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 $L_9(3^{15})$ 正交设计,研究了 B_5 培养基营养组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:培养基的所有营养组分中,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^{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, Fe^{2+} had the greatest effect on the propagation 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 nitrogen, Ca^{2+}, 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[1,2], physiology and ecology[3-6], pests and diseases[7-10], heredity and breeding[11,12], cell, tissue and organ culture[13-16], planting techniques and utilization[17, 18]). 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[19].

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[19-21]. 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[22]. Moreover, the establishment of milk vetch enhanced the degree of vegetative cover and altered the community composition of pastures on steep slope[22]. Guan *et al.*[23] observed that short-rooted cereal pastures grew well after milk vetch pastures declined, however, long-rooted legume forages grew poorly. Liu *et al.*[24] 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.*[25] 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[26].

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[27]. Allelopathy in plants is influenced by environmental factors[28], especially soil microorganisms[29,30]. 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[31,32]. 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 alleleopathic 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℃ with (40 ± 2) μmol/m^2·s of light.

Seedling roots were excised and put into a 200 mL flask containing 50 mL of B_5 medium (pH 5.7) with 2.0% sucrose and 2.148 μmol/L of NAA. The excised roots were cultured in the dark on a rotary shaker ((70 ± 1) r/min) at 25℃ 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_5. 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[33] (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_5 medium (pH 5.7). After incubation in a rotary shaker ((70 ± 1) r/min) at 25℃ 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 alleleopathic 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

Table 1　Factors and levels of $L_9(3^{15})$ for the regulation of the nutritional components in B_5 medium

Levels	Factors														
	Sucrose (%)	Nitrogen (mmol/L)	$H_2PO_4^+$ (mmol/L)	Ca^{2+} (mmol/L)	Mg^{2+} (mmol/L)	Fe^{2+} (mmol/L)	Mn^{2+} (μmol/L)	Cu^{2+} (μmol/L))	Zn^{2+} (μmol/L)	BO_3^{3-} (μmol/L)	MoO_4^{2-} (μmol/L)	Co^{2+} (μmol/L)	I^- (μmol/L)	Basic organic supplements	NAA (μmol/L)
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	1.0	13.400	0.550	0.500	0.510	0.025	15.695	0.039	1.735	12.095	0.258	0.026	1.130	α	1.074
2	2.0	26.800	1.100	1.000	1.020	0.100	62.780	0.156	6.940	48.380	1.030	0.105	4.520	β	2.148
3	3.0	40.200	1.650	1.500	1.530	0.200	125.560	0.312	13.880	96.760	2.060	0.210	9.040	γ	3.222

The second level of each component is the same as the standard level of B_5 media; The first and the third level of each macro-element and basic organic supplement is 0.5 and 1.5 strength the standard level, respectively; The first and the third level of each micro-element is 0.25 and 2 strength the standard level, respectively; The nitrogen content is the sum of NH_3^+ and NO_3^- content and the both ratio is 1:12; Basic organic supplements include $C_8H_{12}ClNO_3$, $C_{12}H_{18}Cl_2N_4OS$, $C_6H_5O_2N$, $C_6H_{12}O_6$; α, β, γ represents 2.432 μmol/L $C_8H_{12}ClNO$ + 14.825 μmol/L $C_{12}H_{18}Cl_2N_4OS$ + 4.062 μmol/L $C_6H_5O_2N$ + 0.278 mmol/L $C_6H_{12}O_6$, 4.863 μmol/L $C_8H_{12}ClNO$ + 29.650 μmol/L $C_{12}H_{18}Cl_2N_4OS$ + 8.123 μmol/L $C_6H_5O_2N$ + 0.555 mmol/L $C_6H_{12}O_6$, 7.115 μmol/L $C_8H_{12}ClNO$ + 44.475 μmol/L $C_{12}H_{18}Cl_2N_4OS$ + 12.185 μmol/L $C_6H_5O_2N$ + 0.833 mmol/L $C_6H_{12}O_6$, respectively

Table 2　Orthogonal design of $L_9(3^{15})$ for the regulation of the nutritional components in B_5 medium

Treatment No.	Codes of factors														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
2	(1)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
3	(1)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
4	(2)	(1)	(2)	(1)	(3)	(3)	(1)	(2)	(3)	(2)	(1)	(2)	(3)	(2)	(1)
5	(2)	(2)	(3)	(2)	(1)	(2)	(3)	(1)	(1)	(3)	(2)	(3)	(1)	(3)	(2)
6	(2)	(3)	(1)	(3)	(2)	(1)	(2)	(3)	(2)	(1)	(3)	(1)	(2)	(1)	(3)
7	(3)	(1)	(2)	(1)	(3)	(3)	(1)	(2)	(3)	(2)	(1)	(2)	(3)	(2)	(1)
8	(3)	(2)	(3)	(2)	(1)	(2)	(3)	(1)	(1)	(3)	(2)	(3)	(1)	(3)	(2)
9	(3)	(3)	(1)	(3)	(2)	(1)	(2)	(3)	(2)	(1)	(3)	(1)	(2)	(1)	(3)

The numbers in brackets indicate the levels of individual factors in each treatment

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[34]. 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℃. 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_5 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_5 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.

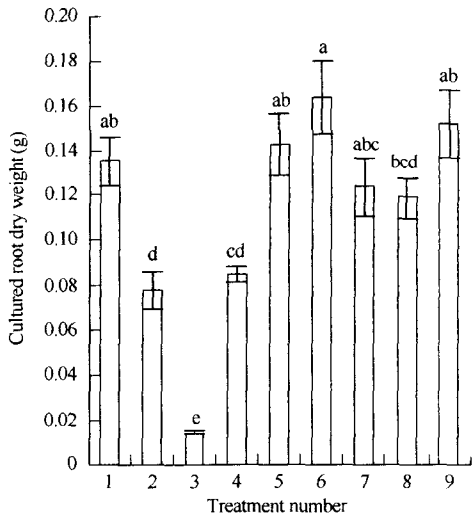

Fig. 1 Effect of nutrient levels in the B_5 medium on the dry weight of cultured milk vetch roots

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

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 + 0.510mmol/L $(NH_4)_2SO_4$ + 12.380mmol/L KNO_3 + 0.550mmol/L NaH_2PO_4 H_2O + 0.500mmol/L $CaCl_2$ $2H_2O$ + 0.510mmol/L $MgSO_4$ $7H_2O$ + 0.025mmol/L $FeSO_4$ $7H_2O$ + 62.780μmol/L $MnSO_4$ $4H_2O$ + 0.039μmol/L $CuSO_4 5H_2O$ + 1.735μmol/L $ZnSO_4$ $7H_2O$ + 12.095μmol/L H_3BO_3 + 0.258μmol/L Na_2MoO_4 $2H_2O$ + 0.026μmol/L $CoCl_2$ $6H_2O$ + 1.130μmol/L KI + 2.432μmol/L $C_8H_{12}ClNO_3$ + 14.825μmol/L $C_{12}H_{18}Cl_2N_4OS$ + 4.062μmol/L $C_6H_5O_2N$ + 0.278mmol/L $C_6H_{12}O_6$ + 1.074μmol/L NAA] was the optimum improved medium for the propagation of cultured milk vetch roots. Compared to standard B_5 medium, the optimum

Table 3　Range analysis for cultured milk vetch root dry weight (g)

Level No.	Codes of factors														
	Sucrose and macro-elements					Micro-elements					Organic supplement				
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*
K_1	0.2272	0.3431	0.4501	0.3431	0.3953	0.4501	0.3431	0.3953	0.3953	0.4501	0.3431	0.4501	0.3953	0.4501	0.3431
K_2	0.3900	0.3383	0.2861	0.3383	0.3931	0.3383	0.3931	0.2861	0.3931	0.2861	0.3383	0.2861	0.3931	0.2861	0.3383
K_3	0.3944	0.3302	0.2754	0.3302	0.2232	0.2232	0.2754	0.3302	0.2232	0.2754	0.3302	0.2754	0.2232	0.2754	0.3302
X_1	0.0757	0.1144	0.1500	0.1144	0.1318	0.1500	0.1144	0.1318	0.1318	0.1500	0.1144	0.1500	0.1318	0.1500	0.1144
X_2	0.1300	0.1128	0.0954	0.1128	0.1310	0.1128	0.1310	0.0954	0.1310	0.0954	0.1128	0.0954	0.1310	0.0954	0.1128
X_3	0.1315	0.1101	0.0918	0.1101	0.0744	0.0744	0.0918	0.1101	0.0744	0.0918	0.1101	0.0918	0.0744	0.0918	0.1101
R	0.0558	0.0043	0.0582	0.0043	0.0574	0.0756	0.0392	0.0364	0.0574	0.0582	0.0043	0.0582	0.0574	0.0582	0.0043

Table 4　Effects of SCFE on seed germination of selected plants

Treatment No.	Germination rate (%)								
	Radish			Wheat			Milk vetch		
	12 h	16 h	22 h	16 h	26 h	40 h	16 h	26 h	40 h
CK	33.33 ±4.69 a	63.33 ±6.58 a	81.67 ±7.34 a	41.67 ±3.28 a	93.33 ±3.83 ab	95.00 ±2.15 ab	16.67 ±3.77 b	33.33 ±4.85 b	53.33 ±4.69 b
1	28.33 ±3.85 ab	53.33 ±6.45 ab	68.33 ±7.15 a	41.67 ±3.67 a	98.33 ±0.75 a	98.33 ±0.25 ab	28.33 ±5.28 a	58.33 ±5.03 a	73.33 ±5.24 a
2	26.67 ±3.54 abc	43.33 ±4.88 bc	68.33 ±6.44 a	20.00 ±1.96 b	83.33 ±5.44 abc	93.33 ±2.86 ab	3.33 ±0.44 c	6.67 ±0.72 cd	21.67 ±3.06 de
3	20.00 ±3.12 bcd	45.00 ±5.14 bc	70.00 ±7.05 a	10.00 ±0.73 bc	65.00 ±5.39 def	98.33 ±0.84 ab	0.00 ±0.00 c	6.67 ±0.54 cd	38.33 ±4.32 bcd
4	10.00 ±1.68 d	25.00 ±3.23 d	68.33 ±6.87 a	18.33 ±2.15 b	78.33 ±6.24 bcd	98.33 ±0.58 b	1.67 ±0.37 c	3.33 ±0.26 cd	16.67 ±20.3 e
5	20.00 ±3.63 bcd	40.00 ±5.36 bcd	71.67 ±7.22 a	1.67 ±0.21 c	68.33 ±7.08 cde	90.00 ±3.87 ab	0.00 ±0.00 c	1.67 ±0.13 d	40.00 ±4.23 bcd
6	16.67 ±3.09 cd	33.33 ±4.12 cd	71.67 ±6.39 a	11.67 ±1.05 bc	66.67 ±6.52 de	91.67 ±3.33 ab	5.00 ±0.61 c	11.67 ±1.34 c	46.67 ±3.87 bc
7	16.67 ±3.26 cd	35.00 ±4.78 cd	76.67 ±7.57 a	6.67 ±0.82 bc	56.67 ±6.47 efg	99.36 ±0.36 a	1.67 ±0.23 c	3.33 ±0.39 cd	28.33 ±3.15 cde
8	13.33 ±2.52 d	28.33 ±3.51 cd	73.33 ±6.55 a	0.00 ±0.00 c	50.00 ±5.25 fg	95.00 ±2.06 ab	1.67 ±0.15 c	1.67 ±0.15 cd	28.33 ±2.50 cde
9	11.67 ±2.16 d	23.33 ±3.33 d	68.33 ±5.66 a	3.33 ±0.26 c	41.67 ±4.67 g	93.33 ±2.45 ab	0.00 ±0.00 c	3.33 ±0.38 cd	25.00 ±3.34 de

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

improved medium contains 3.0% sucrose, 1.074 μmmol/L NAA, plus 1/2 strength macronutrient concentration, 1/4 strength microelement concentration (with the exception of $MnSO_4$ 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.

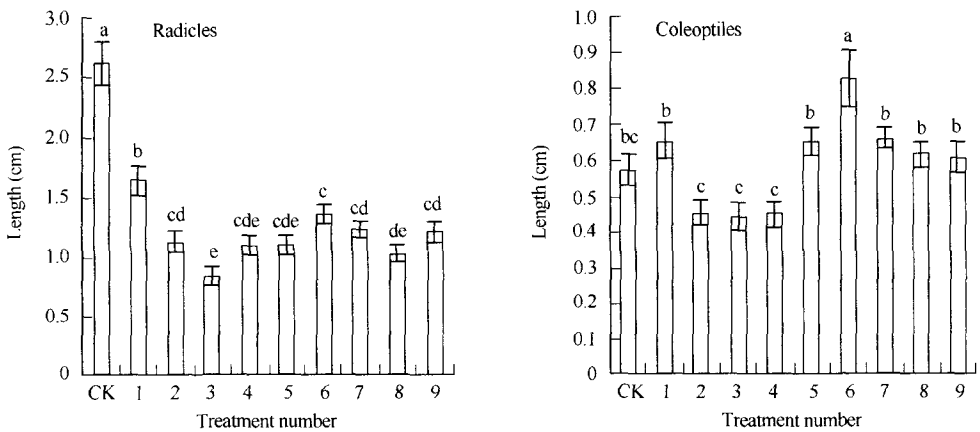

Fig. 2 Effect of SCFE on seedling growth of radish

Data are mean ± 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

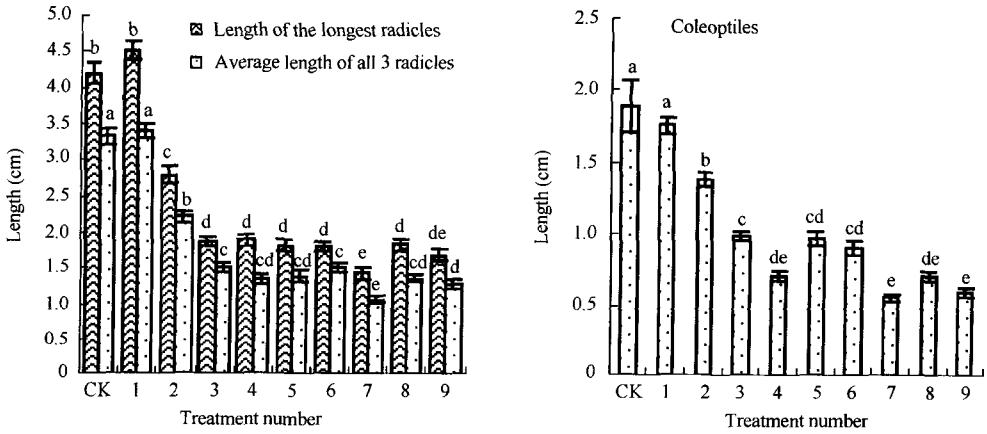

Fig. 3　Effect of SCFE on seedling growth of wheat

Generally, one seedling of wheat had 3 radicles after 64 h incubation

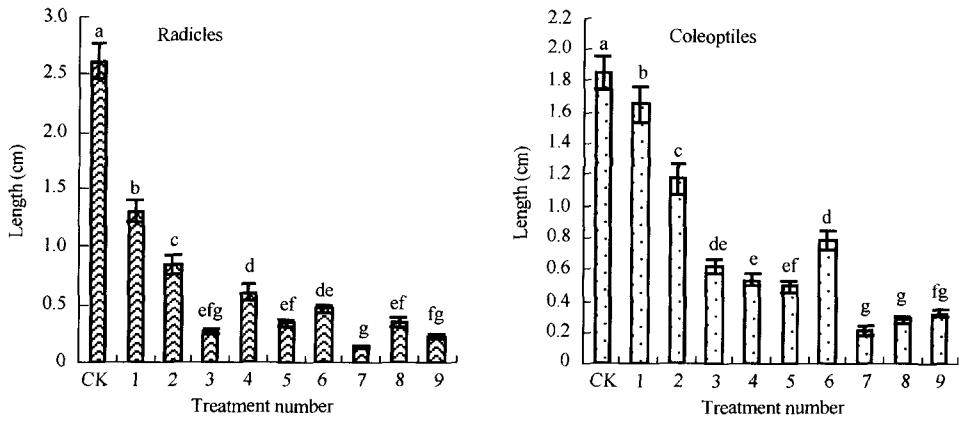

Fig. 4　Effect of SCFE on seedling growth of milk vetch

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_5 media, Fe^{2+} had the strongest effect on milk vetch root culture. This is consistent with results from previous studies. In contrast, the small effect of MoO_4^{2-} 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 $C_8H_{12}ClNO_3$, $C_{12}H_{18}Cl_2N_4OS$, $C_6H_5O_2N$ and $C_6H_{12}O_6$ 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_5 medium is generally considered to be the best medium for tissue culture of hedysarum plants[36], 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_5 medium was adjusted in the following way: 1/2 strength macroelement concentration, 1/4 strength microelement concentration (with the exception of $MnSO_4$ 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 alleolopathic 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[37, 38]. 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 reseach 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 allelolpathic 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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