 糙隐子草 <i>C. squarrosa</i> , 羊草 <i>L. chinensis</i>	93.18 ± 12.42
			MD	N 43°34'05.4" E 116°36'16.0"	1225	中度放牧 Medium grazing	糙隐子草 <i>C. squarrosa</i> , 大针茅 <i>S. grandis</i> , 星毛委陵菜 <i>P. acaulis</i>	95.07 ± 25.73
			HD	N 43°33'09.2" E 116°33'16.8"	1180	重度放牧 Heavily grazing	糙隐子草 <i>C. squarrosa</i> , 大针茅 <i>S. grandis</i> , 猪毛菜 <i>Salsola nitaria</i>	64.19 ± 9.96
砧子山样地 Zhanzi Shan plot	羊草甸草原 <i>L. chinensis</i> meadow steppe	暗栗钙土 Dark chestnut soil	UD	N 43°27'47.7" E 116°38'18.9"	1288	围封打草场 Enclosed grassland	羊草 <i>L. chinensis</i> , 山葱 <i>Allium L.</i> , 大针茅 <i>S. grandis</i>	148.66 ± 16.6
			LD	N 43°27'06.9" E 116°38'59.6"	1294	轻度放牧 Lightly grazed	羊草 <i>L. chinensis</i> , 糙隐子草 <i>C. squarrosa</i> , 冷蒿 <i>A. frigida</i>	94.14 ± 6.12
			MD	N 43°26'41.5" E 116°39'21.6"	1285	中度放牧 Medium grazed	糙隐子草 <i>C. squarrosa</i> , 羊草 <i>L. chinensis</i> , 冷蒿 <i>A. frigida</i>	76.77 ± 13.94
			HD	N 43°25'40.7" E 116°40'18.3"	1272	重度放牧 Heavily grazed	冷蒿 <i>Atemisia frigida</i> , 星毛委陵菜 <i>P. acaulis</i> , 寸草苔 <i>Carex duriuscula</i>	65.01 ± 8.02

UD: 未退化群落, LD: 轻度退化群落, MD: 中度退化群落, HD: 重度退化群落. 地上生物量用平均值 ± 标准差表示 UD: Un-degenerated habitat, LD: lightly degenerated habitat, MD: moderately degenerated habitat, HD: heavily degenerated habitat. Above-ground biomass productivity was presented as mean ± standard deviation

treatments.

We put double layers of filter paper (9 cm diam.) in the bottom of Petri dishes (10 cm diam.) and then added a 3 mL aliquot from one of the SCFE treatments to the paper<sup>[34]</sup>. Ten radish seeds, or ten wheat seeds, or thirty milk vetch seeds were put into the dishes. Enough dishes were prepared so that each SCFE treatment was applied to 60 seeds/species.

Treated seeds were incubated in darkness at 25°C. Distilled water was added to the dishes as needed to maintain moist conditions. The radish seeds were divided into groups of ten and the germination rate for each group was determined at 12, 16 h and 22 h. Germination rates for wheat and milk vetch were determined in a similar manner except that observations were made at 16, 26, and 40 h. Radish radicle and coleoptile lengths were measured at 48 h; wheat radicle and coleoptile lengths were determined at 64 h; and milk vetch radicle and coleoptile lengths were measured at 94 h. Statistical indices for seed germination and seedling growth were compared using the least significant difference (LSD) test calculated with JMP 4.0 software.

## 2 Results

### 2.1 Effect of growth medium composition on the propagation of cultured milk vetch roots

Results indicated that differences in the nutritional components in B<sub>5</sub> media influenced the dry weight of cultured milk vetch roots after 18 d of incubation in a rotary shaker (Fig. 1).

The numerical analysis showing the effect of different nutrient concentrations in the improved B<sub>5</sub> media on milk vetch roots is given in Table 3. Range comparison for the effect of nutrient concentration on the dry weight of cultured roots declined in the order  $R_F(0.0756) > R_C(0.0582) = R_J = R_L = R_N > R_E(0.0574) = R_I = R_M > R_A(0.0558) > R_G(0.0392) > R_H(0.0364) > R_B(0.0043) = R_D = R_K = R_O$ . This indicated that among all the nutritional components tested in this study,  $Fe^{2+}$  had the greatest effect on the growth of cultured milk vetch roots. The effects of sucrose,  $H_2PO_4^+$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $BO_3^{3-}$ ,  $Co^{2+}$ ,  $I^-$ , and  $C_8H_{12}ClNO_3 + C_{12}H_{18}Cl_2N_4OS + C_6H_5O_2N + C_6H_{12}O_6$  were intermediate, and the effects of N,  $Ca^{2+}$ ,  $MoO_4^{2-}$  and NAA were lowest.

The results also showed that the dry weight of the cultured roots declined in the order  $A_3 > A_2 > A_1$ ,  $B_1 > B_2 > B_3$ ,  $C_1 > C_2 > C_3$ ,  $D_1 > D_2 > D_3$ ,  $E_1 > E_2 > E_3$ ,  $F_1 > F_2 > F_3$ ,  $G_2 > G_1 > G_3$ ,  $H_1 > H_3 > H_2$ ,  $I_1 > I_2 > I_3$ ,  $J_1 > J_2 > J_3$ ,  $K_1 > K_2 > K_3$ ,  $L_1 > L_2 > L_3$ ,  $M_1 > M_2 > M_3$ ,  $N_1 > N_2 > N_3$  and  $O_1 > O_2 > O_3$  (Table 3). From these, we determined that the combination of  $A_3B_1C_1D_1E_1F_1G_2H_1I_1J_1K_1L_1M_1N_1O_1$  [ $3.0\%$  sucrose +  $0.510\text{mmol/L } (NH_4)_2SO_4 + 12.380\text{mmol/L } KNO_3 + 0.550\text{mmol/L } NaH_2PO_4 \cdot H_2O + 0.500\text{mmol/L } CaCl_2 \cdot 2H_2O + 0.510\text{mmol/L } MgSO_4 \cdot 7H_2O + 0.025\text{mmol/L } FeSO_4 \cdot 7H_2O + 62.780\mu\text{mol/L } MnSO_4 \cdot 4H_2O + 0.039\mu\text{mol/L } CuSO_4 \cdot 5H_2O + 1.735\mu\text{mol/L } ZnSO_4 \cdot 7H_2O + 12.095\mu\text{mol/L } H_3BO_3 + 0.258\mu\text{mol/L } Na_2MoO_4 \cdot 2H_2O + 0.026\mu\text{mol/L } CoCl_2 \cdot 6H_2O + 1.130\mu\text{mol/L } KI + 2.432\mu\text{mol/L } C_8H_{12}ClNO_3 + 14.825\mu\text{mol/L } C_{12}H_{18}Cl_2N_4OS + 4.062\mu\text{mol/L } C_6H_5O_2N + 0.278\text{mmol/L } C_6H_{12}O_6 + 1.074\mu\text{mol/L } NAA$ ] was the optimum improved medium for the propagation of cultured milk vetch roots. Compared to standard B<sub>5</sub> medium, the optimum

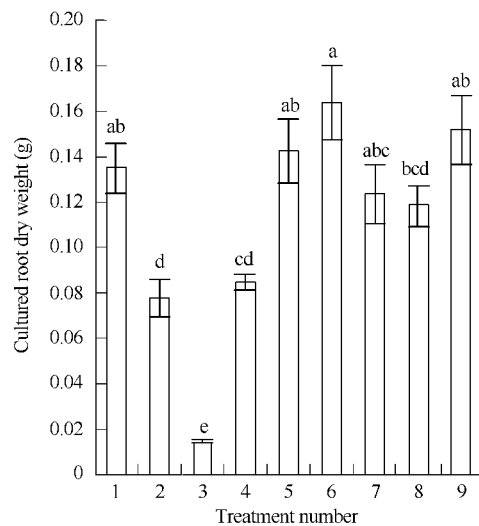


Fig. 1 Effect of nutrient levels in the B<sub>5</sub> medium on the dry weight of cultured milk vetch roots

Data are mean  $\pm$  SE of 6 replications; The same letters indicate no significant difference at  $p = 0.05$  (LSD)

表 1 寿光市不同农业利用方式下土壤铜和锌含量的描述性统计量  
Table 1 Descriptive data of Cu and Zn concentration in soils under various agricultural land use types in Shouguang City through statistical analysis

重金属 Heavy metal	土壤类型 Soil type	样本数 Number of samples	算术 Arithmetic		分布类型 Distribution type	几何 Geometric		最小值 Min.	最大值 Max.	变异系数 C·V(%)	极差 Range
			均值 Mean (mg·kg <sup>-1</sup> )	标准差 Standard deviation (mg·kg <sup>-1</sup> )		均值 Mean (mg·kg <sup>-1</sup> )	标准差 Standard deviation (mg·kg <sup>-1</sup> )				
Cu	设施菜地 Greenhouse vegetable land	62	33.91a *	13.26	正态 Normal distribution	31.72	1.436	13.83	79.50	39.1	65.67
	露天菜地 Uncovered vegetable land	29	24.64b	11.40	正态 Normal distribution	23.34	1.336	15.58	80.39	46.3	64.81
	小麦/玉米/棉花地 Wheat/maize/cotton land	29	22.08b	7.106	正态 Normal distribution	21.07	1.362	13.20	41.72	32.2	28.52
	自然土壤 Natural land	7	21.76b	9.022	正态 Normal distribution	20.29	1.526	11.28	35.58	41.5	24.3
	全体 Total	127	28.57	12.64	对数正态 Logarithm normal distribution	26.43	1.465	11.28	80.39	44.2	69.11
Zn	设施菜地 Greenhouse vegetable land	62	124.2a *	71.39	正态 Normal distribution	115.6	1.387	60.2	268.2	57.5	208.0
	露天菜地 Uncovered vegetable land	29	83.63b	10.11	正态 Normal distribution	81.58	1.144	67.47	111.4	12.1	43.93
	小麦/玉米/棉花地 Wheat/maize/cotton land	29	81.48b	18.78	正态 Normal distribution	79.59	1.241	57.45	131.1	23.0	73.65
	自然土壤 Natural land	7	73.72b	10.93	正态 Normal distribution	73.05	1.160	60.67	82.64	14.8	21.97
	全体 Total	127	103.9	56.1	对数正态 Logarithm normal distribution	80.50	1.257	57.45	627.9	54.0	210.8

\*  $p < 0.05$ ; 不同和相同字母分别表示差异显著和不显著 The same letter means no significant difference, and different letter means significant difference

improved medium contains 3.0% sucrose, 1.074  $\mu\text{mmol/L}$  NAA, plus 1/2 strength macronutrient concentration, 1/4 strength microelement concentration (with the exception of  $\text{MnSO}_4$  which was full strength), and 1/2 strength basic organic supplements. Culturing 0.1 g (fresh weight) of milk vetch roots on the optimum improved medium for 18 d resulted in the production of 0.2538 g (dry weight) roots.

## 2.2 Effect of culture filtrate extracts on seed germination and seedling growth of other plant species

### 2.2.1 Effect of culture filtrate extracts on seed germination of other plant species

Statistical analysis of the germination rate indicated that the SCFE affected seed germination of all three test species (Table 4). However, the effect was not the same for all treatments. The more details can be described as follows:

The SCFE from all treatments inhibited radish germination, but the effect decreased with time. There was no difference among the treatments in radish germination at 22 hours, which suggested that the inhibition effect was disappearing.

Wheat germination was stimulated by SCFE from treatment 1, however, SCFE from treatments 2, 5, 6, and 9 inhibited germination. The SCFE from treatments 3, 4, and 7 initially inhibited wheat germination, but later stimulated germination. Similar to our observation for radish, the effect of SCFE on wheat germination decreased with time. There was little difference in germination rates among the treatments at 40 hours.

The SCFE from treatment 1 sharply stimulated milk vetch germination, but SCFE from all other treatments inhibited milk vetch germination. Large differences in milk vetch germination rates at 40 hours showed that SCFE had a prolonged effect on milk vetch.

### 2.2.2 Effects of culture filtrate extracts on seedling growth of other plant species

Radicle and coleoptile length were measured for each test species to determine the effect of the SCFE on seedling growth. The results are given in Fig. 2, Fig. 3, and Fig. 4.

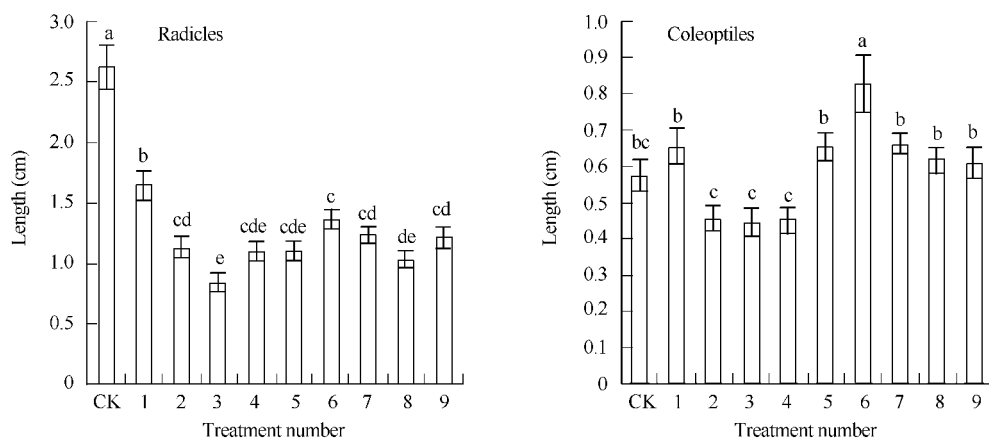


Fig. 2 Effect of SCFE on seedling growth of radish

Data are mean  $\pm$  SE of 60 replications; The same letters indicate no significant difference at  $p = 0.05$  (LSD). The same pattern is used in Fig. 3 and Fig. 4

The SCFE from all treatments inhibited the growth of radish radicles (Fig. 2). In contrast, SCFE from treatments 1, 5, 6, 7, 8, and 9 stimulated the elongation of radish coleoptiles. The SCFE from the other treatments inhibited radish coleoptile elongation.

The growth of wheat radicles (especially the longest radicles) was stimulated by SCFE from treatment 1 (Fig. 3). The SCFE from all other treatments inhibited the growth of wheat radicles. The growth of wheat coleoptiles was

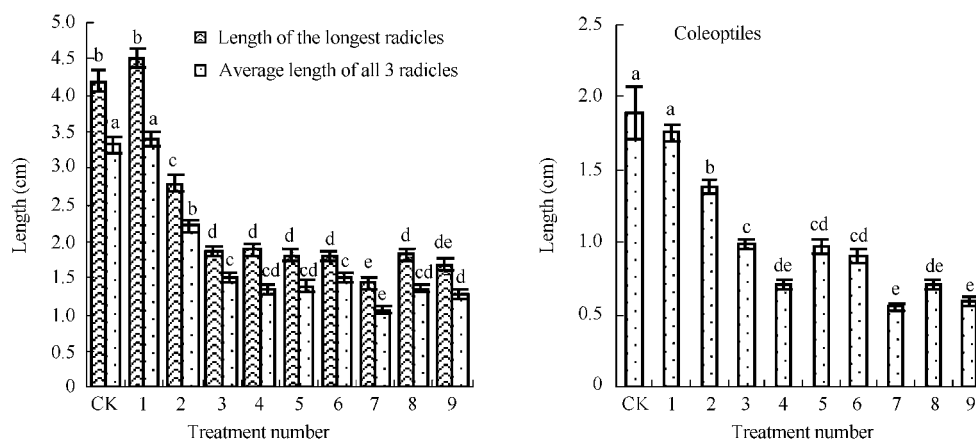


Fig. 3 Effect of SCFE on seedling growth of wheat  
Generally, one seedling of wheat had 3 radicles after 64 h incubation

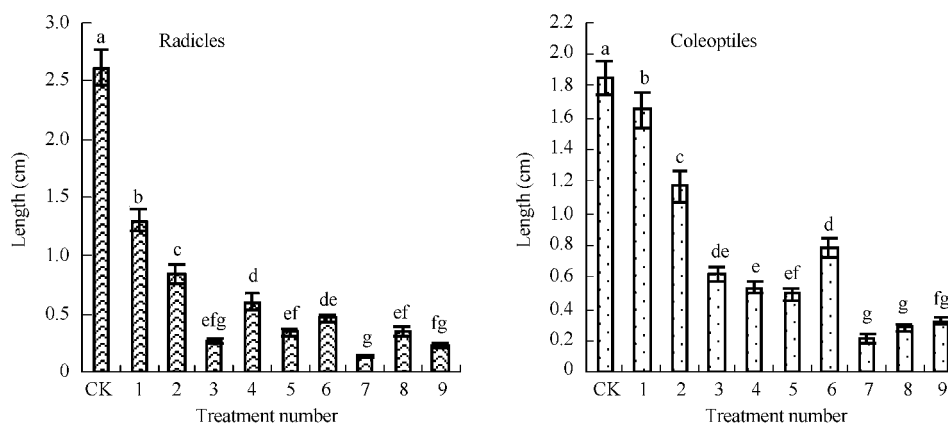


Fig. 4 Effect of SCFE on seedling growth of milk vetch

inhibited by SCFE from all treatments. Wheat seedlings treated with SCFE from treatment 1 had the longest radicles and coleoptiles. In contrast, wheat seedlings treated with SCFE from treatment 7 had the shortest radicles and coleoptiles.

Data in Fig. 4 indicated that SCFE from all treatments inhibited the elongation of milk vetch radicles and coleoptiles. However, the degree of inhibition varied among the treatments. For example, SCFE from treatment 7 had a strong inhibitory effect on both radicle and coleoptile elongation. In contrast, the inhibitory effect of SCFE from treatment 1 on radicle and coleoptile elongation was weak.

### 3 Discussion

#### 3.1 Propagation of cultured milk vetch root and its response to nutrient regulation

Sterile roots cultures are ideal for testing allelopathy in milk vetch. One purpose of this study was to determine the optimum growth medium for the rapid propagation of cultured milk vetch roots. The results suggest that cultured milk vetch roots responded differently to adjustments in the sucrose, macroelement, microelement and organic supplement content in the culture media. Among all nutritional components in the improved B<sub>3</sub> media, Fe<sup>2+</sup> had the strongest effect on milk vetch root culture. This is consistent with results from previous studies. In contrast, the small effect of MoO<sub>4</sub><sup>2-</sup> on the root cultures is different from the results of Jia *et al.* [35]. The success of tissue culture depends primarily on having the correct type and amount of hormone, however organic supplements such as C<sub>8</sub>H<sub>12</sub>ClNO<sub>3</sub>, C<sub>12</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>4</sub>OS, C<sub>6</sub>H<sub>5</sub>O<sub>2</sub>N and C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> also have an important role. These nutritional compounds

were not studied as individual factors in this experiment, but they will be considered in the future.

Although B<sub>5</sub> medium is generally considered to be the best medium for tissue culture of hedysarum plants<sup>[36]</sup>, it was not very effective for the culture of milk vetch roots. Results from this study showed that better results were obtained when the nutrient concentration of standard B<sub>5</sub> medium was adjusted in the following way: 1/2 strength macroelement concentration, 1/4 strength microelement concentration (with the exception of MnSO<sub>4</sub> which was at normal concentration), and 1/2 strength basic organic supplements. In addition, 3.0% sucrose and 1.074 μmmol/L NAA was added to the growth medium. The concentration of sucrose was very high compared to the relatively low concentration of the other basic nutrients in the medium. This indicates that the successful propagation of cultured milk vetch roots depends highly on the carbon source. Rapid propagation of cultured milk vetch roots was possible when the optimal improved media was used. This is significant because it means that the quantity of milk vetch root that can be produced is large enough for the isolation and identification of allelopathic chemicals.

### 3.2 Allelopathic effect of cultured milk vetch roots

Though SCFE influenced germination and seedling growth of the selected species, we can not determine if the effect is due to allelopathic effect. It is possible that residual, organic nutrients in the SCFE could have influenced seed germination and seedling growth. Preliminary results showed that the residual organic nutrient content in all SCFEs was extremely low (data omitted) and therefore probably would not have affected seed germination and seedling growth in this study. However, this needs to be investigated more carefully. In this way, we can determine if the influence of SCFE on seed germination and seedling growth of selected species is an allelopathic effect.

Certainly, some new substances could have been excreted during the course of the root culture. Not all treatments had the same effect. It may be that the nutrient content of the culture medium affected the type or amount of allelopathic chemicals excreted by the cultured roots. This suggests that different nutrient combinations can affect the intensity of the allelopathic effect in milk vetch roots, which is consistent with some previous studies<sup>[37, 38]</sup>. The effects of SCFE on seed germination and seedling growth differed significantly between plants species. This must be related to differences in the biological characteristics among the three test species. In addition, the results suggest that the three species have different tolerances to the allelopathic effect of milk vetch root. The effect of SCFE from the culture of milk vetch root on milk vetch seed germination and seedling growth indicated that this plant has an auto-toxicity effect.

### 3.3 Problems for future research

This work provides important preliminary information regarding the propagation and use of cultured roots for determining allelopathic activity in milk vetch, however several research questions still remain. Specifically, allelopathic chemicals produced by cultured milk vetch roots should be identified in future. Furthermore, allelopathic effects of milk vetch in the natural environment, especially the dynamic variation of allelopathy and the relationship between allelopathy and the environment should be analyzed. The relevance of this study could be enhanced by testing allelopathic activity on receptor species which commonly grow in the same area as milk vetch. In addition, it is important to study the allelopathic effect of milk vetch on some target species (e. g. *Stellera chamaejasme*). The studies described above would provide significant advancement in the study of allelopathy in milk vetch.

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