Chemical Components and Hypoglycemic Activity of Large Scale Micropropagated *Cremastra Appendiculata* (D.Don) Makino

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Research Article

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Abstract

Cremastra appendiculata (D. Don) Makino (Orchidaceae) is a valuable species which is in ornamental field and pharmaceutical industry, inefficient propagations limit the utilization of C. appendiculata. Here, we reported an improved and efficient method for large-scale micropropagation including seed germination, initiation and proliferation of protocorm like-bodies (PLBs), “shoot cluster” induction, rooting and acclimatization. Factually, the highest shoot multiplication coefficient 3.16 was achieved on 1/4 MS medium supplemented with 1.5 mg·L -1 thidiazuron (TDZ), along with 0.4 mg·L -1 indole-3-acetic acid (IAA), 3% sucrose and additives. Likewise, the best medium for protocorm proliferation was 1.0 mg·L -1 indole-3-butyric acid (IBA), 1.0 mg·L -1 6-benzyladenine (6-BA) and 0.2 mg·L -1 adenylate cyclase (AC) with proliferation of 170.07%, followed by root induction on 1/4MS with 0.5 mg·L -1 α naphthaleneacetic acid (NAA), 0.5 mg·L -1 AC and 3% sucrose. Besides, the achieved micropropagation plants grew vigorously and were steady with a survival rate of 85.3% in vermiculite: humus (1: 2). Assessment of the chemical compositions by high performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS) displayed that the composition of dried pseudobulbs from micropropagation plantlets and naturally grown plants were similar, containing colchicine, quercetin, rutin, adenosine, oleanolic acid, myristate, rhein and emodin. Furthermore, the hypoglycemic activity in vitro indicated micropropagation plantlets were superior to naturally grown plants. Consequently, the described protocol could be used for the large-scale propagation and conservation of this endangered plant, which might be natural resource for the diabetes patients.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and accessed as a PDF.

Tables

Table 1

Characterization of nine compounds in *C. appendiculata*.by UPLC-ESI-MS.

Compound	RT (min)	ESI ion mode	Precursor ion (m/z)	Molecular weight	Molecular formula	Identified compounds
1	1.05	+	268	267	$C_{10}H_{13}N_5O_4$	adenosine[a]
2	2.15	-	609	610	$C_{14}H_{28}O_2$	rutin[a]
3	5.69	+	400	399	$C_{22}H_{25}NO_6$	colchicine[a]
4	6.12	-	301	302	$C_{15}H_{10}O_7$	quercetin[a]
5	11.75	-	269	270	$C_{15}H_{10}O_5$	emodin[a]
6	13.37	-	253	254	$C_{15}H_8O_6$	rhein[a]
7	14.00	-	283	284	$C_{16}H_{12}O_5$	physcion[a]
8	15.37	-	227	228	$C_{14}H_{28}O_2$	myristate[a]
9	15.53	-	455	456	$C_{30}H_{48}O_3$	oleanolic acid[a]

Note: RT, retention time. [a] Compared with reference standards.

Table 2

Enzyme inhibitory activities of micropropagation plantlets and naturally grown plants of *C. appendiculata*.

Samples	α-amylase inhibitory (IC50 mg·mL^{-1})	α-glucosidase inhibitory (IC50 mg·mL^{-1})
Micropropagation plantlets	0.3796 ± 0.19 b	0.4253 ± 0.23 b
Naturally grown plants	0.4221 ± 0.38 a	0.5776 ± 0.26 a
Acarbose	0.3479 ± 0.03 c	0.3382 ± 0.04 c

Note: The values followed by different letters within columns are significantly different from each other at 5% level, and values expressed are means ± SE. of three parallel measurements. According to the Tukey's honestly significant difference post hoc test at 5% significance level. IC50 inhibition concentration (mg·mL^{-1}) at which 50% of the α-amylase and α-glucosidase activities were inhibited.

Figures

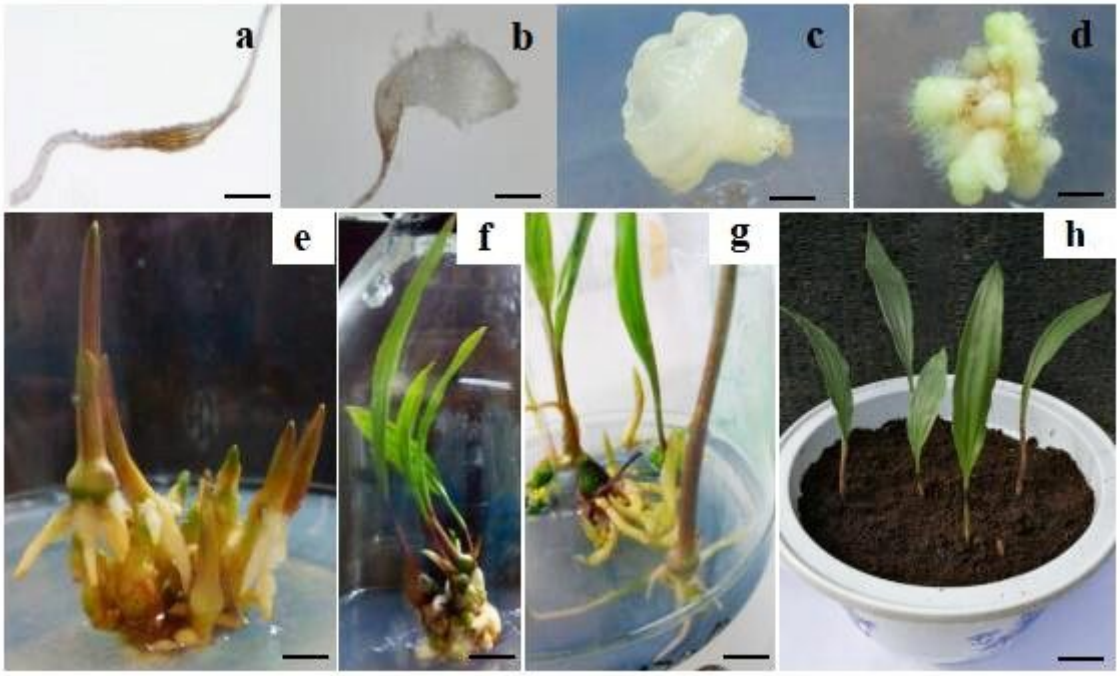

Figure 1

Plant regeneration stages of C. appendiculata. (a) Seed with embryo at pre-germination stage (bar 10 µm). (b) Embryo breasted seed coats (bar 5 µm). (c) Protocorm formation (bar 1 mm). (d) Proliferation protocorms (bar 1 cm). (e) “shoot cluster” (bar 1 cm). (f) “shoot cluster” with stretching leaves (bar 3 cm). (g) Seedling with roots (bar 1 cm). (h) Transplanted seedlings (bar 3 cm).

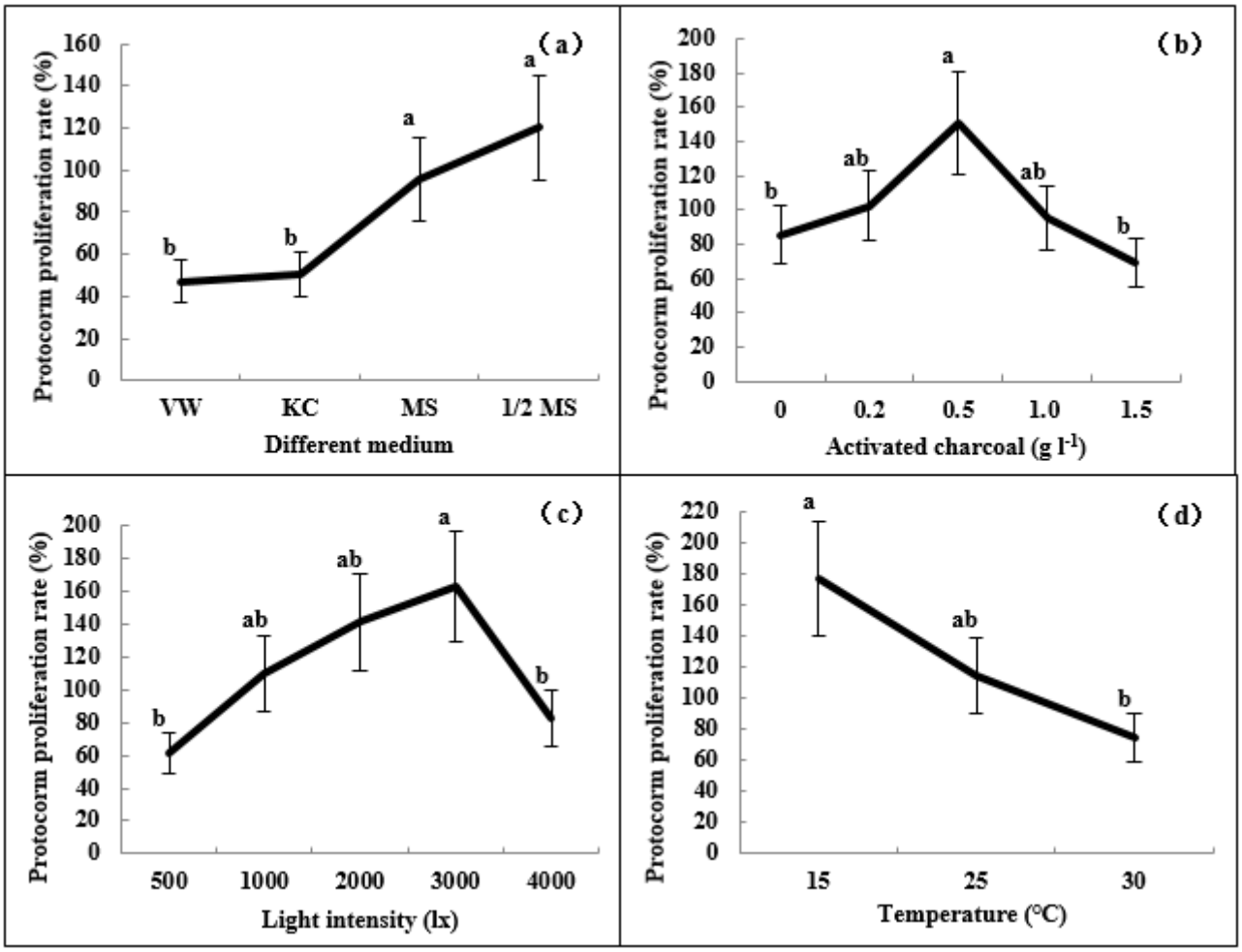

Figure 2

Effect of different medium (a), activated charcoal (b), light intensity (c) and temperature (d) on proliferation rate of C. appendiculata.

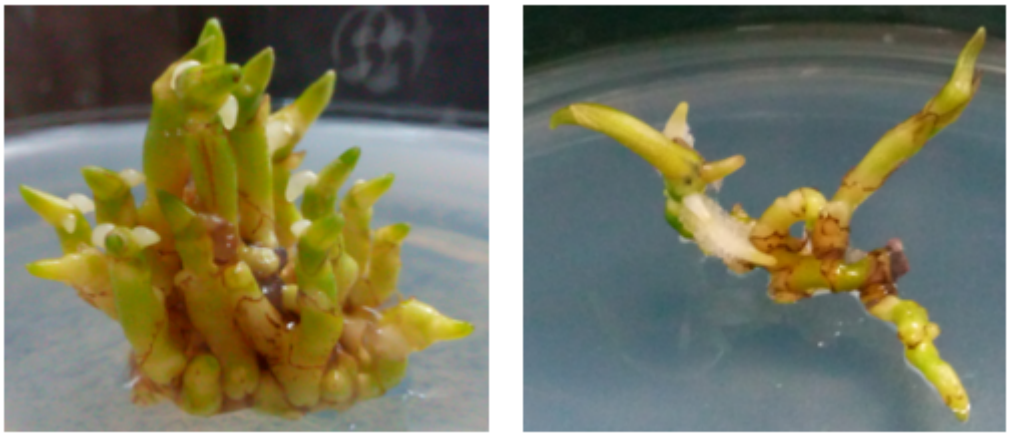

Figure 3

Different shoots of C. appendiculata. (a) Long internodes shoots. (b) Shorter internodes “shoot cluster”.

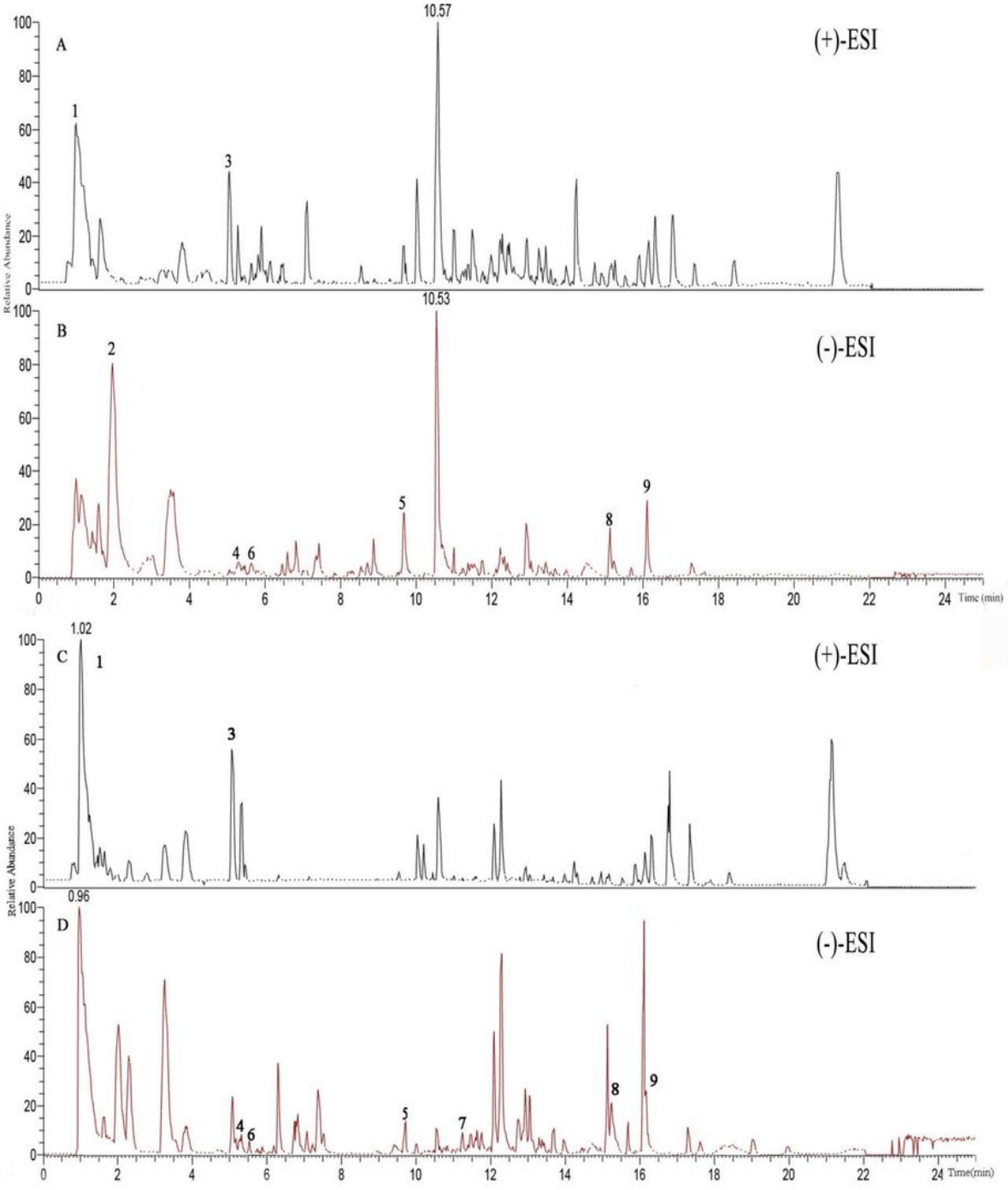

Figure 4

HPLC-ESI-MS of nine components of C. appendiculata. Micropropagation plantlets at positive ion mode (A), and negative ion mode (B). Naturally grown plants at positive ion mode (C) and negative ion mode (D). Compound 1: adenosine (m/z = 268, (+) ES, 1.60E8). Compound 2: rutin (m/z = 609, (-) ES, 2.02E8). Compound 3: colchicine (m/z = 399, (+) ES, 5.70E9). Compound 4: quercetin (m/z= 301, (-) ES, 1.96E7). Compound 5: emodin (m/z = 269, (-) ES, 2.00E6). Compound 6: rhein (m/z = 253, (-) ES, 8.27E6).

Compound 7: physcion (m/z = 283, (-) ES, 1.23E7). Compound 8: myristate (m/z = 227, (-) ES, 1.08E6). Compound 9: oleanolic acid (m/z = 455, (-) ES, 1.87E6).